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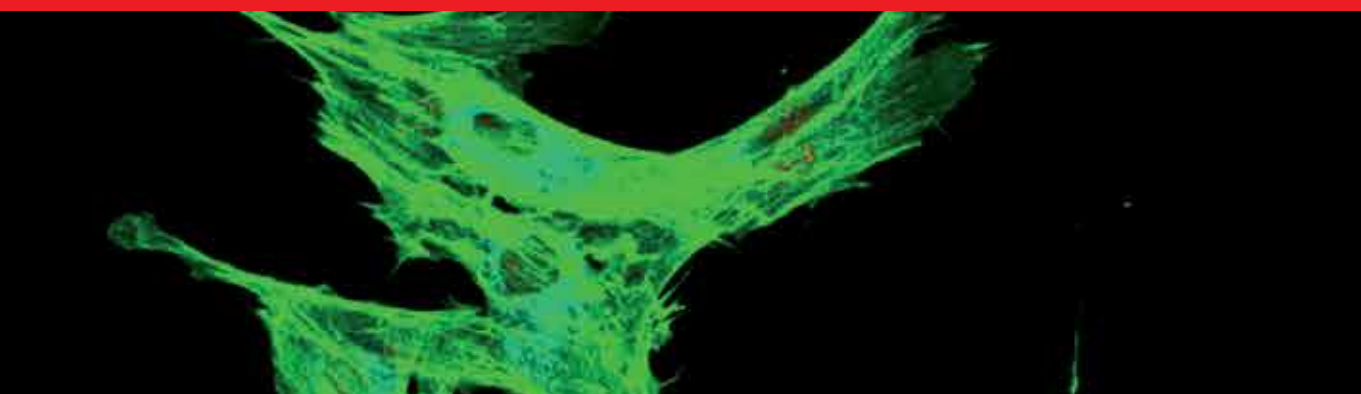
Pancreatic Cancer

Insights into Molecular Mechanisms and Novel Approaches to Early Detection and Treatment

Edited by Kelly McCall



PANCREATIC CANCER - INSIGHTS INTO MOLECULAR MECHANISMS AND NOVEL APPROACHES TO EARLY DETECTION AND TREATMENT

Edited by **Kelly McCall**

Pancreatic Cancer - Insights into Molecular Mechanisms and Novel Approaches to Early Detection and Treatment
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Contributors

Emmanuelle Meuillet, Mary Jo Cantoria, Hitendra Patel, Laszlo Boros, Radu Albulescu, Cristiana Pistol-Tanase, Shiro Urayama, Frank Schwartz, Anthony L Schwartz, Kelly D. McCall, Volker Ellenrieder, Elisabeth Hessmann, Andrada Seicean

Notice

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Meet the editor

Dr. Kelly D. McCall is an Associate Professor of Endocrinology at the Ohio University Heritage College of Osteopathic Medicine specializing in Toll-Like Receptor involvement in the pathogenesis of cancer and autoimmune/inflammatory diseases. Dr. McCall received a BS in Biological Sciences and a PhD in Molecular and Cellular Biology from Ohio University and did her postdoctoral work with world-renowned physician-scientists Drs. Leonard Kohn, MD and Frank Schwartz, MD, FACE. Dr. McCall has published more than 37 peer-reviewed scientific manuscripts, chapters, and abstracts, is an active member of multiple eminent Associations and Societies, and serves as a reviewer for various distinguished scientific journals. Dr. McCall was recently honored by TechGrowth Ohio as the 2013 recipient of the Outstanding Woman in Innovation Award.

Contents

Preface

Pancreatic cancer is difficult to diagnose early, treat effectively, and has a particularly poor prognosis, making it one of the most lethal forms of human cancers. In fact, pancreatic cancer is currently the fourth leading cause of cancer-related deaths in the United States. Despite its steady increasing incidence, the underlying molecular mechanisms that contribute to the development of this devastating malignancy are still largely unknown. Consequently, there is a considerable effort currently underway to uncover the molecular basis for the development and progression of pancreatic cancer.

The major purpose of this book is to provide the reader with a better understanding of the underlying molecular mechanisms and novel linkages that contribute to pancreatic carcinogenesis and metastasis, the identification of novel approaches for improved earlier detection of pancreatic cancer, and evidence for a potentially novel approach to treatment.

This book presents an update on recent findings related to the underlying molecular mechanisms responsible for the development and progression of pancreatic cancer. This book also informs the reader about the link between obesity and pancreatic cancer and how Metformin, a well-known and widely used anti-diabetic drug, may also be a novel approach to the treatment of pancreatic cancer. Moreover, insight into novel approaches for improved earlier detection of pancreatic cancer are also found herein.

As with previous books in this series, we hope that this book will be informative and useful to patients, scientists, clinicians and others, and that it will serve as an invaluable source of information in the quest for a better understanding of the basic biology and clinical approaches to the earlier diagnosis and novel approaches to the treatment of pancreatic malignancies.

Kelly D. McCall, PhD
Department of Specialty Medicine
Ohio University Heritage College of Osteopathic Medicine
Athens, Ohio
USA

Section 1

Molecular Mechanisms

Chapter 1

The Molecular Frame of Pancreatic Carcinogenesis

Elisabeth Heßmann, Sandra Baumgart,
Nai ming Chen, Shiv Singh, Garima Singh,
Alex König, Albrecht Neeße and Volker Ellenrieder

Additional information is available at the end of the chapter

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1. Introduction

Annually, approximately 43,140 people are diagnosed (incidence 10-12/100000) with pancreatic ductal adenocarcinoma (PDAC) in the United States and the mortality rate of 36800 almost equals this number [1]. PDAC ranks fourth on the list of cancer-related causes of death and despite extensive scientific and clinical effort, the prognosis of this exceptionally lethal disease has not improved significantly over the past decades [2]. Surgical resection, for which only a minority (less than 20%) of the patients qualify due to late diagnosis in advanced stages, is currently the only chance of cure, improving 5-year survival rates from <4% if left untreated to 20-30% after resection [3]. Unresectable tumors are characterized by early invasion and metastases as well as by an extreme chemoresistance. Despite subtle progress over the years in terms of therapeutic strategies in many malignancies, no major conventional treatment options have come forward from numerous clinical trials in pancreatic cancer.

Considering its bad prognosis much effort was put into revealing the hidden secrets of pancreatic cancer that explain the severity of this disease. Among the different fields of tumor biology in pancreatic cancer research, this chapter will focus on the morphological and molecular features that cause and accompany pancreatic carcinogenesis.

2. Morphological features of pancreatic carcinogenesis

Although there was little improvement in pancreatic cancer treatment during the past decades, much effort has been made in understanding the pathogenesis of pancreatic cancer. In contrast to its rapid progress after diagnosis, recent published data clearly show that the clonal

evolution of the earliest alterations in cancer initiating cells towards frankly invasive and metastasized PDAC takes at least more than a decade [4, 5]. This creates an important window of opportunity for early detection and much effort is put into attempts to map the molecular and morphological changes resulting in pancreatic cancer formation.

The current model of pancreatic carcinogenesis describes a stepwise process from healthy acinar cells to frank pancreatic adenocarcinoma: Recent lineage-tracing studies have shown that acinar cells harboring molecular alterations are induced to transdifferentiate, generating duct-like cells through a process known as acinar-to-ductal metaplasia (ADM) [6]. ADM lesions then convert to precancerous pancreatic intraepithelial neoplasia (PanIN) that progress to PDAC over time [7]. PanIN lesions are found in the smaller pancreatic ducts and are classified in four grades based on the degree of dysplasia reflected in the cytonuclear atypia and architectural change of the epithelial cell: PanIN-1A, -1B, -2 and -3 [7]. The lowest grade PanIN lesions can be flat (-1A) or papillary (-1B), but are characterized by absence of nuclear atypia and retained nuclear polarity. PanIN-2 lesions show micropapillary features with evidence of nuclear atypia and infrequent mitoses. PanIN-3 lesions demonstrate all hallmarks of cancer, including a widespread loss of polarity, nuclear atypia and frequent mitoses and are considered as *Carcinoma in situ* [1, 8]. Yet, the lesion is confined within the basement membrane and no invasive growth is present. The increasing grades of dysplasia in the various PanIN lesions manifest the morphological steps of tumor progression that precede invasive PDAC. These consecutive steps of tumor progression are accompanied by a cumulative occurrence of molecular alterations.

3. Molecular characteristics of pancreatic carcinogenesis

3.1. Genetic alterations in pancreatic carcinogenesis

For many decades pancreatic cancer was described as an exclusively genetic disease. In 2008 Jones and colleagues discovered 1561 somatic gene mutations within more than 20000 analyzed genes, yielding an average rate of 63 genetic abnormalities per pancreatic cancer, emphasizing the extreme complexity of this disease [9]. These genetic alterations can be clustered in 12 partially overlapping signaling pathways (compare Fig. 1). Five of the pathways comprise specific cellular functions: apoptosis, DNA-damage repair, G1/S phase cell cycle progression, cell-cell adhesion and invasion.

Apoptosis or programmed cell death, plays an essential role in carcinogenesis since resistance to apoptosis is a key factor of the survival of a cancer cell [1]. In PDAC, genes implicated in the apoptosis pathway (Bcl2, Mcl-1, p53, NF-kB among others) were found altered in all tumors studied and many reports document impaired apoptotic signaling in this disease [10, 11]. For example, a high fraction of apoptotic cells has been correlated with longer overall survival as well as absence of nodal involvement [12]. Moreover, resistance to chemotherapeutics is mostly a result of defective apoptosis pathways.

DNA damage control genes code for proteins that repair any damage that occurs in the cell during its lifespan and thus are responsible for safeguarding the integrity of DNA [1]. For

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instance, the BRCA2 protein is involved in DNA damage repair, especially after occurrence of interstrand brakes [13]. Germline BRCA2 gene mutations are responsible for approximately 10% of familial pancreatic cancers [14]. Mismatch repair family (MMR) genes target base substitution mismatches as well as intersection deletions that arise as a result of errors occurring regularly during replication. Alterations in these mismatch repair genes lead to genetic instability and make the genome vulnerable for additional, more severe genetic alterations [15].

One of the most important and best studied proteins involved in DNA damage repair is the tumor suppressor protein p53. p53 is responsible for the cellular response to genotoxic stress as it mediates apoptosis and cell cycle arrest [16]. p53 is frequently disrupted in many human malignancies and the tumor suppressor is lost in 50-75% of PDACs [17].

Cell cycle regulation and progression is affected in virtually all transformed pancreatic cells. Enhanced activation of genes promoting G1/S-phase transition or loss of cell cycle inhibitors results in uncontrolled cell division which facilitates tumor progression and unrestrained tumor growth [1].

In normal pancreatic tissue, cells are anchored to each other and their surroundings via multiple connections. A decrease in these interactions can allow cells to detach from their surroundings and allows transformation, migration and metastasis. As such, cell to cell adhesion and interaction plays an important role in carcinogenesis [18, 19].

The other pathways discovered by Jones and colleagues which proofed to be frequently affected by genetic alterations in pancreatic cancer are signaling cascades that can be divided into three groups: embryonic signaling pathways, MAPKinase signaling pathways and TGFß-signaling pathways [9]. The transforming growth factor ß (TGFß) pathway has been linked to PDAC for many years. TGFß signaling is involved in a wide range of cellular processes including differentiation, proliferation, apoptosis and angiogenesis [20]. As discussed in detail later in this chapter, TGFß signaling functions as a double-edged sword as it comprises tumor-suppressive as well as oncogenic qualities.

All MAPK signaling pathways consist of the same basic kinase components. Stimulation of an upstream MAP2K kinase by growth factors, stress or other extracellular signals leads to phosphorylation of one of the terminal MAPK: extracellular signal-regulated kinase (Erk), c-Jun N-terminal kinase (JNK) or p38 [8]. These signaling cascades result in the activation of multiple oncogenic cellular functions.

One growth factor receptor responsible for many signaling events in early carcinogenesis is the Epidermal Growth Factor Receptor (EGFR). EGFR is located in the cell membrane and is activated by binding of its specific ligands, including epidermal growth factor (EGF) and Transforming Growth Factor alpha (TGFα) [6, 21]. Upon activation, EGFR undergoes dimerization, thus stimulating its intrinsic intracellular protein-tyrosine kinase activity resulting in autophosphorylation of several tyrosine residues in the C-terminal region of the receptor. This autophosphorylation elicits activation of numerous downstream kinases and signal transduction cascades that modulate cancer associated phenotypes as cell proliferation, migration and adhesion [6]. Recent work has proven a high impact of EGFR signaling on induction of

pancreatic metaplasia, and overexpression of the receptor already occurs in early pancreatic precursor lesions [6, 21]. The relevance of EGFR dependent signal cascades was emphasized by a therapeutic beneficial effect of the EGFR inhibitor Erlotinib in a subgroup of pancreatic cancer patients [22].

Since embryogenesis shares many characteristics with carcinogenesis, not surprisingly many embryonic pathways are involved in tumor development. The three embryonic pathways operative in pancreatic carcinogenesis are the Hedgehog-, Notch- and Wnt-signaling cascades [9]. Several studies have shown upregulation of these pathways during pancreatic carcinogenesis and in invasive pancreatic cancer and their inhibition results in decreased tumor proliferation and enhanced apoptosis [1]. For instance, activation of the Notch signaling pathway is involved in cell proliferation and angiogenesis in a variety of human cancers, including pancreatic cancer [23]. Notch signaling is initiated when Notch ligand binds to its receptor between adjacent cells. Upon activation, Notch is cleaved and releases the Notch intracellular domain (NICD) via a cascade of proteolytic enzymes including Y-secretase. Finally, NICD translocates into the nucleus and activates its target genes such as Hes-1, Hey-1, Cyclin D1 and cMyc [24]. Additional to its growth promoting functions accumulating evidence shows a molecular link between Notch and epithelial-to-mesenchymal transition (EMT) in pancreatic cancer [25]. During the EMT process, epithelial cells gain a mesenchymal phenotype accompanied by the cumulative expression of the mesenchymal markers Vimentin, Slug, Snail and ZEB1 and reduced expression of the epithelial marker E-cadherin. EMT-type cells harbor an increased migratory and invasive capacity resulting in invasion and spread of tumor cells even during early carcinogenesis [26]. Inhibition of Notch-signaling leads to reduction of EMT resulting in a better clinical outcome [25].

Similar to Notch-signaling, the hedgehog pathway belongs to the developmental programs of pancreatogenesis. The hedgehog gene was originally identified in Drosophila when a large-scale screening for mutations revealed an altered segmentation pattern of larvae, resulting in a short, fat larva covered in a “lawn” of denticles resembling a hedgehog [27]. Early in development, around embryonic day 8.5-9.0, the hedgehog ligands Indian Hedgehog (Ihh) and Sonic hedgehog (Shh) are expressed throughout the endodermal epithelium of the primitive gut but are noticeably absent in the developed organ [28]. Sonic hedgehog signaling is reactivated in the case of pancreatic regeneration, for example in response to inflammation-associated pancreatic injury [29]. Through inappropriate activation of these pathways, chronic injury might contribute to misdirection of tissue repair, ultimately resulting in neoplasia. Aberrant expression of members of the hedgehog-pathway in chronic pancreatitis and pancreatic carcinogenesis was first noted by Kayed and colleagues [30]. Subsequent research proved that the ligand Shh is expressed aberrantly in pancreatic cancer and its precursor lesions and that Shh functions as a mediator of cancer initiation and growth [31]. Mice with transgenic misexpression of Shh in the pancreatic endoderm develop lesions resembling PanIN, and hedgehog inhibition induces apoptosis and blockes proliferation in pancreatic cancer cells *in vivo* and *in vitro* [31]. Thus, hedgehog signaling can be described as an early and late mediator of pancreatic ductal adenocarcinoma.

Conceptually, these data suggest that pancreatic cancer is substantially a disease of pathways. But research into these pathways rendered clearly that these cascades must ultimately engage the function of epigenetic regulators to influence gene expression in a heritable manner. Thus studies into epigenetics in pancreatic cancer demonstrate a logical extension to the genetic paradigm of this malignant disease.

Signaling pathway	Affected genes
Apoptosis	p53, NF-ϰB, PI3K/Akt
DNA damage repair	p53, BRCA2, MMR-genes
G1/S transition	$p16^{Ink4a}$, $p14^{arf}$, $p15^{Ink4b}$, Cyclin D
Regulation of invasion	TGFß, Integrin signaling
Embryonic signaling	Notch, Hedgehog, Wnt
MAPK signaling	Erk, Jnk, p38
TGFß signaling	TGFß, Smad-proteins

Figure 1. The commonly altered signaling pathways in PDAC accompanied by affected genes from these pathways (adopted from [1]).

4. Epigenetic mechanisms in pancreatic carcinogenesis

Epigenetics are defined as any heritable genomic mechanism unrelated to changes in the DNA sequence [32]. Epigenetic modifications are involved in normal cellular development and maintenance, but they are also responsible for deregulation of gene expression, resulting in diseased cellular phenotypes. Most notably, deregulation of epigenetic mechanisms can contribute to cancer development [33-38]. The past years have witnessed an explosive increase in our knowledge about epigenetic features in pancreatic carcinogenesis. Several well-known epigenetic mechanisms are active in pancreatic cancer, sub-divided into DNA methylation, histone modification and microRNAs, all of them affecting the cell by induction or suppression of gene expression [39-42]. For instance, the introduction of genome-wide screening techniques has accelerated the discovery of a growing list of genes with abnormal methylation patterns in the transforming pancreatic epithelial cell that play a role in the neoplastic process [43]. Hypermethylation of promoter cytosine-phospho-guanine (CpG) islands is closely linked to gene silencing and loss of tumor suppressor function in many cancer entities [44]. Since the first detailed analysis of DNA hypermethylation in pancreatic cancer was reported in 1997 by Schutte et al., many tumor-suppressor or cancer-related genes that undergo aberrant methylation during pancreatic cancer development have been identified, including APC, RUNX3, SOCS-1, $p16^{Ink4a}$, Cyclin D2 and CHD13 [44, 45].

By influencing the structure of chromatin, in addition to DNA methylation, posttranslational modifications of histone tail residues highly affect the transcriptional activity of genes. While acetylation of histones is primarily associated with transcriptional activation, methylation of histones can lead to both, activation and repression, depending on the modified residue [46,

47]. For instance, Polycomb proteins, which are known for their crucial role in induction of repressive histone modifications, embody oncogenic properties in many human cancers. Polycomb proteins can be divided into two functional biochemical categories, Polycomb repressive complexes (PRC) 1 and 2. While members of the PRC 2 complex initiate gene repression by catalysation of H3K27 trimethylation, proteins belonging to PRC1 maintain the repressive state [48, 49]. Under physiological conditions, the activity of Polycomb proteins is crucial in development as well as in maintenance and proliferation of pluripotent progenitor cells in a variety of tissues. Overexpression of these proteins may promote tumorigenesis by fostering a self-renewing population of cells [50, 51]. Indeed, overexpression of Polycomb proteins is responsible for malignant progression and poor prognosis in breast [52], bladder [53] and prostate [54] cancer and shows strong association with hallmarks of cancer, including induced cellular proliferation [55], angiogenesis [56], survival [57] and migration [58]. Enhancer of Zeste Homolog 2 (EZH2) is the only PRC2 protein member thus far studied in pancreatic cancer. Strong nuclear accumulation of EZH2 was found in 55% of well differentiated tumors and 98% of poorly differentiated samples in a comprehensive immunohistochemical analysis of PDACs, indicating a significant correlation between EZH2 expression and dedifferentiation in pancreatic cancer [59]. Additionally, EZH2 overexpression participates in epithelial-to-mesenchymal transition (EMT) and invasion through repression of epithelial proteins like E-cadherin [60].

The third group among the epigenetic players in pancreatic carcinogenesis comprises the MicroRNA (miRNA) family, a class of small non-protein coding RNAs which participate in post-transcriptional control of gene expression in eukaryotic organisms [61]. In the last years, advanced global screening technologies have enabled large scale analyses of miRNA profiles in diverse tissue samples, indicating that miRNAs can function as either oncogenes or tumor suppressors in the development of various cancer types, including pancreatic cancer [62, 63]. The analysis of miRNA expression patterns has let to completely novel insights into pancreatic cancer biology. Specific miRNAs, such as the miR-200 family, miR-34a and miR-155 are involved in PDAC-biology by regulating genes associated with metastasis and cell stemness [64, 65].

The era of epigenetics in pancreatic cancer has emerged strongly within the last years and deepened our understanding of pancreatic cancer biology. One of the most important characteristics of epigenetic mechanisms which clearly demarcates them from genetics is their reversibility. This feature provides new targets for novel therapeutic interventions in pancreatic cancer and other epithelial tumors.

The manifold genetic and epigenetic events observed in pancreatic carcinogenesis mirror the complexity of this malignancy and lead to the assumption that targeting one molecular feature of pancreatic carcinogenesis is not sufficient for successful pancreatic cancer treatment. Though inaccessible for therapeutic options, there exists at least one molecular event found in virtually all invasively growing pancreatic tumors and their precursor lesions: The constitutive activation of oncogenic Kras probably demonstrates the most important and best studied event in pancreatic carcinogenesis.

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5. Impact of Kras activation on pancreatic carcinogenesis

The mutation of Kras belongs to the earliest events in pancreatic carcinogenesis. Kras proteins comprise a family of signal-transducing GTPases that mediate a wide variety of cellular functions including proliferation, differentiation and survival and are frequently mutated in human cancers [66]. Although Kras is a GTPase, its intrinsic activity is inefficient and requires GTPase activating proteins to promote GTP hydrolysis and attenuate downstream signaling [1]. Oncogenic mutation of Kras ($Kras^{G12D}$) is generally accepted to represent the initial key event in pancreatic carcinogenesis and found in virtually all invasively growing pancreatic tumors [7]. Due to its prominent role in pancreatic carcinogenesis Kras is considered to be an attractive therapeutic target of PDAC-treatment, but specific biochemical properties of the protein have made this an elusive goal [67]. Activating Kras point mutations at codon 12 (from GGT to GAT or GTT and more rarely CGT) result in substitution of glycine with aspartate, valine or arginine. Oncogenic Kras mutations lock the protein in its GTP-bound form thus permitting its constitutive interaction with and activation of multiple effectors, independent on growth factor stimulation [67].

The activation of Kras engaged effector pathways, like the RAF-mitogen-activated kinase (MAPK)-cascade, phosphoinositide-3-kinase- (PI3K) signaling and the Ral GDS pathway results in stimulation of proliferation, invasion, metastases and survival thus enabling pancreatic cancer progression [3]. Given the aforementioned limitations in Kras inhibition, these downstream targets may provide alternative effective points of therapeutic intervention and thus are the focus of ongoing studies in pancreatic specific systems.

The impact of constitutive Kras activation is not limited on the epithelial cell but also participates in the modulation of the tumor environment. One hallmark of PDAC is an extensive stromal remodeling, the most prominent features of which are the recruitment of inflammatory and mesenchymal cells as well as fibrotic replacement of pancreatic parenchyma [68]. Recent studies revealed that even early stages of PanIN development are associated with a stromal reaction, which is characterized by a robust desmoplastic response and recruitment of immune cells. Subclasses of these immune cells, immature myeloid cells, suppress infiltrating T cells and thus establish an immune privilege in the tumor microenvironment promoting pancreatic carcinogenesis [69, 70]. Mechanistically, constitutive activation of Kras in pancreatic ductal cells triggers the production of the cytokine GM-CSF, which, in turn, promotes the expansion of immunosuppressive myeloid cells, leading to the evasion of $CD8^+$ T-cell-driven-antitumor immunity [69, 70].

Due to its high biological relevance for pancreatic carcinogenesis, a genetically engineered mouse model (GEMM) with pancreas specific Kras mutation was created, allowing detailed investigations of morphological as well as molecular features of this disease [71]. This transgenic mouse model bares a mutation of the endogenous murine Kras gene specifically in pancreatic progenitor cells by crossing mice with a conditionally activated Kras allel (LSL-$Kras^{G12D}$) to transgenic strains that express Cre recombinase in pancreatic lineages (PdxCre or p48Cre). These “KC” mice develop low and high grade PanIN lesions recapitulating pancreatic carcinogenesis in the human situation but only slowly progress

to PDAC at an advanced age [71]. This mouse model taught us that in spite of the requirement of Kras-activation for pancreatic cancer development oncogenic Kras mutation alone fails to transform precursor lesions into invasive cancer due to activation of powerful fail-safe mechanisms (compare Fig. 2).

Counteracting transformation and growth, cellular senescence, a permanent cell growth arrest, is increasingly recognized as one of the most critical fail-safe programs in pancreatic carcinogenesis [72]. A major cause of this permanent growth arrest was found in telomeres, which are non-coding nucleoprotein complexes positioned in the extremes of chromosomes [73]. During successive cellular divisions, telomeres in normal human cells shorten progressively and, when telomeres erode down below a threshold length, the cell ceases to divide itself and becomes senescent. Importantly, senescence can also be observed in the absence of any detectable telomere shortening or dysfunction in numerous conditions such as cellular stress or oncogene activation. Oncogene induced senescence (OIS) has emerged as a powerful tumor suppressor mechanism protecting cells from unrestrained proliferation imposed by oncogenic signaling [74]. It has been proven that normal cells, when forced to express high levels of oncogenic Ras, undergo a permanent and irreversible cell cycle arrest [75]. OIS is frequently found in premalignant lesions but is essentially absent in advanced cancers, suggesting that malignant tumor cells can find ways to bypass or escape senescence [76].

Pancreas specific expression of oncogenic $Kras^{G12D}$ promotes an initial burst of proliferation accompanied by the development of PanIN precursor lesions before cells stop dividing. These precursor lesions then exhibit many features of senescence including positive senescence-associated ß-galactosidase staining and induction of cell cycle inhibitors [77]. Successful progression of PanIN lesions towards frank adenocarcinoma requires evasion from senescence. This can result from additional genetic or epigenetic events concerning major tumor suppressor pathways, namely the $p19^{Arf}$-p53 pathway and the $p16^{Ink4a}$-Rb cascade [74].

6. Role of tumor suppressor inactivation in pancreatic carcinogenesis

The p53 protein plays a central role in modulating cellular responses to cytotoxic stress by contributing to both, cell cycle arrest and programmed cell death [3]. Signals of mitogenic oncogenes, such as cMyc or Kras lead to activation of p53, which depending on cell type and stimulus induces either apoptosis or senescence and consequently leads to the elimination of cells with oncogenic activation. p53 is integrated in a complex network of upstream sensors and downstream effectors. An important sensor of oncogenic signals for p53 is $p19^{Arf}$, which is encoded in an alternative reading frame (ARF) by the tumor suppressor locus CDKN2A [78]. Activation of $p19^{Arf}$ antagonizes the effect of the E3 ubiquitin ligase MDM2 that acts upon p53 to initiate its proteasomal degradation, thereby contributing to the stabilization of the tumor suppressor gene [74]. In the nucleus, stabilized p53 binds to promoters of more than 300 target genes with implications for cell growth control. One such important p53 downstream target is p21. p21 binds to and inhibits the activity of Cyclin-CDK2 and Cyclin-CDK1 complexes and thus functions as a negative regulator of cell cycle progression at the G1 phase [79].

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In agreement with its key role in senescence and tumor suppression, mutational p53 inactivation is associated with accelerated carcinogenesis in many tumor entities [80]. In the pancreas, p53 inactivation on chromosome 17 has been reported in 50-75% of carcinomas [1]. In the murine pancreas carcinoma model, genetic loss of p53 allows Kras to bypass senescence resulting in 100% penetrance at an early age, thus recapitulating human PDAC including histopathological similarities in neoplastic cells, desmoplasia and occurrence of liver and lung metastases [81].

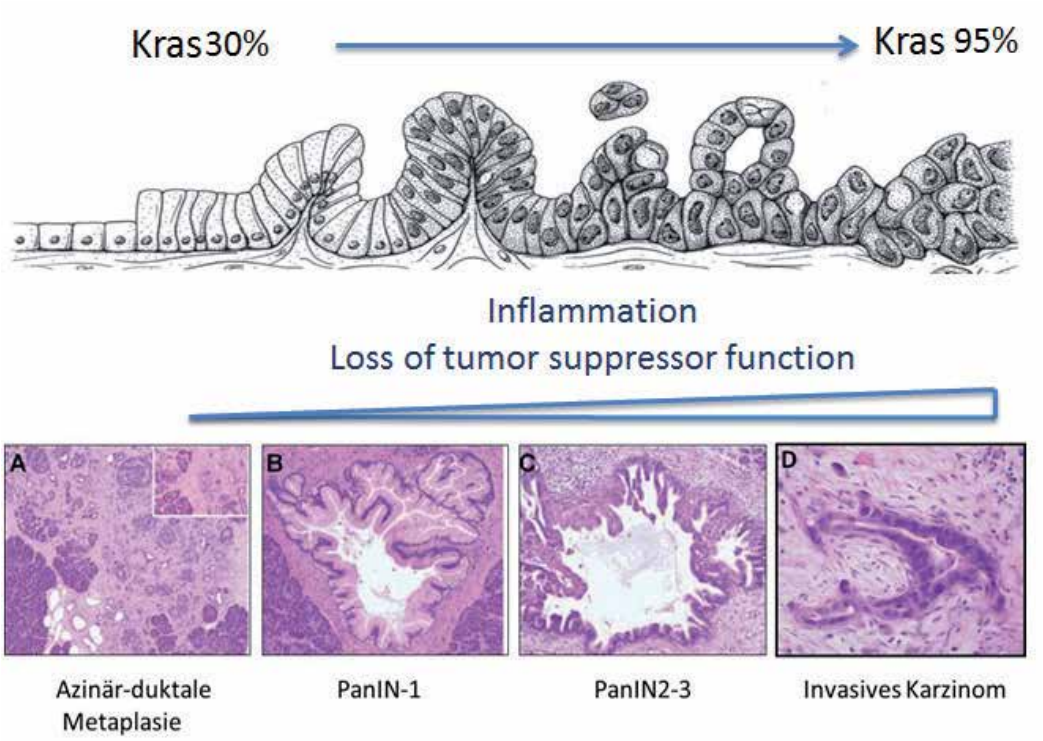

Figure 2. Current model of pancreatic carcinogenesis: on the background of Kras mutation ADM lesions progress to PanIN-precursor lesions and invasive carcinoma depending on additional signals as loss of tumor suppressor function or activation of inflammatory pathways. A: Acinar-ductal metaplasia, B: PanIN-1, C: PanIN-2-3, D: Invasive pancreatic cancer.

The $p16^{Ink4a}$ gene, located on the short arm of chromosome 9, is one of the most frequently inactivated tumor suppressor genes in pancreatic cancer [1, 2]. Remarkably, virtually all pancreatic carcinomas bare loss of $p16^{Ink4a}$ function, in 40% of pancreatic cancer through homozygous deletion, in 40% by intragenic mutation coupled with loss of the second allele, and in 15% by hypermethylation of the $p16^{Ink4a}$ gene promoter [8].

The protein $p16^{Ink4a}$ belongs to the cyclin D-dependent kinase (CDK) inhibitor family and functions to prevent the phosphorylation of Rb-1 by CDK 4 and 6, resulting in a blockage of G1/S-phase transition of the cell cycle [82]. This event is a decisive step in the inhibition of cell

cycle progression and also in senescence initiation. In contrast to that, loss of $p16^{Ink4a}$ results in inappropriate phosphorylation of Rb-1, thereby facilitating progression of the cell cycle through enhanced G1/S transition [1-3, 74].

Additional to inactivation of tumor suppressor genes, Kras-initiated pancreatic carcinogenesis can be promoted by signals from the inflammatory environment [69, 70]. This type of proinflammatory environment can be provided by chronic pancreatitis, the most relevant risk factor for PDAC development in human [83]. Chronic pancreatitis supports the initiation and progression of this malignancy by direct modification of gene expression networks in pancreatic epithelial cells. For instance, pancreatitis contributes to tumor progression by abrogating the senescence barrier characteristic of low-grade PanIN lesions [84]. Most importantly, chronic pancreatitis induces a wide range of proteins, predominantly inflammatory transcription factors. The majority of these inflammatory transcription factors inhabits oncogenic potential, mediated by inhibition of tumor suppressor genes or synergism with $Kras^{G12D}$ signaling to promote pancreatic carcinogenesis.

By introducing the inflammatory family of Nuclear factor of activated T cells (NFAT) proteins, the following part of the chapter will cite an example how deregulated oncogenes participate in and cooperate with $Kras^{G12D}$ mediated signaling in every single step of pancreatic carcinogenesis, beginning from induction of ADM over progression of pancreatic precursor lesions to frank invasive pancreatic ductal adenocarcinoma.

6.1. NFAT proteins and their role in pancreatic carcinogenesis

6.1.1. The family of NFAT transcription factors and their cellular regulation

The NFAT family, first described as a regulator of T cell activation and differentiation, comprises four calcium-responsive isoforms named NFATc1, NFATc2, NFATc3 and NFATc4 as well as a more distant relative, NFAT5 [85]. In resting cells, NFAT factors are located in the cytoplasm in a highly phosphorylated, inactive state [85, 86]. Ligand binding to many receptors results in the activation of phospholipase C (PLC), the release of IP_3 and in a transient release of Ca^{2+} from intracellular stores through IP_3 receptors. This initial release of Ca^{2+} demonstrates the prerequisite for increased influx of Ca^{2+} through specialized Ca^{2+} released activated channels (termed CRAC) [86]. CRACs provide the persistent Ca^{2+} signal that is necessary for sufficient activation of the phosphatase calcineurin that targets and dephosphorylates moderately conserved serine rich motifs in the N-terminal homology region of NFAT proteins to unmask its nuclear localization signals [87]. Subsequently, NFAT proteins shuttle into the nucleus where they are either ubiquitinated for HDM2-dependent proteasomal degradation or stabilized by GSK3ß-mediated phosphorylation (compare Fig. 3) [88]. Upon stabilization the transcription factor recognizes its GGAAA consensus sequence within target gene elements and binds DNA either as homodimer or heterodimer [85-88]. In fact, NFAT proteins frequently cooperate with other transcription factors to elicit high-affinity binding on common target genes. GATA Proteins, FoxP3 and members of the MEF family are only few among a wide range of NFAT partner proteins [89]. Additionally, NFAT recruits other signaling regulated transcription factors (e.g. Smad3 and NKkB) to integrate pathway specific signals to

Ca^{2+}/calcineurin regulated transcription [90]. Thus, NFAT transcription complexes function as signal integrators and detectors. One signal has to be Ca^{2+}/calcineurin, while the second one can have developmental origin or can embody oncogenic qualities as the Ras-MAP kinase pathway [89, 90]. Doing so, the cooperation between NFAT and its partners helps controlling the specificity of NFAT target gene binding and the resulting mode of action.

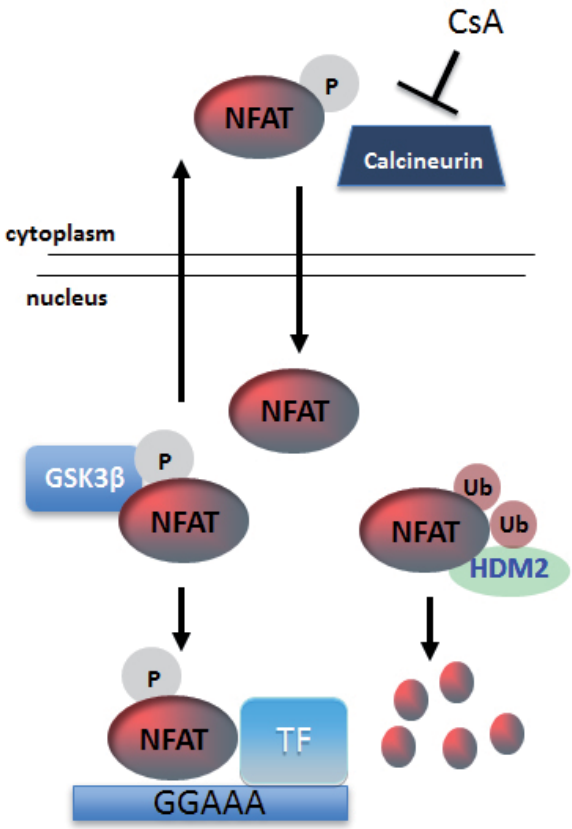

Figure 3. NFAT signaling in pancreatic cancer. Upon Ca^{2+} - dependent activation of Calcineurin, NFAT becomes dephosphorylated and shuttles into the nucleus. The calcineurin-inhibitor Cycosporin A (CsA) prevents NFAT activation. In the nucleus GSK3ß-dependent phosphorylation of NFAT either leads to its nuclear export or allows binding to target genes in association with partner transcription factors. Ubiquitination of NFAT proteins labels them for proteosomal degradation by HDM2.

7. Oncogenic potential of NFAT signaling

The NFAT family of transcription factors was originally identified as a group of inducible nuclear proteins which regulate transcription during T lymphocyte activation [91]. Following

their initial discovery, a multitude of studies quickly established that NFAT proteins are also expressed outside the immune system where they participate in the regulation of the expression of genes influencing cell growth and differentiation [86]. One of the first studies implicating NFAT factors in cell proliferation was performed in fibroblasts, in which constitutively active NFATc1 induces cell transformation and colony formation [92]. Similarly, in pancreatic tumor cells proliferation and anchorage-independent growth is - at least in part - dependent on calcineurin activity and nuclear translocation of NFAT proteins [93]. This is consistent with high levels of nuclear NFAT in pancreatic cancer cells and in particular in those cells with accelerated growth. Nowadays, ectopic activation of NFAT members is recognized as an important aspect of oncogenic transformation in several human malignancies, most notably in pancreatic cancer [88, 93]. Proliferation and anchorage-independent growth of cultured pancreatic cancer cells is significantly attenuated by inhibition of Ca^{2+}/Calcineurin signaling with Cyclosporin A or siRNA-technology-mediated depletion of NFATc1 [94]. Besides proliferation and growth, NFAT proteins incorporate additional features of tumor biology. Being downstream mediators of α6ß4 integrin signaling NFATc2 and NFAT5 promote cancer invasion in breast and colon cancer [95]. Stimulation of angiogenesis through upregulation of VEGF and enhancement of tumor cell migration via transcriptional activation of Cox2 are additional oncogenic features of NFAT proteins [86, 96].

GEMM with constitutive activation of NFATc1 revealed increased cellular proliferation in pancreata of young mice but mice baring a constitutive activation of NFATc1 failed to develop advanced PanIN lesions within a one-year observations span. In contrast to mice bearing an isolated transgenic induction of NFATc1, mice carrying combined constitutive activation of Kras and NFATc1, a situation found in 70% of human PDACs, surprise with a dramatically shortened survival compared to the $Kras^{G12D}$ animals [Baumgart et al., unpublished data]. Further resembling human PDAC, $Kras^{G12D}$;NFATc1 mice develop severe cachexia and abdominal distension caused by the accumulation of sanguineous ascites and bile duct obstruction. At necropsy, the pancreata from $Kras^{G12D}$;NFATc1 mice are enlarged by tumor mass, which contains both solid and cystic regions. Notably, pancreata from $Kras^{G12D}$;NFATc1 mice express nuclear NFATc1 throughout carcinogenesis and at equivalent levels to those observed in human PDAC.

Beyond doubt, the experience with the described transgenic mouse model which recapitulates human PDAC disease in a very accurate manner clearly shows that activation of NFAT proteins works synergistically with Kras signaling and leads to acceleration of pancreatic carcinogenesis. Further investigations shot light on the NFAT dependent mechanisms facilitating and hastening pancreatic carcinogenesis.

8. NFATc1 function in ADM

The cellular origin of PDAC has been a controversial topic for many decades. PDAC has long been considered to be a disease of pancreatic ducts. However, early efforts to model the disease by forcing Kras expression in pancreatic duct cells did not yield discernable pathology [97]. In

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recent years, increasing evidence arised that PanIN precursor lesions and invasive PDAC originate from differentiated acinar cells. The development of duct-like PanIN lesions from acinar cells requires massive remodeling of these cells, both morphologically and with respect to gene expression profiles. The transition from acinar to ductal cell properties has been termed acinar-to-ductal metaplasia (ADM) and lineage tracing experiments have confirmed that this process is a result of direct transdifferentiation from adult acinar cells that convert to a ductal phenotype upon expression of constitutive active Kras [97, 98]. In murine and in human samples, ADM development has been shown to precede PanIN formation, suggesting that ADM represents the first step of pancreatic carcinogenesis.

Appreciating the relevance of ADM for pancreatic cancer development, much effort was put into research on the molecular mechanisms facilitating ADM. As a transcription factor that is involved in differentiation processes in many tissues NFAT constitutes a promising candidate to mediate ADM. Indeed, NFATc1 is highly operative in pancreatic ADM, while only rare expression of the transcription factor can be found in acinar cells. *In vitro* and *in vivo* studies have revealed that $Kras^{G12D}$ driven ADM requires ligand-dependent activation of the Epidermal growth factor receptor [6, 21]. Careful molecular studies have proven that EGFR signaling – at least in part – is mediated via NFATc1. Most importantly, in spite of active EGFR signaling, pharmacological or genetic inactivation of NFATc1 in acinar cell explants extracted from $Kras^{G12D}$ mice reduces duct formation *in vitro*. Furthermore, $Kras^{G12D}$ mice harboring a pancreas specific transgenic inactivation of NFATc1 are less susceptible to inflammation induced ADM and show a significant delay of pancreatic carcinogenesis [unpublished data]. These findings clearly indicate a key role of NFAT signaling in the initial steps of pancreatic carcinogenesis.

9. NFATc1 and STAT3 cooperation in pancreatic carcinogenesis

Recent investigations established that NFATc1 cooperates with the signal transducer and activator of transcription-3 (STAT3) [Baumgart et al., unpublished data]. Like NFAT proteins, STAT3 is also regulated primarily at the level of its subcellular localization [90]. In resting cells, STAT3 resides in a non-phosphorylated version in the cytoplasm. However, following cytokine or growth factor stimulation, STAT3 proteins are inducibly phosphorylated on critical regulatory tyrosine residues promoting their homodimerization and subsequent translocation into the nucleus where they control gene transcription [99]. Interestingly, genetic depletion of STAT3 attenuates the transformation capacity of NFATc1, suggesting a cooperative function of both transcription factors in pancreatic cancer. From the mechanistic point of view, NFATc1 interacts with STAT3 to form enhancer-promoter communications at jointly regulated genes involved in inflammation and oncogenesis, e.g. EGFR and Wnt-family members. The NFATc1-STAT3 transcription pathway is operative in pancreatitis-mediated carcinogenesis as well as in established human pancreatic cancer [Baumgart et al., under review].

10. Impact of NFAT proteins on the inflammatory tumor environment

Cancer-associated inflammation plays an important role in restraining anti-tumor immunity, particularly in pancreatic cancer for which a massive infiltration of immunosuppressive leukocytes into the tumor stroma is an early and consistent event in carcinogenesis [84]. In contrast to many other solid tumors, intratumoral T cells are rare in pancreatic cancer, which is associated with an immune escape and bad prognosis [70]. In PDAC, increasing evidence suggests, that oncogenic Kras drives an inflammatory program that establishes immune privilege in the tumor microenvironment [69, 70]. The immune surveillance of pancreatic cancer demonstrates the response to signals from the transformed epithelial pancreatic cell. Cytokines like GM-CSF are secreted by ductal pancreatic cells to modulate the inflammatory tumor environment. Recent work suggests an essential role of NFAT proteins in the transcriptional induction of a core of cytokines associated with encapsulation of the transformed cell from physiological immune response [100, unpublished data]. Thus, NFAT inactivation might represent a promising possibility to restore pancreatic cancer response to tumor suppressive immune signals.

11. NFAT mediated TGFß switch from tumor suppressor to oncogene in pancreatic carcinogenesis

As mentioned above, an emerging model in cancer biology supports a dual role for TGFß signaling in tumorigenesis, acting as a tumor suppressor in early carcinogenesis and as a strong promoter of cell proliferation, migration and invasion in advanced tumor stages [101, 102]. TGFß blocks cell proliferation in untransformed cells through the induction of a cell cycle arrest at late G1 phase. Two critical molecular events underlie TGFß anti-proliferative response: the transcriptional repression of cMyc and subsequent induction of cell cycle inhibitors like p21 and $p15^{Ink4b}$ [102, 103]. As an immediate early transcription factor proto-oncogenic cMyc functions as a master regulator of G1-S-cell cycle progression and growth promotion in pancreatic cancer [93, 103]. cMyc repression by TGFß requires the activation of a Smad3-4 complex to transduce its stimulus into the nucleus. Here, Smad proteins complex with the transcription factors E2F4/5 and DP1 and corepressor p107 to repress cMyc promoter via binding to its TGFß-inhibitory element (TIE) [104].

During pancreatic carcinogenesis, tumor cells change their transcriptional responsiveness to TGFß and become resistant to the growth inhibitory effects due to functional inactivation of the TGFß-Smad pathway [103]. Depending on the cell type and the activation status of a cell, TGFß then signals through Smad-independent pathways (e.g. PI3K and MAPK pathways) to promote the acquisition of a mesenchymal phenotype and stimulate tumor cell migration [102, 103].

TGFß induces expression of NFATc1 and c2, which accumulate in the nucleus and displace pre-existing Smad3 repressor complexes from the cMyc TIE element. Mechanistically, NFATc1 binding to the serum responsive element within the proximal cMyc promoter initiates p300-

dependent histone acetylation rendering the promoter transcriptionally active. Hyperacetylation of the cMyc promoter is required for recruitment of the Ets-like gene 1 (ELK-1), a protein signaling downstream of Kras, responsible for maximal activation of cMyc [94]. The functional significance of this pathway is emphasized by restoration of TGFß growth suppressor function in cancer cells and impaired cMyc expression indicated by reduced tumor growth and G1-arrest following the pharmacological or genetic inactivation of NFAT proteins [94, 102].

12. NFAT dependent silencing of tumor suppressor genes by formation of heterochromatin complexes

Activation of NFAT proteins does not only lead to target gene activation in pancreatic cancer, but also contributes to gene silencing. Being a member of the Ink4 family, $p15^{Ink4b}$ impedes the activation and function of Cyclin dependent kinases (CDK) 4 and 6 which leads to cell cycle inhibition and diminished G1-S phase transition [105]. Therefore, $p15^{Ink4b}$ incorporates important functions as a tumor suppressor in numerous malignancies, most importantly in pancreatic cancer, where $p15^{Ink4b}$ inactivation by genetic or epigenetic events occurs in over 90% of all tumors [9]. NFATc2 targets $p15^{Ink4b}$ for inducible and sequential heterochromatin formation and gene silencing. Sequential Chromatinimmunprecipitation revealed that NFATc2 binding to its putative binding side on the $p15^{Ink4b}$ promoter leads to recruitment of the histone methyltransferase Suv39H1. Local trimethylation of Lysine 9 on histone 3 (H3K9trime) allows docking of heterochromatin protein 1 y (HP1y) which results in stabilization of the heterochromatin complex on the $p15^{Ink4b}$ promoter. Conflicting with that, inactivation of NFATc2 disrupts the repressor complex and results in restoration of $p15^{Ink4b}$ expression and function [106].

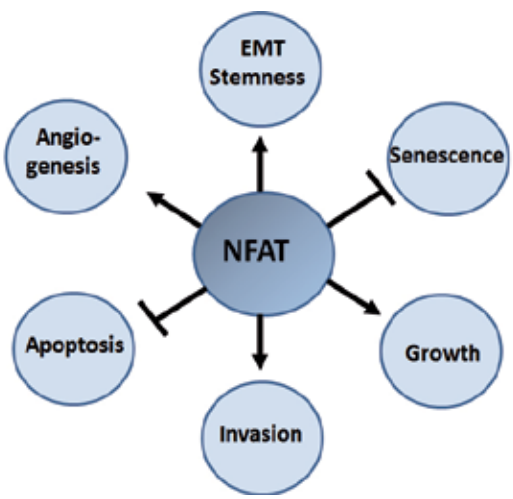

Figure 4. NFAT transcription factors and their impact on hallmarks of cancer

13. Perspective

These examples of NFAT dependent alterations in signaling pathways and transcriptional processes promoting pancreatic carcinogenesis only demonstrate a small insight into how oncogenic transcription factors contribute to pancreatic cancer development. Via transduction of EGFR signaling to downstream targets, by cooperation with other pre inflammatory oncogenes, by modulation of the tumor microenvironment, induction of cell cycle promoting genes as well as via silencing of important tumor suppressor genes, NFAT proteins are highly involved in all phases of pancreatic carcinogenesis reaching from early acinar-to-ductal-metaplasia over establishment of precursor lesions to frank invasive pancreatic adenocarcinoma.

As dismal as pancreatic cancer presents itself clinically, as complex and multi-layered are the histopathological and molecular mechanisms responsible for pancreatic carcinogenesis. As the molecular main reason for pancreatic cancer development - the constitutive activation of Kras - evades any pharmacological approach, targeting oncogenic factors like NFAT proteins represents a promising option approaching success in pancreatic cancer treatment.

Author details

Elisabeth Heßmann[1], Sandra Baumgart[2], Nai ming Chen[1], Shiv Singh[2], Garima Singh[2], Alex König[1], Albrecht Neeße[1] and Volker Ellenrieder[1]

1 Department of Gastroenterology II, Georg-August-University, Göttingen, Germany

2 Signaling and Transcription Laboratory, Department of Gastroenterology, Philipps University, Marburg, Germany

References

[1] Ottenhof NA, Wilde RF, Maitra A, Hruban RH, Offerhaus GJA. Molecular characteristics of pancreatic ductal adenocarcinoma. Pathology Research International. 2011 Article ID: 620601:1-16

[2] Hidalgo M. Pancreatic cancer. The New England Journal of Medicine 2010;362:1605-17

[3] Maitra A, Kern SE, Hruban RH. Molecular pathogenesis of pancreatic cancer. Best practice and research: Cinical Gastroenterology 2005. Vol. 9, No. 9, pp. 1191-1206

[4] Campbell PJ, Yachida S, Mudie LJ, Stephens PJ, Pleasance ED, Stebbings LA, Morsberger LA, Latimer C, McLaren S, Lin ML, McBride DJ, Varela I, Nik-Zainal SA, Leroy C, Jia M, Menzies A, Butler AP, Teague JW, Griffin CA, Burton J, Swerdlow H,

Quail MA, Stratton MR, Iacobuzio-Donahue C, Futreal PA. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature. 2010 Oct 28;467(7319):1109-13.

[5] Hruban RH, Takaori K, Klimstra DS, Adsay NV, Albores-Saavedra J, Biankin AV, Biankin SA, Compton C, Fukushima N, Furukawa T, Goggins M, Kato Y, Klöppel G, Longnecker DS, Lüttges J, Maitra A, Offerhaus GJ, Shimizu M, Yonezawa S.An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. American journal of Surgical Pathology, 2004 Aug;28(8):977-87

[6] Navas C, Hernandez-Porras I, Schuhmacher AJ, Sibilia M, Guerra C, Barbacid M. EGF receptor signalling is essential for K-ras oncogene-driven Pancreatic Adenocarcinoma. Cancer Cell 2012. 22, 318-30

[7] Hruban RH, Maitra A, Goggins M. Update on Pancreatic Intraepithelial Neoplasia. International Journal of Clinical and Experimanetal Pathology 2007;1:306-16

[8] Singh M, Maitra A. Precursor lesions of pancreatic cancer: molecular pathology and clinical applications. Pancreatology 2007;7(1):9-19

[9] Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee, EM, Goggins M, maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Cor signaling pathways in human pancreatic cancer revealed by global genomic analyses. Science 2008;321(5897):1801-6

[10] Vikajärvi N, Pääkkö P, Soini Y. Apoptotic index and apoptosis influencing proteins bcl-2, mcl-1, bax and caspases 3, 6 and 8 in pancreatic carcinoma. Histopathology 1998;33(5):432-9

[11] Evans JD, Cornford PA, Dodson A, Greenhalf W, Foster CS, Neoptolemos JP. Detailed tissue expression of bcl-2, bax, bak and bcl-x in the normal human pancreas and in chronic pancreatitis, ampullary and pancreatic ductal adenocarcinomas. Pancreatology 2001;1(3):254-62

[12] Magistrelli P, Coppola R, Tonini G, Vincenzi B, Santini D, Borzomati D, Vecchio F, Valeri S, Castri F, Antinori A, Nuzzo G, Caraglia M, Picciocchi A. Apoptotic index or a combination of Bax/Bcl-2 expression correlate with survival after resection of pancreatic adenocarcinoma. J Cell Biochem 2006. 1;97(1): 98-108

[13] Schutte M, da Costa LT, Hahn SA, Moskaluk C, Hoque AT, Rozenblum E, Weinstein CL, Bittner M, Meltzer PS, Trent JM, et al. Identification by representational difference analysis of a homozygous deletion in pancreatic carcinoma that lies within the BRCA2 region. Proc Natl Acad Sci USA 1995.20;92(13):5950-4

[14] Goggins M, Schutte M, Lu J, Moskaluk CA, Weinstein CL, Petersen GM, Yeo CJ, Jackson CE, Lynch HT, Hruban RH, Kern SE. Germline BRCA2 gene mutations in patients with apparently sporadic pancreatic carcinomas. Cancer Res 1996. 1;56(23): 5360-4

[15] Yamamoto H, Itoh F, Nakamura H, Fukushima H, Sasaki S, Perucho M, Imai K. Genetic and clinical features of human pancreatic ductal adenocarcinomas with widespread microsatellite instability. Cancer Res. 2001. 1;61(7):3139-44

[16] Redston MS, Caldas C, Seymour AB, Hruban RH, da Costa L, Yeo CJ, Kern SE. p53 mutations in pancreatic carcinoma and evidence of common involvement of homocopolymer tracts in DNA microdeletions. Cancer Res. 1994 Jun 1;54(11):3025-33.

[17] Barton CM, Staddon SL, Hughes CM, Hall PA, O'Sullivan C, Klöppel G, Theis B, Russell RC, Neoptolemos J, Williamson RC, et al. Abnormalities of the p53 tumour suppressor gene in human pancreatic cancer. Br J Cancer. 1991 Dec;64(6):1076-82.

[18] Joo YE, Rew JS, Park CS, Kim SJ. Expression of E-cadherin, alpha- and beta-catenins in patients with pancreatic adenocarcinoma. Pancreatology. 2002;2(2):129-37.

[19] Li YJ, Ji XR. Relationship between expression of E-cadherin-catenin complex and clinicopathologic characteristics of pancreatic cancer. World J Gastroenterol. 2003 Feb;9(2):368-72.

[20] Shi Y, Massagué J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell. 2003 Jun 13;113(6):685-700.

[21] Ardito CM, Grüner BM, Takeuchi KK, Lubeseder-Martellato C, Teichmann N, Mazur PK, Delgiorno KE, Carpenter ES, Halbrook CJ, Hall JC, Pal D, Briel T, Herner A, Trajkovic-Arsic M, Sipos B, Liou GY, Storz P, Murray NR, Threadgill DW, Sibilia M, Washington MK, Wilson CL, Schmid RM, Raines EW, Crawford HC, Siveke JT. EGF receptor is required for KRAS-induced pancreatic tumorigenesis. Cancer Cell. 2012 Sep 11;22(3):304-17. doi: 10.1016/j.ccr.2012.07.024.

[22] Vaccaro V, Bria E, Sperduti I, Gelibter A, Moscetti L, Mansueto G, Ruggeri EM, Gamucci T, Cognetti F, Milella M. First-line erlotinib and fixed dose-rate gemcitabine for advanced pancreatic cancer. World J Gastroenterol. 2013 Jul 28;19(28):4511-9. doi: 10.3748/wjg.v19.i28.4511.

[23] Mullendore ME, Koorstra JB, Li YM, Offerhaus GJ, Fan X, Henderson CM, Matsui W, Eberhart CG, Maitra A, Feldmann G. Ligand-dependent Notch signaling is involved in tumor initiation and tumor maintenance in pancreatic cancer. Clin Cancer Res. 2009 Apr 1;15(7):2291-301. doi: 10.1158/1078-0432.CCR-08-2004. Epub 2009 Mar 3.

[24] Ma J, Xia J, Miele L, Sarkar FH, Wang Z. Notch signaling pathway in pancreatic cancer progression. Pancreat Disord Ther. 2013. 3(114): 1-5

[25] Wang Z, Li Y, Kong D,Sarkar FH. The role of Notch signaling pathway in epithelial-mesenchymal transition (EMT) during development and tumor aggressiveness. Curr Drug Targets. 2010. 11:745-51

[26] Gnoni A, Licchetta A, Scarpa A, Azzariti A, Brunetti AE, Simone G, Nardulli P, Santini D, Aieta M, Delcuratolo S, Silvestris N. Carcinogenesis of pancreatic adenocarcinoma: precursor lesions. Int J Mol Sci. 2013 Sep 30;14(10):19731-62.

[27] Rosow DE, Liss AS, Strobel O, Fritz S, Bausch D, Valsangkar NP, Alsina J, Kulemann B, Park JK, Yamaguchi J, LaFemina J, Thayer SP. Sonic Hedgehog in pancreatic cancer: from bench to bedside, then back to the bench. Surgery. 2012 Sep;152(3 Suppl 1):S19-32. Epub 2012 Jul 6.

[28] Aubin J, Déry U, Lemieux M, Chailler P, Jeannotte L. Stomach regional specification requires Hoxa5-driven mesenchymal-epithelial signaling. Development. 2002 Sep; 129(17):4075-87.

[29] Fendrich V, Esni F, Garay MV, Feldmann G, Habbe N, Jensen JN, Dor Y, Stoffers D, Jensen J, Leach SD, Maitra A. Hedgehog signaling is required for effective regeneration of exocrine pancreas. Gastroenterology. 2008 Aug;135(2):621-31.

[30] Kayed H, Kleeff J, Keleg S, Büchler MW, Friess H. Distribution of Indian hedgehog and its receptors patched and smoothened in human chronic pancreatitis. J Endocrinol. 2003 Sep;178(3):467-78.

[31] Thayer SP, di Magliano MP, Heiser PW, Nielsen CM, Roberts DJ, Lauwers GY, Qi YP, Gysin S, Fernández-del Castillo C, Yajnik V, Antoniu B, McMahon M, Warshaw AL, Hebrok M. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature. 2003 Oct 23;425(6960):851-6.

[32] Sato N, Goggins M. The role of epigenetic alterations in pancreatic cancer. J Hepatobilary Pancreat Surg. 2006;13:382-95

[33] Esteller M. Epigenetic gene silencing in cancer: the DNA hypermethylome. Hum. Mol Genet. 2007;16:R50-9

[34] Eden A, Gaudet F, Waghmare A, Jaenisch R. Chromosomal instability and tumors promoted by DNA hypomethylation. Science 2003;300:455

[35] Tufarelli C, Stanley JA, Garrick D, Sharpe JA, Ayyub H, Wood WG, Higgs DR. Transcription of antisense RNA leading to gene silencing and methylation as a novel cause of human genetic disease. Nat Genet 2003;34:157-65

[36] Fabbri M, Garzon R, Cimmuno A, Liu Z, Zanesi N, Callegari E, Liu S, Adler H, Costinean S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chan KK, Marcucci G, Calin GA, Huebner K, Croce CM. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA-methyltransferases 3A and 3B. Proc Natl Acad Sci USA 2007;104:15805-10

[37] Yamada N, Nishida Y, Tsutsumida H, Hamada T, Goto M, Higashi M, Nomoto M, Yonezawa S. Muc1 expression is regulated by DNA methylation and histone H3 lysine 9 modification in cancer cells. Cancer Res 2008;68:2708-16

[38] Vincent A, Ducourouble MP, van Seuningen I. Epigenetic regulation of the human mucin gene Muc4 in epithelial cancer cell lines involves both DNA methylation and histone modifications mediated by DNA methylatransferases and histone deacetylases. Faseb J 2008;22:3035-45

[39] Zagni C, Chiacchio U, Rescifina A. Histone methyltransferase inhibitors: novel epigenetic agents for cancer treatment. Curr Med Chem 2013; 20:167-85

[40] Lorincz AT. The promise and the Problem of Epigenetics Biomarkers in Cancer. Expert Opin Med Diagn. 2011; 5 (5): 375-79

[41] Lujambio A, Calin GA, Villanueva A, Ropero S, Sanchez-Cespedes M, Blanco D, Montuenga LM, Rossis S, Nicoloso MS, Faller WJ, Gallagher WM, Eccles SA, Croce CM, Esteller M. A microRNA DNA methylation signature for human cancer metastasis. Proc Natl Acad Sci USA. 2008;105:13556-61

[42] Berger SL. Histone modifications in transcriptional regulation. Curr Opin Genet Dev. 2002;12:142-148

[43] Lopez-Serra L, Esteller M. Proteins that bind methylated DNA and human cancer: reading the wrong words. Br J Cancer 2008; 12:1881-5

[44] Sato N, Fukushima N, Hruban RH, Goggins M. CpG island methylation profile of pancreatic intraepithelial neoplasia. Mod Pathol. 2008 Mar;21(3):238-44. Epub 2007 Dec 21.

[45] Schutte M, Hruban RH, Geradts J, Maynard R, Hilgers W, Rabindran SK, Moskaluk CA, Hahn SA, Schwarte-Waldhoff I, Schmiegel W, Baylin SB, Kern SE, Herman JG. Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. Cancer Res. 1997 Aug 1;57(15):3126-30.

[46] Jenuwein T, Allis CD. Translating the histone code. Science 2001;293:1074-80

[47] Lachner M, Jenuwein T. The many faces of histone lysine methylation. Curr Opin Cell Biol. 2002;14:286-98

[48] Czermin B, Melfi R, McCabe D, Seitz V, Imhof A, Pirrotta V. Drosophila Enhancer of zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. Cell 2002. 111(2):185-96

[49] Shao Z, Raible F, Mollaaghababa R, Guyon JR, Wu CT, Bender W, Kingston RE. Stabilization of chromatin structure by PRC1, a Polycomb complex. Cell. 1999;98(1)37-46

[50] Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, Isono K, Koseki H, Fuchikami T, Abe K, Murray HL, Zucker JP, Yuan B, Bell GW, Herbolsheimer E, Hannett NM, Sun K, Odom DT, Otte AP, Vol-

kert TL, Bartel DP, Melton DA, Gifford DK, Jaenisch R, Young RA. Control of developmental regulators by Polycomb in human embryonic stem cells. Cell. 2006;125(2): 301-13

[51] Molovsky AV, Pardal R, Iwashita T, Park IK, Clarke MF, Morrison SF. Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. Nature. 2003;425(6961):962-7

[52] Saeki M, Kobayashi D, Tsuji N, Kuribayashi K, Watanabe N. Diagnostic importance of overexpression of Bmi-1 mRNA in early breast cancers. Int J Incol. 2009;35(3):511-5

[53] Qin ZK, Yang JA, Ye YL, Zhang X, Xu LH, Zhou FJ, Han H, Liu ZW, Song LB, Zeng MS. Expression of BMI-1 is a prognostic marker in bladder cancer. BMC Cancer. 2009;9:61

[54] Xu K, Wu ZJ, Groner AC, He HH, Cai C, Lis RT, WU X, Stack EC, Loda M, Liu T, Xu H, Cato L, Thornton JE, Gregory RI, Morrissey C, Vessella RL, Montironi R, Magi-Galluzzi C, Kantoff PW, Balk SP, Liu XS, Brown M. EZH2 oncogenic activity in castration-resistant prostate cancer cells is Polycomb-independent. Science. 2012;338(6113):1465-9

[55] Tonini T, Bagella L, D´Andrilli G, Claudio PP, Giodano A. Ezh2 reduces the ability of HDAC1-dependence pRb2/p130 transcriptional repression of cyclin A. Oncogene. 2004;23(28)(:4930-7

[56] Lu C, Han HD, Mangala LS, Ali-Fehmi R, Newton CS, Ozbun L, Armaiz-Pena GN, Hu W, Stone RL, Munkarah A, Ravoori MK, Shahzad MM, Lee JW, Mora E, Langley RR, Carroll AR, Matsuo K, Spannuth WA, Schmandt R, Jennings NB, Goodman BW, Jaffe RB, Nick AM, Kim HS, Guven EO, Chen YH, Li LY, Hsu MC, Coleman RL, Calin GA, Denkbas EB, Lim JY, Lee JS, Kundra V, Birrer MJ, Hung MC, Lopez-Berestein G, Sood AK. Regulation of tumor angiogenesis by EZH2. Cancer Cell. 2010;18(2): 185-97

[57] Zeidler M, Kleer CG. The polycomb protein Enhancer of Zeste 2: its link to DNA repair and breast cancer. J Mol Histol. 2006;37(5-7):219-23

[58] Cao Q, Yu J, Dhanasekaran SM, Kim JH, Mani RS, Tomlins SA, Mehra R, Laxman B, Cao X, Yu J, Kleer CG, Varambally S, Chinnaiyan AM. Repression of E-cadherin by the polycomb group protein EZH2 in cancer. Oncogene. 2008;27(58):7274-84

[59] Ougolkov AV, Bilim VN, Billadeau DD. Regulation of pancreatic tumor cell proliferation and chemoresistance by the histone methyltransferase enhancer of zeste homologue 2. Clin Cancer Res. 2008;14(21):6790-6

[60] Winter JM, Ting AH, Vilardell F, Gallmeier E, Baylin SB, Hruban RH, Kern SE, Iacobuzio-Donahue CA. Absence of E-cadherin expression distinguishes noncohesive from cohesive pancreatic cancer. Clin Cancer Res. 2008;14(2):412-8

[61] McCleary-Wheeler AL, Lomberk GA, Weiss FU, Schneider G, Fabbri M, Poshusta TL, Dusetti NJ, Baumgart S, Iovanna JL, Ellenrieder V, Urrutia R, Fernandez-Zapico ME.

Insights into the epigenetic mechanisms controlling pancreatic carcinogenesis. Cancer Lett. 2013;328(2):212-21.

[62] Szafranska AE, Davison TS, John J, Cannon T, Sipos B, Maghnouj A, Labourier E, Hahn SA. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. Oncogene. 2007 Jun 28;26(30):4442-52.

[63] Lee EJ, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, Morgan DL, Postier RG, Brackett DJ, Schmittgen TD. Expression profiling identifies microRNA signature in pancreatic cancer. Int J Cancer. 2007 Mar 1;120(5):1046-54.

[64] Burk U, Schubert J, Wellner U, Schmalhofer O, Vincan E, Spaderna S, Brabletz T. A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. EMBO Rep. 2008 Jun;9(6):582-9.

[65] Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, Feldmann G, Yamakuchi M, Ferlito M, Lowenstein CJ, Arking DE, Beer MA, Maitra A, Mendell JT. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell. 2007 Jun 8;26(5):745-52.

[66] Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. Nat Rev Cancer. 2003 Jun;3(6):459-65.

[67] Downward J. Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer. 2003 Jan;3(1):11-22. Review.

[68] Maitra A, Hruban RH. Pancreatic cancer. Annu Rev Pathol. 2008;3:157-88

[69] Pylayeva-Gupta Y, Lee KE, Hajdu CH, Miller G, Bar-Sagi D. Oncogenic Kras-induced GM-CSF production promotes the development of pancreatic neoplasia. Cancer Cell 2012;21(6):836-47

[70] Bayne LJ, Beatty GL, Jhala N, Clark CE, Rhim AD, Stanger BZ, Vonderheide RH. Tumor-derived granulocyte-macrophage colony-stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer. Cancer Cell. 2012 Jun 12;21(6):822-35.

[71] Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, Ross S, Conrads TP, Veenstra TD, Hitt BA, Kawaguchi Y, Johann D, Liotta LA, Crawford HC, Putt ME, Jacks T, Wright CV, Hruban RH, Lowy AM, Tuveson DA. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell. 2003 Dec;4(6):437-50.

[72] Larsson LG. Cellular senescence--a barrier against tumor development? Semin Cancer Biol. 2011 Dec;21(6):347-8.

[73] Blackburn EH. Telomeres: no end in sight. Cell. 1994 Jun 3;77(5):621-3.

[74] Singh S, Ellenrieder V. Senescence in pancreatic carcinogenesis: from signalling to chromatin remodelling and epigenetics. Gut 2013;62:1364-72

[75] DeNicola GM, Tuveson DA. RAS in cellular transformation and senescence. Eur J Cancer. 2009 Sep;45 Suppl 1:211-6

[76] Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, Benguría A, Zaballos A, Flores JM, Barbacid M, Beach D, Serrano M. Tumour biology: senescence in premalignant tumours. Nature. 2005 Aug 4;436(7051):642.

[77] Collado M, Serrano M. Senescence in tumours: evidence from mice and humans. Nat Rev Cancer. 2010 Jan;10(1):51-7.

[78] Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000 Nov 16;408(6810):307-10.

[79] Coqueret O. New roles for p21 and p27 cell-cycle inhibitors: a function for each cell compartment? Trends Cell Biol. 2003 Feb;13(2):65-70.

[80] Stiewe T. The p53 family in differentiation and tumorigenesis. Nat Rev Cancer. 2007 Mar;7(3):165-8.

[81] Hingorani SR, Wang L, Mutani AS, Combs C, Deramaudt TB, Hruban RH. Rustgi AK, Chang S, Tuveson DA. TRP53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastic pancreatic ductal adenocarcinoma in mice. Cancer Cell. 2005; 7(5):469-83

[82] Rozenblum E, Schutte M, Goggins M, Hahn SA, Panzer S, Zahurak M, Goodman SN, Sohn TA, Hruban RH, Yeo CJ, Kern SE. Tumor-suppressive pathways in pancreatic carcinoma. Cancer Res. 1997 May 1;57(9):1731-4.

[83] Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology. 2013;144(6):1252-61

[84] Guerra C, Collado M, Navas C, Schuhmacher AJ, Hernández-Porras I, Cañamero M, Rodriguez-Justo M, Serrano M, Barbacid M. Pancreatitis-induced inflammation contributes to pancreatic cancer by inhibiting oncogene-induced senescence. Cancer Cell. 2011 Jun 14;19(6):728-39.

[85] Buchholz M, Ellenrieder V. An emerging role for Ca2+/calcineurin/NFAT signaling in cancerogenesis. Cell Cycle. 2007;6(1):16-19

[86] Mancini M, Toker A. NFAT proteins: emerging roles in cancer progression. Nat Rev Cancer. 2009 Nov;9(11):810-20.

[87] Hogan PG, Chen L, Nardone J, Rao A. Transcriptional regulation by calcium, calcineurin, and NFAT. Genes & Development 2003. 17:2205-32

[88] Singh SK, Baumgart S, Singh G, König AO, Reutlinger K, Hofbauer LC, Barth P, Gress TM, Lomberk G, Urrutia R, Fernandez-Zapico ME, Ellenrieder V. Disruption

of a nuclear NFATc2 protein stabilization loop confers breast and pancreatic cancer growth suppression by zoledronic acid. J Biol Chem. 2011 Aug 19;286(33):28761-71.

[89] Baumgart S, Ellenrieder V, Fernandez-Zapico ME. Oncogenic transcription factors: cornerstones of inflammation-linked pancreatic carcinogenesis. Gut. 2013 Feb;62(2): 310-6.

[90] König A, Fernandez-Zapico ME, Ellenrieder V. Primers on molecular pathways--the NFAT transcription pathway in pancreatic cancer. Pancreatology. 2010;10(4):416-22.

[91] Diehl S, Chow CW, Weiss L, Palmetshofer A, Twardzik T, Rounds L, Serfling E, Davis RJ, Anguita J, Rincon M. Induction of NFATc2 expression by interleukin 6 promotes T helper type 2 differentiation. J. Exp. Med. 2002;196(1):39-49

[92] Neal JW, Clipstone NA. A constitutively active NFATc1 mutant induces a transformed phenotype in 3T3-L1 fibroblasts. J Biol Chem. 2003 May 9;278(19):17246-54.

[93] Buchholz M, Schatz A, Wagner M, Michl P, Linhart T, Adler G, Gress TM, Ellenrieder V. Overexpression of c-myc in pancreatic cancer caused by ectopic activation of NFATc1 and the Ca2+/calcineurin signaling pathway. EMBO J. 2006;25(15):3714-24

[94] König A, Linhart T, Schlengelmann K, Reutlinger K, Wegele J, Adler G, Singh G, Hofmann L, Kunsch S, Büch T, Schäfer E, Gress TM, Fernandez-Zapico ME, Ellenrieder V. NFAT-induced Histone Acetylation Relay Switch promotes c-Myc-dependent growth in Pancreatic Cancer Cells. Gastroenterology. 2010;138:1189-99

[95] Jauliac S, López-Rodriguez C, Shaw LM, Brown LF, Rao A, Toker A. The role of NFAT transcription factors in integrin-mediated carcinoma invasion. Nat Cell Biol. 2002 Jul;4(7):540-4.

[96] Duque J, Fresno M, Iñiguez MA. Expression and function of the nuclear factor of activated T cells in colon carcinoma cells: involvement in the regulation of cyclooxygenase-2. J Biol Chem. 2005 Mar 11;280(10):8686-93.

[97] Shi A, DiRenzo D, Qu C, Barney D, Miley D, Konieczny SF. Maintanance of acina cell organization iscritical to preventing Kras-induced acinar-ductal metaplasia. Oncogene 2013. 32, 1950-8

[98] Shi C, Hong SM, Lim P, Kamiyama H, Khan M, Anders RA, Goggins M, Hruban RH, Eshleman JR. KRAS2 mutations in human pancreatic acinar-ductal metaplastic lesions are limited to those with PanIN: implications for the human pancreatic cancer cell of origin. Mol Cancer Res. 2009 Feb;7(2):230-6

[99] Huang C, Yang G, Jiang T, Cao J, Huang KJ, Qiu ZJ. Down-regulation of STAT3 expression by vector-based small interfering RNA inhibits pancreatic cancer growth. World J Gastroenterol. 2011 Jul 7;17(25):2992-3001.

[100] Shang C, Attema J, Cakouros D, Cockerill PN, Shannon MF. Nuclear factor of activated T cells contributes to the function of the CD28 response region of the granulocyte

macrophage-colony stimulating factor promoter. Int Immunol. 1999 Dec;11(12): 1945-56.

[101] Ellenrieder V, Fernandez Zapico ME, Urrutia R. TGFbeta-mediated signaling and transcriptional regulation in pancreatic development and cancer. Curr Opin Gastroenterol. 2001 Sep;17(5):434-40.

[102] Fernandez-Zapico ME, Ellenrieder V. NFAT transcription factors, the potion mediating "Dr. Jekill-Mr. Hyde" transformation of the TGFβ pathway in cancer cells. Cell Cycle. 2010 Oct 1;9(19):3838-9.

[103] Singh G, Singh SK, König A, Reutlinger K, Nye MD, Adhikary T, Eilers M, Gress TM, Fernandez-Zapico ME, Ellenrieder V. Sequential activation of NFAT and c-Myc transcription factors mediates the TGF-beta switch from a suppressor to a promoter of cancer cell proliferation. J Biol Chem. 2010 Aug 27;285(35):27241-50

[104] Ellenrieder V. TGFbeta regulated gene expression by Smads and Sp1/KLF-like transcription factors in cancer. Anticancer Res. 2008 May-Jun;28(3A):1531-9

[105] Gil J, Peters G. Regulation of the In4b-ARF-Ink4a tumour suppressor locus: all for one and one for all. Nature Reviews. 2006;7:667-77

[106] Baumgart S, Glesel E, Singh G, Chen NM, Reutlinger K, Zhang J, Billadeau DD, Fernandez-Zapico ME, Gress TM, Singh SK, Ellenrieder V. Restricted Heterochromatin Formation links NFATc2 repressor activity with Growth promotion in Pancreatic Cancer. Gastroenterology. 2012;142:388-98

Chapter 2

Stem Cells in Pancreatic Cancer

Cristiana Pistol Tanase, Ana-Maria Enciu, Maria Linda Cruceru, Laura Georgiana Necula, Ana Iulia Neagu, Bogdan Calenic and Radu Albulescu

Additional information is available at the end of the chapter

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1. Introduction

Pancreatic cancer is the fourth most frequent cause of cancer-related deaths; it also represents one of the most aggressive cancer types, with a high incidence of distant metastasis and mortality [1]. The detection of pancreatic cancer at early stages, the prediction of the potential resectability, or the response to therapy are the current major challenges in improving the clinical outcome of pancreatic ductal adenocarcinoma (PDAC) [2]. The main issue against successful therapy is represented by the absence of early diagnostic and prognostic markers, as well as the unresponsiveness to radiation and chemotherapies [3]. Among other factors that contribute to the lack of success in the therapy of pancreatic malignancies, cancer stem cells (CSCs) appear to have a major role. Cancer is characterized by cellular heterogeneity; CSCs, which represent a distinct subpopulation of cells, seem to be responsible for tumor initiation and persistency, due to their properties of self-renewal and multilineage differentiation. CSCs are considered as best candidates responsible for tumorigenesis, metastasis, and chemo-and radio-resistance [4]. Understanding and properly addressing the challenge represented by CSCs appears as a logical, yet difficult task in anti-cancer strategies.

2. Cancer stem cells: Involvement in the progression, invasion and metastasis

2.1. Pancreatic cancer stem stells (CSCs) phenotyping and isolation

Cancer stem cells from epithelial tissues were identified for the first time in breast cancer in 2003, when Al-Hajj et al. reported that a distinct population of cells, CD44+CD24−/low

epithelial-specific antigen (ESA+), develops tumors in immunodeficient mice [5]. In pancreatic cancer, the presence of CSCs was reported in 2007 by Li C *et al*, who showed that CD44+CD24+ESA+cells possess highly tumorigenic potential [6].

Similar to other types of cancer, pancreatic tumor cells apparently grow around a population of CSCs which are capable of promoting tumor growth and progression through many mechanisms, including alteration of adjacent stromal cells and evasion of conventional therapies [7]. Therefore, their identification, isolation and further *in vitro* studies represent the field that provided the most important breakthroughs in pancreatic cancer. The phenotypic characterization of CSCs is an ongoing process, however, there are some biomarkers that are recognized as significant for the stemness phenotype: CD133, Nestin, Notch1-4, Jagged 1 and 2, ABCG2 and aldehyde dehydrogenase (ALDH1) [8]. Following the model of breast cancer stem cells [5], a pancreatic CSC subpopulation was shown to be epithelial-specific antigen $(ESA)^+/CD44^+$, but unlike the first, also $CD24^+$[6]. $CD44^+CD24^+ESA^+$cells represent 0.5% to 1.0% of all pancreatic cancer cells [4] and show self-renewal capacity *in vitro*, are capable of forming tumor spheres, and can be passaged multiple times without loss of tumor sphere-forming capability [9, 10].

CD133 is a biomarker for putative CSC in several solid tumors [11] and it was used as a marker for flow cytometry to select a subpopulation of tumor cells able to generate tumors in athymic mice [12]; it has been reconfirmed in later studies, by immunohistochemistry, to be present in ductal adenocarcinomas [13]. Furthermore, double positive $CD133^+/CXCR4^+$seem to be preferentially located in the migration front of pancreatic tumors [12] and demonstrate increased metastatic abilities [14].

Along with CD133, aldehyde dehydrogenase 1 (ALDH1) is also considered a useful marker of stemness, both of which are currently being used for flow cytometry sorting of stem-enriched side populations [15]. Increased activity of ALDH1 was associated with CSCs and has been correlated with invasion, migration and poor overall survival in patients with pancreatic cancer [16]. Therefore, ALDH (+) cells have stem and mesenchymal cell features and are more tumorigenic than $CD44^+/CD24^+$cells [17]. An intriguing and somewhat discouraging observation is that only 0.015% of all tumor cells are concomitantly ALDH+and $CD44^+/CD24^+$, yet ALDH+cells alone have potent tumorigenic activity, thus, several subsets of tumor-initiating cells might be present within a pancreatic tumor [18].

The majority of CSCs is not positive for cytokeratins (intermediate filament proteins present in differentiated epithelial cells) [12], but for Nestin – an intermediate filament protein and a stem cell marker associated with cell integrity, migration, and differentiation. In pancreatic carcinoma, one third of tumor cells present nestin expression which is correlated with tumor staging and metastasis. Nestin-expressing cells are involved in epithelial-to-mesenchymal transition (EMT) and seem to be the origin of pancreatic intraepithelial neoplasia lesions [19]. Recently, presence of Nestin in various types of malignancy was associated with tumoral angiogenesis and was proposed as an angiogenic marker [20].

Within a recent study, authors comparatively analyzed cancer stem cell markers in normal pancreas and pancreatic ductal adenocarcinoma, yielding surprising results: although

expression was increased, neither CD133, nor Notch proteins or ALDH1 reached statistical significance; in turn, Jagged 1 was shown to be a robust marker, along with Nestin [8].

Mouse models of ductal pancreatic neoplasia seem to harbor a subpopulation of cells expressing high levels of doublecortin-like kinase 1(DCLK1), alpha tubulin acetyltransferase 1(ATAT1), hairy and enhancer of split-1(HES1), hairy/enhancer-of-split related with YRPW motif 1(HEY1), Insulin-like growth factor 1 receptor (IGF1R), and Abelson murine leukemia viral oncogene homolog 1 (ABL1) with cancer-initiating properties. As this subpopulation is identifiable at very early stages during adenocarcinoma development, it provides new targets for early diagnostic and drug testing [21].

All the studies suggest the importance of CSCs in the prognostic and therapeutic responses of pancreatic cancer patients and underline the necessity of stem cell surface marker characterization. In this regard, it is useful to better understand the basic genetic and epigenetic processes of cancer stem cell transformation from highly regulated stem cells and also the interaction between stem cells and the tumor niche [22].

2.2. Epithelial-to-mesenchymal transition

Recent studies suggest the involvement of CSCs in the progression, aggressiveness and epithelial-to-mesenchymal transition (EMT) in pancreatic cancer [23, 24].

The epithelial-to-mesenchymal transition concept was first described 40 years ago, in relation to the development of the embryo and germ layer formation [25]. Since then, EMT has been shown to be a key player in several normal biological processes or pathologies, such as: embryogenesis, wound healing or cancer progression. The process is essentially defined by phenotypic changes of epithelial cells towards mesenchymal cells. During embryogenesis, EMT represents the biological process in which cells from the epithelial compartment detach, migrate and acquire a mesenchymal phenotype required for the formation of the mesoderm [26]. EMT also plays a key role upon wounding; the wound healing process is marked by epithelial cell migration to the site following EMT signals from the surrounding tissues and acquisition of the mesenchymal-like phenotype [27]. During this process, changes occur in the expression of specific genes, epithelial cell down-regulation of adherent and tight junction proteins (Claudin1 and 7, Occludin and E-cadherin) and matrix metalloproteinase-increased activity, resulting in increased mobility [28]. The major embryonic signaling pathways Wnt, Notch, Hedgehog and Transforming growth factor beta (TGF-β) are involved in upregulation of EMT-activating transcription factors, including Snail, Twist and Slug families [29].TGF-β signaling, associated with other signaling pathways like Ras/MAPK, is essential for EMT process by repressing junction components like E-cadherin, Claudins, and Occludin via Snail transcription factors. TGF-β is also involved in carcinogenesis, playing dual roles by acting as a tumor suppressor in early tumor development, and paradoxically, by promoting tumor cell invasion in later stages [30].

Wnt signaling is also involved in theEMT program, by stabilizing Snail and β-catenin levels and by blocking Glycogen synthase kinase 3 (GSK-3β) activity, processes also related to

cancer metastasis. On the other hand, Snail can interact with β-catenin and it enhances Wnt signaling [31].

Notch signaling is responsible for cell fate, proliferation, differentiation, apoptosis and the maintenance of stem cells and also for hypoxia, which can activate EMT in cancer [32]. It is also considered that Notch can regulate endothelial and mesenchymal markers to sustain mesenchymal transformation [33]. Notch pathways have been shown to increase cellular migration by activating Nuclear factor kappa β (NF-κB), Matrix metalloproteinase 9 and Vascular endothelial growth factor (VEGF) in pancreatic cancer cells [34]. More studies suggest that Notch inhibition can reverse EMT in the Mesenchymal-to-Epithelial Transition (MET) and can be considered a promising therapeutic strategy in cancer treatment [35].

Hedgehog signaling is also involved in embryonic cell growth and organogenesis as well as in regulating genes associated with cell proliferation, differentiation, and cell motility [36]. Some studies showed that the Hedgehog pathway, normally quiescent in adult organs, is very active in cancer where it can increase stromal hyperplasia, myofibroblast differentiation, and production of extracellular matrix, enabling the EMT process in cancer cells [37].

A solid body of literature shows that the EMT process is actively implicated in tumor metastasis and tumor recurrence and that cancer stem cells that have undergone EMT display resistance to therapy [38, 39]. The accepted theory is that CSCs from solid tumors acquire migratory potential together with mesenchymal transition, migrate from the primary tumor, colonize other tissues and form a new metastatic tumor with similar characteristics as the initial one (Figure 1) [40, 41]. *In vitro* and *in vivo* studies support EMT involvement in early steps of carcinogenesis, by identifying EMT-associated markers such as mesenchymal-specific markers (i.e. Vimentin and Fibronectin), epithelial specific markers (i.e. E-cadherin and Cytokeratin), and transcription factors (i.e. Snail and Slug) in tumor samples [42]. Moreover, the expression of EMT-specific genes has been identified at the level of the invasive front of primary tumors [32] and reversely, the expression of CSCs markers can be induced by overexpressing Snail or Twist, the most important transcription factors involved in the EMT process [43]. From the other point of view, cancer cells from metastasis after the EMT process can show a CSC phenotype and TGF-β signaling is considered to be a crucial factor involved in these processes [44].

Cellular migratory potential is also increased by up-regulation of Mucin-4 (MUC4) and fibroblast growth factor receptor 1 (FGFR-1) stabilization [45]. Other studies show that the process in pancreatic cancer can also be regulated by Forkhead box protein M1 (FoxM1)-caveolin [46], GLI-Kruppel family member GLI1 (GLI1) [47], hepatocyte growth factor (HGF) or platelet-derived growth factor (PDGF) [48]. Taken into account these observations, EMT-type pancreatic tumor cells represent a highly important research focus for the therapies aiming at reducing or preventing invasion, metastasis and therapeutic resistance in pancreatic cancer.

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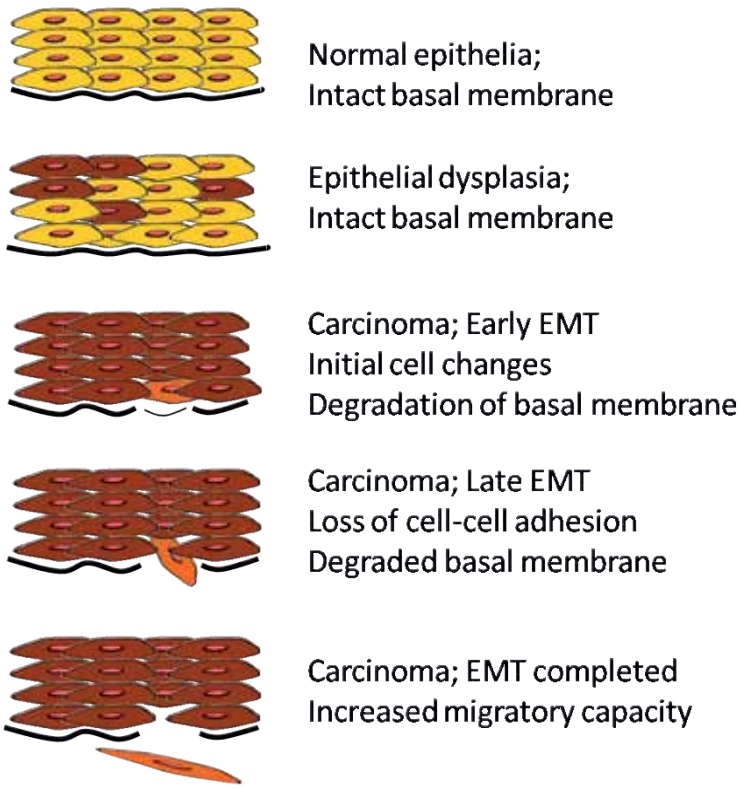

Figure 1. Epithelial-to-mesenchymal transition process

2.3. Regulatory pathways in pancreatic cancer stem cells

Analysis of expression of CSC-related genes in a purified subpopulation of putative pancreatic CSCs showed that up to 46 canonical pathways are upregulated, including human embryonic stem cell pluripotency, tight junction signaling, NF-kB signaling, Wnt/β-catenin signaling, integrin signaling, and Ephrin signaling networks [49].

In particular, out of most signaling pathways involved in maintaining self-renewal in normal stem cells, pancreatic CSCs are characterized by overexpression of Sonic Hedgehog (Shh), Wnt, Notch, AKT, NF-kB, and BMI1 Polycomb Ring Finger Oncogene(BMI-1). Further, signaling pathways which are not dysregulated in metastatic tumors are overexpressed in the pancreatic CSCs [4, 50].

Hedgehog, Notch, Wnt (Figure 2) are shown to be of particular importance in pancreatic cancer stem cells, due to their role in pancreatic embryonic development and differentiation [51]. These signaling pathways are altered in CSCs and EMT-like cells in pancreatic cancer, being involved in self-renewal of CSCs, tumor growth, invasion, metastasis, and resistance to therapy [52].

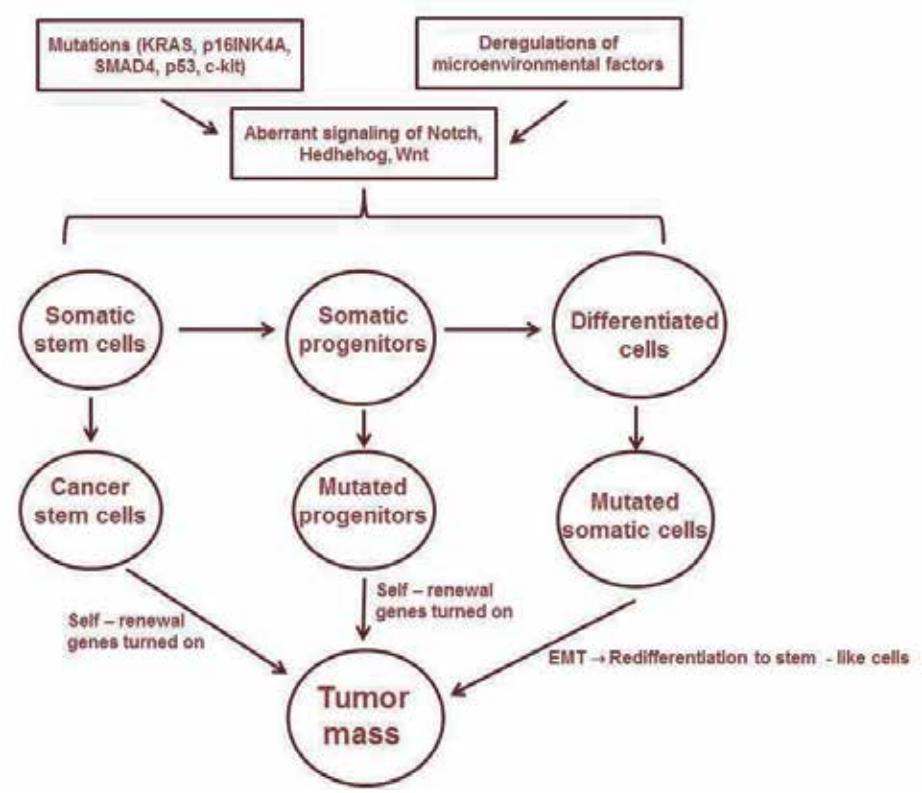

Figure 2. Factors involved in occurrence of cancer stem cells. The emergence of mutations and aberrant signaling in normal stem cells, progenitors, or differentiated cells triggers the transformation of normal cells into cancer stem cells, losing control of cell division.

Notch signaling is involved in the early developmental stages of pancreatic cancer by maintaining epithelial cells in a progenitor state. Tumor cells present an overexpression of Notch signaling, high levels of Notch-1 and Notch-2 while normal pancreas shows a weak expression of pathway-related molecules [53, 54]. Notch signaling is involved in cell proliferation, survival, apoptosis and differentiation of pancreatic cells and can promote EMT by controlling some transcription factors and growth factors like Snail, Slug, and TGF-β. Among Notch target genes are found Akt, cyclin D1, c-myc, cyclooxygenase-2 (COX-2), extracellular signal-regulated kinase (ERK), matrix metalloproteinase-9 (MMP-9), mammalian target of rapamycin (mTOR), NF-κB, VEGF, p21cip1, p27kip1, and p53, all involved in development and progression of human cancer. Gemcitabine-resistant pancreatic cancer cells present overexpression of Notch-2 and Jagged-1, while Notch1, a key downstream mediator of Kirsten rat sarcoma viral oncogene homolog(KRAS), is responsible for pancreatosphere formation [7, 51, 53]. Overexpression of Notch ligand Delta like ligand 4 (Dll-4) in pancreatic cancer cells promotes expression of octamer-binding transcription factor 4(Oct4) and Homeobox Transcription Factor Nanog(Nanog) (transcription factors essential for both early embryonic development and pluripotency maintenance in ES cells) and thus increases the number of CSCs [55, 56].

Many studies found that pancreatic cancer stem cell resistance to chemotherapy is linked to activated Notch signaling, but the exact mechanism remains unclear [57, 58]. There is more evidence showing that the Notch signaling pathway is essential in supporting KRAS ability to transform normal cells into tumor stem cells. Notch-1 inhibition with specific siRNA or treatment with γ-secretase inhibitors increases apoptosis and decreases proliferative rates, cell migration and invasive properties of pancreatic cancer cells [53]. In this regard, in pancreatic cancer treatment, Notch signaling inhibition can be quite attractive, as long as there is no data arguing that Notch signaling has a critical role in normal adult pancreatic homeostasis [59]. Targeting Notch signaling as a treatment for metastatic pancreatic cancer could prevent the acquisition of the EMT phenotype and resistance to therapy [60].

Hedgehog signaling is another self-renewal pathway, allowing normal stem cells to become independent of control signals; as a result of mutations in this signaling, transformed cells can use Hedgehog for tumor initiation, progression, and metastasis. *In vivo* studies showed that compared to normal pancreatic epithelial cells, CD44+CD24+ESA+pancreatic cancer stem cells present with an up-regulation of Sonic Hedgehog (Shh) transcripts (a ligand of Hedgehog signaling) [61]. Moreover, 70% of pancreatic cancer tissue presents overexpression of Shh, suggesting that Hedgehog signaling may be involved in pancreatic carcinogenesis [51]. Many studies showed that Shh signaling can activate pancreatic stellate cells, promotes fibroblast infiltration, and increases secretion of fibronectin, collagen type I, MMPs, and TGF-β [62]. Studies in the pancreatic cancer cell line PANC-1 showed that inhibition of Hedgehog signaling by Smoothened (Smo) suppression can reverse EMT, induce apoptosis via PI3K/AKT inhibition, and inhibit the invasion of pancreatic cancer cells [63]. Moreover, combination of focal irradiation with Hedgehog signaling inhibition reduces lymph node metastasis in an orthotopic animal model [64].

Wnt/β-catenin signaling is involved in cell proliferation, migration, apoptosis, differentiation, and stem cell self-renewal in several types of cancer [65]. Wnt/β-catenin signaling pathway dysregulation is also associated with chemoresistance in pancreatic cancer and recent studies suggest that nuclear β-catenin is essential for the EMT [66, 67]. *In vitro* and *in vivo* studies suggest that activated β-catenin may decrease differentiation of epidermal stem cells, increase self-renewal capacity, and develop epithelial cancers in transgenic mice [68]. Kong D et al. showed that there are some connections between Wnt signaling and Snail, a major regulator of the EMT process. Thus, overexpression of Snail could increase expression of Wnt target genes by interaction with β-catenin [69].

In 2013, Sun L et al. showed that one of the most active signaling pathways in pancreatic cancer stem cells is NF-kB, whose inhibition leads to loss of stem cell properties. This study also showed that aberrant epigenetic processes, like CpG promoter methylation, can be involved in carcinogenesis mediated by cancer stem cells [70]. These results were confirmed by studies conducted on PANC1 and HPAC pancreatic cancer cell lines [51]. Activity of the pro-inflammatory NF-κB induces expression of Shh by pancreatic cancer cells and stromal cells, leading to activation of the Hedgehog pathway [71].

Another possible marker for pancreatic CSCs is Met Proto-Oncogene (c-Met), whose inhibition has been correlated with a decrease of tumor growth and with preventing the development of

metastases [1, 72]. c-Met is a receptor tyrosine kinases involved in cell survival, growth, angiogenesis and metastasis. c-Met activates many signaling pathways, including Ras-MAPK, PI3K/Akt NF-kB, and Wnt/GSK-3β/β-Catenin and is overexpressed in pancreatic cancer [73].

2.4. MicroRNAs in pancreatic adenocarcinoma

MicroRNAs (miRNAs) are potent regulators of cell function via their role as translational regulators for the synthesis of key proteins. Most often, several miRNAs display different expression profiles in cancer cells, including pancreatic cancers.

MiR-21, miR-155 and miR-17−5p appear upregulated in tumoral cells, and these miRs are often called oncogenic miRNAs [60, 74]. Similarly, a series of miRNAs, referred to as tumor suppressor miRs (miR-34, miR-15a, miR-16−1 and let-7) are downregulated in cancers [54, 75]. Key cell differentiation programs during development are controlled by the members of lethal-7 (Let-7) and miR-200 families. In cancer, loss of Let-7 leads to disease progression and de-differentiation. The EMT process is also regulated by miRNA-dependent mechanisms and the same Let-7 family appears as a regulator of EMT and of stem cell maintenance. According to Hasselman et al [75], inhibition of maturation of Let-7 by nuclear receptor for the cytotoxic ligand TNFSF10/TRAIL (TRAILR2) in pancreatic cancer cell lines, increases their proliferation. This is consistent with high levels of nuclear TRAIL2 in tissue samples from poor outcome patients.

Pancreatic neoplasms seem also to exhibit their own pattern of miR overexpression, when compared to normal pancreatic tissue: upregulation of miR-93, miR-95, miR-135b, miR-181c, miR-181d, miR-182, miR-183, miR-190, miR-196b and miR-203, miR-767 and miR-1269 and downregulation of miR-20a and miR-29c [76]. In human pancreatic cancer, DCLK1 regulates EMT by a mechanism dependent on miR-200a [77].

MiRNAs were recently considered to have a role in regulation of CSCs [51]. The population of BxPC-3-LN cells (lymph node metastatic pancreatic cells) contains a 5-fold increased population of CD133+/CXCR4+cells (stem-like cells) compared with the parental (non-metastatic) BxPC-3 cells. Remarkably, a different miRNA pattern is displayed in CSC-like compared with the regular cells: up-regulated miR-572, miR-206, miR-449a, miR-489 and miR-184 were found, as well as downregulated let-7g-3p, let-7i-3p, let-7a-3p, miR-107, miR-128 and miR-141−5p[14].

The miR-200 family members are identified as key regulators of cell maintenance and EMT. It is considered possible that tumor progression is a process resulting in progressive de-differentiation towards a cell type having a stem cell-like phenotype. This process appears to be regulated by miRNA-dependent mechanisms. DCLK1 (a putative marker for pancreatic and intestinal cancer stem cells) regulates EMT in human pancreatic cancer cells via a miR-200a-dependent mechanism [77]; it also acts as a regulator of Let-7a in pancreatic and colorectal cancer cells, supporting the concept that these miRNAs may be novel and relevant targets in solid tumor cancers [78]. Sureban et al demonstrated that DCLK1 inhibition results in up-regulation of miRNAs that negatively regulate some key angiogenic and pluripotency

factors [79]. In AsPC1 tumor xenografts, downregulation of c-MYC and KRAS via let-7a was observed by a similar mechanism demonstrated in pancreatic cancer cells.

Repression of two tumor-suppressor miRs, miR-143 and miR-145, is reported in pancreatic cancer, as well as in other cancers [80]; moreover, experimental restoration of miR 143/145 levels using nano-vector delivery was demonstrated to inhibit pancreatic cancer cell growth [81]. The miR-143/145 cluster cooperates and inhibits the expression of KRAS2 and ras responsive element binding protein 1 (RREB1), its downstream effector [80]. MiR-145 was demonstrated to inhibit cell proliferation in lung adenocarcinoma, by targeting epidermal growth factor receptor (EGFR). In many cancers, including pancreatic cancer, EGFR is upregulated [82], while inhibition of EGF signaling inhibits cancer initiation and progression [83]. Also a suppressive effect of EGFR on miR-143 and miR-145 was demonstrated on models of colon cancer [84]. These findings are indicators of a negative feedback loop between EGFR and miR-143/145, which is similar to KRAS/RREB1 − miR-143/145.

The major role of vascular endothelial growth factor (VEGF) signalling via its receptors, VEGFR1 and VEGFR2, was demonstrated in tumor vascular growth, angiogenesis, and metastasis, while upregulated angiogenic factors in various cancers-colorectal, breast, renal, liver, and ovarian-have been correlated with poor prognosis.Pancreatic ductal adenomacarcinoma (PDAC) exhibits endothelial cell proliferation, a mechanisms that increases angiogenesis. Inhibition of VEGF-A, VEGFR1 and VEGFR2 resulted in inhibition of tumor growth and angiogenesis in mouse models of PDAC. Studies and computational analysis outlined a putative binding site for miR-200 (miR-200a, b and c) in the 3′ UTR of VEGFR1 and VEGFR2 [85].

Identification of dysregulated expression of various miRNAs, the existence of regulatory loops between miRNAs and protein regulators of key processes (such as cell growth, angiogenesis, differentiation) suggested the need and potential effectiveness of strategies aiming to restore the ”normal phenotype” expression pattern of miRNAs for cancer treatment. Various approaches are developed and investigated, such as the delivery of tumor suppressor miRNAs [86], suppression of expression or action of oncomirs [87], targeting the expression of key regulators (such as DCLK1, adenosine monophosphate activated kinase α1(AMPKα1)[88], leading to miRNAs modulation or even to simultaneous modulation of multiple miRNAs, suggesting that using miRNAs as therapeutic agents or addressing miRNAs as targets represents a potential solution for the therapy of critical cancers.

2.5. CSCs and tumor environment

Although the presence of stromal tissue is described and accepted as a fact in all types of solid cancers, pancreatic adenocarcinoma displays a particularly dense atmosphere of connective tissue, known as “desmoplastic reaction”. Since the new cancer paradigm of “stroma-cancer interaction”, more thorough investigations have focused on the pancreatic tumor environment, and it is now accepted that the dense connective tissue surrounding malignant cells is at least partially responsible for hindering drug delivery. The pancreatic cancer stroma is now the focus of a new therapeutic approach called “stroma depletion”, which can be achieved

through Hedgehog inhibitors [89]. What stromal cells are responsible for Hedgehog signaling responsiveness is currently under investigation, as it would designate them as new anti-cancer targets. Stromal cells are also of importance when considering the concept of stem cell niche- a unique microenvironment involved in generating hierarchies to maintainself-renewal and to control cell fate. The relationship between CSCs and a putative malignant niche is less well stated than for normal stem cells. CSCs are capable of migrating from the original tumor to distance, behavior that is not common for adult, normal stem cells, but is well documented for the hematopoietic stem cell. Stroma of hematopoietic tissue is a particular one, based on reticular connective tissue, unlike most malignant stromas, rich in dense irregular connective tissue. This would possibly indicate the partial independence of CSC from stem-cell niche [90].

a. Pancreatic stellate cells

There is a proven interaction between the CSCs and the tumor stroma, at least in part responsible for increased metastatic abilities of cancer cells. Tumor-stroma interaction is the new cancer paradigm and in the particular case of pancreatic cancer is supported by the presence of pancreatic stellate cells (PSCs) – a subpopulation of desmin-positive periacinar cells, found as well, but in inactive state, in the normal pancreas [91]. Studied at first in relationship with pancreatic fibrosis [92], they were more recently increasingly investigated in the progression of pancreatic cancer [93-95]. In the activated form, stellate cells secrete an array of pro-inflammatory cytokines and promote an immunosuppresive microenvironment [96], secrete various growth factors (e.g. platelet-derived growth factor, stromal-derived factor 1, epidermal growth factor, insulin-like growth factor 1, fibroblast growth factor) [97], as well as matrix adhesion molecules (collagen type I, secreted protein acidic and rich in cysteine (SPARC), small leucine-rich proteoglycans, periostin) and matrix metalloproteinases (MMP-2 and MMP-9), that have been associated with the invasive phenotype of pancreatic cancer cell lines [41]. This particular pattern of pancreatic cell secretome mediates effects on tumor growth, invasion, metastasis and resistance to chemotherapy and is modulated by CSCs, through release of mitogenic and fibrogenic stimulants, such as Transforming Growth Factor β1 platelet-derived growth factor, sonic hedgehog, galectin 3, endothelin 1 and serine protease inhibitor nexin 2 [97]. Recognition of their importance in tumoral behaviour led efforts to isolate, cultivate and immortalize them for further manipulation with therapeutic purposes [98-100]. Upon activation, pancreatic stellate cells suffer a shift of phenotype towards myofibroblast morphology and a subsequent switch of protein expression [101]. Indirect co-culture of pancreatic cancer cells with PSCs seem to favor the stem phenotype of cancer cells, as evaluated by Hamada et al. by the spheroid-forming ability of cancer cells and expression of cancer stem cell-related genes ABCG2, Nestin and LIN28. In addition, co-injection of PSCs enhanced tumorigenicity of pancreatic cancer cells *in vivo* [90]. The presence of α smooth muscle actin (αSMA) in activated pancreatic stellate cells leads to association with cancer-associated fibroblasts (CAFs) – a cancer modified subpopulation of fibroblasts, identified by the very same marker, that was shown to sustain tumor cells metabolism and favor tumor progression [102]. CAFs also mediate EMT of tumor cells, possibly through a pro-inflammatory signature [103] – secretome that has also been reported in pancreatic stellate cells, not only in cancer but also in chronic pancreatitis [104].

From tumor-stroma interactions new lessons were learned in diagnostics and therapeutics of pancreatic cancer. Secreted Protein, Acidic, Cysteine-Rich (SPARC) (a member of the family of matricellular glycoproteins that is highly expressed in PSCs and the tumour/stroma interface) is now proposed as marker for accurate diagnostic, as 80% of pancreatic ductal adenocarcinomas seem to express it [105]. Due to its ability to bind to basement membrane collagen IV and fibrillar collagens I, III, V and also to bind albumin [106], it has been used to increase distribution of the chemotherapeutic agent paclitaxel within the tumoral mass [107].

Changes within the stem niche, such as hypoxia, are "tuning" the behavior of stem cells, inducing the activation of survival, proliferation, differentiation and angiogenesis.

b. Mesenchymal stem cells – dual facets in cancer

Mesenchymal stem cells (MSCs) are pluripotent cells with homing abilities that are involved in tissue repair, including outside their native niche, that reside primarily in the bone marrow, but also exist in other sites such as adipose tissue, peripheral blood, cord blood, liver, and fetal tissues [108]. They also exhibit a natural tendency of homing into tumors – ability that is starting to be exploited in anticancer treatment, using these versatile cells as cargo delivery for cytotoxic drugs or gene therapy [109]. This behavior has been also reported in pancreatic cancer, by the use of genetically engineered labeled MSCs that efficiently accumulatewithin the pancreatic tumor, when injected into tumor-bearing mice [110].

Pro-tumor effect of MSCs

Very recent reports have demonstrated that mesenchymal stem cells (MSCs) can function as precursors for CAFs [111, 112]. Interestingly, not all types of MSCs have this particular ability, a recent report from Subramanian et al. arguing that this is not a feature of umbilical-cord derived pluripotent cells[113]. In pancreatic cancer, like in any other type of cancer, these myofibroblast-like cells contribute to inducing EMT in side population cells, maintain tumor-initiating stem cell-like characteristics, including augmenting expression levels of various stemness-associated genes, enhancing sphere-forming activity, promoting tumor formation in a mouse xenograft model, and showing resistance to anticancer drugs [114].

Bone marrow derived progenitor cells were found to participate to neovascularization of tumors [115], a process that was shown to be dependent on Hedgehog signaling [116]. The recruitment of these progenitors is accomplished by CAFs through stroma-cell derived factor 1(SDF-1) signaling [117].

Anti-tumor activity

An increasing number of reports show that MSCs have the ability of negatively influencing tumor behaviour, in terms of proliferation and invasiveness. Cell cultures co-cultivated or treated with MSCs conditioned media showed inhibited growth [118-120] and co-injection of tumor cells and MSCs in nude animals showed that tumor growth was significantly inhibited [120]. Some authors explain this activity by MSCs to inhibit the expression of Wnt signaling pathway-related factors in tumor cells, consequently unbalancing cellular proliferation and apoptosis [121].

To conclude, the presence of MSC within the tumor site is a fact, but its role is still to be determined.

3. CSCs and therapy outcomes

In pancreatic cancer, surgery is usually accompanied by other complementary treatments such as multi-chemotherapy regimens and radiotherapy. Despite clear progress in detection and treatment of cancer, current strategies fail to completely remove the tumor and prevent recurrence and metastasis. Existing therapies are toxic and non-specific, being directed towards both normal cells and tumor cells. Most chemotherapeutic regimens are based on gemcitabine, but provided a modest improvement in median survival. The response rate was increased by using more than two chemotherapeutic agents [122]. Human pancreatic cancer tissue contains CSCs defined by CD133 and CXCR4 expression and these cells are highly resistant to standard chemotherapy and are involved in metastasis [12]. Features of CSCs have also been confirmed in brain and colon cancers [9].Therapy failure for other highly malignant tumors has been explained, at least partially, by the chemo-[10, 123] and radio-resistant [124] nature of CSCs. Cancer stem cells therapy resistance is considered to be the result of inappropriate activation of several proliferative signaling pathways, including EGFR, PDGFR(platelet-derived growth factor receptor), stem cell factor (SCF) receptor KIT [125], and activation of Hedgehog and Wnt/β-catenin signaling [50]. Another well sustained argument for chemotherapy resistance is the expression of multidrug resistance-linked genes, out of which most are ATP-binding cassette (ABC) drug transporters [126]. High levels ofABC transporters were documented in pancreatic CSCs and chemotherapeutic agents such as etoposide, doxorubicin, vincristine and paclitaxel are direct substrates of ABC transporters [127]. Gemcitabine uptake, the golden standard for pancreatic adenocarcinoma chemiotherapy, seems to be negatively influenced by expression of ABCG2, though there is no clear evidence that ABC transporters directly efflux gemcitabine or its metabolites in pancreatic cancer cells [90]. Several reports indicate that conventional chemotherapy itself could propagate the CSC population in pancreatic cancer, through exerting a positive selection pressure of CD24/CD44/ESA triple positive CSC fraction [12, 128].

Differential expression of some CSCs biomarkers can be indicative of particular characteristics, such as responsiveness to different therapies or outcomes.

3.1. CSCs as therapeutic targets

Different strategies are developed to target specifically CSCs, thus eliminating this particular set of cells. Several key regulatory pathways operating in the stem cells have been proposed and demonstrated to considerably improve the therapy outcomes; relevant examples are Sonic Hedgehog, Notch/Jagged, CD133, TGF beta signaling; specifically addressing such pathways, by small molecule inhibitors, monoclonal antibodies or siRNAs results in increasing the efficacy of therapies, as suggested by *in vitro* studies, as well as by clinical outcomes.

Some *in vitro* studies showed that blocking *cis*-acting elements, that are common for pluripotency maintaining Transcription Factor SOX-2 (Sox2), Oct4, and proto-Oncogene C-Myc (c-Myc), dramatically decreased CSCs proliferation and their ability to generate tumors in nude mice [15]. Equally, simultaneous knockdown of OCT4 and its target Nanog led to decreased proliferation, migration, invasiveness and tumorigenesis of putative pancreatic cancer stem cells [129]. Inhibition of the Nodal/Activin receptor Alk4/7 in CSCs decreased almost to zero their self-renewal capacity and tumorigenicity, and reversed the resistance of CSCs to gemcitabine. Concordant with previous reports on stroma-tumor interaction, Lonardo *et al.* also found the response to gemcitabine was dependent on the amount of stroma which hindered drug delivery. The addition of a stroma-targeting hedgehog pathway inhibitor (HHI) enhanced delivery of the Nodal/Activin inhibitor and translated into long-term, progression-free survival [130].

The *Hedgehog* signaling pathway is usually targeted in experimental designs as adjuvant to classic chemotherapy. The combined blockade of Shh and mTOR signaling together with gemcitabine is capable of eliminating pancreatic CSCs [131]. Inhibition of Smoothen (Smo), combined with gemcitabine and mTOR inhibitor rapamycin, led to abrogation of cancer stem cells and the authors reported a long-term disease stabilization or regression and subsequent long-term survival [132].

Notch pathway inhibition by selective γ-secretase inhibitors, such as PF-03084014, a selective γ-secretase inhibitor, alone and in combination with gemcitabine, inhibited the cleavage of nuclear Notch 1 intracellular domain and Notch targets Hes-1 and Hey-1 and induced tumor regression in xenograft tumor models. The authors argue that the observed effects are due to PF-03084014 targeting of putative aggressive cancer stem cells [59]. Another potent and selective γ-secretase inhibitor, MRK-003, also led to downregulation of nuclear Notch1 intracellular domain, inhibition of anchorage-independent growth, and reduction of tumor-initiating cells capable of extensive self-renewal. Pretreatment of a pancreatic adenocarcinoma cell line with MRK-003 significantly inhibited the subsequent engraftment in immunocompromised mice and mixed regimen MRK-003 and gemcitabine of engrafted mice reduced tumor cell proliferation, and induced both apoptosis and intratumoral necrosis [133].However, some of such pathways are common to normal and CSCs, raising the problem of increasing the selectivity towards cancer stem cells.

3.2. Clinical studies

Most clinical studies addressing molecular therapies in pancreatic cancer report usage of monoclonal antibodies, for several simple rationales: i) they are already tested as drugs in other types of pathologies, tumoral or not; ii) they block proliferative oversignaling – a characteristic feature of malignancy; iii) some of them address phenotypic anomalies given by genetic dysregulations, such as EFGR overexpression/ oversignaling. However, these antibodies do not address specifically stem cells, but the larger category of cancer cells. There are some constructs that are, however, effective on the side population of CSCs. A combination of tigatuzumab, a fully humanized death receptor5 (DR5) agonist monoclonal antibody, with gemcitabine proved to be more efficacious in killing both CSCs and adenocarcinoma bulk cells.

The combination therapy produced remarkable reduction in pancreatic CSCs, tumor remissions, and significant improvements in time to tumor progression [134]. Signaling pathways can also be inhibited by small molecule kinase inhibitors that act downstream of the extracellular domain of the receptor. Sunitinib targets multiple receptor tyrosine kinases, including stem cell factor receptor (c-KIT) and it has been shown to have antitumor efficacy in *in vivo*. The combination of gemcitabine with sunitinib could not surpass the effects of the single agent sunitinib [135]. Cabozantinib – a small kinase inhibitor that targets c-Met and VEGFR2-inhibited viability and spheroid formation and induced apoptosis in pancreatic malignant cells with minor effects in non-malignant cells. In primary, CSC-enriched spheroidal cultures cabozantinib downregulated CSC markers SOX2, c-Met and CD133 and induced apoptosis [73]. Most clinical studies, so far, do not seem to report any significant improvement with various regimens employed [136]. Early clinical data for the Shh inhibitor, GDC-0449 (vismodegib), in combination with either gemcitabine or erlotinib, indicate that these regimens are feasible and well tolerated [137]. However, a phase II trial of gemcitabine plus saridegib versus gemcitabine plus placebo in previously untreated patients with metastatic pancreatic cancer was halted early based on a shorter overall survival rate in the gemcitabine plus saridegib arm [106].

A very interesting new trend in advanced, chemotherapy-resistant cancers, aiming for a different approach, tests personalized peptide vaccination (PPV) – a method to generate an immune response against tumor-associated antigens and so far employed for aggressive cancers such as lung cancer [138] and biliary tract cancer [139]. For advanced pancreatic cancer a phase II clinical trial was also conducted in which vaccine antigens were selected and administered based on the pre-existing IgG responses to 31 different pooled peptides [140]. Other vaccines are aimed at increasing the patient's immune response against tumor cells – targeting cancer markers with the aid of specialized antigen-presenting cells such as dendritic cells. Currently, there are several vaccines for human pancreatic cancer in clinical trials including: i) whole-cell vaccines, ii) combined dendritic cells with antigen to present to patient leukocytes iii) peptide and DNA vaccines, iv) Ras peptide vaccine; v) vaccine against common cancer mutations, targetable by CD4/8 T cells; vi) Telomerase peptide vaccine; vii) carcinoembrionar antigen (CEA) and Mucin 1; viii) Survivin-targeted vaccine [141]. Also, it was shown that boosting the immune response by additional treatment with dendritic cells (LANEX-DC®) is highly effective and extends the median survival times up to 8.9 months [142].

Lack of response to all of the above mentioned types of therapies led to an investigation of *non-conventional therapies*. Salinomycin, an anti-protozoa agent that was recently shown to preferentially kill breast CSCs [143], and later investigated in other types of malignancies, was shown to inhibit growth of pancreatic adenocarcinoma CSCs *in vitro. In vivo* xenografting studies showed that salinomycin combined with gemcitabine could eliminate the engraftment of human pancreatic cancer more effectively than the individual agents [144]. Adamantyl-substituted retinoid-related molecules (ARRs) inhibit growth and induce apoptosis in the pancreatic stem-like cell population, possibly through decreased IGF-1R and β-catenin expression [145]. Isothiocyanate sulforaphane (SF) was used as sensitizer of pancreatic CSCs to tumor necrosis factor–related apoptosis inducing ligand (TRAIL)-induced apoptosis, by quercetin and sorafenib. The combination of SF with a cytotoxic drug efficiently induced

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apoptosis along with inhibition of self-renewing potential, ALDH1 activity, clonogenicity, xenograft growth and relapse of gemcitabinetreated tumor cells in nude mice [146]. The flavonoid Quercetin enhances TRAIL-mediated apoptosis, acts as a chemosensitizer for the ABC pump-proteins, and can enhance the effects of sulforaphane in inhibiting the pancreatic CSC characteristics [147].

4. Nanotheragnostics in pancreatic cancer

Targeted therapeutic delivery is a way to ensure that drugs reach the designated target at the highest concentration within safety margins, limiting in the same time undesired side effects resulting from unspecific diffusion in well vascularized tissues. This aim is now being resolved with the use of nanomedicine –a multidisciplinary field that aims to utilize nanoscale (up to 100 nm) particles to improve delivery of chemotherapeutics [148]. These constructs fall into several categories – micelles, microemulsions, liposomes, polymers [149] silica and carbon-based nanoparticles [150] and dendrimers [151]. This coating of a nanoparticle can be improved with stabilizing agents (such as polyethylene glycol – PEG) or ligands to direct them to a specific target (such as an antibody towards a cancer cell type). Liposome delivery of active agents has been recently paired with ultrasound technology, by development of ultrasound-responsive stable liposomes. Ultrasound-induced heating triggers phase transition in the phospholipid membrane, leading to drug release in the targeted region [152]. To date, there are at least twelve FDA (Food and Drug Administration) approved liposome-based drugs, most of them being chemotherapeutics for breast, ovarian and pancreatic cancer [153].

Generation of magnetic/metallic nanoparticles was considered a step-forward in magnetic resonance imaging and diagnostics [154], adding a new utility to biomedical nanoscience. Another type of imaging strategy using nanoparticles is optical, through use of carbon nanomaterials that display natural fluorescence emission [155], or use of other infrared light emission agents [156], forming upconversion nanoparticles [157], or incorporated in a wide variety of coating surfaces, such as gold [158] and polymer-based [159]. Photoacoustic imaging is another nanomedical promising technology that combines the benefits of optical imaging methods with the clinically available and cost-effective ultrasound imaging modality [160]. Originally used for investigation of vascularization pattern, based on high endogenous contrast of blood *versus* surrounding tissues [161] and or/vascular wall/lumen alterations [162], it has been increasingly used in tumor assessment, providing further molecular information on cancer, given by the chemical composition of tissues and by targeted nanoparticles that can interact with extravascular tissues at the receptor level [163].

By incorporating active drugs into imaging nanoparticles, a dual therapeutic and diagnostic agent was generated, thus the emerging field of "theragnostic", is widely used especially in cancer research. Most nanoparticles accumulate in tumors due to their intense and leaky neovascularization, but some can be retained there with the use of cancer-specific antigens [164] and stimulated into releasing their chemotherapeutic cargo. Cancer diagnostic and concomitant treatment through nanoparticles benefits from real-time assessment of drug bioavailability and more accurate monitoring of tumor evolution.

Pancreatic cancer treatement benefits from development of biomedical nanotechnology, in both clinical practice and fundamental research. A PEGylated polymeric nanoparticle containing a potent antagonist of the Hedgehog transcription factor Gli1 combined with gemcitabine significantly impeded the growth of orthotopic pancreatic cancer xenografts [165]. In *in vivo* studies, squalene-conjugated gemcitabine nanoparticles decreased tumor growth significantly, prevented tumor cell invasion, and prolonged the survival time of mice bearing orthotopic pancreatic tumors [166]. Liposomal delivery of tissue transglutaminase 2 siRNA effectively blocked the growth of pancreatic adenocarcinoma in nude mice [167]. EGFR monoclonal antibody or peptidylglycine alpha-amidating monooxygenase (PAM4)-conjugated gold nanoparticles induced significant tumor destruction in a murine model of pancreatic carcinoma after radiofrequency radiation [168]. Paclitaxel, one of first-line chemotherapeutic agents before the gemcitabine era, is now available as a positively charged lipid-based complex (known as EndoTAG-1) [169] that in combination with gemcitabine was able to inhibit the incidence of metastasis in pancreatic cancer animal models [170]. A controlled phase II clinical trial for pancreatic cancer showed significantly increased survival rates of patients treated with EndoTAG®-1 and gemcitabine combination therapy [171]. An ongoing phase I study (NCT00968604) of advanced pancreatic cancer is currently investigating the effects of intravenous injection of the liposome nanoparticle BikDD, which contains a pro-apoptotic agent [172].

4.1. Nanoparticles for cancer stem cell targeted therapy

In the same manner that nanoparticles are targeted for the bulk tumor, they can be targeted for CSCs, through the use of antigens against specific CSCs markers (e.g CD-133). Such targeted therapy has already been tested *in vitro*, against targeting CD133-expressing cancer cells of colon and pancreatic origin, with encouraging results [56]. Breast CSCs-targeted nanoparticle delivery of doxorubicin reduced their mammosphere formation capacity and cancer initiation activity, eliciting tumor growth inhibition in animal models[173].

Apart from cytotoxic drug delivery, nanoparticles can be used to target and modify certain characteristics of CSCs, such as activation of signaling pathways that confer renewal properties, targeting metabolism and inhibiting drug efflux transporters in an attempt to sensitize them to therapy [174]. Multi-lamellar vesicle liposomes targeted against CSCs, containing a steroid nucleus, were formulated to disrupt mitochondrial integrity and to facilitate release of cytochrome c to attain programmed cell death [175].

5. Conclusions

CSCs represent key components in the heterogeneous cellular system represented by pancreatic tumors. Their biological features configure them as one of the major players and major targets for investigation; they offer sets of additional and reliable biomarkers for prognosis and stratification. Discovery of target mechanisms and molecules within cancer stem cells is plausible to provide the needed boost for therapy improvement.

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Author details

Cristiana Pistol Tanase[1*], Ana-Maria Enciu[1,2], Maria Linda Cruceru[2],
Laura Georgiana Necula[1,3], Ana Iulia Neagu[1,3], Bogdan Calenic[1,2] and Radu Albulescu[1,4]

*Address all correspondence to: bioch@vbabes.ro

1 Victor Babes National Institute of Pathology, Dept. of Biochemistry-Proteomics, Splaiul Independentei, Bucharest, Romania

2 Carol Davila University of Medicine and Pharmacy, Eroilor Sanitari, Bucharest, Romania

3 Stefan S. Nicolau Institute of Virology, Bucharest, Romania

4 National Institute for Chemical Pharmaceutical Research and Development, Bucharest, Romania

References

[1] Herreros-Villanueva M, Zubia-Olascoaga A, Bujanda L. c-Met in pancreatic cancer stem cells: therapeutic implications. World journal of gastroenterology : WJG. 2012;18(38):5321-3.

[2] Dima SO TC, Albulescu R, Botezatu A and Popescu I (2012). Novel Biomarkers in Pancreatic Cancer, Pancreatic Cancer-Clinical Management, Prof. Sanjay Srivastava (Ed.), ISBN: 978-953-51-0394-3, InTech, DOI: 10.5772/29001. Available from: http://www.intechopen.com/books/pancreatic-cancer-clinical-management/novel-biomarkers-in-pancreatic-cancer.

[3] Kaur S, Baine MJ, Jain M, Sasson AR, Batra SK. Early diagnosis of pancreatic cancer: challenges and new developments. Biomarkers in medicine. 2012;6(5):597-612.

[4] Lee CJ, Dosch J, Simeone DM. Pancreatic cancer stem cells. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2008;26(17):2806-12.

[5] Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(7):3983-8.

[6] Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic cancer stem cells. Cancer research. 2007;67(3):1030-7.

[7] Abel EV, Simeone DM. Biology and clinical applications of pancreatic cancer stem cells. Gastroenterology. 2013;144(6):1241-8.

[8] Vizio B, Mauri FA, Prati A, Trivedi P, Giacobino A, Novarino A, et al. Comparative evaluation of cancer stem cell markers in normal pancreas and pancreatic ductal adenocarcinoma. Oncology reports. 2012;27(1):69-76.

[9] Simeone DM. Pancreatic cancer stem cells: implications for the treatment of pancreatic cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008;14(18):5646-8.

[10] Izumiya M, Kabashima A, Higuchi H, Igarashi T, Sakai G, Iizuka H, et al. Chemoresistance is associated with cancer stem cell-like properties and epithelial-to-mesenchymal transition in pancreatic cancer cells. Anticancer research. 2012;32(9):3847-53.

[11] Grosse-Gehling P, Fargeas CA, Dittfeld C, Garbe Y, Alison MR, Corbeil D, et al. CD133 as a biomarker for putative cancer stem cells in solid tumours: limitations, problems and challenges. The Journal of pathology. 2013;229(3):355-78.

[12] Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell stem cell. 2007;1(3):313-23.

[13] Immervoll H, Hoem D, Sakariassen PO, Steffensen OJ, Molven A. Expression of the "stem cell marker" CD133 in pancreas and pancreatic ductal adenocarcinomas. BMC cancer. 2008;8:48.

[14] Luo G, Long J, Cui X, Xiao Z, Liu Z, Shi S, et al. Highly lymphatic metastatic pancreatic cancer cells possess stem cell-like properties. International journal of oncology. 2013;42(3):979-84.

[15] Wang X, Liu Q, Hou B, Zhang W, Yan M, Jia H, et al. Concomitant targeting of multiple key transcription factors effectively disrupts cancer stem cells enriched in side population of human pancreatic cancer cells. PloS one. 2013;8(9):e73942.

[16] Fredebohm J, Boettcher M, Eisen C, Gaida MM, Heller A, Keleg S, et al. Establishment and characterization of a highly tumourigenic and cancer stem cell enriched pancreatic cancer cell line as a well defined model system. PloS one. 2012;7(11):e48503.

[17] Rasheed ZA, Yang J, Wang Q, Kowalski J, Freed I, Murter C, et al. Prognostic significance of tumorigenic cells with mesenchymal features in pancreatic adenocarcinoma. Journal of the National Cancer Institute. 2010;102(5):340-51.

[18] Kim MP, Fleming JB, Wang H, Abbruzzese JL, Choi W, Kopetz S, et al. ALDH activity selectively defines an enhanced tumor-initiating cell population relative to CD133 expression in human pancreatic adenocarcinoma. PloS one. 2011;6(6):e20636.

[19] Su HT, Weng CC, Hsiao PJ, Chen LH, Kuo TL, Chen YW, et al. Stem Cell Marker Nestin Is Critical for TGF-beta1-Mediated Tumor Progression in Pancreatic Cancer. Molecular cancer research : MCR. 2013.

[20] Matsuda Y, Hagio M, Ishiwata T. Nestin: a novel angiogenesis marker and possible target for tumor angiogenesis. World journal of gastroenterology : WJG. 2013;19(1): 42-8.

[21] Bailey JM, Alsina J, Rasheed ZA, McAllister FM, Fu YY, Plentz R, et al. DCLK1 Marks a Morphologically Distinct Subpopulation of Cells with Stem Cell Properties in Preinvasive Pancreatic Cancer. Gastroenterology. 2013.

[22] Giuffrida D, Rogers IM. Targeting cancer stem cell lines as a new treatment of human cancer. Recent patents on anti-cancer drug discovery. 2010;5(3):205-18.

[23] Xu L. Cancer stem cell in the progression and therapy of pancreatic cancer. Frontiers in bioscience : a journal and virtual library. 2013;18:795-802.

[24] Xia J, Chen C, Chen Z, Miele L, Sarkar FH, Wang Z. Targeting pancreatic cancer stem cells for cancer therapy. Biochimica et biophysica acta. 2012;1826(2):385-99.

[25] Hay ED. Organization and fine structure of epithelium and mesenchyme in the developing chick embryo. Epithelial-mesenchymal interactions. 1968:31-55.

[26] Kelleher FC, Fennelly D, Rafferty M. Common critical pathways in embryogenesis and cancer. Acta oncologica. 2006;45(4):375-88.

[27] Leopold PL, Vincent J, Wang H. A comparison of epithelial-to-mesenchymal transition and re-epithelialization. Seminars in cancer biology. 2012;22(5-6):471-83.

[28] Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009;139(5):871-90.

[29] Garg M. Epithelial-mesenchymal transition-activating transcription factors-multifunctional regulators in cancer. World journal of stem cells. 2013;5(4):188-95.

[30] Vincent T, Neve EP, Johnson JR, Kukalev A, Rojo F, Albanell J, et al. A SNAIL1-SMAD3/4 transcriptional repressor complex promotes TGF-beta mediated epithelial-mesenchymal transition. Nature cell biology. 2009;11(8):943-50.

[31] Wang Y, Zhou BP. Epithelial-mesenchymal transition in breast cancer progression and metastasis. Chinese journal of cancer. 2011;30(9):603-11.

[32] Ishida T, Hijioka H, Kume K, Miyawaki A, Nakamura N. Notch signaling induces EMT in OSCC cell lines in a hypoxic environment. Oncology letters. 2013;6(5):1201-6.

[33] Wang Z, Li Y, Kong D, Sarkar FH. The role of Notch signaling pathway in epithelial-mesenchymal transition (EMT) during development and tumor aggressiveness. Current drug targets. 2010;11(6):745-51.

[34] Wang Z, Banerjee S, Li Y, Rahman KM, Zhang Y, Sarkar FH. Down-regulation of notch-1 inhibits invasion by inactivation of nuclear factor-kappaB, vascular endothelial growth factor, and matrix metalloproteinase-9 in pancreatic cancer cells. Cancer research. 2006;66(5):2778-84.

[35] Li Y, Ma J, Qian X, Wu Q, Xia J, Miele L, et al. Regulation of EMT by Notch Signaling Pathway in Tumor Progression. Current cancer drug targets. 2013.

[36] Behnsawy HM, Shigemura K, Meligy FY, Yamamichi F, Yamashita M, Haung WC, et al. Possible role of sonic hedgehog and epithelial-mesenchymal transition in renal cell cancer progression. Korean journal of urology. 2013;54(8):547-54.

[37] Lei J, Ma J, Ma Q, Li X, Liu H, Xu Q, et al. Hedgehog signaling regulates hypoxia induced epithelial to mesenchymal transition and invasion in pancreatic cancer cells via a ligand-independent manner. Molecular cancer. 2013;12:66.

[38] Singh A, Settleman J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene. 2010;29(34):4741-51.

[39] Liu M, Li CF, Chen HS, Lin LQ, Zhang CP, Zhao JL, et al. Differential expression of proteomics models of colorectal cancer, colorectal benign disease and healthy controls. Proteome science. 2010;8:16.

[40] Kalluri R. EMT: when epithelial cells decide to become mesenchymal-like cells. The Journal of clinical investigation. 2009;119(6):1417.

[41] Franco-Chuaire ML, Magda Carolina SC, Chuaire-Noack L. Epithelial-mesenchymal transition (EMT): principles and clinical impact in cancer therapy. Investigacion clinica. 2013;54(2):186-205.

[42] Iwatsuki M, Mimori K, Yokobori T, Ishi H, Beppu T, Nakamori S, et al. Epithelial-mesenchymal transition in cancer development and its clinical significance. Cancer science. 2010;101(2):293-9.

[43] Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. 2008;133(4):704-15.

[44] Singh A, Settleman J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene. 2010;29(34):4741-51.

[45] Rachagani S, Macha MA, Ponnusamy MP, Haridas D, Kaur S, Jain M, et al. MUC4 potentiates invasion and metastasis of pancreatic cancer cells through stabilization of fibroblast growth factor receptor 1. Carcinogenesis. 2012;33(10):1953-64.

[46] Huang C, Qiu Z, Wang L, Peng Z, Jia Z, Logsdon CD, et al. A novel FoxM1-caveolin signaling pathway promotes pancreatic cancer invasion and metastasis. Cancer research. 2012;72(3):655-65.

[47] Joost S, Almada LL, Rohnalter V, Holz PS, Vrabel AM, Fernandez-Barrena MG, et al. GLI1 inhibition promotes epithelial-to-mesenchymal transition in pancreatic cancer cells. Cancer research. 2012;72(1):88-99.

[48] Sarkar FH, Li Y, Wang Z, Kong D. Pancreatic cancer stem cells and EMT in drug resistance and metastasis. Minerva chirurgica. 2009;64(5):489-500.

[49] Van den Broeck A, Vankelecom H, Van Delm W, Gremeaux L, Wouters J, Allemeersch J, et al. Human pancreatic cancer contains a side population expressing cancer stem cell-associated and prognostic genes. PloS one. 2013;8(9):e73968.

[50] Takebe N, Harris PJ, Warren RQ, Ivy SP. Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. Nature reviews Clinical oncology. 2011;8(2): 97-106.

[51] Li Y, Kong D, Ahmad A, Bao B, Sarkar FH. Pancreatic cancer stem cells: emerging target for designing novel therapy. Cancer letters. 2013;338(1):94-100.

[52] Castellanos JA, Merchant NB, Nagathihalli NS. Emerging targets in pancreatic cancer: epithelial–mesenchymal transition and cancer stem cells. Onco Targets Ther. 2013;6:1261.

[53] Wang Z, Ahmad A, Li Y, Azmi AS, Miele L, Sarkar FH. Targeting notch to eradicate pancreatic cancer stem cells for cancer therapy. Anticancer research. 2011;31(4): 1105-13.

[54] Ji Q, Hao X, Zhang M, Tang W, Yang M, Li L, et al. MicroRNA miR-34 inhibits human pancreatic cancer tumor-initiating cells. PloS one. 2009;4(8):e6816.

[55] Radtke F, Raj K. The role of Notch in tumorigenesis: oncogene or tumour suppressor? Nature reviews Cancer. 2003;3(10):756-67.

[56] Bostad M, Berg K, Hogset A, Skarpen E, Stenmark H, Selbo PK. Photochemical internalization (PCI) of immunotoxins targeting CD133 is specific and highly potent at femtomolar levels in cells with cancer stem cell properties. Journal of controlled release : official journal of the Controlled Release Society. 2013;168(3):317-26.

[57] Gungor C, Hofmann BT, Wolters-Eisfeld G, Bockhorn M. Pancreatic Cancer. British journal of pharmacology. 2013.

[58] Long J, Zhang Y, Yu X, Yang J, LeBrun DG, Chen C, et al. Overcoming drug resistance in pancreatic cancer. Expert opinion on therapeutic targets. 2011;15(7):817-28.

[59] Yabuuchi S, Pai SG, Campbell NR, de Wilde RF, De Oliveira E, Korangath P, et al. Notch signaling pathway targeted therapy suppresses tumor progression and metastatic spread in pancreatic cancer. Cancer letters. 2013;335(1):41-51.

[60] Bao B, Wang Z, Ali S, Kong D, Li Y, Ahmad A, et al. Notch-1 induces epithelial-mesenchymal transition consistent with cancer stem cell phenotype in pancreatic cancer cells. Cancer letters. 2011;307(1):26-36.

[61] Rangwala F, Omenetti A, Diehl AM. Cancer stem cells: repair gone awry? Journal of oncology. 2011;2011:465343.

[62] Bailey JM, Swanson BJ, Hamada T, Eggers JP, Singh PK, Caffery T, et al. Sonic hedgehog promotes desmoplasia in pancreatic cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008;14(19):5995-6004.

[63] Hao K, Tian XD, Qin CF, Xie XH, Yang YM. Hedgehog signaling pathway regulates human pancreatic cancer cell proliferation and metastasis. Oncology reports. 2013;29(3):1124-32.

[64] Gu D, Liu H, Su GH, Zhang X, Chin-Sinex H, Hanenberg H, et al. Combining hedgehog signaling inhibition with focal irradiation on reduction of pancreatic cancer metastasis. Molecular cancer therapeutics. 2013;12(6):1038-48.

[65] Li Y, Wicha MS, Schwartz SJ, Sun D. Implications of cancer stem cell theory for cancer chemoprevention by natural dietary compounds. The Journal of nutritional biochemistry. 2011;22(9):799-806.

[66] Cui J, Jiang W, Wang S, Wang L, Xie K. Role of Wnt/beta-catenin signaling in drug resistance of pancreatic cancer. Current pharmaceutical design. 2012;18(17):2464-71.

[67] Yao H, Ashihara E, Maekawa T. Targeting the Wnt/beta-catenin signaling pathway in human cancers. Expert opinion on therapeutic targets. 2011;15(7):873-87.

[68] Liu S, Dontu G, Wicha MS. Mammary stem cells, self-renewal pathways, and carcinogenesis. Breast cancer research : BCR. 2005;7(3):86-95.

[69] Kong D, Wang Z, Sarkar SH, Li Y, Banerjee S, Saliganan A, et al. Platelet-derived growth factor-D overexpression contributes to epithelial-mesenchymal transition of PC3 prostate cancer cells. Stem Cells. 2008;26(6):1425-35.

[70] Sun L, Mathews LA, Cabarcas SM, Zhang X, Yang A, Zhang Y, et al. Epigenetic Regulation of SOX9 by the NF-kappaB Signaling Pathway in Pancreatic Cancer Stem Cells. Stem Cells. 2013;31(8):1454-66.

[71] Hindriksen S, Bijlsma MF. Cancer Stem Cells, EMT, and Developmental Pathway Activation in Pancreatic Tumors. Cancers. 2012;4(4):989-1035.

[72] Avan A, Quint K, Nicolini F, Funel N, Frampton AE, Maftouh M, et al. Enhancement of the antiproliferative activity of gemcitabine by modulation of c-Met pathway in pancreatic cancer. Current pharmaceutical design. 2013;19(5):940-50.

[73] Hage C, Rausch V, Giese N, Giese T, Schonsiegel F, Labsch S, et al. The novel c-Met inhibitor cabozantinib overcomes gemcitabine resistance and stem cell signaling in pancreatic cancer. Cell death & disease. 2013;4:e627.

[74] Albulescu R, Neagu M, Albulescu L, Tanase C. Tissular and soluble miRNAs for diagnostic and therapy improvement in digestive tract cancers. Expert review of molecular diagnostics. 2011;11(1):101-20.

[75] Haselmann V, Kurz A, Bertsch U, Hubner S, Olempska-Muller M, Fritsch J, et al. Nuclear Death Receptor TRAILR2 Inhibits Maturation of Let-7 and Promotes Proliferation of Pancreatic and Other Tumor cells. Gastroenterology. 2013.

[76] Jia J, Parikh H, Xiao W, Hoskins JW, Pflicke H, Liu X, et al. An integrated transcriptome and epigenome analysis identifies a novel candidate gene for pancreatic cancer. BMC medical genomics. 2013;6(1):33.

[77] Sureban SM, May R, Lightfoot SA, Hoskins AB, Lerner M, Brackett DJ, et al. DCAMKL-1 regulates epithelial-mesenchymal transition in human pancreatic cells through a miR-200a-dependent mechanism. Cancer research. 2011;71(6):2328-38.

[78] Jung DE, Wen J, Oh T, Song SY. Differentially expressed microRNAs in pancreatic cancer stem cells. Pancreas. 2011;40(8):1180-7.

[79] Sureban SM, May R, Qu D, Weygant N, Chandrakesan P, Ali N, et al. DCLK1 Regulates Pluripotency and Angiogenic Factors via microRNA-Dependent Mechanisms in Pancreatic Cancer. PloS one. 2013;8(9):e73940.

[80] Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, et al. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nature cell biology. 2009;11(12):1487-95.

[81] Pramanik D, Campbell NR, Karikari C, Chivukula R, Kent OA, Mendell JT, et al. Restitution of tumor suppressor microRNAs using a systemic nanovector inhibits pancreatic cancer growth in mice. Molecular cancer therapeutics. 2011;10(8):1470-80.

[82] Cioffi M, Dorado J, Baeuerle PA, Heeschen C. EpCAM/CD3-Bispecific T-cell engaging antibody MT110 eliminates primary human pancreatic cancer stem cells. Clinical cancer research : an official journal of the American Association for Cancer Research. 2012;18(2):465-74.

[83] Padhye SS, Guin S, Yao HP, Zhou YQ, Zhang R, Wang MH. Sustained expression of the RON receptor tyrosine kinase by pancreatic cancer stem cells as a potential targeting moiety for antibody-directed chemotherapeutics. Molecular pharmaceutics. 2011;8(6):2310-9.

[84] Zhu H, Dougherty U, Robinson V, Mustafi R, Pekow J, Kupfer S, et al. EGFR signals downregulate tumor suppressors miR-143 and miR-145 in Western diet-promoted murine colon cancer: role of G1 regulators. Molecular cancer research : MCR. 2011;9(7):960-75.

[85] Choi YC, Yoon S, Jeong Y, Yoon J, Baek K. Regulation of vascular endothelial growth factor signaling by miR-200b. Molecules and cells. 2011;32(1):77-82.

[86] Biray Avci C, Ozcan I, Balci T, Ozer O, Gunduz C. Design of polyethylene glycol-polyethylenimine nanocomplexes as non-viral carriers: mir-150 delivery to chronic myeloid leukemia cells. Cell biology international. 2013;37(11):1205-14.

[87] Sicard F, Gayral M, Lulka H, Buscail L, Cordelier P. Targeting miR-21 for the therapy of pancreatic cancer. Molecular therapy : the journal of the American Society of Gene Therapy. 2013;21(5):986-94.

[88] Zhao G, Zhang JG, Liu Y, Qin Q, Wang B, Tian K, et al. miR-148b functions as a tumor suppressor in pancreatic cancer by targeting AMPKalpha1. Molecular cancer therapeutics. 2013;12(1):83-93.

[89] Garber K. Stromal depletion goes on trial in pancreatic cancer. Journal of the National Cancer Institute. 2010;102(7):448-50.

[90] Hamada S, Shimosegawa T. Pancreatic cancer stem cell and mesenchymal stem cell. In: Grippo P, Munshi H, editors. Pancreatic Cancer and Tumor Microenvironment. Trivandrum (India): Transworld Research Network; 2012.

[91] Apte MV, Haber PS, Applegate TL, Norton ID, McCaughan GW, Korsten MA, et al. Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. Gut. 1998;43(1):128-33.

[92] Haber PS, Keogh GW, Apte MV, Moran CS, Stewart NL, Crawford DH, et al. Activation of pancreatic stellate cells in human and experimental pancreatic fibrosis. The American journal of pathology. 1999;155(4):1087-95.

[93] Apte MV, Park S, Phillips PA, Santucci N, Goldstein D, Kumar RK, et al. Desmoplastic reaction in pancreatic cancer: role of pancreatic stellate cells. Pancreas. 2004;29(3): 179-87.

[94] Xu Z, Vonlaufen A, Phillips PA, Fiala-Beer E, Zhang X, Yang L, et al. Role of pancreatic stellate cells in pancreatic cancer metastasis. The American journal of pathology. 2010;177(5):2585-96.

[95] Kozono S, Ohuchida K, Eguchi D, Ikenaga N, Fujiwara K, Cui L, et al. Pirfenidone inhibits pancreatic cancer desmoplasia by regulating stellate cells. Cancer research. 2013;73(7):2345-56.

[96] Mace TA, Bloomston M, Lesinski GB. Pancreatic cancer-associated stellate cells: A viable target for reducing immunosuppression in the tumor microenvironment. Oncoimmunology. 2013;2(7):e24891.

[97] Duner S, Lopatko Lindman J, Ansari D, Gundewar C, Andersson R. Pancreatic cancer: the role of pancreatic stellate cells in tumor progression. Pancreatology. 2010;10(6):673-81.

[98] Kruse ML, Hildebrand PB, Timke C, Folsch UR, Schafer H, Schmidt WE. Isolation, long-term culture, and characterization of rat pancreatic fibroblastoid/stellate cells. Pancreas. 2001;23(1):49-54.

[99] Masamune A, Satoh M, Kikuta K, Suzuki N, Shimosegawa T. Establishment and characterization of a rat pancreatic stellate cell line by spontaneous immortalization. World journal of gastroenterology : WJG. 2003;9(12):2751-8.

[100] Vonlaufen A, Phillips PA, Yang L, Xu Z, Fiala-Beer E, Zhang X, et al. Isolation of quiescent human pancreatic stellate cells: a promising in vitro tool for studies of human pancreatic stellate cell biology. Pancreatology. 2010;10(4):434-43.

[101] Apte MV, Pirola RC, Wilson JS. Pancreatic stellate cells: a starring role in normal and diseased pancreas. Frontiers in physiology. 2012;3:344.

[102] Weiland A, Roswall P, Hatzihristidis TC, Pietras K, Ostman A, Strell C. Fibroblast-dependent regulation of the stem cell properties of cancer cells. Neoplasma. 2012;59(6):719-27.

[103] Giannoni E, Bianchini F, Calorini L, Chiarugi P. Cancer associated fibroblasts exploit reactive oxygen species through a proinflammatory signature leading to epithelial mesenchymal transition and stemness. Antioxidants & redox signaling. 2011;14(12): 2361-71.

[104] Masamune A, Watanabe T, Kikuta K, Shimosegawa T. Roles of pancreatic stellate cells in pancreatic inflammation and fibrosis. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2009;7(11 Suppl):S48-54.

[105] Infante JR, Matsubayashi H, Sato N, Tonascia J, Klein AP, Riall TA, et al. Peritumoral fibroblast SPARC expression and patient outcome with resectable pancreatic adenocarcinoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2007;25(3):319-25.

[106] Heinemann V, Reni M, Ychou M, Richel DJ, Macarulla T, Ducreux M. Tumour-stroma interactions in pancreatic ductal adenocarcinoma: Rationale and current evidence for new therapeutic strategies. Cancer treatment reviews. 2013.

[107] Gradishar WJ. Albumin-bound paclitaxel: a next-generation taxane. Expert opinion on pharmacotherapy. 2006;7(8):1041-53.

[108] Liu ZJ, Zhuge Y, Velazquez OC. Trafficking and differentiation of mesenchymal stem cells. Journal of cellular biochemistry. 2009;106(6):984-91.

[109] Bao Q, Zhao Y, Niess H, Conrad C, Schwarz B, Jauch KW, et al. Mesenchymal stem cell-based tumor-targeted gene therapy in gastrointestinal cancer. Stem cells and development. 2012;21(13):2355-63.

[110] Zischek C, Niess H, Ischenko I, Conrad C, Huss R, Jauch KW, et al. Targeting tumor stroma using engineered mesenchymal stem cells reduces the growth of pancreatic carcinoma. Annals of surgery. 2009;250(5):747-53.

[111] Gu J, Qian H, Shen L, Zhang X, Zhu W, Huang L, et al. Gastric cancer exosomes trigger differentiation of umbilical cord derived mesenchymal stem cells to carcinoma-associated fibroblasts through TGF-beta/Smad pathway. PloS one. 2012;7(12):e52465.

[112] Cho JA, Park H, Lim EH, Lee KW. Exosomes from breast cancer cells can convert adipose tissue-derived mesenchymal stem cells into myofibroblast-like cells. International journal of oncology. 2012;40(1):130-8.

[113] Subramanian A, Shu-Uin G, Kae-Siang N, Gauthaman K, Biswas A, Choolani M, et al. Human umbilical cord Wharton's jelly mesenchymal stem cells do not transform to tumor-associated fibroblasts in the presence of breast and ovarian cancer cells unlike bone marrow mesenchymal stem cells. Journal of cellular biochemistry. 2012;113(6):1886-95.

[114] Kabashima-Niibe A, Higuchi H, Takaishi H, Masugi Y, Matsuzaki Y, Mabuchi Y, et al. Mesenchymal stem cells regulate epithelial-mesenchymal transition and tumor progression of pancreatic cancer cells. Cancer science. 2013;104(2):157-64.

[115] Gao D, Nolan D, McDonnell K, Vahdat L, Benezra R, Altorki N, et al. Bone marrow-derived endothelial progenitor cells contribute to the angiogenic switch in tumor growth and metastatic progression. Biochimica et biophysica acta. 2009;1796(1):33-40.

[116] Nakamura K, Sasajima J, Mizukami Y, Sugiyama Y, Yamazaki M, Fujii R, et al. Hedgehog promotes neovascularization in pancreatic cancers by regulating Ang-1 and IGF-1 expression in bone-marrow derived pro-angiogenic cells. PloS one. 2010;5(1):e8824.

[117] Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005;121(3):335-48.

[118] Qiao L, Xu Z, Zhao T, Zhao Z, Shi M, Zhao RC, et al. Suppression of tumorigenesis by human mesenchymal stem cells in a hepatoma model. Cell research. 2008;18(4): 500-7.

[119] Qiao L, Xu ZL, Zhao TJ, Ye LH, Zhang XD. Dkk-1 secreted by mesenchymal stem cells inhibits growth of breast cancer cells via depression of Wnt signalling. Cancer letters. 2008;269(1):67-77.

[120] Ahn JO, Lee HW, Seo KW, Kang SK, Ra JC, Youn HY. Anti-Tumor Effect of Adipose Tissue Derived-Mesenchymal Stem Cells Expressing Interferon-beta and Treatment with Cisplatin in a Xenograft Mouse Model for Canine Melanoma. PloS one. 2013;8(9):e74897.

[121] Hou L, Wang X, Zhou Y, Ma H, Wang Z, He J, et al. Inhibitory effect and mechanism of mesenchymal stem cells on liver cancer cells. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2013.

[122] Belli C, Cereda S, Anand S, Reni M. Neoadjuvant therapy in resectable pancreatic cancer: a critical review. Cancer treatment reviews. 2013;39(5):518-24.

[123] Du Z, Qin R, Wei C, Wang M, Shi C, Tian R, et al. Pancreatic cancer cells resistant to chemoradiotherapy rich in "stem-cell-like" tumor cells. Digestive diseases and sciences. 2011;56(3):741-50.

[124] Diehn M, Clarke MF. Cancer stem cells and radiotherapy: new insights into tumor radioresistance. Journal of the National Cancer Institute. 2006;98(24):1755-7.

[125] Mimeault M, Hauke R, Mehta PP, Batra SK. Recent advances in cancer stem/progenitor cell research: therapeutic implications for overcoming resistance to the most aggressive cancers. Journal of cellular and molecular medicine. 2007;11(5):981-1011.

[126] Moitra K, Lou H, Dean M. Multidrug efflux pumps and cancer stem cells: insights into multidrug resistance and therapeutic development. Clinical pharmacology and therapeutics. 2011;89(4):491-502.

[127] Januchowski R, Wojtowicz K, Andrzejewska M, Zabel M. Expression of MDR1 and MDR3 gene products in paclitaxel-, doxorubicin-and vincristine-resistant cell lines. Biomed Pharmacother. 2013.

[128] Jimeno A, Feldmann G, Suarez-Gauthier A, Rasheed Z, Solomon A, Zou GM, et al. A direct pancreatic cancer xenograft model as a platform for cancer stem cell therapeutic development. Molecular cancer therapeutics. 2009;8(2):310-4.

[129] Lu Y, Zhu H, Shan H, Lu J, Chang X, Li X, et al. Knockdown of Oct4 and Nanog expression inhibits the stemness of pancreatic cancer cells. Cancer letters. 2013;340(1): 113-23.

[130] Lonardo E, Hermann PC, Mueller MT, Huber S, Balic A, Miranda-Lorenzo I, et al. Nodal/Activin signaling drives self-renewal and tumorigenicity of pancreatic cancer stem cells and provides a target for combined drug therapy. Cell stem cell. 2011;9(5): 433-46.

[131] Mueller MT, Hermann PC, Witthauer J, Rubio-Viqueira B, Leicht SF, Huber S, et al. Combined targeted treatment to eliminate tumorigenic cancer stem cells in human pancreatic cancer. Gastroenterology. 2009;137(3):1102-13.

[132] Hermann PC, Trabulo SM, Sainz B, Jr., Balic A, Garcia E, Hahn SA, et al. Multimodal Treatment Eliminates Cancer Stem Cells and Leads to Long-Term Survival in Primary Human Pancreatic Cancer Tissue Xenografts. PloS one. 2013;8(6):e66371.

[133] Mizuma M, Rasheed ZA, Yabuuchi S, Omura N, Campbell NR, de Wilde RF, et al. The gamma secretase inhibitor MRK-003 attenuates pancreatic cancer growth in preclinical models. Molecular cancer therapeutics. 2012;11(9):1999-2009.

[134] Rajeshkumar NV, Rasheed ZA, Garcia-Garcia E, Lopez-Rios F, Fujiwara K, Matsui WH, et al. A combination of DR5 agonistic monoclonal antibody with gemcitabine targets pancreatic cancer stem cells and results in long-term disease control in human pancreatic cancer model. Molecular cancer therapeutics. 2010;9(9):2582-92.

[135] Awasthi N, Zhang C, Ruan W, Schwarz MA, Schwarz RE. Evaluation of poly-mechanistic antiangiogenic combinations to enhance cytotoxic therapy response in pancreatic cancer. PloS one. 2012;7(6):e38477.

[136] Strimpakos AS, Syrigos KN, Saif MW. Novel agents in early phase clinical studies on refractory pancreatic cancer. JOP : Journal of the pancreas. 2012;13(2):166-8.

[137] Sarris EG, Syrigos KN, Saif MW. Novel agents and future prospects in the treatment of pancreatic adenocarcinoma. JOP : Journal of the pancreas. 2013;14(4):395-400.

[138] Yoshiyama K, Terazaki Y, Matsueda S, Shichijo S, Noguchi M, Yamada A, et al. Personalized peptide vaccination in patients with refractory non-small cell lung cancer. International journal of oncology. 2012;40(5):1492-500.

[139] Takahashi R, Yoshitomi M, Yutani S, Shirahama T, Noguchi M, Yamada A, et al. Current status of immunotherapy for the treatment of biliary tract cancer. Human vaccines & immunotherapeutics. 2013;9(5):1069-72.

[140] Yutani S, Komatsu N, Yoshitomi M, Matsueda S, Yonemoto K, Mine T, et al. A phase II study of a personalized peptide vaccination for chemotherapy-resistant advanced pancreatic cancer patients. Oncology reports. 2013;30(3):1094-100.

[141] DeVito NC, Saif MW. Advances in immunotherapy for pancreatic cancer: 2013. JOP : Journal of the pancreas. 2013;14(4):347-53.

[142] Gansauge F, Poch B, Kleef R, Schwarz M. Effectivity of long antigen exposition dendritic cell therapy (lanex-dc(R)) in the palliative treatment of pancreatic cancer. Current medicinal chemistry. 2013.

[143] Gupta PB, Onder TT, Jiang G, Tao K, Kuperwasser C, Weinberg RA, et al. Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell. 2009;138(4):645-59.

[144] Zhang GN, Liang Y, Zhou LJ, Chen SP, Chen G, Zhang TP, et al. Combination of salinomycin and gemcitabine eliminates pancreatic cancer cells. Cancer letters. 2011;313(2):137-44.

[145] Farhana L, Dawson MI, Das JK, Murshed F, Xia Z, Hadden TJ, et al. Adamantyl Retinoid-Related Molecules Induce Apoptosis in Pancreatic Cancer Cells by Inhibiting IGF-1R and Wnt/beta-Catenin Pathways. Journal of oncology. 2012;2012:796729.

[146] Kallifatidis G, Labsch S, Rausch V, Mattern J, Gladkich J, Moldenhauer G, et al. Sulforaphane increases drug-mediated cytotoxicity toward cancer stem-like cells of pancreas and prostate. Molecular therapy : the journal of the American Society of Gene Therapy. 2011;19(1):188-95.

[147] Srivastava RK, Tang SN, Zhu W, Meeker D, Shankar S. Sulforaphane synergizes with quercetin to inhibit self-renewal capacity of pancreatic cancer stem cells. Front Biosci (Elite Ed). 2011;3:515-28.

[148] Blanco E, Hsiao A, Mann AP, Landry MG, Meric-Bernstam F, Ferrari M. Nanomedicine in cancer therapy: innovative trends and prospects. Cancer science. 2011;102(7): 1247-52.

[149] Narang AS, Chang RK, Hussain MA. Pharmaceutical development and regulatory considerations for nanoparticles and nanoparticulate drug delivery systems. Journal of pharmaceutical sciences. 2013;102(11):3867-82.

[150] Wang LS, Chuang MC, Ho JA. Nanotheranostics--a review of recent publications. Int J Nanomedicine. 2012;7:4679-95.

[151] Lee CC, MacKay JA, Frechet JM, Szoka FC. Designing dendrimers for biological applications. Nature biotechnology. 2005;23(12):1517-26.

[152] Rapoport N, Kennedy AM, Shea JE, Scaife CL, Nam KH. Ultrasonic nanotherapy of pancreatic cancer: lessons from ultrasound imaging. Molecular pharmaceutics. 2010;7(1):22-31.

[153] Chang HI, Yeh MK. Clinical development of liposome-based drugs: formulation, characterization, and therapeutic efficacy. Int J Nanomedicine. 2012;7:49-60.

[154] Corot C, Robert P, Idee JM, Port M. Recent advances in iron oxide nanocrystal technology for medical imaging. Advanced drug delivery reviews. 2006;58(14):1471-504.

[155] Chao JI, Perevedentseva E, Chung PH, Liu KK, Cheng CY, Chang CC, et al. Nanometer-sized diamond particle as a probe for biolabeling. Biophysical journal. 2007;93(6): 2199-208.

[156] Mader HS, Kele P, Saleh SM, Wolfbeis OS. Upconverting luminescent nanoparticles for use in bioconjugation and bioimaging. Current opinion in chemical biology. 2010;14(5):582-96.

[157] Cheng L, Wang C, Liu Z. Upconversion nanoparticles and their composite nanostructures for biomedical imaging and cancer therapy. Nanoscale. 2013;5(1):23-37.

[158] Wu X, He X, Wang K, Xie C, Zhou B, Qing Z. Ultrasmall near-infrared gold nanoclusters for tumor fluorescence imaging in vivo. Nanoscale. 2010;2(10):2244-9.

[159] Cho H, Kwon GS. Polymeric micelles for neoadjuvant cancer therapy and tumor-primed optical imaging. ACS nano. 2011;5(11):8721-9.

[160] Homan K, Shah J, Gomez S, Gensler H, Karpiouk A, Brannon-Peppas L, et al. Silver nanosystems for photoacoustic imaging and image-guided therapy. Journal of biomedical optics. 2010;15(2):021316.

[161] Wang LV. Prospects of photoacoustic tomography. Med Phys. 2008;35(12):5758-67.

[162] Douma K, Megens RT, van Zandvoort MA. Optical molecular imaging of atherosclerosis using nanoparticles: shedding new light on the darkness. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2011;3(4):376-88.

[163] Wilson KE, Wang TY, Willmann JK. Acoustic and photoacoustic molecular imaging of cancer. J Nucl Med. 2013;54(11):1851-4.

[164] Kim TH, Lee S, Chen X. Nanotheranostics for personalized medicine. Expert review of molecular diagnostics. 2013;13(3):257-69.

[165] Chenna V, Hu C, Pramanik D, Aftab BT, Karikari C, Campbell NR, et al. A polymeric nanoparticle encapsulated small-molecule inhibitor of Hedgehog signaling (NanoHHI) bypasses secondary mutational resistance to Smoothened antagonists. Molecular cancer therapeutics. 2012;11(1):165-73.

[166] Rejiba S, Reddy LH, Bigand C, Parmentier C, Couvreur P, Hajri A. Squalenoyl gemcitabine nanomedicine overcomes the low efficacy of gemcitabine therapy in pancreatic cancer. Nanomedicine. 2011;7(6):841-9.

[167] Verma A, Guha S, Diagaradjane P, Kunnumakkara AB, Sanguino AM, Lopez-Berestein G, et al. Therapeutic significance of elevated tissue transglutaminase expression in pancreatic cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008;14(8):2476-83.

[168] Glazer ES, Zhu C, Massey KL, Thompson CS, Kaluarachchi WD, Hamir AN, et al. Noninvasive radiofrequency field destruction of pancreatic adenocarcinoma xenografts treated with targeted gold nanoparticles. Clinical cancer research : an official journal of the American Association for Cancer Research. 2010;16(23):5712-21.

[169] Schuch G. EndoTAG-1. MediGene. Curr Opin Investig Drugs. 2005;6(12):1259-65.

[170] Eichhorn ME, Ischenko I, Luedemann S, Strieth S, Papyan A, Werner A, et al. Vascular targeting by EndoTAG-1 enhances therapeutic efficacy of conventional chemotherapy in lung and pancreatic cancer. Int J Cancer. 2010;126(5):1235-45.

[171] Lohr JM, Haas SL, Bechstein WO, Bodoky G, Cwiertka K, Fischbach W, et al. Cationic liposomal paclitaxel plus gemcitabine or gemcitabine alone in patients with advanced pancreatic cancer: a randomized controlled phase II trial. Ann Oncol. 2012;23(5):1214-22.

[172] Ansari D, Chen BC, Dong L, Zhou MT, Andersson R. Pancreatic cancer: translational research aspects and clinical implications. World journal of gastroenterology : WJG. 2012;18(13):1417-24.

[173] Sun TM, Wang YC, Wang F, Du JZ, Mao CQ, Sun CY, et al. Cancer stem cell therapy using doxorubicin conjugated to gold nanoparticles via hydrazone bonds. Biomaterials. 2014;35(2):836-45.

[174] Zhao Y, Alakhova DY, Kabanov AV. Can nanomedicines kill cancer stem cells? Advanced drug delivery reviews. 2013.

[175] Doss CG, Debajyoti C, Debottam S. Disruption of Mitochondrial Complexes in Cancer Stem Cells Through Nano-based Drug Delivery: A Promising Mitochondrial Medicine. Cell Biochem Biophys. 2013.

Chapter 3

miRNAs in Pancreatic Cancer

Radu Albulescu, Adrian Claudiu Popa, Elena Codrici, Daniela Ionela Popescu, Simona Mihai and Cristiana Tanase

Additional information is available at the end of the chapter

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1. Introduction

Pancreatic cancer and especially PDAC (Pancreatic Ductal AdenoCarcinoma) is among the most difficult to treat cancer, characterized by invasiveness, metastatic potential and bad outcomes.

miRNAs emerged in recent years as potent regulators of cellular activities, playing a central role in controlling the protein expression at the post-transcriptional level. They have significant implication in pathology in general and most relevantly in cancers. Their main role is the control of the process of proteosynthesis at the translational level, by leading their target mRNAs as a miRNA-mRNA dimer to a degradative complex.

Deregulation in expression levels of miRNAs and some genetic alterations were demonstrated in various cancers, including PDAC. Investigations on tissue samples provided a considerable amount of knowledge, leading to the identification of miRNAs with altered expression associated with tumorigenesis and tumor progression. Tumor-inducing and tumor-promoting miRNAs were significantly up-regulated, while sets of tumor-suppressor miRNAs are down-regulated or suppressed. By targeting major protein players in cell regulatory networks, some miRNAs appear to have the ability to shift the balance towards tumorigenesis, while other miRNAs are seen inhibiting or even reversing the process.

Tissular and soluble miRNAs were demonstrated as potential biomarkers, serving as diagnostic, stratification or prognostic tools, while other representatives were identified as "candidate" therapeutic targets or "candidate" therapeutic tools.

MicroRNAs (miRs, miRNAs) form a class of small-sized but powerful cell regulators. Presently, the family of human miRNAs comprises 1872 precursors and 2578 mature forms, but

the discovery process is adding further members at a rapid rate [1, 2]. The conventional nomenclature of miRNAs establishes some rules: a mature miRNA is designated in the form hsa-miR-121, where the first three characters encode the species. The letters that may occur at the end of the name refer to the different locations where the coding gene is located (the same miRNA can be encoded on multiple chromosomes and on either + or – strands). At the same time, the precursors are designated in the form hsa-mir-121. The rate of discovery is quite fast, so usually the numbers are assigned in the sequential order of discovery. However, if a new miRNA has a similar sequence to an existing one, it will acquire identical names, the differentiation being made by the letter. For historical reasons, the first miRNAs discovered, let-7 and lin-4, are exempt from the rule.

miRNAs act in the post-transcriptional regulation of protein expression and their involvement was demonstrated in normal processes as well as in pathology. Most of them are "multivalent", so that one single miRNA is able to "target" multiple genes, thus regulating the expression of several proteins. miRNAs are non-coding RNA molecules, 18-28 nucleotides lengths in the mature form, that regulate a variety of cellular processes including cell differentiation, cell cycle progression and apoptosis. miRNAs can function either as oncogenes or tumor suppressors [3]; oncogenic miRNAs (oncomiRs) are up-regulated in cancer cells [1].

In cancer, several miRNAs are situated "upstream" of the carcinogenesis process – acting as triggers for carcinogenesis or for progression; other miRNAs are situated "downstream" of the carcinogenic process, their modified expression appearing as the outcome of carcinogenetic transformation or progression. miRNAs play major roles in the multistep processes of carcinogenesis, either by oncogenic or tumor-suppressor functions. The study of miRNAs has been extended into many kinds of tumors, including those of the pancreas [4, 5]. Those studies have revealed that miRNAs may be potential diagnostic or prognostic tools for cancer [6, 7]. miRNAs are important tools due to their suitability for detection in both tissues [either fresh or Formalin-Fixed-Paraffin-Embedded (FFPE)] and in other biological samples (blood, serum, plasma, saliva, feces).

The discovery of miRNAs opened new opportunities for non-invasive tests for the early diagnosis of cancer [8, 9]. It has been recently revealed that, once detected, the miRNAs (being differentially expressed in blood) can be used as diagnostic and prognostic circulating biomarkers [10].

In the present chapter we summarize some of the existing knowledge regarding miRNAs involved in tumorigenesis and progression of pancreatic cancer. We have focused on the possible diagnostic role of miRNAs and their tissue-related expression in correlation with their soluble forms. We have summarized recent evidence regarding the assessment of their diagnostic value in pancreatic cancer patients.

2. miRNAs: Biogenesis and mechanisms of action

miRNAs act as post-transcriptional regulators of gene expression in eukaryotic cells. Their biological roles in development, normal cell function and in pathology, including cancer,

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have been described and several reviews thoroughly describe the processes involving miRNAs [11, 12].

A brief description of the basic mechanisms of biogenesis may be given as follows:

- miRNAs are encoded in various locations (both in protein-coding and non-coding gene sequences); often, the location of these coding sequences is in fragile chromosomal regions; therefore, they are highly susceptible to molecular modification.
- miRNA sequences are transcribed by RNA polymerase II as larger primary-microRNA molecules, which are further processed by Rnase III endonucleases Drosha and DGCR8 to form precursor miRNAs (pre-microRNAs, stem-loop structures containing about 70 nucleotides).
- Exportin 5 transfers pre-microRNAs to the cytoplasm.
- Processing in the cytoplasm is performed by Dicer (an RNAse III endonuclease), which removes the loop of the pre-miRNA and generates an imperfect duplex, formed by the mature miRNA sequence and a fragment of similar size derived from the opposite side of the loop, (miRNA*).
- The counter strand is separated and most often degraded; however, in many cases this counter strand can also function as a regulator.

Gene expression is controlled by regulation of mRNA translation and degradation:

- Perfect or near-perfect complementarity targets mRNA for degradation by RISC (RNA-Induced Silencing Complex).
- Imperfect complementarity blocks translation by the ribosome.

The majority (if not all) of miRNAs are multivalent. That is, almost every miRNA has the ability to interfere with multiple genes. Often a "cross talk" between miRNAs and other cell-regulatory or effector proteins is encountered, generating a mutual modification of expression, resulting in negative regulatory loops.

A novel pathway, translation activation, was demonstrated by Vasudevan et al. (2007) for miR-369-3. Cell cycle arrest by serum starvation transforms the TNFα AU-rich element (ARE) into a translation activator signal. AGO2 (Argonaute2) and FXR1 (fragile X mental retardation–related protein 1 (FXR1) are associated with ARE on translation activation; both proteins are required to increase translation efficiency. The seed sequence (the nucleotides 2-8 at the 5′ end of the miRNA [13] of miR-369-3 was demonstrated to be able to form base-pairs with two target sites on the minimal TNFα ARE required for translation activation. The formation of base-pairs between mir-369-3 and the target sites was demonstrated to be required for translation activation by knock-down experiments and by experiments using mutant ARE, as well as modified sequences of miR-369-3 (in order to restore complementarity to modified targets on mutant ARE) [14].

3. miRNAs in tumor progression

3.1. Cell growth and proliferation

Low levels of expression of miR-34a, 34b and 34c were found in cultivated pancreatic cancer cells (MiaPaCa2 and BxpC3), while the levels of the target genes Bcl2 (Apoptosis regulator Bcl-2) and Notch1 (Neurogenic locus notch homolog protein 1) were elevated. Restoration of miR-34 levels by transfection with miR-34 mimics down-regulation of the target genes, inhibits clonogenic growth and activates apoptosis via the caspase-3 pathway [15].

miR-21 over-expression is demonstrated in PDAC. Its presence and over-expression is associated with poor survival, invasiveness and resistance to gemcitabine. The findings relating to miR-21's role and mechanism in tumor tissue were confirmed *in vitro*, on primary cultures and cancer cell lines, fibroblasts and normal pancreatic ductal cell lines [16]. Enhancement of miR-21 levels (by pre-miR-21 transfection) decreased the anti-proliferative and anti-apoptotic effects of gemcitabine and up-regulated the expression of MMP2 (Matrix-MetalloProteinase 2) and MMP9 (Matrix-MetalloProteinase 9) [16].

3.2. Tumorigenesis

In the case of pancreatic cancer, as is the case in other cancers, distinct patterns of expression of miRNAs occur, depending on disease stage. The expression changes during progression. From these miRNAs, some are common to many cancers, while a few are tissue-specific and can help to track more precisely the tissue in which a carcinogenic process takes place [17]. There is a clear distinction between pre-malignant lesions, primary tumors and metastasis in the pattern of expression of miRNAs. Moreover, some of these distinctions can also be made in exosomal miRNAs.

Deregulated expression of miRNAs may represent an early modification in pancreatic tumorigenesis, generating progression of PanIN (Pancreatic Intraepithelial Neoplasia) lesions to more invasive forms. Ryu et al. investigated three candidates (selected on the basis of previous reports as over-expressed in pancreatic cancer). mir-155 was significantly over-expressed in PanIN-2 and 3 (2.6-fold, $p=0.02$ and 7.4-fold $p=0.049$, respectively); miR-21 was over-expressed only in PanIN-3 (2.5-fold, $p=0.02$), while no modification was found for miR-221 in PanIN lesions compared to normal duct epithelium [18]. Another set of miRNAs were investigated by du Rieu et al. in laser-dissected tissue samples from PanIN lesions (from a mouse model and from human patients). miR-21, 205 and 200 paralleled PanIN progression in mouse models. mir-21 and miR-205 preceded phenotypic changes of the duct. In precursor lesions, miR-21 achieved the highest relative concentrations. In human samples, miR-21,-221, 222 and let-7a increased with lesion grade, with maximal expression in two thirds of lesions. Up-regulation of miR-21 was controlled by KRAS and EGFR in PDAC-(Pancreatic Ductal AdenoCarcinoma) derived cell lines [19].

Another complex investigation of miRNA signatures during tumorigenesis and progression in pancreatic cancer was reported by Olson et al. The study stressed the down-regulation of the miR-200 family in metastases and metastasis-like primary tumors. Also, multiple changes

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in microRNA expression in tumor stages were investigated [20]. A synthesis of these data is presented in table 1.

miRNA	Modification	Significance	Ref.
let-7	Down-regulated		[21, 22]
let-7d	Up-regulated		[23, 24]
let-7f-1	Up-regulated		[21]
miR-10a	Up-regulated	Metastasis	[22] [20, 25]
miR-10b	Up-regulated		[25]
miR-15b	Up-regulated	Metastasis, Hyperplasia	[20, 25]
miR-16-1	Up-regulated		[24]
miR-17-5p	Up-regulated	Angiogenesis, Hyperplasia	[20]
miR-18a	Up-regulated		[26]
miR-19b	Up-regulated	Angiogenesis	[20]
miR-20a	Up-regulated	Hyperplasia, angiogenesis	[20]
miR-21	Up-regulated	Angiogenesis	[24, 26]
miR-23a	Up-regulated		[25]
miR-23b	Up-regulated	Metastasis	[20, 25]
miR-24	Up-regulated	Metastasis	[20]
miR-24-1,2	Up-regulated		[24]
miR-25	Up-regulated	Hyperplasia, dysplasia	[20]
miR-27b	Up-regulated	Metastasis	[20]
miR-29c	Down-regulated		[26]
miR-31	Up-regulated		[26]
Mir-92	Up-regulated	Hyperplasia, dysplasia, metastasis	[20]
miR-92-1	Up-regulated		[24]
miR-93	Up-regulated		[26]
miR-95	Up-regulated		[26]
miR-96	Down-regulated		[24]
miR-99	Up-regulated		[25]
miR-100	Up-regulated		[24, 25]
miR-100-1/2	Up-regulated		[25]
miR-103-2	Up-regulated		[25]
miR-106a	Up-regulated	Hyperplasia, dysplasia	[20]
miR-107	Up-regulated		[24, 25]
miR-124a	Up-regulated	Tumor signature, metastasis	[20]
miR-125a	Up-regulated		[25]
miR-125b-1	Up-regulated		[24, 25]
miR-126	Up-regulated	Metastasis	[20]

miRNA	Modification	Significance	Ref.
miR-126*	Up-regulated	Angiogenesis	[20]
miR-129	Up-regulated	Metastasis	[20]
miR-129-3p	Up-regulated	Angiogenesis	[20]
miR-130b	Down-regulated		[24]
miR-132	Up-regulated	Metastasis, tumor signature	[20]
miR-137	Up-regulated	Metastasis	[20]
miR-139	Down-regulated		[24]
miR-141	Down-regulated	Metastasis	[20]
miR-142-p	Down-regulated		[24]
miR-142-3p	Down-regulated	Hyperplasia, angiogenesis, tumor signature	[20]
miR-142-5p	Up-regulated Down-regulated	Angiogenesis Tumor signature	[20]
miR-143	Down-regulated	Metastasis	[25],18,[26]
miR-145	Down-regulated	Metastasis	[20, 24, 26]
miR-146	Up-regulated		[25]
miR-146a	Down-regulated	Angiogenesis Metastasis	[20, 26]
miR-148a	Down-regulated	Metastasis	[25, 26]
miR-148b	Down-regulated	Metastasis	[25, 26]
miR-150	Down-regulated	Tumor signature	[20, 26]
miR-152	Down-regulated	Metastasis	[20]
miR-155	Up-regulated	Hyperplasia/dysplasia	[20, 25, 26]
miR-181a	Up-regulated	Metastasis	[20, 24, 25]
miR-181b	Up-regulated	Metastasis	[20, 25]
miR-181b-1	Up-regulated		[25]
miR-181b-2	Up-regulated		[25]
miR-181c	Up-regulated		[25]
miR-181d	Up-regulated		[25]
MiR-182	Down-regulated	Metastasis	[20]
miR-184	Down-regulated	Tumor signature	[20]
miR-186	Up-regulated		[27]
miR-189	Up-regulated	Metastasis	[20]
miR-190	Up-regulated		[27]
miR-196a	Up-regulated		[26]
miR-196b	Up-regulated		[26]
miR-199a-1	Up-regulated		[25]
miR-199a-2	Up-regulated		[25]
miR-200a	Down-regulated	Metastasis	[20]

miRNA	Modification	Significance	Ref.
miR-200b	Down-regulated	Metastasis	[20, 27]
miR-200c	Up-regulated	Metastasis	[20]
miR-203	Up-regulated		[26]
miR-205	Up-regulated		[25, 26]
miR-210	Up-regulated		[25]
miR-212	Up-regulated		[24]
miR-213	Up-regulated		[25]
miR-216	Down-regulated		[26]
miR-217	Down-regulated		[26]
miR-220	Up-regulated		[25]
miR-221	Up-regulated		[24-27]
miR-222	Up-regulated		[25-27]
miR-223	Up-regulated		[25]
miR-224	Up-regulated		[26]
miR-301	Up-regulated		[24]
miR-329	Up-regulated	Metastasis	[20]
miR-335	Down-regulated	Tumor signature	[20]
miR-344	Up-regulated	Metastasis	[20]
miR-345	Down-regulated		[24]
miR-365	Down-regulated	Metastasis	[24]
miR-375	Down-regulated		[25, 26]
miR-376a	Up-regulated		[24]
miR-376	Up-regulated	Tumor signature	[24]
409-3p	Up-regulated	Tumor signature	[20]
miR-410	Down-regulated Up-regulated	Hyperplasia Metastasis	[20]
miR-424	Up-regulated	Angiogenesis	[20, 24]
miR-429	Down-regulated	Metastasis	[20]
miR-431	Up-regulated	Metastasis	[20]
miR-434-3p	Up-regulated	Tumor signature	[20]
miR-449	Up-regulated	Metastasis	[20]

Table 1. miRNAs involved in tumorigenesis of the pancreas

3.3. Apoptosis, cell viability

miR-21, miR-155 and miR-221 over-expression was reported by Lee et al. (2007) for pancreatic tumors compared to paired normal samples. Since the same miRNAs were also over-expressed in other cancers, the authors hypothesized that deregulation of these miRNAs represents a common feature in cancer. For other miRNAs, the pattern of differential expression appeared

different in pancreatic cancer compared to other cancers; modification of miR-376a and miR-301 expression was reported as a distinctive feature of pancreatic cancer [24].

Inhibiting miR-21 and miR-221 with antisense nucleotides resulted in reduced proliferation and increased apoptosis [28].

Hanoun et al. reported the identification of 29 miRNA encoding genes that are susceptible to inactivation by hypermethylation. "In-depth" investigations on miR-148a showed that its production was repressed due to hypermethylation. Hypermethylation analysis was demonstrated as a potential tool for differential diagnostics for PDAC and pancreatitis [29].

Down-regulation of miR-146 was demonstrated by Li et al. (2010) in pancreatic cancer cells compared with normal duct epithelium. Re-expression of miR-146 inhibits the invasive capacity of cancer cells, concomitant with down-regulation of EGFR (Epidermal Growth Factor Receptor) and IRAK-1 (Interleukin 1 Receptor-Associated Kinase). Treatment with two natural compounds, diindolylmethane and isoflavone, were demonstrated to activate miR-146a and inhibit invasion [30].

3.4. Tumor suppressors

Mees et al. (2009) applied microarray, TLDA (Taq-Man Low Density Array) and RT-PCR (Real Time Polymerase Chain Reaction) methods to investigate microRNA profiles in pancreatic cancer cell lines. Fifty-six miRNAs with modified expression were identified: 27 (by microarray) and 19 (by TLDA) miRNAs were over-expressed in highly metastatic cell lines compared to less metastatic ones. Down-regulation (investigated by TLDA) revealed 35 down-regulated microRNAs. Eight of these were tumor-suppressor gene-related miRNAs: miR-21 (PTEN-Phosphatase and TENsin Homolog), miR-26b (EP300-E1A binding protein p300, PTEN), miR-194 (EP300), miR-200b (EP300), miR-200c (EP300), miR-320 (PTEN), miR-374 (EP300) and miR-429 (EP300) [31].

The influence of miR-10a on the behavior of pancreatic tumors was investigated by Weiss et al. in a zebrafish animal model (zebrafish with transplanted tumors) [22]. miR-10a promotes metastatic potential and miR-10 repression is sufficient to inhibit invasions and metastasis. mir-10a is a retinoic acid (RA) target and RA receptor antagonists are effective repressors of miR-10a expression. The anti-metastatic effect is blocked by the knockdown of HOXB1 (Homeodomain containing DNA-binding Box protein 1) and HOXB3 (HOXB3=Homeodomain containing DNA-binding Box protein 3) genes. The epithelial to mesenchymal cell transition (EMT) program triggers cellular mobility and promotes invasion and metastasis. ZEB1 (zinc finger E box binding homeobox1), an EMT activator, promotes cell mobility by disrupting stemness maintenance and promoting mobile, migrating stem cells. ZEB1 was demonstrated to inhibit miR-200 family members and miR-203 [32].

A specific miRNA signature differentiates between pancreatic adenocarcinoma, normal pancreas and chronic pancreatitis [25]. In total, 21 over-expressed and four down-regulated miRNAs allow a differential diagnosis among these three pathologic conditions. In addition, Szafranska et al. reported that miR-196a and-196b levels are high in pancreatic ductal adenocarcinoma but not in normal or inflamed pancreatic tissues [26].

4. miRNAs in tumor stem cells

In gemcitabine-resistant cells with fibroblast morphology, high levels of vimentin and ZEB1 and low levels of E-cadherin, miR-200b, miR-200c, let-7b, 7c, 7d and 7e were found to be down-regulated, according to Li et al. (2009). As in the case of miR-146, DIM (3, 3′ DiIndolylMethane) and isoflavone were demonstrated to restore a less invasive phenotype [33]. ZEB1 was demonstrated to repress expression of stemness-inhibiting miR-203; candidate targets of miR-200 family members are also stem cell factors, such as Sox2 (Transcription factor SOX-2) and Klf4 (Krueppel-like factor 4). miR-200c, miR-203 and miR-183 cooperate to suppress expression of stem cell factors in cancer cells and mouse embryonic stem (ES) cells, as demonstrated for the polycomb repressor Bmi1 (Polycomb complex protein BMI-1) [32].

Key cell differentiation programs during development are controlled by the members of let-7 and miR-200 families. In cancer, loss of let-7 leads to disease progression and de-differentiation [33]. The same let-7 family appears as a regulator of EMT and of stem cell maintenance. The EMT process is regulated by miRNA-dependent mechanisms. In human pancreatic cancer, DCLK1 (Serine/threonine-protein kinase DCLK1) regulates EMT by a mechanism dependent on miR-200a [33-35]. According to Hasselman et al. [36], inhibition of maturation of let-7 by nuclear TRAIL-R2 (TNF-Related Apoptosis-Inducing Ligand Receptor 2) in pancreatic cancer cell lines increases their proliferation. This is consistent with high levels of nuclear TRAIL-R2 in tissue samples from poor outcome patients [36].

The population of BxPC-3-LN cells (lymph node metastatic pancreatic cells) contains a five-fold increased population of CD133+/CXCR4+cells (stem cell-like cells) compared with the parental (non-metastatic) BxPC-3 cells. Remarkably, a different miRNA pattern is displayed in CSC-like cells compared with the regular cells: up-regulated miR-572, miR-206, miR-449a, miR-489 and miR184 were found, as well as down-regulated let-7g-3p, let-7i-3p, let-7a-3p, miR-107, miR-128 and miR-141-5p [37].

The miR-200 family members are identified as key regulators of cell maintenance and EMT. It is considered possible that tumor progression is a process resulting in progressive de-differentiation towards a cell type which has a stem cell-like phenotype. This process appears to be regulated by miRNA-dependent mechanisms. DCLK1 (a putative marker for pancreatic and intestinal cancer stem cells) regulates EMT in human pancreatic cancer cells via a miR-200a-dependent mechanism [38]; it also acts as a regulator of let-7a in pancreatic and colorectal cancer cells, supporting the idea that these miRNAs may be novel and relevant targets in solid tumor cancers [33-35]. Sureban et al. [39] demonstrated that DCLK1 inhibition results in up-regulation of miRNAs that negatively regulate some key angiogenic and pluripotency factors. In AsPC1 (metastatic adenocarcinoma cell line) tumor xenografts, the down-regulation of c-MYC (Myc Proto-oncogene Protein) and KRAS (GTP-ase Kras) via let-7a was observed, by a similar mechanism demonstrated in pancreatic cancer cells.

5. miRNA Polymorphisms

Single nucleotide polymorphisms (SNPs) were demonstrated to affect the functional capacity of miRNAs, influencing MIR processing and miR-mRNA interactions. SNPs in miR-196a2 and miR-146a were differentially expressed between patients with T1/T2 stage pancreatic tumors compared with T3/T4 stages [40].

6. Circulating miRNAs

Some serum tumor markers, such as carcinoembryonic antigen and carbohydrate antigen 19–9, are used as convenient diagnostic markers. Other factors involved in cancer progression, among which are angiogenic factors such as VEGF (Vascular Endothelial Growths Factor) and bFGF (Basic Fibroblast Growth Factor), have drawn attention for the detection of pancreatic cancers [41, 42]. However, these conventional serum markers lack the sensitivity and specificity to facilitate the early detection of cancer. Several studies have identified tumor-specific alterations in plasma/serum nucleic acids in cancer patients and have shown the potential of plasma-circulating nucleic acids to act as new non-invasive biomarkers in patients with various cancers [43]. Recently, several studies have demonstrated that miRNAs are stably detectable in plasma/serum and have discussed key aspects regarding experimental design, such as extraction from biological material, different techniques for miRNA evaluation (TLDA, arrays, etc.) [44-46]. Mitchell et al. clearly showed that circulating miRNAs originate from cancer tissues and are protected from endogenous RNase activity. They also demonstrated that the circulating plasma miRNAs are not associated with circulating tumor cells [45].

Li et al. investigated a set of 735 miRNAs by RT-qPCR (Reverse-Transcriptase quantitative Polymersase Chain Reaction) using microarrays. Eighteen candidates were further validated. The best classifier was miR-1290, with ROC-AUC (Receiver Operator Characteristics Area Under the Curve) of 0.96, while other miRNAs (miR-24, miR-134, miR-146a, miR-378, miR-484, miR-628-3p and miR-1825) also displayed considerable accuracy (ROC-AUC > 0.7). miR-1290 could differentiate between normal pancreas, chronic pancreatitis, pancreatic adenocarcinoma and pancreatic neuroendocrine tumors. Remarkably, miR-1290 is a better classifier than the classical biomarker CA 19-9, distinguishing with greater accuracy low-grade pancreatic cancer from normal subjects [47].

Morimura et al. demonstrated the value of miR-18a as a biomarker for pancreatic cancer; they demonstrated higher levels of expression of this miRNA in cancer tissue and cancer cell lines (compared to normal tissue) and also reported higher plasma levels in patients with PC, with ROC-AUC of 0.9369 [46].

A signature of seven miRNAs was established as a good biomarker for early detection by Liu R et al. [48]. The panel comprised miR-20a, miR-21, miR-24, miR-25, miR-99a, miR-185 and miR-191; the levels of overexpression in plasma ranged between 2.1 and 5.08.

Exosomal miRNAs represents a more recent field of investigation. Exosomes are 40-100 nm vesicles derived from the fusion of multivesicular bodies with the plasma membrane. They

appear in all body fluids and interest in studying them has increased since they contain functional proteins, mRNA and miRNAs. Thus, exosomal populations of different origins may be identified by their protein and miRNA signatures. Moreover, they appear to be actively involved in cell communication. In the case of cancer, they will be, for instance, involved in tumorigenesis, differentiation of stem cells, metastasis and angiogenesis [49]. Most of the reports on exosomes so far concern other cancers, like ovarian [50], prostate [51] and glioblastoma [52]; however, there is also a study concerning exosomes in pancreatic cancer [53].

7. miRNAs: Therapeutic targets and drugs

miRNAs, already described as potent regulators of genes, can be viewed as both therapeutic targets and therapy agents [54]. Recently, the potential to target miRNAs was demonstrated by a series of *in vitro* studies.

In pancreatic cancer (and other epithelial tumors as well), a loss of epithelial differentiation and acquisition of the mesenchymal phenotype occurs, leading to enhanced invasion and migration [55]. Another feature of pancreatic cancer is its drug resistance characteristics, often associated with epithelial-to-mesenchymal transition (EMT) [56].

miR-21 overexpression is associated with resistance to gemcitabine and is generally associated with poor survival [57]. Inhibition of miR-21 decreases cell proliferation and promotes apoptosis [58] but also correlates with 5-FU (5-Fluoro-Uracyl) sensitivity [59]. Another study points out that miR-200 down-regulation and over-expression of miR-21 associates with gemcitabine resistance and their restoration renders the pancreatic cell lines responsive to gemcitabine [60].

miR-34 is a tumor-suppressor miRNA, which can restore chemo-and radio-sensitivity in tumor cell lines; overexpression of miR-34 reduces the tumor-initiating cells and tumor-sphere formation significantly [61].

Another candidate is miR-155, which also appears down-regulated in pancreatic cancers; its overexpression appears to suppress tumor growth [18, 62]. Similar findings are also published for miR-20 and miR-146, with regard to their impact on invasiveness and chemo-sensitivity [63, 64].

The published literature dealing with miRNAs as therapeutic targets in digestive tract cancers does not abound and relies mostly on results obtained on cell lines. miRNAs as therapeutic targets are foreseen in chemotherapy resistance [65-67], silencing oncogenic miRNAs and intervention on tumor-suppressive miRNAs.

Another important set of studies focuses on miRNAs' oncogenic function and on the modalities of intervening using miRNA silencing, antisense blocking and miRNA modifications [54].

The miRNAs with tumor-suppression functions can represent new strategies for inhibiting tumor growth in pancreatic cancer, liver cancer and colorectal cancer [68], while miRNAs as oncogenes can be targeted leading to controlling multiple genes [69].

Recently, the inhibition of miR-21 and miR-17-92 activity was reported as being associated with reduced tumor growth, invasion, angiogenesis and metastasis in PDAC [70, 71].

As therapeutic targets, miRNAs can be manipulated with silencing methodology or recovery of altered microRNAs. Using miRNAs as therapeutic targets may result in several clinical goals: prevent recurrence of the disease, control the growth of advanced metastatic tumors and sensitize tumor cells to chemo-and radiotherapy. There are few studies on *in vivo* experimental models and no clinical trial have commenced using these small molecules as targets [54]. Thus, miRNAs may be possible drugs and/or drug targets and they could potentially be used as molecular therapeutic agents that could inhibit oncogenes or restore the expression of silenced tumor-suppressor genes [1, 72].

Taking into account the updated findings, miRNA-based cancer gene therapy is to be used as follows: RNA or DNA drugs against messenger RNA-encoding genes involved in the pathogenesis of cancers or by directly targeting ncRNAs (non-coding RNAs) that participate in cancer pathogenesis, as reported in colorectal cancers [73].

The reported stages of miRNAs as drugs are generally at the preclinical phase. Groups are using cell lines or even primary cells in a workflow comprising *in vitro* treatment and afterwards detecting the alteration of proliferation, increase in apoptosis and/or abolishment of cancer stem cell characteristics. In animal models treating tumor-bearing mice with specific siRNA, the overall effect on tumor development and survival was tested along with the excretion route of the drug. Very few clinical studies are reported and mostly only in phase I [74, 75]

Up to now, the reported inhibitory RNAs drugs have been: antisense oligonucleotides (ASOs), ribozymes or DNAzymes, siRNAs, microRNAs mimetics, LNAs (Locked Nucleic Acids), anti-miRNAs or antagomiRs (small synthetic oligonucleotides blocking the binding of miRNAs to their targets [28, 76].

siRNAs represent a double strand RNA homologous to the mRNA of a target gene. These siRNAs are incorporated into a multiprotein RNA-induced silencing complex (RISC). The antisense strand guides this complex to its homologous mRNA target for endo-nucleasic cleavage of messenger RNA. ERBB2 (ErbB2 protein encoding gene) amplification was demonstrated in gastrointestinal adenocarcinomas, while in cellular and animal models, siRNA was used to knock down ERBB2 in cell lines, demonstrating that this treatment decreased ERBB2 protein levels and apoptotic pathways were triggered [77].

siRNA specific for bcl-2 (Apoptosis regulator Bcl-2) was also used as a possible therapeutic tool in pancreatic cancer in cells lines and xenografts and the bcl-2 gene was inhibited [78].

MicroRNAs mimics represent small single-strand 19–24 nucleotide RNA produced from the cleavage of a hairpin structure by RNAse III enzymes. These possible therapeutic agents act by the inhibition of protein production by either mRNA degradation or translational block after the formation of miRNA<mRNA duplexes.

miR-34 can target p53, Notch, HMGA2 (High Mobility Group Protein HMGI-C) and Bcl-2, genes mainly involved in cancer stem cells characteristics. There are no clinical trials published

so far of these possible drugs, therefore only preclinical studies were reported, showing that upon the insertion of a functional miR-34, inhibition of cell growth, chemo-sensitization and apoptosis are triggered along with the abolishment of cancer stem cell characteristics [79].

8. Conclusions

Modification in the expression of miRNAs is consistently associated with the process of tumorigenesis. Such deregulation of miRNAs encompasses early-stage or tumor-initiating events, triggers invasion and metastasis, or alternatively it may also represent the outcome of complex alterations specific to tumor cells. Deregulated miRNAs were demonstrated to have potential and several have been already validated as biomarkers for cancer diagnostics or prognosis, including several for pancreatic cancer, especially in tissue-based investigations.

A great number of miRNAs have similar expression patterns in cancers, but several have been demonstrated to be tumor-specific.

A considerable effort is directed towards the development of miRNA-based instruments for diagnostics, prognostics or monitoring the disease and great hope is placed in the exosomal miRNAs. Assays based on exosomal or plasma miRNAs have potential clinical uses in screening patients at risk of cancer or monitoring recurrence post-resection.

They also prove useful in evaluating the completeness of tumor resection and the evaluation of adjuvant therapy. As biomarkers, they show important advantages over other nucleic acids. Compared to the mRNA, in the case of miRNAs a considerably smaller number of molecules can establish an effective screen to differentiate normal from tumoral disease. At the same time, circulating miRNAs are more stable for detection, by comparison with other classes of markers, like mRNAs or proteins.

Meanwhile, the presence of specific miRNAs in pathological tissue opens a new perspective in therapy. As has already been proved, targeting deregulated miRNAs with specific instruments, like miR-mimics, antago-miRs or miRNAs, restores the phenotype from tumoral to normal and the results so far suggest that controlling the expression of miRNA modifies clinical features of tumor cells, such as growth rate, apoptotic susceptibility, drug resistance, mobility and invasiveness of metastatic potential.

9. Abbreviations

Proteins:

AGO2=Protein Argonaute2

ARE=TNFα AU-rich element

Bcl2=Apoptosis regulator Bcl-2

bFGF=Basic Fibroblast Growth Factor

Bmi1=Polycomb complex protein BMI-1

c-MYC=Myc Proto-oncogene Protein

DCLK1=Serine/threonine-protein kinase DCLK1

DGCR8=Integral membrane protein DGCR2/IDD

EGFR=Epidermal Growth Factor Receptor

EP300=E1A binding protein p300

ERBB2=ErbB2 protein encoding gene

EMT=Epithelial-Mesenchymal Transition

FXR1=Fragile X Mental Retardation–related Protein 1

HMGA2=High Mobility Group Protein HMGI-C

HOXB1=Homeodomain containing DNA-binding Box protein 1

HOXB3=Homeodomain containing DNA-binding Box protein 3

IRAK-1=Interleukin 1 Receptor-Associated Kinase

Klf4=Krueppel-like factor 4

KRAS=GTP-ase Kras

MMP2=Matrix-MetalloProteinase 2

MMP9=Matrix-MetalloProteinase 9

Notch1=Neurogenic locus notch homolog protein 1

PTEN=Phosphatase and TENsin homolog

Sox2=Transcription factor SOX-2

TRAIL2=TNF-Related Apoptosis-Inducing Ligand 2

TRAILR2=TNF-Related Apoptosis-Inducing Ligand Receptor 2

VEGF=Vascular Endothelial Growths Factor

ZEB1=Zinc finger E box Binding homeobox1

Other Abbreviations

5-FU=5 Fluoro-Uracil

CSC=Cancer Stem Cells

DIM=3, 3′ DiIndolylMethane

FFPE=Formalin-Fixed, Paraffin Embedded

LNA=Locked Nucleic Acids: conformationally-restricted nucleic acid analogue in which the ribose ring is 'locked' with a methylene bridge connecting the 2′-O atom with the 4′-C atom.

PanIN=Pancreatic Intraepithelial Neoplasia

PDAC=Pancreatic Duct AdenoCarcinoma

RA=Retinoic Acid

RISC=RNA-Induced Silencing Complex

ROC-AUC=Receiver Operator Characteristics Area Under the Curve

SNPs=Single Nucleotide Polymorphisms

TLDA=Taq-Man Low Density Array

Author details

Radu Albulescu[1,2], Adrian Claudiu Popa[3], Elena Codrici[1], Daniela Ionela Popescu[1], Simona Mihai[1] and Cristiana Tanase[1]

1 National Institute of Pathology "Victor Babes", Bucharest, Romania

2 National Institute for Chemical Pharmaceutical R&D, Bucharest, Romania

3 Army Center for Medical Research, Bucharest, Romania

References

[1] Saito Y, Friedman JM, Chihara Y, Egger G, Chuang JC, Liang G. Epigenetic therapy upregulates the tumor suppressor microRNA-126 and its host gene EGFL7 in human cancer cells. Biochemical and biophysical research communications. 2009;379(3): 726-31. Epub 2009/01/01.

[2] 2013; Available from: http://www.mirbase.org/.

[3] Wiemer EA. The role of microRNAs in cancer: no small matter. Eur J Cancer. 2007;43(10):1529-44. Epub 2007/05/29.

[4] Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, et al. Micro-RNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA : the journal of the American Medical Association. 2008;299(4):425-36. Epub 2008/01/31.

[5] Schepeler T, Reinert JT, Ostenfeld MS, Christensen LL, Silahtaroglu AN, Dyrskjot L, et al. Diagnostic and prognostic microRNAs in stage II colon cancer. Cancer research. 2008;68(15):6416-24. Epub 2008/08/05.

[6] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature. 2005;435(7043):834-8. Epub 2005/06/10.

[7] Waldman SA, Terzic A. MicroRNA signatures as diagnostic and therapeutic targets. Clinical chemistry. 2008;54(6):943-4. Epub 2008/05/30.

[8] Heneghan HM, Miller N, Kelly R, Newell J, Kerin MJ. Systemic miRNA-195 differentiates breast cancer from other malignancies and is a potential biomarker for detecting noninvasive and early stage disease. The oncologist. 2010;15(7):673-82. Epub 2010/06/26.

[9] Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. International journal of cancer Journal international du cancer. 2010;127(1):118-26. Epub 2009/10/31.

[10] Fabbri M. miRNAs as molecular biomarkers of cancer. Expert review of molecular diagnostics. 2010;10(4):435-44. Epub 2010/05/15.

[11] Mendell JT. miRiad roles for the miR-17-92 cluster in development and disease. Cell. 2008;133(2):217-22. Epub 2008/04/22.

[12] Esquela-Kerscher A, Slack FJ. Oncomirs-microRNAs with a role in cancer. Nature reviews Cancer. 2006;6(4):259-69. Epub 2006/03/25.

[13] Olive V, Jiang I, He L. mir-17-92, a cluster of miRNAs in the midst of the cancer network. The international journal of biochemistry & cell biology. 2010;42(8):1348-54. Epub 2010/03/17.

[14] Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. Science. 2007;318(5858):1931-4. Epub 2007/12/01.

[15] Ji Q, Hao X, Zhang M, Tang W, Yang M, Li L, et al. MicroRNA miR-34 inhibits human pancreatic cancer tumor-initiating cells. PloS one. 2009;4(8):e6816. Epub 2009/08/29.

[16] Giovannetti E, Funel N, Peters GJ, Del Chiaro M, Erozenci LA, Vasile E, et al. MicroRNA-21 in pancreatic cancer: correlation with clinical outcome and pharmacologic aspects underlying its role in the modulation of gemcitabine activity. Cancer research. 2010;70(11):4528-38. Epub 2010/05/13.

[17] Albulescu R, Neagu M, Albulescu L, Tanase C. Tissular and soluble miRNAs for diagnostic and therapy improvement in digestive tract cancers. Expert review of molecular diagnostics. 2011;11(1):101-20. Epub 2010/12/22.

[18] Ryu JK, Hong SM, Karikari CA, Hruban RH, Goggins MG, Maitra A. Aberrant MicroRNA-155 expression is an early event in the multistep progression of pancreatic adenocarcinoma. Pancreatology. 2010;10(1):66-73. Epub 2010/03/25.

[19] du Rieu MC, Torrisani J, Selves J, Al Saati T, Souque A, Dufresne M, et al. MicroRNA-21 is induced early in pancreatic ductal adenocarcinoma precursor lesions. Clinical chemistry. 2010;56(4):603-12. Epub 2010/01/23.

[20] Olson P, Lu J, Zhang H, Shai A, Chun MG, Wang Y, et al. MicroRNA dynamics in the stages of tumorigenesis correlate with hallmark capabilities of cancer. Genes & development. 2009;23(18):2152-65. Epub 2009/09/18.

[21] Torrisani J, Bournet B, du Rieu MC, Bouisson M, Souque A, Escourrou J, et al. let-7 MicroRNA transfer in pancreatic cancer-derived cells inhibits in vitro cell proliferation but fails to alter tumor progression. Human gene therapy. 2009;20(8):831-44. Epub 2009/03/28.

[22] Weiss FU, Marques IJ, Woltering JM, Vlecken DH, Aghdassi A, Partecke LI, et al. Retinoic acid receptor antagonists inhibit miR-10a expression and block metastatic behavior of pancreatic cancer. Gastroenterology. 2009;137(6):2136-45 e1-7. Epub 2009/09/15.

[23] Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA: a cancer journal for clinicians. 2010;60(5):277-300. Epub 2010/07/09.

[24] Lee EJ, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, et al. Expression profiling identifies microRNA signature in pancreatic cancer. International journal of cancer Journal international du cancer. 2007;120(5):1046-54. Epub 2006/12/07.

[25] Bloomston M, Frankel WL, Petrocca F, Volinia S, Alder H, Hagan JP, et al. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. JAMA : the journal of the American Medical Association. 2007;297(17):1901-8. Epub 2007/05/03.

[26] Szafranska AE, Davison TS, John J, Cannon T, Sipos B, Maghnouj A, et al. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. Oncogene. 2007;26(30):4442-52. Epub 2007/01/24.

[27] Zhang Y, Li M, Wang H, Fisher WE, Lin PH, Yao Q, et al. Profiling of 95 microRNAs in pancreatic cancer cell lines and surgical specimens by real-time PCR analysis. World journal of surgery. 2009;33(4):698-709. Epub 2008/11/26.

[28] Park JK, Lee EJ, Esau C, Schmittgen TD. Antisense inhibition of microRNA-21 or-221 arrests cell cycle, induces apoptosis, and sensitizes the effects of gemcitabine in pancreatic adenocarcinoma. Pancreas. 2009;38(7):e190-9. Epub 2009/09/05.

[29] Hanoun N, Delpu Y, Suriawinata AA, Bournet B, Bureau C, Selves J, et al. The silencing of microRNA 148a production by DNA hypermethylation is an early event in pancreatic carcinogenesis. Clinical chemistry. 2010;56(7):1107-18. Epub 2010/05/01.

[30] Li Y, Vandenboom TG, 2nd, Wang Z, Kong D, Ali S, Philip PA, et al. miR-146a suppresses invasion of pancreatic cancer cells. Cancer research. 2010;70(4):1486-95. Epub 2010/02/04.

[31] Mees ST, Schleicher C, Mardin WA, Senninger N, Colombo-Benkmann M, Haier J. Analyzing miRNAs in ductal adenocarcinomas of the pancreas. The Journal of surgical research. 2011;169(2):241-6. Epub 2010/01/19.

[32] Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, et al. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nature cell biology. 2009;11(12):1487-95. Epub 2009/11/26.

[33] Li Y, VandenBoom TG, 2nd, Kong D, Wang Z, Ali S, Philip PA, et al. Up-regulation of miR-200 and let-7 by natural agents leads to the reversal of epithelial-to-mesenchymal transition in gemcitabine-resistant pancreatic cancer cells. Cancer research. 2009;69(16):6704-12. Epub 2009/08/06.

[34] Peter ME. Let-7 and miR-200 microRNAs: guardians against pluripotency and cancer progression. Cell Cycle. 2009;8(6):843-52. Epub 2009/02/18.

[35] Watanabe S, Ueda Y, Akaboshi S, Hino Y, Sekita Y, Nakao M. HMGA2 maintains oncogenic RAS-induced epithelial-mesenchymal transition in human pancreatic cancer cells. The American journal of pathology. 2009;174(3):854-68. Epub 2009/01/31.

[36] Haselmann V, Kurz A, Bertsch U, Hubner S, Olempska-Muller M, Fritsch J, et al. Nuclear Death Receptor TRAIL-R2 Inhibits Maturation of Let-7 and Promotes Proliferation of Pancreatic and Other Tumor Cells. Gastroenterology. 2014;146(1):278-90. Epub 2013/10/15.

[37] Luo G, Long J, Cui X, Xiao Z, Liu Z, Shi S, et al. Highly lymphatic metastatic pancreatic cancer cells possess stem cell-like properties. International journal of oncology. 2013;42(3):979-84. Epub 2013/01/23.

[38] Sureban SM, May R, Lightfoot SA, Hoskins AB, Lerner M, Brackett DJ, et al. DCAMKL-1 regulates epithelial-mesenchymal transition in human pancreatic cells through a miR-200a-dependent mechanism. Cancer research. 2011;71(6):2328-38. Epub 2011/02/03.

[39] Sureban SM, May R, Qu D, Weygant N, Chandrakesan P, Ali N, et al. DCLK1 regulates pluripotency and angiogenic factors via microRNA-dependent mechanisms in pancreatic cancer. PloS one. 2013;8(9):e73940. Epub 2013/09/17.

[40] Pavlakis E, Papaconstantinou I, Gazouli M, Theodosopoulos T, Karamanolis G, Genatas K, et al. MicroRNA gene polymorphisms in pancreatic cancer. Pancreatology. 2013;13(3):273-8. Epub 2013/05/31.

[41] Tanase CP, Neagu M, Albulescu R, Codorean E, Dima SO. Biomarkers in the diagnosis and early detection of pancreatic cancer. Expert opinion on medical diagnostics. 2009;3(5):533-46. Epub 2009/09/01.

[42] Pistol-Tanase C, Raducan E, Dima SO, Albulescu L, Alina I, Marius P, et al. Assessment of soluble angiogenic markers in pancreatic cancer. Biomarkers in medicine. 2008;2(5):447-55. Epub 2008/10/01.

[43] Chan KC, Lo YM. Circulating tumour-derived nucleic acids in cancer patients: potential applications as tumour markers. British journal of cancer. 2007;96(5):681-5. Epub 2007/02/22.

[44] Kroh EM, Parkin RK, Mitchell PS, Tewari M. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods. 2010;50(4):298-301. Epub 2010/02/12.

[45] Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(30):10513-8. Epub 2008/07/30.

[46] Morimura R, Komatsu S, Ichikawa D, Takeshita H, Tsujiura M, Nagata H, et al. Novel diagnostic value of circulating miR-18a in plasma of patients with pancreatic cancer. British journal of cancer. 2011;105(11):1733-40. Epub 2011/11/03.

[47] Li A, Yu J, Kim H, Wolfgang CL, Canto MI, Hruban RH, et al. MicroRNA array analysis finds elevated serum miR-1290 accurately distinguishes patients with low-stage pancreatic cancer from healthy and disease controls. Clinical cancer research : an official journal of the American Association for Cancer Research. 2013;19(13):3600-10. Epub 2013/05/24.

[48] Liu R, Chen X, Du Y, Yao W, Shen L, Wang C, et al. Serum microRNA expression profile as a biomarker in the diagnosis and prognosis of pancreatic cancer. Clinical chemistry. 2012;58(3):610-8. Epub 2011/12/24.

[49] Zoller M. Pancreatic cancer diagnosis by free and exosomal miRNA. World journal of gastrointestinal pathophysiology. 2013;4(4):74-90. Epub 2013/12/18.

[50] Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecologic oncology. 2008;110(1):13-21. Epub 2008/07/01.

[51] Bryant RJ, Pawlowski T, Catto JW, Marsden G, Vessella RL, Rhees B, et al. Changes in circulating microRNA levels associated with prostate cancer. British journal of cancer. 2012;106(4):768-74. Epub 2012/01/14.

[52] Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nature cell biology. 2008;10(12):1470-6. Epub 2008/11/18.

[53] Wang H, Rana S, Giese N, Buchler MW, Zoller M. Tspan8, CD44v6 and alpha6beta4 are biomarkers of migrating pancreatic cancer-initiating cells. International journal of cancer Journal international du cancer. 2013;133(2):416-26. Epub 2013/01/23.

[54] Slaby O, Svoboda M, Michalek J, Vyzula R. MicroRNAs in colorectal cancer: translation of molecular biology into clinical application. Molecular cancer. 2009;8:102. Epub 2009/11/17.

[55] Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nature reviews Cancer. 2002;2(6):442-54. Epub 2002/08/22.

[56] Sabbah M, Emami S, Redeuilh G, Julien S, Prevost G, Zimber A, et al. Molecular signature and therapeutic perspective of the epithelial-to-mesenchymal transitions in epithelial cancers. Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy. 2008;11(4-5):123-51. Epub 2008/08/23.

[57] Dillhoff M, Liu J, Frankel W, Croce C, Bloomston M. MicroRNA-21 is overexpressed in pancreatic cancer and a potential predictor of survival. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2008;12(12):2171-6. Epub 2008/07/22.

[58] Hwang JH, Voortman J, Giovannetti E, Steinberg SM, Leon LG, Kim YT, et al. Identification of microRNA-21 as a biomarker for chemoresistance and clinical outcome following adjuvant therapy in resectable pancreatic cancer. PloS one. 2010;5(5):e10630. Epub 2010/05/26.

[59] Moriyama T, Ohuchida K, Mizumoto K, Yu J, Sato N, Nabae T, et al. MicroRNA-21 modulates biological functions of pancreatic cancer cells including their proliferation, invasion, and chemoresistance. Molecular cancer therapeutics. 2009;8(5):1067-74. Epub 2009/05/14.

[60] Ali S, Ahmad A, Banerjee S, Padhye S, Dominiak K, Schaffert JM, et al. Gemcitabine sensitivity can be induced in pancreatic cancer cells through modulation of miR-200 and miR-21 expression by curcumin or its analogue CDF. Cancer research. 2010;70(9):3606-17. Epub 2010/04/15.

[61] Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Molecular cell. 2007;26(5):745-52. Epub 2007/06/02.

[62] Gironella M, Seux M, Xie MJ, Cano C, Tomasini R, Gommeaux J, et al. Tumor protein 53-induced nuclear protein 1 expression is repressed by miR-155, and its restoration inhibits pancreatic tumor development. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(41):16170-5. Epub 2007/10/04.

[63] Singh S, Chitkara D, Kumar V, Behrman SW, Mahato RI. miRNA profiling in pancreatic cancer and restoration of chemosensitivity. Cancer letters. 2013;334(2):211-20. Epub 2012/10/18.

[64] Lin F, Wang X, Jie Z, Hong X, Li X, Wang M, et al. Inhibitory effects of miR-146b-5p on cell migration and invasion of pancreatic cancer by targeting MMP16. Journal of Huazhong University of Science and Technology Medical sciences=Hua zhong ke ji da xue xue bao Yi xue Ying De wen ban=Huazhong keji daxue xuebao Yixue Yingdewen ban. 2011;31(4):509-14. Epub 2011/08/09.

[65] Zhou J, Zhou Y, Yin B, Hao W, Zhao L, Ju W, et al. 5-Fluorouracil and oxaliplatin modify the expression profiles of microRNAs in human colon cancer cells in vitro. Oncology reports. 2010;23(1):121-8. Epub 2009/12/04.

[66] Song B, Wang Y, Xi Y, Kudo K, Bruheim S, Botchkina GI, et al. Mechanism of chemoresistance mediated by miR-140 in human osteosarcoma and colon cancer cells. Oncogene. 2009;28(46):4065-74. Epub 2009/09/08.

[67] Khan S, Ansarullah, Kumar D, Jaggi M, Chauhan SC. Targeting microRNAs in pancreatic cancer: microplayers in the big game. Cancer research. 2013;73(22):6541-7. Epub 2013/11/10.

[68] Li M, Marin-Muller C, Bharadwaj U, Chow KH, Yao Q, Chen C. MicroRNAs: control and loss of control in human physiology and disease. World journal of surgery. 2009;33(4):667-84. Epub 2008/11/26.

[69] Boni V, Bandres E, Zarate R, Colucci G, Maiello E, Garcia-Foncillas J. MicroRNAs as a new potential therapeutic opportunity in gastrointestinal cancer. Oncology. 2009;77 Suppl 1:75-89. Epub 2009/01/01.

[70] Krichevsky AM, Gabriely G. miR-21: a small multi-faceted RNA. Journal of cellular and molecular medicine. 2009;13(1):39-53. Epub 2009/01/30.

[71] Dews M, Homayouni A, Yu D, Murphy D, Sevignani C, Wentzel E, et al. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nature genetics. 2006;38(9):1060-5. Epub 2006/08/01.

[72] DeSano JT, Xu L. MicroRNA regulation of cancer stem cells and therapeutic implications. The AAPS journal. 2009;11(4):682-92. Epub 2009/10/21.

[73] Hu G, Chen D, Li X, Yang K, Wang H, Wu W. miR-133b regulates the MET protooncogene and inhibits the growth of colorectal cancer cells in vitro and in vivo. Cancer biology & therapy. 2010;10(2):190-7. Epub 2010/05/28.

[74] Okamoto K, Murawaki Y. The therapeutic potential of RNA interference: novel approaches for cancer treatment. Current pharmaceutical biotechnology. 2012;13(11): 2235-47. Epub 2011/05/25.

[75] Tsuda N, Ishiyama S, Li Y, Ioannides CG, Abbruzzese JL, Chang DZ. Synthetic microRNA designed to target glioma-associated antigen 1 transcription factor inhibits division and induces late apoptosis in pancreatic tumor cells. Clinical cancer research : an official journal of the American Association for Cancer Research. 2006;12(21):6557-64. Epub 2006/11/07.

[76] Frampton AE, Castellano L, Colombo T, Giovannetti E, Krell J, Jacob J, et al. MicroRNAs Cooperatively Inhibit a Network of Tumor Suppressor Genes to Promote Pancreatic Tumor Growth and Progression. Gastroenterology. 2014;146(1):268-77 e18. Epub 2013/10/15.

[77] Arrington AK, Dahlberg PS, Davydova J, Vickers SM, Yamamoto M. ERBB2 suppression decreases cell growth via apoptosis in gastrointestinal adenocarcinomas. Surgery. 2009;146(2):213-9. Epub 2009/07/25.

[78] Ocker M, Neureiter D, Lueders M, Zopf S, Ganslmayer M, Hahn EG, et al. Variants of bcl-2 specific siRNA for silencing antiapoptotic bcl-2 in pancreatic cancer. Gut. 2005;54(9):1298-308. Epub 2005/08/16.

[79] Ji Q, Hao X, Meng Y, Zhang M, Desano J, Fan D, et al. Restoration of tumor suppressor miR-34 inhibits human p53-mutant gastric cancer tumorspheres. BMC cancer. 2008;8:266. Epub 2008/09/23.

Chapter 4

Linking Obesity and Pancreatic Cancer

Kelly McCall, Anthony L Schwartz and
Frank L Schwartz

Additional information is available at the end of the chapter

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1. Introduction

Cancer of the pancreas is the tenth most common form of cancer in the United States and the fourth leading cause of cancer-related death with a stunningly low 5-year survival rate of less that 6% [1-4]. Although there are genetic links with pancreatic cancer (10-15% of patients diagnosed will have a family history) [5], chronic pancreatitis [6], cigarette smoking and smokeless tobacco [7, 8], obesity [9-11], and type 2 diabetes mellitus (T2DM) [12-14] are the strongest environmental risk factors linked to this malignancy. Recently high fructose corn syrup (HFCS) consumption which also contributes to obesity, T2DM, and non-alcoholic fatty liver disease (NAFLD), has also been directly linked to pancreatic cancer [15]. The development of the industrial age and subsequent loss of the "hunter-gatherer" life-style has resulted in a world-wide epidemic of obesity and its associated chronic diseases including: atherosclerotic heart disease, stroke, diabetes, and multiple obesity-associated malignancies including cancer of the pancreas. Epidemiologic studies have demonstrated that as underdeveloped countries progress into industrialized economies and life-styles change (especially consumption of high density fat/carbohydrate diets coupled with decreased physical activity), the prevalence of obesity and obesity-related chronic diseases increases. The direct link between obesity, chronic inflammation, and oncogenesis is becoming increasingly more appreciated and the underlying cellular mechanisms involved this process are currently intensively being investigated and reviewed [16, 17]. In addition to the direct role of obesity in oncogenesis, obese individuals also demonstrate worse outcomes and shorter cancer survival compared to persons with normal body mass indexes (BMIs) [16]. These observations suggest that the abnormal hormonal and inflammatory milieu of obesity is directly involved in oncogenesis, promotes tumor growth, spread, and metastasis while possibly also increasing resistance to therapeutic intervention [16]. This chapter is meant to review the links between obesity, abnormal adipose tissue function, induction of abnormal hormonal and chronic inflammatory signaling path-

ways involved pancreatic cancer origin, growth, spread, and resistance to treatment. Our research efforts have been focused on the role of pathologic expression of toll like receptors (TLRs) in this process which links increasing visceral obesity to these processes.

2. Genetic linkage to pancreatic cancer

Family aggregation of pancreatic cancers suggests a genetic linkage and several important pancreatic cancer susceptibility genes have been identified including high-penetrance genes: **BRCA2, PALB2, PRSS1, SPINK1, STK11** have recently been reviewed [5], and DNA miss-match repair genes. Genome-wide association studies (GWAS) are also finding single-gene polymorphisms (snps) that are also associated with increased risk for pancreatic cancer including: **ABO, 1q32.1, 13q22.1, CLPTM1/TERT, CFTR** [18, 19].

Chronic pancreatitis is the strongest independent risk factor for cancer of the pancreas and there are environmentally induced forms as well as rare inherited forms. Autosomal dominant mutations of the cationic trypsinogen gene **PRSS1** causes a hereditary form of chronic pancreatitis [20] while an autosomal recessive defect in the serine protease inhibitor gene **SPINK1** also causes hereditary pancreatitis [21]. These familial forms of chronic pancreatitis exhibit the greatest risk for pancreatic cancer (50-fold increase compared to the general population) and these individuals also experience the longest duration of chronic pancreatitis as well. As life expectancy from cystic fibrosis (CF) has increased from childhood into adulthood, individuals with the cystic fibrosis transmembrane conductance regulator (**CFTR**) gene now exhibit a 5-fold increased risk for pancreatic cancer from their early onset exocrine pancreatic disease and chronic pancreatitis [22, 23]. These are the major genes associated with risk for pancreatic cancer to date and most investigators anticipate that gene-gene and gene-environmental interactions coupled with the chronic inflammation are cooperatively involved in the pathogenesis of such complex cancers.

3. Environmental causes of chronic pancreatitis

Patients with chronic pancreatitis from any cause are at increased risk for pancreatic cancer with severity and duration of chronic pancreatitis (>20 years), age of the patient, and concomitant tobacco use being the major associated co-factors. Although alcohol abuse is causally linked to the development of chronic pancreatitis, interestingly it does not appear to be an independent risk factor for pancreatic cancer which has been confirmed by multiple recent epidemiologic met-analysis studies [24, 25]. **Cigarette smoke contains numerous carcinogenic compounds** including nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone **(NNK) [26]**. One of the most well-known features of NNK is the ability of its metabolites to bind to DNA and induce activating point mutations in the RAS gene [26]. Nicotine itself has also been shown to stimulate Src kinase activity which facilitates the induction of the inhibitor of differentiation-1 (Id1) transcription factor which promotes pancreatic tumor growth, meta-

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stasis, and resistance to chemotherapeutics. Cigarette smoking also increases the risk for T2DM by inducing insulin resistance as well. Finally, as will be reviewed below, increasing BMI and obesity are also clearly risk factors for the development of hyperlipidemia, T2DM, chronic pancreatitis, and a 2-fold higher prevalence of pancreatic cancer.

4. Epidemiology of obesity, T2DM, and pancreatic cancer

There are multiple epidemiologic studies in the US and world-wide linking the epidemic of obesity and higher BMI to increa`sed risk for multiple malignancies including carcinoma of the pancreas [10, 27]. The American Cancer Society calculates that of the 1.5 million new cancer cases diagnosed each year, at least 20% are due to obesity [2]. The risk of pancreatic cancer in both men and women is increased in those who have a BMI > 25 but is most pronounced in those with a BMI of 35 or greater [11, 28]. The risk has been shown to increase by 10 per cent for every five-point increase in BMI. The strongest environmental risk factors related to pancreatic cancer as stated previously are cigarette smoking [8] and obesity [9]; both of which are also linked to inducing chronic inflammation, insulin resistance and T2DM [14]. As stated previously, individuals with T2DM are also twice as likely to develop acute pancreatitis and pancreatic cancer compared to non-diabetics [9, 10]. Studies looking at the components of diet and pancreatic cancer link increased risk with consumption of high fat diets, processed and/or organ meats, the glycemic index of food, and recently high-fructose corn syrup (HFCS) as important factors contributing to obesity, T2DM and risk for carcinoma of the pancreas [15]. As many as 40-50 % of patients with chronic pancreatitis will develop diabetes mellitus (DM) from the chronic destruction of beta cell function as well (insulin deficiency rather than the hyperinsulinemia discussed later). Furthermore, 40% of patients with carcinoma of the pancreas develop insulin deficiency from tumor replacement of beta cells and the DM often precedes the diagnosis of the cancer.

In contrast, there is a reciprocal relationship between the amount of exercise and risk for obesity, T2DM, and pancreatic cancer. Exercise alone burns calories and reduces the risk and/or severity of obesity, reduces insulin resistance, and promotes the production of anti-inflammatory cytokines which counter all of the proinflammatory and oncogenic processes which are discussed below [10].

5. Molecular pathways linking obesity, inflammation, diabetes, and pancreatic cancer

When caloric intake exceeds normal metabolic demand there is a need to store this excess energy and that is the principle function of the adipocyte. Adipose tissue however, is more than just a storage depot. Adipose tissue (especially **visceral fat**) is composed of multiple cell types (adipocytes, pre-adipocytes, macrophages, fibroblasts, and blood vessels), and is now recognized as a significant endocrine organ that expresses and secretes multiple hormones

(leptin, adiponectin, resistin), inflammatory cytokines (TNF-α, IL-6, and IFN-β), components of complement, plasminogen activator inhibitor-1 (PAI-1), vascular endothelial growth factor (VEGF) and other proteins such as monocyte chemoattractant protein (MCP-1). These adipose tissue-derived factors (Figure 1) are now thought to contribute dramatically to the induction of chronic inflammation which is expressed as insulin resistance [29], hyperinsulinemia, T2DM, hyperlipidemia, hypertension, and atherosclerosis [30], and also contributing to the oncogenesis of many solid tumors [11, 16]. Visceral obesity is the fat depot most closely associated with the production of these substances and the subsequent development of insulin resistance, T2DM, and pancreatic cancer oncogenesis.

5.1. Dietary contributions

a. High Fat Diets (HFDs) and Excess Free Fatty Acids (FFAs):

Dietary fats (triglycerides, glycerol, and FFAs) are directly absorbed from the small intestine as chylomicrons into the thoracic duct into the subclavian vein and then into the general circulation. Chylomicrons are taken up by adipocytes and hepatocytes [31]. However, once the adipocyte storage capacity is exceeded, excess TG's and FFA's stimulate adipogenesis and are deposited ectopically into the liver where these excess fats accumulate in small vacuoles within hepatocytes which is the first stage of fatty liver disease (steatosis) [32, 33]. There is also increased *de novo* hepatic lipogenesis with consequent endogenous over-production of triglycerides (TGs) and free fatty acids (FFAs). Excess fats are also deposited in skeletal muscle and other insulin target tissues (even beta cells of pancreas) where they initiate acute inflammatory processes (lipotoxicity) with the activation of multiple inflammatory cytokines [16]. Inflammatory cytokines in turn, directly contribute to the induction of insulin resistance through down regulation of the insulin receptor (IR) and post-receptor signaling pathways in insulin target tissues [33]. In the liver, the ectopic dietary fat also initiates an inflammatory response (steatohepatitis) which contributes to the development of non-alcoholic fatty liver disease (NAFLD) [33].

Within visceral fat cells themselves, FFAs (palmitate, etc.) directly induce the release of inflammatory cytokines [16] and also trigger the pathologic signaling of toll-like receptors (TLRs); activation of TLR4, in particular, increases additional inflammatory cytokine production, contributing to the initiation of insulin resistance [34] and adipogenesis, further increasing adipocyte mass, and the chronic inflammatory state now associated with obesity, T2DM, and oncogenesis.

b. High Fructose Corn Syrup (HFCS):

Fructose is a dietary carbohydrate normally derived from plant sources (tree and vine fruits, flowers, berries, and most root vegetables) which is much sweeter than glucose or sucrose. It is commonly used commercially in prepared foods due to its sweetness, effects on prepared food texture, and browning of baked foods. Commercially it is derived from sugar cane, sugar beets, and corn. HFCS is a mixture of glucose and fructose as monosaccharaides and as a food supplement it is now being vilified for its role in the obesity epidemic as well as induction of insulin resistance, T2DM and non-alcoholic fatty liver disease (NAFLD) [35-38]. NAFLD is

now the leading cause of cirrhosis of the liver and primary hepatocellular cancer. Diets high in HFCS have also been linked directly to increased risk for pancreatic cancer [39]. Mechanisms by which HFCS induces insulin resistance are thought to be due to its unique metabolism in the liver via pathways identical to alcohol. Fructose binds to only one of the glucose transporters (GLUT 5) which is present only in enterocytes of the intestine and in the liver. Thus, although it is selectively concentrated in the liver, fructose cannot be utilized as a carbohydrate for energy in any other cell or organ of the body. Acutely, fructose ingestion results in the shunting of fructose-1-phosphate into dihydroxyacetone-phosphate and glyceraldehyde which enters the TCA cycle from pyruvate and citrate to excessively increase *de novo* hepatic lipogenesis and the over-production of TGs and FFAs [40]. Fructose-1-phosphate also directly induces janus kinase-1 (**JNK-1**) signaling, increasing serine phosphorylation of insulin receptor substrate-1 (**IRS-1**) in the liver and preventing normal insulin-stimulated tyrosine phosphorylation of IRS-1 [41]. TG and FFAs derived from HFCS intake also induce insulin resistance in the liver as the FFAs precipitate in hepatocytes (lipid droplet accumulation), also stimulating excessive TLR4 signaling and further amplification of multiple inflammatory cytokine pathways. Dihydroxyacetone-phosphate and glyceraldehyde are also both directly hepatotoxic while the excessive accumulation of lipid droplets in the liver induces steatosis further amplifying inflammatory cytokine release. All of these processes are thought to contribute to progressive development of hepatic fibrosis, cirrhosis, and primary hepatic cancer. Elevated TGs and FFAs produced by the liver or which cannot be cleared from the portal vein by the liver accumulate in the peripheral circulation, exerting similar effects on the insulin receptor signaling in other target tissues such as adipose tissue, skeletal muscle, and the exocrine pancreas [40].

With regard to pancreatic cancer, there is increasing evidence of a specific dose-dependent linkage between HFCS intake and its occurrence and this risk is independent of obesity or BMI [15]. Furthermore, fructose directly stimulates increased nucleic acid synthesis through the pentose phosphate pathway (catalyzed by transketolase) which is necessary for proliferation of malignant cells and consumption of HFCS is now linked both to oncogenesis as well as tumor spread and metastasis [15].

c. Carcinogens in Foods:

High intake of processed meats containing heterocyclic amines and benzo (a) pyrines or have been prepared at high temperatures (fried or grilled) have been linked to pancreatic cancer [42] as have other foods containing aflatoxins [43] and other mutagens, however their link to pancreatic cancer are fairly weak at this time.

5.2. Molecular pathways triggered by dietary constituents

a. Adipocyte-Derived Inflammatory Proteins:

Inflammatory cytokines (adipokines) such as **TNF-α, IL-6, IL8, VEGF, and IFN-β** have been shown to be elevated in states of visceral obesity [16], as well as acute and chronic pancreatitis, and pancreatic cancer [11]. Visceral adipocytes/macrophages are major sources of the obesity-associated cytokines which are thought to promote insulin resistance [29] (see below) as well

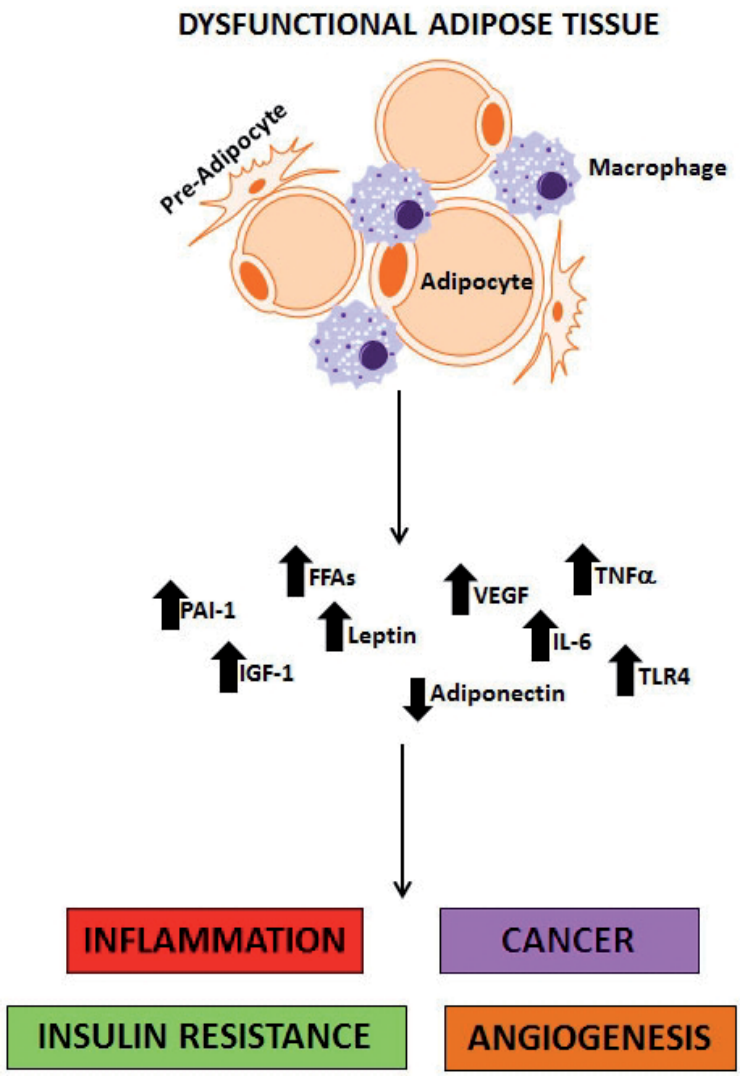

Figure 1. The role of dysfunctional adipose tissue in obesity. Dysfunctional adipose tissue is a critical source of molecules that mediate inflammation, cancer, insulin resistance and angiogenesis. PAI-1 (plasminogen activator inhibitor-1); FFAs (free fatty acids); IGF-1 (insulin-like growth factor 1); VEGF (vascular endothelial growth factor); IL-6 (interleukin 6); TNF-α (tumor necrosis factor alpha); TLR4 (toll-like receptor 4).

as directly contribute to oncogenesis via several pathways [16] including other growth factor receptors, cytokine receptors, or non-receptor tyrosine kinases. Each of these pathways can increase Janus kinase (JAK)/signal transduction and activator of transcription Signal Transducer and Activator of Transcription (STATs) of which **STAT3** [44, 45] is directly linked to cancer of the pancreas. Both of these pathways can stimulate cellular proliferation—transformation through (1) up-regulation of genes encoding cell cycle regulators (cyclins D1/D2, c-Myc), (2) increasing the probability of mutation, (e.g., cellular proto-oncogenes, DNA, and

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cellular repair mechanisms), (3) inhibition of apoptosis (*Bcl-xL*, *Mcl-1*), (4) decreased cellular adhesion, and/or (5) stimulation of angiogenesis (VEGF) [46].

Leptin is also secreted by adipocytes and plays a key role in regulating metabolism and appetite. Leptin is known as the satiety hormone however serum leptin levels are elevated in obesity due to central leptin receptor resistance (by mechanisms similar to insulin discussed below). Leptin has mitogenic actions in many cancer cell lines which appear to be via mitogen-activated-protein-kinase (**MAPK**) mediated pathways; however in certain pancreatic cancer cell lines it inhibits growth [47] so its role in this cancer is unclear at present [48, 49].

Adiponectin is exclusively secreted by adipocytes and has both anti-inflammatory and insulin-sensitizing effects. Known as the "good adipokine" serum levels of leptin are inversely related to BMI and levels are reduced obese patients and in many cancers. High levels of adiponectin are inversely related to the incidence of pancreatic cancer [49].

PAI-1 is a serine protease inhibitor produced by adipocytes and stromal cells in visceral fat, is associated with tumor cell invasion, metastasis, and angiogenesis of many malignancies, and over-expression of PAI-1 has been demonstrated in many obesity-related tumors suggesting it contributes to the spread of malignancies [50]. Interestingly, high expression of the plasminogen activator inhibitor-2 (PAI-2) was a predictor of improved survival in patients with pancreatic adenocarcinoma [51].

VEGF is another adipocyte-derived polypeptide that has been implicated in cancer growth, shown to be over-expressed in many pancreatic cancers, and its expression in these tumors is linked to poorer survival [52, 53].

b. Insulin Resistance, Hyperinsulinemia, and Increased Insulin/IGF-1 Receptor Signaling Pathways

The FFA's and inflammatory cytokines produced by visceral obesity discussed earlier directly induce insulin resistance at the insulin receptor (IR) level [34, 54] resulting in compensatory beta cell insulin secretion (hyperinsulinemia) in an attempt to maintain euglycemia. The hyperinsulinemia becomes a self-perpetuating vicious cycle, in turn, as it directly contributes to insulin resistance by down-regulating its own receptor. Insulin resistance can originate anywhere in the insulin-action cascade; from a direct reduction in IR number or affinity, to reduced phosphorylation/activation of the insulin receptor itself, to down-regulation of the intracellular protein-kinase cascade normally triggered by insulin action following interaction with the IR (post-receptor signaling) [55]. Over-stimulation of the IR by hyperinsulinemia itself results in high levels of **STAT3** activation, which then up-regulates suppressors of cytokine signaling-3 (***socs-3***); which in turn, inhibits post-receptor insulin signaling as a negative "feedback" inhibitory mechanism, thereby down-regulating its own receptor system [56]. We have shown that excessive TLR4 signaling and inflammatory cytokine release up-regulates *socs-3* which contributes to insulin resistance [34]. Overall decreased insulin signaling then leads to decreased activation of GLUT4 transporters and decreased insulin-stimulated suppression of hepatic gluconeogenesis and glucose uptake into peripheral target tissues such as adipocytes and skeletal muscle which leads to the development of T2DM. Although IR-mediated pathways associated with carbohydrate and fat metabolism are down-regulated,

other signaling pathways are not suppressed but rather continuously stimulated by insulin resulting in activation of the Ras/Raf/mitogen-activated-protein-kinase (**MAPK**) system and **mTOR** pathways which are known to promote abnormal cell growth and proliferation [57, 58]. Thus, in states of obesity and FFA/TLR4/cytokine-mediated insulin resistance, the principle functions of insulin action via the IR (glucose transport and suppression of gluconeogenesis) are impaired while insulin-stimulated abnormal cell growth and proliferation in target tissues continues [58]. Secondly, hyperinsulinemia induces the synthesis of insulin-like growth factor-1 (**IGF-1**) in liver and the high serum levels of free IGF-1 also results in overstimulation of its own receptor (IGF-1R). Excess IGF-1R signaling also stimulates abnormal cell proliferation through the same downstream signaling networks which are being chronically stimulated by insulin; including the phosphatidylinositol 3-kinase **(PI3-K)-AKT** system [58]. Thus obesity induced insulin resistance results in excess insulin and IGF-1 promotion of abnormal cell growth and proliferation in multiple organ systems. Expression of IGF-1 receptors has also been demonstrated in multiple malignant tumors including pancreatic cancer, and IGF-1 contributes to cell migration and invasion in some human pancreatic carcinomas.

c. Hyperglycemia Induces Pancreatic Cancer Epidermal Growth Factor Expression

As we have previously discussed in this chapter, diabetes is associated with an increased risk of pancreatic cancer by a variety of cytokine and hormone receptor signaling pathways and that large numbers of patients with pancreatic cancer develop diabetes and elevated glucoses. The direct effect of hyperglycemia on oncogenesis, pancreatic cancer growth and spread is of interest as well. Epidemiologic studies have demonstrated that glucose control in patients with pancreatic cancer results in improved survival, suggesting that high glucose levels might directly promote tumor growth and progression [59]. Recent *in vitro* cell culture studies have demonstrated that glucose in a dose-dependent manner promotes different pancreatic cancer cell line growth and perineural invasion through the regulation of expression of glial cell line-derived neurotropic factor (GDNF) and epidermal growth factor (EGF) via increased epidermal growth factor receptor (EGFR) transactivation [60]. These observations support intensive glucose control as a potential target for improving patient survival in pancreatic cancer.

6. Obesity, toll-like receptors, and pancreatic oncogenesis

Toll-Like Receptors (**TLRs**) are pathogen recognition receptors (PRRs) critical for the activation of the innate and adaptive immune responses to foreign pathogens. Functional TLRs are not only expressed in immune cells but also in many non-immune cells [61]. Their activation, signaling, and proinflammatory responses have been shown to be mediators of multiple inflammatory and autoimmune diseases, as well as, contribute to oncogenesis, tumor growth and metastasis. Pathologic signaling of multiple TLRs have been implicated in many cancers including; melanoma, breast, prostate cancer, colorectal, lung, cervical, liver, and pancreatic cancer [62-64]. Obesity and T2DM are associated with an increased risk for many of these same malignancies; especially pancreatic cancer. FFA's are capable of activating TLR4 signaling in

adipocytes which stimulates adipocyte differentiation, high fat diet (HFD)-mediated induction of visceral obesity, TLR4-mediated cytokine signaling, insulin resistance, and glucose intolerance [34, 65]. This in turn stimulates insulin/IGF-1 signaling pathways which also promote tumor growth. Fructose also stimulates abnormal TLR4 signaling [36] and as mentioned earlier, HFCS diets are associated with induction of visceral obesity, T2DM, chronic pancreatitis, and cancer of the pancreas as well. Since both FFA's and fructose are potent ligands for TLR4 and both are present in high concentrations in the diets of developed countries it is logical that they could promote pancreatic oncogenesis via TLR mediated pathways to be described. Finally, as just mentioned hyperglycemia in the form of glucose intolerance and overt T2DM also stimulates abnormal TLR4 signaling [66] as well as EGFR transactivation in pancreatic tissue in a glucose dependent manner thus also serving as a ligand to promote tumor growth and spread.

Chronic inflammation has been shown to be an important risk factor for the onset and progression of multiple cancers, including pancreatic cancer [67-72] [72-75]. Chronic inflammation is thought to induce malignant transformation via activation of oncogenes, induction of immunosuppression, and inhibition of tumor suppressor genes and lymphocytes. Pathologic activation of TLRs play a critical role in the inflammatory response induced by high fat diets and HFCS by inducing the production of multiple pro-inflammatory cytokines and they have been shown to be important for the induction, proliferation, survival, metastasis, and escape from immune surveillance of many of these cancers as well [70, 76]. Some of the most important TLR-induced cytokines implicated in cancer include **TNF-α, IL-1, IL-6, IL-8, IL-10 and IL-23.** Proinflammatory cytokine production then leads to the activation of many tumor promoting transcription factors and anti-apoptotic genes. Nuclear factor kappa beta **(NF-κB**) and **signal** transducer and activator of transcription 3 (**STAT3**) are two of the most well studied oncogenic transcription factors.

7. Pathologic toll-like receptor signaling, pancreatic cancer growth, and resistance to therapy

We have previously described the relationship between obesity and pancreatic cancer risk as well as the direct correlation between increasing BMI and hyperglycemia to lower responses to treatment and over-all worse outcomes in this all too common disease. Obesity-induced TLR activation of NF-κB and STAT3 signaling pathways are major mediators of this process in multiple cancers including pancreatic cancer. NF-κB and STAT3 are activated by a variety of similar stimuli (stressors, cytokines, etc.) and both control expression of proliferation-enhancing, anti-apoptotic, angiogenic, and immune-modulating genes; however they are regulated by entirely different signaling mechanisms. NF-κB's pro-inflammatory cytokine receptors such as; **TNF-α** and **IL-1** [77-80] promote not only tumor transformation, but also proliferation, angiogenesis, invasion, metastasis, and chemo/radio resistance [81-89]. STAT3 activation by TLR-mediated cytokines also activates the **IL-6 family** (IL-6, IL-11, IL-27, etc.), **IL-10 family** (IL-10, IL-22, IL-19, IL-20), and the epidermal growth factor (**EGF**) family (VEGF, IL-21, IL-23, HGF) of growth factors which also stimulate tumor transformation, growth and

resistance to therapy. NF-κB and STAT3 activate anti-apoptotic genes such as Bcl-xL, Bcl-2, and c-IAP2 [90-92] and also interact and mediate crosstalk between tumor cells and inflammatory cells within the tumor microenvironment to promote the development and progression of multiple types of human cancers including but not limited to pancreatic, colon, gastric, skin, head and neck, and liver cancers [44, 90, 93-96]. Finally, Wnt5a a member of the Wnt family has also been implicated in carcinogenesis and inflammation. Non-canonical Wnt5a activates β-catenin-independent pathways important for cell migration and polarity. Wnt5a has been found in tissue samples of pancreatic adenocarcinomas [97] and is highly expressed in advanced pancreatic cancer [98]. Recently, a TLR / IL-6 / STAT3 / Wnt5a signaling loop was described [62, 99].

8. TLRs as a potential therapeutic target

Several recent studies have evaluated the potential therapeutic use of TLR activators and inhibitors in multiple cancer models. The theory for activation of TLR signaling pathways in a tumor environment is that it would possibly induce tumor cell apoptosis or inhibit the production of various factors described in this review that control tumor growth. In addition, it induction of TLR signaling could elicit an antitumor immune response that could lead to tumor cell destruction by the host's immune system. Treatment with TLR agonists have shown to induce an antitumor response by enhancing dendritic cell (DC) vaccination or T cell adoptive therapies. A recent study reported that the use of TLR agonists such as poly(I:C) or CpG combined with adoptive transfer immunotherapy directly to a B16F10 melanoma model inhibited tumor growth [100]. Also, in a mouse breast xenograft model, the antitumor effect of the TLR3 activator was shown to be dependent on the expression of TLR3 expression in tumor cells. This was further validated in humans where treatment with dsRNA improved outcomes in patients harboring TLR3-positive breast tumors [101]. Similarly, CpG treatment via TLR9 activation induced tumor cell death in human neuroblastoma cells, and tumor-targeted delivery of this TLR9 agonist increased survival in a xenograft model of neuroblastoma [102].

In contrast, it has also been shown that TLR agonists can promote cancer cell survival and migration, and tumor progression. For example, TLR agonists have been shown to increase tumor viability and metastasis of human lung cancer (TLR7/8) [103] ; proliferation of human myeloma (TLR3) [104] ; adhesion and metastasis of human colorectal cancer (TLR4) [105] ; and migration of human glioblastoma (TLR4) or human breast cancer (TLR2) [106]. In regards to pancreatic cancer, TLR7 was recently reported not only be highly expressed in mouse and human pancreatic cancers, but ligation of TLR7 led to accelerated tumor progression through the STAT3 growth pathways previously discussed. Thus, there appears to be a double edged sword between reducing or promoting tumor growth using agonists based therapies for different TLRs.

On the other hand, the use of TLR antagonists has shown to be beneficial at inhibiting tumor growth in animal models in which the tumor microenvironment promotes survival and

metastasis via TLR signaling. TLR antagonists might also decrease the level of activation of stromal cells such as tumor-associated macrophages. Macrophages express an array of TLRs and are able to produce several growth factors via TLR signaling [107]. Moreover, abrogation of TLR-4 signaling in tumor-associated macrophages decrease tumor growth *in vivo* [108].

Our group demonstrated that in papillary thyroid carcinoma cells, IL-6, a TLR3 signaling product, activates STAT3, results in overexpression of **Wnt5a** which mediates tumor growth and spread [62]. Further, we demonstrated that phenylmethimazole (C10), a small molecule derivative of methimazole, blocked TLR3 signaling, and subsequent IL-6 production, STAT3 activation, Wnt5a overexpression, and subsequent growth and migration of papillary thyroid carcinoma cells [62]. Toll-like receptors were first implicated in the pathogenesis of pancreatic cancer in 2009. Our laboratory demonstrated that TLR3 and Wnt5a were coordinately constitutively expressed in a human pancreatic cell line (PANC-1), activation of signaling also played a key role in the regulation of pancreatic cancer growth and migration and that C10, inhibited its growth and migration both in vitro and *in vivo* [63]. Another study reported that activation of the TLR4 signaling pathway-increased invasiveness of pancreatic cancer cells while blockade of TLR4 signaling decreased invasive ability [109]. These studies were the first to implicate both TLR3 or TLR4 expression and signaling as playing a role in pancreatic tumor growth and migration and demonstrated that inhibition of TLR signaling pathways were potential therapeutic targets. Gemcitabine is currently the standard of care chemotherapeutic for pancreatic cancer; however, its efficacy is diminished due to toxicity and the chemoresistance of the tumors. Recently, another group combined TLR4/NF-κB antagonist with gemcitabine in an orthotopic model of pancreatic cancer and the combination therapy significantly delayed tumor growth and decreased tumor size compared to gemcitabine alone or the control groups. Thus, TLR antagonists, when combined with other chemotherapeutic agents may prove to be effective adjunctive therapies to suppress the inflammatory cytokine/growth factor microenvironment which contributes to the induction and/or support of tumor growth and progression and reduce the dose/toxicity of established agents.

9. Prevention of obesity associated pancreatic cancer

There is now compelling evidence that obesity, chronic inflammation, and the associated secretion of numerous inflammatory cytokines, hormones and growth factors described herein contribute both directly and indirectly to the increased risk for pancreatic cancer, more aggressive tumor growth, as well as poor response to therapeutic intervention. Thus, in addition to smoking cessation and moderation in alcohol consumption, life-style modification with exercise, maintenance of normal BMI's, consumption of higher amounts of fresh fruits and vegetables, less animal fat and processed foods; especially those fortified with HFCS are obvious recommendations. In addition, there is increasing evidence that other anti-inflammatory agents such as the non-steroidal anti-inflammatory drugs (NSAIDS) [110], the Statin lipid-lowering medications, and T2DM medications such as the thiazolidinediones (TZD's) [111] and metformin [112, 113] have specific protective effects against oncogenesis as well as tumor growth and response to treatment.

10. Conclusion

Obesity contributes to increased risk for multiple solid cancers including pancreatic cancer. For pancreatic cancer in particular, obesity promotes a proinflammatory environment which promotes oncogenesis, tumor growth, metastatic spread as well as resistance to therapy through a variety of molecular pathways. The principle obesity-linked pathways include increases in TNF-α, IL-1, IL-6, IL-8, IL-10 and IL-23 as well as activation of NF-κB and STAT3. The current diets of industrialized nations which contain too much low glycemic-index carbohydrates, saturated fats, and HFCS are major environmental triggers of pathologic TLR3 and TLR4 signaling pathways in adipocytes which then contribute to the development of insulin resistance, ectopic fat deposition in multiple tissues including the pancreas which in turn amplify the growth and signaling pathways described herein which lead to oncogenesis and tumor spread.

Author details

Kelly McCall, Anthony L Schwartz and Frank L Schwartz*

*Address all correspondence to: schwartf@ohio.edu

Ohio University Heritage College of Osteopathic Medicine, Ohio, USA

References

[1] Hariharan D, Saied A, Kocher HM. Analysis of mortality rates for pancreatic cancer across the world. HPB (Oxford). 2008;10(1):58-62. PubMed PMID: 18695761. Pubmed Central PMCID: 2504856. Epub 2008/08/13. eng.

[2] American Cancer Society: Facts and Figures. 2010:4 for incidence & 19 for survival.

[3] Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin. Jul-Aug;61(4):212-36. PubMed PMID: 21685461. Epub 2011/06/21. eng.

[4] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. Mar-Apr;61(2):69-90. PubMed PMID: 21296855. Epub 2011/02/08. eng.

[5] Klein AB. Genetic Susceptibility to Pancreatic Cancer. Molec Carcinog. 2012;51(1): 14-24.

[6] Lowenfels AB, Maisunneuve, P., DiMagno, E.P., Elitser, Y., Gates, L. K., Perralt, J., Whticomb, D. C. Heriditary Pancreatitis and risk for Pancreatic Cancer. J Nat Cancer Institute. 1997 Mar 19, 1997;89(6):443-6.

[7] Trevino JG, Pillai S, Kunigal S, Singh S, Fulp WJ, Centeno BA, et al. Nicotine induces inhibitor of differentiation-1 in a Src-dependent pathway promoting metastasis and chemoresistance in pancreatic adenocarcinoma. Neoplasia. Dec;14(12):1102-14. PubMed PMID: 23308043. Pubmed Central PMCID: 3540937. Epub 2013/01/12. eng.

[8] Alguacil J, Silverman DT. Smokeless and other noncigarette tobacco use and pancreatic cancer: a case-control study based on direct interviews. Cancer Epidemiol Biomarkers Prev. 2004 Jan;13(1):55-8. PubMed PMID: 14744733. Epub 2004/01/28. eng.

[9] Martinez J, Sanchez-Paya J, Palazon JM, Suazo-Barahona J, Robles-Diaz G, Perez-Mateo M. Is obesity a risk factor in acute pancreatitis? A meta-analysis. Pancreatology. 2004;4(1):42-8. PubMed PMID: 14988657. Epub 2004/02/28. eng.

[10] Bracci PM. Obesity and pancreatic cancer: overview of epidemiologic evidence and biologic mechanisms. Mol Carcinog. Jan;51(1):53-63. PubMed PMID: 22162231. Pubmed Central PMCID: 3348117. Epub 2011/12/14. eng.

[11] Gumbs A. Obesity, Pancreatitis, and Pancreatic Cancer. Obes Rev. 2008;18:1183-87.

[12] Everhart J, Wright D. Diabetes mellitus as a risk factor for pancreatic cancer. A meta-analysis. JAMA. 1995 May 24-31;273(20):1605-9. PubMed PMID: 7745774. Epub 1995/05/24. eng.

[13] Huxley R, Ansary-Moghaddam A, Berrington de Gonzalez A, Barzi F, Woodward M. Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies. Br J Cancer. 2005 Jun 6;92(11):2076-83. PubMed PMID: 15886696. Pubmed Central PMCID: 2361795. Epub 2005/05/12. eng.

[14] Wang F, Herrington M, Larsson J, Permert J. The relationship between diabetes and pancreatic cancer. Mol Cancer. 2003 Jan 6;2:4. PubMed PMID: 12556242. Pubmed Central PMCID: 149418. Epub 2003/01/31. eng.

[15] Liu H, Huang D, McArthur DL, Boros LG, Nissen N, Heaney AP. Fructose induces transketolase flux to promote pancreatic cancer growth. Cancer Res. Aug 1;70(15): 6368-76. PubMed PMID: 20647326. Epub 2010/07/22. eng.

[16] Gilbert CA, Slingerland JM. Cytokines, obesity, and cancer: new insights on mechanisms linking obesity to cancer risk and progression. Annu Rev Med.64:45-57. PubMed PMID: 23121183. Epub 2012/11/06. eng.

[17] van Kruijsdijk RC, van der Wall E, Visseren FL. Obesity and cancer: the role of dysfunctional adipose tissue. Cancer Epidemiol Biomarkers Prev. 2009 Oct;18(10): 2569-78. PubMed PMID: 19755644. Epub 2009/09/17. eng.

[18] Li D, Tanaka M, Brunicardi FC, Fisher WE, Gibbs RA, Gingras MC. Association between somatostatin receptor 5 gene polymorphisms and pancreatic cancer risk and

survival. Cancer. Jul 1;117(13):2863-72. PubMed PMID: 21692047. Pubmed Central PMCID: 3135720. Epub 2011/06/22. eng.

[19] Dong X, Tang H, Hess KR, Abbruzzese JL, Li D. Glucose metabolism gene polymorphisms and clinical outcome in pancreatic cancer. Cancer. Feb 1;117(3):480-91. PubMed PMID: 20845477. Epub 2010/09/17. eng.

[20] Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet. 1996 Oct;14(2):141-5. PubMed PMID: 8841182. Epub 1996/10/01. eng.

[21] Pfutzer RH, Whitcomb DC. SPINK1 mutations are associated with multiple phenotypes. Pancreatology. 2001;1(5):457-60. PubMed PMID: 12120224. Epub 2002/07/18. eng.

[22] Maisonneuve P, Marshall BC, Lowenfels AB. Risk of pancreatic cancer in patients with cystic fibrosis. Gut. 2007 Sep;56(9):1327-8. PubMed PMID: 17698876. Pubmed Central PMCID: 1954996. Epub 2007/08/19. eng.

[23] Cohn JA, Mitchell RM, Jowell PS. The impact of cystic fibrosis and PSTI/SPINK1 gene mutations on susceptibility to chronic pancreatitis. Clin Lab Med. 2005 Mar; 25(1):79-100. PubMed PMID: 15749233. Epub 2005/03/08. eng.

[24] Lucenteforte E, La Vecchia C, Silverman D, Petersen GM, Bracci PM, Ji BT, et al. Alcohol consumption and pancreatic cancer: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). Ann Oncol. Feb;23(2):374-82. PubMed PMID: 21536662. Pubmed Central PMCID: 3265544. Epub 2011/05/04. eng.

[25] Ye W, Lagergren J, Weiderpass E, Nyren O, Adami HO, Ekbom A. Alcohol abuse and the risk of pancreatic cancer. Gut. 2002 Aug;51(2):236-9. PubMed PMID: 12117886. Pubmed Central PMCID: 1773298. Epub 2002/07/16. eng.

[26] Lin RK, Hsieh YS, Lin P, Hsu HS, Chen CY, Tang YA, et al. The tobacco-specific carcinogen NNK induces DNA methyltransferase 1 accumulation and tumor suppressor gene hypermethylation in mice and lung cancer patients. J Clin Invest. Feb;120(2): 521-32. PubMed PMID: 20093774. Pubmed Central PMCID: 2810088. Epub 2010/01/23. eng.

[27] Larsson SC, Orsini N, Wolk A. Body mass index and pancreatic cancer risk: A meta-analysis of prospective studies. Int J Cancer. 2007 May 1;120(9):1993-8. PubMed PMID: 17266034. Epub 2007/02/03. eng.

[28] Michaud DS, Giovannucci E, Willett WC, Colditz GA, Stampfer MJ, Fuchs CS. Physical activity, obesity, height, and the risk of pancreatic cancer. JAMA. 2001 Aug 22-29;286(8):921-9. PubMed PMID: 11509056. Epub 2001/08/31. eng.

[29] Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest. 2000 Aug;106(4): 473-81. PubMed PMID: 10953022. Pubmed Central PMCID: 380258. Epub 2000/08/23. eng.

[30] Lau D, Dhillon, B Szmitko, P, Verma, S. Adipokines: molecular links between obesity and atherosclerosis. Am J Phys Heart and Circ Physiology. 2005;288:2031-41.

[31] Newsholm EaL, Anthony. Functional Biochemistry in Health and Disease. New York: John Wiley & Sons; 2009.

[32] Ryysy L, Hakkinen AM, Goto T, Vehkavaara S, Westerbacka J, Halavaara J, et al. Hepatic fat content and insulin action on free fatty acids and glucose metabolism rather than insulin absorption are associated with insulin requirements during insulin therapy in type 2 diabetic patients. Diabetes. 2000 May;49(5):749-58. PubMed PMID: 10905483. Epub 2000/07/25. eng.

[33] Yki-Jarvinen H. Ectopic fat accumulation: an important cause of insulin resistance in humans. J R Soc Med. 2002;95 Suppl 42:39-45. PubMed PMID: 12216326. Pubmed Central PMCID: 1308944. Epub 2002/09/10. eng.

[34] McCall KD, Holliday D, Dickerson E, Wallace B, Schwartz AL, Schwartz C, et al. Phenylmethimazole blocks palmitate-mediated induction of inflammatory cytokine pathways in 3T3L1 adipocytes and RAW 264.7 macrophages. J Endocrinol. Dec; 207(3):343-53. PubMed PMID: 20813836. Epub 2010/09/04. eng.

[35] Ouyang X, Cirillo P, Sautin Y, McCall S, Bruchette JL, Diehl AM, et al. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. J Hepatol. 2008 Jun; 48(6):993-9. PubMed PMID: 18395287. Pubmed Central PMCID: 2423467. Epub 2008/04/09. eng.

[36] Spruss A, Kanuri G, Wagnerberger S, Haub S, Bischoff SC, Bergheim I. Toll-like receptor 4 is involved in the development of fructose-induced hepatic steatosis in mice. Hepatology. 2009 Oct;50(4):1094-104. PubMed PMID: 19637282. Epub 2009/07/29. eng.

[37] Lim JS M-SM, Valente A, Schwarz JM, Lustig RH. The role of fuctose in the pathogenesis of NAFLD and the metabolic syndrome. Nat Rev Gastroenterol Hepatol. 2010 May;7(5):251-64.

[38] Tobey TA, Mondon CE, Zavaroni I, Reaven GM. Mechanism of insulin resistance in fructose-fed rats. Metabolism. 1982 Jun;31(6):608-12. PubMed PMID: 7043185. Epub 1982/06/01. eng.

[39] Michelotti GA, Machado MV, Diehl AM. NAFLD, NASH and liver cancer. Nat Rev Gastroenterol Hepatol. Nov;10(11):656-65. PubMed PMID: 24080776. Epub 2013/10/02. eng.

[40] Metin Basaranoglu GB, Tevfik Sabuncu, Hakan Sentürk. Fructose as a key player in the development of fatty liver disease. World J Gastroenterol. 2013;28(19 (8)): 1166-72.

[41] Wei YW, E. Moran, G. Estrada, A. Pagliassotti, M. Fructose-induced stress signaling in the liver involves methylglyoxal. Nutri Metab. 2013;8(10):38-40.

[42] Li D1 DR, Bondy ML, Sinha R, Nguyen NT, Evans DB, Abbruzzese JL, Hassan MM. Dietary mutagen exposure and risk of pancreatic cancer. Cancer Epidemiol Biomarkers Prev. 2007;16(4):655-61.

[43] Suárez-Bonnet E1 CM, Méndez-Ramírez I, Castillo-Urueta P, Cortés-Eslava J, Gómez-Arroyo S, Melero-Vara JM. Aflatoxin (B1, B2, G1, and G2) contamination in rice of Mexico and Spain, from local sources or imported. J Food Sci. 2013;78(11):1822-9.

[44] Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C, et al. Stat3 as an oncogene. Cell. 1999 Aug 6;98(3):295-303. PubMed PMID: 10458605. Epub 1999/08/24. eng.

[45] Bromber JW, T.. Inflammation and cancer: IL-6 and STAT3 complete the link. Cancer Cell. 2009;15:79-80.

[46] Chen J. Multiple signal pathways in obesity-associated cancer. Obes Rev. 2011;12:1063-70.

[47] Somasundar P, Yu AK, Vona-Davis L, McFadden DW. Differential effects of leptin on cancer in vitro. J Surg Res. 2003 Jul;113(1):50-5. PubMed PMID: 12943810. Epub 2003/08/29. eng.

[48] Krechler T, Zeman M, Vecka M, Macasek J, Jachymova M, Zima T, et al. Leptin and adiponectin in pancreatic cancer: connection with diabetes mellitus. Neoplasma. 58(1):58-64. PubMed PMID: 21067267. Epub 2010/11/12. eng.

[49] Gasiorowska A, Talar-Wojnarowska R, Kaczka A, Borkowska A, Czupryniak L, Malecka-Panas E. Role of adipocytokines and its correlation with endocrine pancreatic function in patients with pancreatic cancer. Pancreatology. Jul-Aug;13(4):409-14. PubMed PMID: 23890140. Epub 2013/07/31. eng.

[50] Bajou K, Maillard C, Jost M, Lijnen RH, Gils A, Declerck P, et al. Host-derived plasminogen activator inhibitor-1 (PAI-1) concentration is critical for in vivo tumoral angiogenesis and growth. Oncogene. 2004 Sep 9;23(41):6986-90. PubMed PMID: 15286708. Epub 2004/08/03. eng.

[51] Smith R, Xue A, Gill A, Scarlett C, Saxby A, Clarkson A, et al. High expression of plasminogen activator inhibitor-2 (PAI-2) is a predictor of improved survival in patients with pancreatic adenocarcinoma. World J Surg. 2007 Mar;31(3):493-502; discussion 3. PubMed PMID: 17219285. Epub 2007/01/16. eng.

[52] Zorgetto VA, Silveira GG, Oliveira-Costa JP, Soave DF, Soares FA, Ribeiro-Silva A. The relationship between lymphatic vascular density and vascular endothelial growth factor A (VEGF-A) expression with clinical-pathological features and survival in pancreatic adenocarcinomas. Diagn Pathol.8(1):170. PubMed PMID: 24138811. Pubmed Central PMCID: 3816792. Epub 2013/10/22. eng.

[53] Georgiadou D, Sergentanis TN, Sakellariou S, Filippakis GM, Zagouri F, Vlachodimitropoulos D, et al. VEGF and Id-1 in pancreatic adenocarcinoma: Prognostic signifi-

cance and impact on angiogenesis. Eur J Surg Oncol. Jan 18. PubMed PMID: 24480377. Epub 2014/02/01. Eng.

[54] Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, White MF. Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. J Biol Chem. 2002 Jan 11;277(2):1531-7. PubMed PMID: 11606564. Epub 2001/10/19. eng.

[55] Tanti JF, Jager J. Cellular mechanisms of insulin resistance: role of stress-regulated serine kinases and insulin receptor substrates (IRS) serine phosphorylation. Curr Opin Pharmacol. 2009 Dec;9(6):753-62. PubMed PMID: 19683471. Epub 2009/08/18. eng.

[56] Kim JH, Yoon MS, Chen J. Signal transducer and activator of transcription 3 (STAT3) mediates amino acid inhibition of insulin signaling through serine 727 phosphorylation. J Biol Chem. 2009 Dec 18;284(51):35425-32. PubMed PMID: 19875458. Pubmed Central PMCID: 2790971. Epub 2009/10/31. eng.

[57] A. A. Samani SY, D. LeRoith, and P. Brodt,. The role of the IGF system in cancer growth and metastasis: overview and recent insights. Endocrine Reviews. 2007;28(1): 20-47.

[58] Sung MK, Yeon JY, Park SY, Park JH, Choi MS. Obesity-induced metabolic stresses in breast and colon cancer. Ann N Y Acad Sci. Jul;1229:61-8. PubMed PMID: 21793840. Epub 2011/07/29. eng.

[59] Han L, Ma Q, Li J, Liu H, Li W, Ma G, et al. High glucose promotes pancreatic cancer cell proliferation via the induction of EGF expression and transactivation of EGFR. PLoS One.6(11):e27074. PubMed PMID: 22087246. Pubmed Central PMCID: 3210779. Epub 2011/11/17. eng.

[60] Liu H, Ma Q, Li J. High glucose promotes cell proliferation and enhances GDNF and RET expression in pancreatic cancer cells. Mol Cell Biochem. Jan;347(1-2):95-101. PubMed PMID: 20960036. Epub 2010/10/21. eng.

[61] Medzhitov R. Toll-like receptors and innate immunity. Nat Rev Immunol. 2001 Nov; 1(2):135-45. PubMed PMID: 11905821. Epub 2002/03/22. eng.

[62] McCall KD, Harii N, Lewis CJ, Malgor R, Kim WB, Saji M, et al. High Basal Levels of Functional Toll-Like Receptor 3 (TLR3) and Non-Cannonical Wnt5a Are Expressed in Papillary Thyroid Cancer (PTC) and Are Coordinately Decreased by Phenylmethimazole Together with Cell Proliferation and Migration. Endocrinology. 2007;148(9): 4226-37.

[63] Schwartz AL, Malgor R, Dickerson E, Weeraratna AT, Slominski A, Wortsman J, et al. Phenylmethimazole decreases Toll-like receptor 3 and noncanonical Wnt5a expression in pancreatic cancer and melanoma together with tumor cell growth and

migration. Clin Cancer Res. 2009 Jun 15;15(12):4114-22. PubMed PMID: 19470740. Pubmed Central PMCID: 2765042. Epub 2009/05/28. eng.

[64] Sato Y, Goto Y, Narita N, Hoon DS. Cancer Cells Expressing Toll-like Receptors and the Tumor Microenvironment. Cancer Microenviron. 2009 Sep;2 Suppl 1:205-14. PubMed PMID: 19685283. Pubmed Central PMCID: 2756339. Epub 2009/08/18. eng.

[65] Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest. 2006 Nov;116(11):3015-25. PubMed PMID: 17053832. Pubmed Central PMCID: 1616196. Epub 2006/10/21. eng.

[66] Dasu MR, Jialal I. Free fatty acids in the presence of high glucose amplify monocyte inflammation via Toll-like receptors. Am J Physiol Endocrinol Metab. Jan; 300(1):E145-54. PubMed PMID: 20959532. Pubmed Central PMCID: 3023203. Epub 2010/10/21. eng.

[67] Berstein LM. Clinical usage of hypolipidemic and antidiabetic drugs in the prevention and treatment of cancer. Cancer Lett. 2005 Jun 28;224(2):203-12. PubMed PMID: 15914271.

[68] Otake S, Takeda H, Suzuki Y, Fukui T, Watanabe S, Ishihama K, et al. Association of visceral fat accumulation and plasma adiponectin with colorectal adenoma: evidence for participation of insulin resistance. Clin Cancer Res. 2005 May 15;11(10):3642-6. PubMed PMID: 15897559.

[69] von Hafe P, Pina F, Perez A, Tavares M, Barros H. Visceral fat accumulation as a risk factor for prostate cancer. Obes Res. 2004 Dec;12(12):1930-5. PubMed PMID: 15687393.

[70] Lu H, Ouyang W, Huang C. Inflammation, a key event in cancer development. Mol Cancer Res. 2006 Apr;4(4):221-33. PubMed PMID: 16603636.

[71] Garcia M, Fernandez-Garcia NI, Rivas V, Carretero M, Escamez MJ, Gonzalez-Martin A, et al. Inhibition of xenografted human melanoma growth and prevention of metastasis development by dual antiangiogenic/antitumor activities of pigment epithelium-derived factor. Cancer Res. 2004 Aug 15;64(16):5632-42. PubMed PMID: 15313901.

[72] Kuper H, Adami HO, Trichopoulos D. Infections as a major preventable cause of human cancer. J Intern Med. 2000 Sep;248(3):171-83. PubMed PMID: 10971784. Epub 2000/09/06. eng.

[73] Khatami M. Unresolved inflammation: 'immune tsunami' or erosion of integrity in immune-privileged and immune-responsive tissues and acute and chronic inflammatory diseases or cancer. Expert Opin Biol Ther. 2011 Jun 11. PubMed PMID: 21663532. Epub 2011/06/15. Eng.

[74] Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer. 2004 Aug;4(8):579-91. PubMed PMID: 15286738. Epub 2004/08/03. eng.

[75] Khatami M. Unresolved inflammation: 'immune tsunami' or erosion of integrity in immune-privileged and immune-responsive tissues and acute and chronic inflammatory diseases or cancer. Expert Opin Biol Ther. Jun 11. PubMed PMID: 21663532. Epub 2011/06/15. Eng.

[76] Garcea G, Dennison AR, Steward WP, Berry DP. Role of inflammation in pancreatic carcinogenesis and the implications for future therapy. Pancreatology. 2005;5(6): 514-29. PubMed PMID: 16110250.

[77] Bezbradica JS, Medzhitov R. Integration of cytokine and heterologous receptor signaling pathways. Nat Immunol. 2009 Apr;10(4):333-9. PubMed PMID: 19295629. Epub 2009/03/20. eng.

[78] Dinarello CA. The interleukin-1 family: 10 years of discovery. Faseb J. 1994 Dec;8(15): 1314-25. PubMed PMID: 8001745. Epub 1994/12/01. eng.

[79] Karin M, Gallagher E. TNFR signaling: ubiquitin-conjugated TRAFfic signals control stop-and-go for MAPK signaling complexes. Immunol Rev. 2009 Mar;228(1):225-40. PubMed PMID: 19290931. Epub 2009/03/18. eng.

[80] Kruglov AA, Kuchmiy A, Grivennikov SI, Tumanov AV, Kuprash DV, Nedospasov SA. Physiological functions of tumor necrosis factor and the consequences of its pathologic overexpression or blockade: mouse models. Cytokine Growth Factor Rev. 2008 Jun-Aug;19(3-4):231-44. PubMed PMID: 18502680. Epub 2008/05/27. eng.

[81] Chaturvedi MM, Sung B, Yadav VR, Kannappan R, Aggarwal BB. NF-kappaB addiction and its role in cancer: 'one size does not fit all'. Oncogene. 2011 Apr 7;30(14): 1615-30. PubMed PMID: 21170083. Epub 2010/12/21. eng.

[82] Beg AA, Baltimore D. An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. Science. 1996 Nov 1;274(5288):782-4. PubMed PMID: 8864118. Epub 1996/11/01. eng.

[83] Liu ZG, Hsu H, Goeddel DV, Karin M. Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kappaB activation prevents cell death. Cell. 1996 Nov 1;87(3):565-76. PubMed PMID: 8898208. Epub 1996/11/01. eng.

[84] Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM. Suppression of TNF-alpha-induced apoptosis by NF-kappaB. Science. 1996 Nov 1;274(5288):787-9. PubMed PMID: 8864120. Epub 1996/11/01. eng.

[85] Wang CY, Mayo MW, Baldwin AS, Jr. TNF-and cancer therapy-induced apoptosis: potentiation by inhibition of NF-kappaB. Science. 1996 Nov 1;274(5288):784-7. PubMed PMID: 8864119. Epub 1996/11/01. eng.

[86] Joyce D, Albanese C, Steer J, Fu M, Bouzahzah B, Pestell RG. NF-kappaB and cell-cycle regulation: the cyclin connection. Cytokine Growth Factor Rev. 2001 Mar;12(1): 73-90. PubMed PMID: 11312120. Epub 2001/04/20. eng.

[87] Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008 Jul 24;454(7203):436-44. PubMed PMID: 18650914. Epub 2008/07/25. eng.

[88] Huang TT, Miyamoto S. Postrepression activation of NF-kappaB requires the amino-terminal nuclear export signal specific to IkappaBalpha. Mol Cell Biol. 2001 Jul; 21(14):4737-47. PubMed PMID: 11416149. Pubmed Central PMCID: 87155. Epub 2001/06/21. eng.

[89] Luo JL, Maeda S, Hsu LC, Yagita H, Karin M. Inhibition of NF-kappaB in cancer cells converts inflammation-induced tumor growth mediated by TNFalpha to TRAIL-mediated tumor regression. Cancer Cell. 2004 Sep;6(3):297-305. PubMed PMID: 15380520. Epub 2004/09/24. eng.

[90] Yu H, Jove R. The STATs of cancer--new molecular targets come of age. Nat Rev Cancer. 2004 Feb;4(2):97-105. PubMed PMID: 14964307. Epub 2004/02/18. eng.

[91] Chang L, Kamata H, Solinas G, Luo JL, Maeda S, Venuprasad K, et al. The E3 ubiquitin ligase itch couples JNK activation to TNFalpha-induced cell death by inducing c-FLIP(L) turnover. Cell. 2006 Feb 10;124(3):601-13. PubMed PMID: 16469705. Epub 2006/02/14. eng.

[92] Rebouissou S, Amessou M, Couchy G, Poussin K, Imbeaud S, Pilati C, et al. Frequent in-frame somatic deletions activate gp130 in inflammatory hepatocellular tumours. Nature. 2009 Jan 8;457(7226):200-4. PubMed PMID: 19020503. Pubmed Central PMCID: 2695248. Epub 2008/11/21. eng.

[93] Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, et al. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell. 2004 Aug 6;118(3):285-96. PubMed PMID: 15294155. Epub 2004/08/06. eng.

[94] Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer. 2009 Nov;9(11):798-809. PubMed PMID: 19851315. Epub 2009/10/24. eng.

[95] Lin L, Hutzen B, Zuo M, Ball S, Deangelis S, Foust E, et al. Novel STAT3 phosphorylation inhibitors exhibit potent growth-suppressive activity in pancreatic and breast cancer cells. Cancer Res. Mar 15;70(6):2445-54. PubMed PMID: 20215512. Pubmed Central PMCID: 2843552. Epub 2010/03/11. eng.

[96] Grivennikov SI, Karin M. Dangerous liaisons: STAT3 and NF-kappaB collaboration and crosstalk in cancer. Cytokine Growth Factor Rev. Feb;21(1):11-9. PubMed PMID: 20018552. Pubmed Central PMCID: 2834864. Epub 2009/12/19. eng.

[97] Crnogorac-Jurcevic T, Efthimiou E, Capelli P, Blaveri E, Baron A, Terris B, et al. Gene expression profiles of pancreatic cancer and stromal desmoplasia. Oncogene. 2001 Nov 1;20(50):7437-46. PubMed PMID: 11704875.

[98] Ripka S, Konig A, Buchholz M, Wagner M, Sipos B, Kloppel G, et al. WNT5A--target of CUTL1 and potent modulator of tumor cell migration and invasion in pancreatic cancer. Carcinogenesis. 2007 Jun;28(6):1178-87. PubMed PMID: 17227781.

[99] Katoh M. STAT3-induced WNT5A signaling loop in embryonic stem cells, adult normal tissues, chronic persistent inflammation, rheumatoid arthritis and cancer (Review). Int J Mol Med. 2007 Feb;19(2):273-8. PubMed PMID: 17203201. Epub 2007/01/05. eng.

[100] Amos SM, Pegram HJ, Westwood JA, John LB, Devaud C, Clarke CJ, et al. Adoptive immunotherapy combined with intratumoral TLR agonist delivery eradicates established melanoma in mice. Cancer Immunol Immunother. 2011 May;60(5):671-83. PubMed PMID: 21327636. Epub 2011/02/18. eng.

[101] Salaun B, Zitvogel L, Asselin-Paturel C, Morel Y, Chemin K, Dubois C, et al. TLR3 as a biomarker for the therapeutic efficacy of double-stranded RNA in breast cancer. Cancer Res. 2011 Mar 1;71(5):1607-14. PubMed PMID: 21343393. Epub 2011/02/24. eng.

[102] Brignole C, Marimpietri D, Di Paolo D, Perri P, Morandi F, Pastorino F, et al. Therapeutic targeting of TLR9 inhibits cell growth and induces apoptosis in neuroblastoma. Cancer Res. 2010 Dec 1;70(23):9816-26. PubMed PMID: 20935225. Epub 2010/10/12. eng.

[103] Cherfils-Vicini J, Platonova S, Gillard M, Laurans L, Validire P, Caliandro R, et al. Triggering of TLR7 and TLR8 expressed by human lung cancer cells induces cell survival and chemoresistance. J Clin Invest. 2010 Apr 1;120(4):1285-97. PubMed PMID: 20237413. Pubmed Central PMCID: 2846035. Epub 2010/03/20. eng.

[104] Chiron D, Pellat-Deceunynck C, Amiot M, Bataille R, Jego G. TLR3 ligand induces NF-{kappa}B activation and various fates of multiple myeloma cells depending on IFN-{alpha} production. J Immunol. 2009 Apr 1;182(7):4471-8. PubMed PMID: 19299748. Epub 2009/03/21. eng.

[105] Hsu RY, Chan CH, Spicer JD, Rousseau MC, Giannias B, Rousseau S, et al. LPS-induced TLR4 signaling in human colorectal cancer cells increases beta1 integrin-mediated cell adhesion and liver metastasis. Cancer Res. 2011 Mar 1;71(5):1989-98. PubMed PMID: 21363926. Epub 2011/03/03. eng.

[106] Thuringer D, Hammann A, Benikhlef N, Fourmaux E, Bouchot A, Wettstein G, et al. Transactivation of the epidermal growth factor receptor by heat shock protein 90 via Toll-like receptor 4 contributes to the migration of glioblastoma cells. J Biol Chem. 2010 Feb 4;286(5):3418-28. PubMed PMID: 21127066. Pubmed Central PMCID: 3030348. Epub 2010/12/04. eng.

[107] Pinhal-Enfield G, Ramanathan M, Hasko G, Vogel SN, Salzman AL, Boons GJ, et al. An angiogenic switch in macrophages involving synergy between Toll-like receptors 2, 4, 7, and 9 and adenosine A(2A) receptors. Am J Pathol. 2003 Aug;163(2):711-21. PubMed PMID: 12875990. Pubmed Central PMCID: 1868201. Epub 2003/07/24. eng.

[108] Lee CH, Wu CL, Shiau AL. Toll-like receptor 4 signaling promotes tumor growth. J Immunother. 2009 Jan;33(1):73-82. PubMed PMID: 19952954. Epub 2009/12/03. eng.

[109] Ikebe M, Kitaura Y, Nakamura M, Tanaka H, Yamasaki A, Nagai S, et al. Lipopolysaccharide (LPS) increases the invasive ability of pancreatic cancer cells through the TLR4/MyD88 signaling pathway. J Surg Oncol. 2009 Dec 15;100(8):725-31. PubMed PMID: 19722233. Epub 2009/09/02. eng.

[110] Yanga HHK, J. Ikhlas, A. Khana,C. Walker, L. Shabana, I. Khana, C. Nonsteroidal anti-inflammatory drug activated gene-1 (NAG-1)modulators from natural products as anti-cancer agent. Life Sci. 2014:1-10.

[111] Galli A CE, Crabb DW, Mello T, Salzano R, Grappone C, Milani S, Surrenti E, Surrenti C, Casini A. Antidiabetic thiazolidinediones inhibit invasiveness of pancreatic cancer cells via PPARgamma independent mechanisms. Gut. 2004;53(11):1688-97.

[112] Bao B1 AA, Azmi AS, Ali S, Sarkar FH. Overview of cancer stem cells (CSCs) and mechanisms of their regulation: implications for cancer therapy. Curr Protoc Pharmacol. 2013 (June).

[113] Lonardo E CM, Sancho P, Sanchez-Ripoll Y, Trabulo SM, Dorado J, Balic A, Hidalgo M, Heeschen C. Metformin targets the metabolic achilles heel of human pancreatic cancer stem cells. PloS One. 2013;18(8).

Section 2

Novel Approaches to Earlier Detection

Chapter 5

Endoscopic Ultrasound in Pancreatic Cancer: The New Perspective

Andrada Seicean

Additional information is available at the end of the chapter

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1. Introduction

Pancreatic cancer is one of the most deadly forms of cancer worldwide, with median survival of less than 6 months and a 5-year survival rate of 35%. Endoscopic ultrasound (EUS) was first introduced for assessment of pancreatic pathology more than 30 years ago, as transabdominal imaging yields limited information. EUS has a role in the detection, staging and sampling of pancreatic tumor. Curative-intent surgery, chemotherapy, and radiation therapy of pancreatic cancer are all performed more frequently in patients with EUS evaluation [1]. Palliative EUS-guided treatments are also possible. However, a recent large observational study reported no influence on survival [2].

2. Detection

The detection rate for pancreatic tumors by EUS is 90-100%, with good detection for tumors less than 2 cm in diameter, but EUS does not definitively rule out the presence of malignancy. In certain situations EUS may give false-negative results, especially when there is concomitant chronic pancreatitis, if the examination is performed too soon after an acute episode of acute pancreatitis, or in the presence of diffusely infiltrating carcinoma or a prominent ventral/dorsal split [3]. For patients with false-negative endoscopic ultrasound fine-needle aspiration (EUS-FNA), the risk for malignancy is higher when vascular involvement or lymph nodes are seen, with a mean of 66 days until diagnosis [4].

EUS vs CT

Two studies showed that the detection of small pancreatic tumors (diameter less than 3 cm) by EUS is better than by CT or MRI (accuracy 93% vs 53% vs 67%) [5] or than by CT or US

(accuracy 100% vs 94% vs 65%) [6]. The size of tumors less than 3 cm in diameter is assessed better by EUS than the size of larger tumors (90% vs 30%) [7].

When a mass is not visible on CT, with enlargement of the pancreatic head or dilatation of the pancreatic duct, but without obstructive jaundice, EUS can reliably identify a pancreatic mass in 7-9% of cases [8-11]. If combined bilio-pancreatic dilation is present with obstructive jaundice, the prevalence of pancreatic malignancy is 85% [12]. The risk of positive findings on EUS is higher in patients with weight loss, hyperbilirubinemia, or dilation of the common bile duct [13]. If there is no dilation of the pancreatic duct in a suspected pancreatic mass, the prevalence of malignancy is 17% [14].

EUS vs MRI. Studies carried out before 2000 showed a clear superiority of EUS over MRI in tumor detection [15]. Even after advances in MRI technology, and despite excellent sensitivity of MRI (87-91%), EUS remained superior to MRI [16], albeit non-significantly so in one study [17].

EUS vs PET.EUS is more sensitive than PET in the detection of pancreatic cancer (93% vs 87%) [18]. Another study found similar sensitivities for EUS, CT, and US, with a negative predictive value of 82% on EUS [19]. Due to the high costs, however, EUS is not routinely used for detection.

EUS vs IDUS.Intraductal endoscopic ultrasound (IDUS) identifies the wall of the pancreatic duct as a hyperechoic layer and the surrounding neoplastic tissue as a hypoechoic area. IDUS yielded impressive sensitivity (100%) and specificity (91.7%) for differentiation between pancreatic cancer and chronic pancreatitis in patients with localized stenosis of the main pancreatic duct. The same study compared IDUS with EUS, CT, and ERCP, which had sensitivity of 92.9%, 64.3%, and 85.7% and specificity of 58.3%, 66.7%, and 66.7%, respectively. Another study compared IDUS, EUS, CT, and ERCP, and found higher sensitivities (75% vs 50%, 37%, and 37%, respectively) but lower specificities (67%, 67%, 33%, and 67%) [20]. However, a recent study revealed no difference between EUS and IDUS in pancreatic tumor detection, with sensitivity of 81-89% and specificity of 74-88% [19].

3. Staging

Pancreatic cancer typically has the EUS appearance of a heterogeneous hypoechoic mass with irregular margins, but based on this aspect only 55% are correctly diagnosed [21]. Lymph nodes appear as hypoechoic structures, round and well delineated, usually over 1 cm in diameter. They are found in the peri-aortic space, in peripancreatic locations, in the liver hilum, in the celiac region, or in the mediastinum (in around 10% of the cases). A positive periductal hypoechoic sign, defined as patchy hypoechoic areas adjacent to a dilated pancreatic duct, was predictive for malignancy with accuracy of 80% [22].

The first studies used the 1987 TNM staging, which considered stage T3 as the involvement of adjacent vessels (both arteries and veins) and of neighboring organs, and found T staging accuracy of 73-94% [23-26]. Later studies used the 1997 TNM classification, which defined

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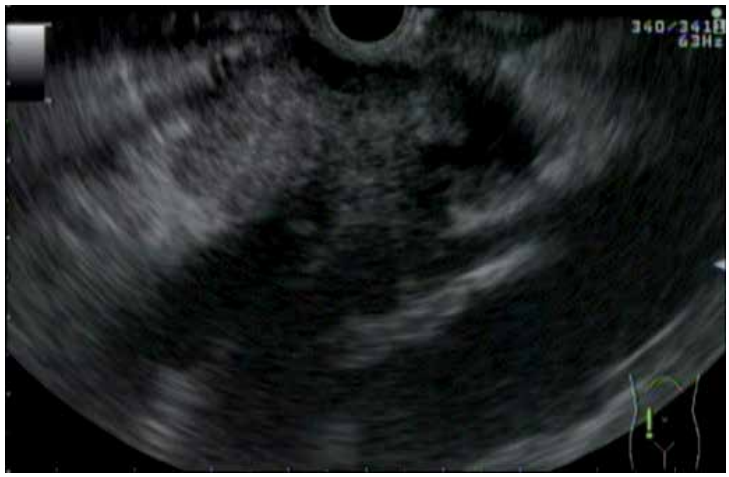

Figure 1. T4 tumor of the pancreatic body. The hypoechoic lesion with invasion of the celiac trunk and gastric wall.

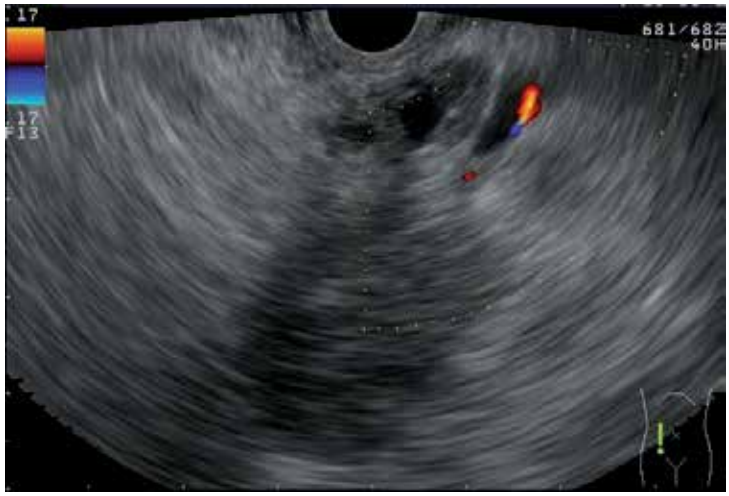

Figure 2. T3 tumor of the pancreatic body. The hypoechoic lesion invades splenic vein and produces the dilation of the Wirsung duct.

invasion of the portal vein, celiac trunk, and mesenteric vessels as stage T4. The results for T stage accuracy were poorer: 61- 74% for stage T3 and 78-88% for stage T4 [7,16,27,28,29]. Currently the 2002 TNM classification is being used. This includes invasion of superior mesenteric artery or celiac artery as stage T4, representing a criterion for irresectability. Using this latest classification, accuracy rises to 85% for T stage and 72% for N stage [30-31](Table 1).

Vascular invasion is the main factor in resectability. Typical findings are the loss of the sonographic interface between the echogenic vessel and the parenchyma, a tumor within the vessel lumen, or the presence of collateral circulation. However, the overall sensitivity when

Primary tumor(T)	
T0	No evidence of primary tumor
T1	Tumor limited to the pancreas, ≤ 2 cm in greatest dimension
T2	Tumor limited to the pancreas, > 2 cm in greatest dimension
T3	Tumor extends beyond the pancreas but without involvement of the celiac axis or the superior mesenteric artery
T4	Tumor involves the celiac axis or the superior mesenteric artery (unresectable primary tumor)
Regional lymph node(N)	
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
Distant metastasis(M)	
M0	No distant metastasis
M1	Distant metastasis

Table 1. TNM Classification for Pancreatic Cancer

using this criterion is modest (43%), with specificity of 91%. In a study published at the turn of the century, the positive and negative predictive values for the parameters chosen to diagnose portal venous involvement were as follows: 42% and 33% for irregular tumor-vessel relationship, 36% and 34% for visualization of tumor in the vascular lumen, 80% and 28% for complete vascular obstruction, and 88% and 18% for collateral vessels [32].

Initial comparative studies of EUS versus surgery indicated that portal vein invasion, but not encasement of the superior mesenteric artery, was reliably assessed by EUS [32-34]. A meta-analysis on pancreatic and peri-ampullary malignancies published in 2007 concluded that EUS diagnoses vascular invasion with sensitivity of 73% and specificity of 90% [35]. Recent data based on images obtained with newer digital echoendoscope, indicate good results for superior mesenteric vessel invasion or hepatic artery invasion [36]. Globally, the accuracy of vascular invasion is 83-93% [36,37].

EUS vs CT. In an early study that compared conventional CT with mechanical EUS and surgical exploration, the results were in favor of EUS, with a global accuracy of 85-98% vs 30-86% for T staging and of 72-84% vs 52-68% for N staging [5,18,24,37,38,40]. In a series of 53 surgical patients, EUS had better accuracy than multidetector CT (67% vs 46%) for T stage and similar results for N stage (44% vs 47%) [29]. A systematic review of 11 prospective studies concluded the superiority of EUS for detection [31] and this was confirmed in recent studies [30]. Newer data show better assessment of arteries, including the superior mesenteric artery, and better assessment of resectability by digital linear EUS than by CT [36,40]. Furthermore, EUS has a significant threefold advantage over CT with regard to T stage and an even higher significant advantage with regard to N stage [40].

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Vascular invasion was predicted better by EUS assessment than by conventional CT evaluation (93-100% vs 45-62%) [37,38]. EUS evaluation of portal vein invasion had results superior to those of US, CT, or angiography (93% vs 67%, 74%, and 79% respectively) [41]. Also, assessment of the portal vein and of the superior mesenteric vein invasion by EUS was better than by CT [18]. However, another study showed that radial EUS predicted resectability in only 46% of cases and that T and N staging accuracy were 69% and 54% [27]. Moreover, other studies found better [15,28] or similar [42] resectability accuracies for CT. The current recommendation is to use EUS for situations where invasion is doubtful as assessed by CT. One study recommended both EUS and CT evaluation for arterial invasion [30], but this would represent a huge volume of investigations and high costs.

EUS vs MRI. The accuracy of MRI for T and N staging is 89% and 76% respectively. Arterial involvement seemed to be best evaluated by MRI in one study on 59 patients [16], but further studies are needed before MRI can be performed routinely in patients with pancreatic cancer.

EUS vs PET. Understaging using EUS and PET was comparable (25% vs 27%) in a small study of 48 surgically explored patients [43], but routine PET examination is not indicated.

EUS vs US. Although hypoechoic masses can be seen during US examination, together with dilation of the pancreatic duct or common bile duct, the accuracy of US in pancreatic cancer diagnosis is modest (sensitivity 67%, specificity 40%) [32]. US and MRI are not accurate enough for the prediction of staging and resectability; CT should be used for this purpose [44].

4. Endoscopic ultrasound fine-needle aspiration

EUS-FNA is indicated for obtaining specimens for cytology and histopathology with regard to palliative radiochemotherapy and for differential from other nodular pancreatic lesions such as chronic pancreatitis nodules, autoimmune pancreatitis, pancreatic metastasis, or neuroendocrine tumors.

The accuracy of diagnosis by FNA is 85-95% and depends on several factors: the type of needle, the number of passes, the presence of cytopathologist in the room, the technical quality of processing, and the experience of the pathologist.

Type of needle. The main advantage of EUS-FNA is the use of thin needles -- 19G, 22G, and 25 G -- to yield cytological smears or core specimens. The Tru-Cut needle and histological needles have the advantage of obtaining tissue samples which maintain the architecture of the pancreas, thus facilitating interpretation by the pathologist, especially for non-adenocarcinoma tumor types or inflammatory masses [45,46]. Cytological smears are associated with description of atypia in 1-14% of cases, similar to reports for thyroid cytology; however, the risk of malignancy in pancreatic smears is higher (25-100%) [47]. The combination of smears and core specimens revealed the diagnosis in 90-100% of cases [45,46,48] and the recommendation of the European Society of Gastrointestinal Endoscopy (ESGE) is to try to obtain material for histology routinely [49]. The overall pancreatic tissue-sampling rate for cytology using 22G needles is variable compared with histology

(82-93% vs 84-87%), while the overall diagnostic accuracy of histology on each pass is only 60% for the 25G needle and 75% for the 22G needle [50].

The accuracy of diagnosis for pancreatic masses using 22G needles is up to 95% [51]. A meta-analysis compared the 22G and 25G needles for pancreatic and peripancreatic masses showed non-significant differences in sensitivity (78% vs 91%), and 100% specificity, with no difference in the number of passes or complications [52]. Repeating EUS-FNA in the case of initial negative cytology increases the diagnostic yield [53-55].

Because the 19G aspiration needles are more rigid [56,57], they are not routinely recommended for head pancreatic biopsies [49]. However, the diagnostic accuracy for body/tail pancreatic lesions is better with 19G needles than with 22G needles [57,58], especially for the differential diagnosis of pancreatic masses.

Tru-Cut biopsy using 19G EUS-TCB needles is recommended when EUS-FNA is nondiagnostic owing to insufficient biopsy material, but cannot be used so readily in the antrum, fundus, and duodenal bulb, where the echoendoscope is angulated [59]). The tendency is to replace the 19G EUS-TCB needle with the flexible 19G needle (Flex 19, Boston Scientific, Natick, MA) or the 19G or 22G histological needle (ProCore, Wilson-Cook, Ireland). A comparison of 22G needles and histological 22G needles reported better diagnostic accuracy for 22G needles [60]. Likewise, a 25G needle showed high sensitivity of 96% when three passes were done [61].

The yield for malignancy is similar with or without use of a stylet (87% vs 83%) [62-64], but in some studies sample adequacy was significantly better when a stylet was used (75% vs 87%) [62]. Also. The amount of blood in the sample was greater when the stylet was used (75% vs 52%) [62-64]. Although no conclusion has yet been drawn, the ESGE recommendations leave it to the discretion of the endosonographer whetherto use a stylet or not [49].

The current recommendation of the ESGE is to use suction for solid masses [49]. Moreover, a prospective comparative trial showed better diagnostic accuracy when suction was applied (85% vs 75%), but more blood was present in the case of sampling with suction [65].

Most studies have used a standard back and forth technique for sampling. In a randomized trial comparing the fanning and standard techniques, the diagnostic accuracy was non-significantly different, although better in the fanning technique (76% vs 96%), with a lower number of passes to establish the diagnosis and better sensitivity after the first pass [66].

Number of passes. The current recommendation for EUS-FNA of solid pancreatic masses is at least five passes with a 22G needle [49]. In a retrospective study, a mean of two passes with combined histology and cytology provided adequate tissue for pancreatic mass diagnosis [45]. When Tru-Cut biopsy is done, more than two passes are usually necessary to improve diagnostic accuracy [67].

Presence of a cytopathologist. It is not clear whether the presence of a cytopathologist improves the diagnostic accuracy over 90%. The cytopathologic on-site rapid assessment of smear slides is reported to be better than that of monolayer prepared slides [68]. The first large prospective study (540 patients) which included cytopathologic assessment found that the agreement

between cytopathology and final diagnosis was very good, but the presence of the pathologist did not significantly increase the accuracy of the diagnosis [69]. Thus, the presence of a cytopathologist does not always guarantee better results.

Features of lesion. The presence of features of chronic pancreatitis was associated with lower accuracy of EUS-FNA for the differential diagnosis of pancreatic masses (73% vs 91%) and may necessitate a higher number of passes to establish the diagnosis [70]. The presence of stents (either plastic or metallic) usually does not impede EUS-FNA [71-73], although the stent has to be placed at least one day before performing EUS-FNA [72]. There is no difference in diagnostic accuracy between lesions less or more than 3 cm in diameter [74], although one study found sensitivity as low as 40% for tumors less than 1 cm in diameter [75].

5. Differential diagnosis of pancreatic masses

A recent meta-analysis found that the sensitivity and specificity of EUS-FNA in differential diagnosis are 86% and 95%, respectively [76]. New imaging methods, such as elastography and contrast-enhanced EUS (CEUS), are considered additive to EUS-FNA in the differential diagnosis of pancreatic masses. Molecular analysis of the specimen obtained by FNA can also help in discrimination of pancreatic masses. Needle-based confocal laser endomicroscopy to provide real-time imaging at microscopic level for pancreatic cancer is still also under evaluation.

Elastography

This method assesses the elasticity of tissue during the ultrasound examination. The blue aspect of pancreatic adenocarcinoma is on elastography due to hard desmoplastic tissue, while the soft normal tissue is red [77]. Based on the elastography pattern, the sensitivity and specificity for differentiation of benign and malignant pancreatic lesions were 92.3% and 80.0% respectively, compared to 92.3% and 68.9% for the conventional B-mode images [78], and the overall accuracy for diagnosis of malignancy was 94% [79]. The hue histogram analysis of elastographic images differentiated malignant from benign nodules (cut-off point: 175) with sensitivity, specificity, and accuracy of 91.4%, 87.9%, and 89.7% respectively [80,81]. Using a second-generation US machine for elastography, the strain ratio can be calculated, comparing the strain value of the mass to a strain value from a control area in the region under study. A strain ratio of 4.65 and elasticity of 0.27% were the cut-off points for differentiation of pancreatic cancer from inflammatory masses [82]. Higher strain ratios were diagnostic for malignancy with an accuracy of 98% [83,84]. Three recent meta-analyses found sensitivity of 95-99%, specificity of 69-76%, and accuracy of 89-96% [85-87]. The combination of power Doppler CEUS and elastography yielded global accuracy of 83%, with better specificity than elastography alone [88].

Contrast-enhanced EUS

The principle of the CEUS technique is based on visualization of microvessels inside the pancreatic tumor; their presence was found useful for predicting efficacy of chemotherapy [89].

The initial indication was achievement of better delineation of pancreatic nodules or better visualization of vascular involvement. However, these aspects seem not to be improved and many studies of CEUS have focused on differential diagnosis of pancreatic masses. The contrast agents are microbubbles of gas included in a hydrophilic shell. The initial studies used Levovist, which is rapidly destroyed in pulmonary capillaries. Second-generation contrast agents, such as Sonovue, Sonazoid, or Definity, have a better lifetime in the vascular flow and are able to pass the pulmonary capillaries. Hypoenhancement on CEUS is considered suggestive of adenocarcinoma, due to the presence of a high proportion of desmoplastic tissue within the tumors, with few microvessels. Using a high mechanical index and Doppler CEUS, the hypovascular aspect was suggestive of adenocarcinoma in 83-94% of patients [88,90-94]. Motion artifacts and blooming effect are frequent, however, and this method has been replaced by harmonic CEUS. This latter procedure uses frequencies resulting from non-linear oscillation of microbubbles, and the low mechanical index of the ultrasound machine allows subtraction of the tissue-derived signal from the microvessel of the tumor [95]. The qualitative interpretation of the contrast image as hypoenhanced was diagnostic for adenocarcinoma in 80-95 % of patients, presenting the prospect of successful diagnosis in the case of false-negative EUS-FNA [96-98](Figure3). Also, CEUS seemed superior to CT scan in detecting lesions under 2 cm in diameter [98].

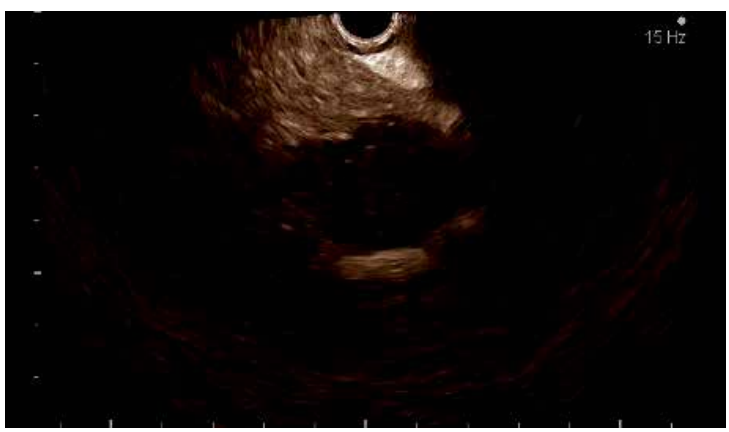

Figure 3. A hypoenhanced lesion of the head of the pancreas during the arterial phase of constrast uptake suggestive for pancreatic adenocarcinoma.

In total, a meta-analysis of both power Doppler and harmonic CEUS showed that hypoenhancement was associated with pooled sensitivity of 94% and specificity of 89% [99]. Qualitative interpretation can be subjective, however, and quantification of contrast uptake is expected to yield new information with improved accuracy. We used a hue histogram analysis and noted that a hypoenhanced aspect can occur even in severe chronic pancreatitis, but the level of contrast enhancement compared with surrounding tissue is much lower in adenocarcinoma than in chronic pancreatitis [100]. Using specialized software to interpret contrast data, our results were confirmed in another study where time to peak (TTP) was associated with

sensitivity of 93% and specificity of 89% [101]. Using Sonazoid in 91 patients, the CEUS accuracy for detection of pancreatic cancer increased from 84% to 94% with quantitative analysis of TTP [102]. Compared with autoimmune pancreatitis, maximum intensity gain rather than TTP was confirmed as significant for pancreatic cancer contrast uptake [103]. In a comparative study of different methods in 58 patients, specificity and sensitivity were 73.7% and 61.5% for B-mode endosonography; 94.7% and 33.4% for elastography; 84.2% and 76.9% for harmonic CEUS; and 89.5% and 92.3% for power Doppler CEUS. These latter results need further evaluation due to artifacts in the power Doppler CEUS procedure [104].

Linear 3D endoscopic ultrasound, considered as a potential means of improving visualization of vessel involvement, allows the reconstruction of tumor volume, but further technical improvement of ultrasound equipment is necessary to establish the practical importance of this technique [105,106].

Digital image analysis can obtain high diagnostic accuracy (94-97%) [107-109]. Detection of chromosomal abnormalities by fluorescence in-situ hybridization (FISH) analysis is useful when the cytology is inconclusive [110].

Molecular analysis of EUS-FNA samples is expected to improve the accuracy of diagnosis. Kras mutation occurred in 10 of 11 cases of pancreatic adenocarcinoma in which DNA amplification was successful, but in none of 16 patients with autoimmune pancreatitis. However, the fractional allelic loss did not differ between the two groups [111]. Another large study (n = 394 EUS-FNA samples) found 87% Kras mutations in pancreatic adenocarcinoma and only 3% in inflammatory masses and improved the accuracy of cancer diagnosis by 6% [112]. A recent meta-analysis showed that Kras detection in inconclusive EUS-FNA cases reduces the false-negative rate by 55.6%, with a false-positive rate of 10.7%, and the combined modality increases diagnostic accuracy from 80% to 88% [113].

In indeterminate pancreatic masses, the combination of Kras mutation detection and serum CA19-9 showed better sensitivity than serum CA19-9 alone (81% vs 54%) [11]. Identification of telomerase activity in pancreatic mass samples increased the sensitivity from 85% to 100%, maintaining 100% specificity [114].

6. Treatment

EUS can be used for direct antitumor therapy by injection, ablation, fiducial implantation to guide radiotherapy, pain treatment, and treatment of jaundice.

6.1. Antitumor therapy

Intratumoral injection for pancreatic cancer has been performed in several trials. Vaccination with dendritic cells as immunotherapy is considered a potential anti-cancer tool, and OK-432 represents a maturation stimulus for dendritic cells [115]. One early trial used concomitant immunotherapy with EUS-guided injection of OK-432, followed by intravenous infusion of lymphokine-activated killer cells stimulated with anti-CD3 monoclonal antibody. The

investigators hypothesized that apoptotic cells induced by gemcitabine treatment could release tumor antigens slowly over time and that this stimulates dendritic cells to process and present tumor antigens [116]. The results were encouraging in five patients, but further studies are needed [117]. Immature dendritic cells and OK-432 were preoperatively injected intratumorally in nine patients with resectable pancreatic cancer; there were no complications, and survival was prolonged in only one patient with distant metastasis [115].

Allogeneic mixed lymphocyte cultures (cytoimplants) were injected in four patients who then survived for a mean 13.2 months, with two partial responses and one minor response. The main side effect was low-grade fever responsive to acetaminophen. No further investigation ensued [118].

Weekly injection of ONYX-015 (dl1520), an E1B-55kD gene-deleted replication-selective adenovirus that preferentially replicates in and kills malignant cells, was performed in 21 patients with irresectable pancreatic adenocarcinoma. More than half had progressive disease and developed treatment toxicity. Sepsis was noted in two patients, and duodenal perforation was seen when the injection was delivered transduodenally [119].

TNFrade is a replication-deficient adenovector containing human tumor necrosis factor (TNF)-α gene, regulated by a radiation-inducible promoter Egr-1 (early growth response). The advantage of this approach is the potential to maximize local antitumor activity and to minimize systemic toxicity. Five once-weekly intratumoral injections of TNFrade before radiotherapy and continuous infusion of 5-FU were reported as beneficial in the management of inoperable pancreatic cancer [120,121], but the phase III randomized controlled trial showed no survival advantage (6.8 months vs 7 months) [122].

EUS-guided local injection for anaplastic carcinoma with chemosensitivity to paclitaxel was associated with complete tumor response 2 years later [123].

6.2. EUS-guided tumor ablation

EUS-guided Tumor ablation, a minimally invasive technique allowing selective ablation of tumor masses, might improve the efficacy of neoadjuvant treatments in patients not suitable for any other kind of treatment. Local ablative therapies such as radiofrequency ablation, photodynamic therapy, and brachytherapy have been applied in animal models or humans.

Tumor destruction by radiofrequency ablation (RFA) results in a scar, surrounded by normal tissue, which shrinks in the course of time. The pancreas is thermosensitive and usually responds with inflammation followed by edema, fibrotic and sometimes cystic transformation. The potential advantage of ablation under EUS control is guidance by real-time imaging into a deeply located target such as the pancreas which is extremely difficult to reach by a percutaneous approach. Moreover, the established precision of EUS in the measurement of the location and size of pancreatic masses could be used to estimate and follow up the area of ablation and then to avoid damage of surrounding structures.

The first report of EUS-guided RFA in the pancreas was in a porcine model, using a modified EUS needle and a commercial RF needle. RFA provided localized tissue ablation in a 1-cm

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zone from the needle catheter. One of the 13 pigs developed pancreatitis [124]. Carrara demonstrated the feasibility and efficacy of EUS-guided RFA using a Cryotherm probe in 14 pigs, with good results in spleen and liver [125]. Other investigators found the technique to be safe in the pig model, with minimal evidence of fat necrosis in intrapancreatic and/or extrapancreatic adipose tissue [126,127].

The EUS-guided RFA technique was recently successfully applied in 16 patients, but in another 6 patients either the wall or the tumor was too stiff to permit passage of the Cryotherm probe. No pancreatitis was noted in the successful group, although an increase in amylases was seen in 3 of the 16 patients [128,129]. However, the impact on survival or tumor size needs further evaluation.

Ablation with a neodymium:yttrium-aluminum-garnet laser was tried in a porcine model, following the results in hepatocellular carcinoma, and no major complications were noted [129].

EUS-guided photodynamic therapy (PDT) with the photosensitizing agent porfimer sodium was used in an animal model again and the extension of necrosis was found to be related to the light dose applied, but no human study has yet been conducted due to lack of controlling the area of necrosis, similar to laser ablation [130].

EUS-guided intraoperative interstitial brachytherapy had a moderate local tumor effect and showed some clinical benefit in one third of 15 patients, with some severe hematological complications, pancreatitis, and pseudocyst formation, but without serious clinical sequelae [131]. Another study involving EUS-guided implantation of seeds in local advanced adenocarcinoma showed improvement in pain control, but no survival benefit [132].

6.3. EUS fiducial implantation

EUS guidance can also be used for the placement of radio-opaque fiducial markers in or near the tumor. Fiducials define the tumor border and serve to guiding radiotherapy. Fiducials vary in shape -- spheres, coils, seeds, etc. -- and their EUS visibility varies [133]. They are deployed into the mass by using the 19G or the less stiff 22G needle, by means of a stylet, or by injecting sterile water into the needle. A mean number of 2-4 fiducial markers per patient have to be placed [134]. The "ideal fiducial geometry" was studied in 77 patients and the placement of fiducials judged to be better by surgery than by EUS; however, this geometry was unnecessary for successful tracking and delivery of radiation [135].There is migration of 0.8-2 mm in relation to bony landmarks [133,136], and in one study the procedure had to be repeated in 7% of the patients [137]. However, no migration-related complications have been reported to date.

6.4. Pain palliation by EUS-guided celiac plexus neurolysis

The NCCN guidelines version 2.2012 for pancreatic adenocarcinoma recommend EUS-guided celiac plexus neurolysis (EUS-CPN) for the treatment of severe tumor-associated pain. In the case of jaundice caused by an unresectable pancreatic head tumor, biliary drainage should be offered first, then EUS-CPN if pain persists. Relative contraindications to EUS-CPN include difficult access owing to anatomical distortion from previous surgery or congenital malfor-

mations. The absolute contraindications for EUS-CPN are the same as for any other invasive procedure: coagulopathy, platelets < 50 000, and patients who are unable or unwilling to cooperate [138].

The mean rate of pain alleviation is 72-80%, with a much lower rate of complete pain response [139-141]. The post-neurolytic residual pain could be related to non-visceral pain owing to invasion of the muscles or surrounding connective tissue. The bilateral technique on both sides of the celiac trunk was associated with a rate of pain alleviation of 45-88% [142-144], while the central technique, with injection above the celiac trunk, showed 68-72% alleviation [145,146]. To date, only one randomized controlled trial has compared the central and bilateral techniques of EUS-CPN; it found no difference in duration of pain relief (11 vs. 14 weeks), complete pain relief (2/29 vs. 2/21 patients), or reduction in pain medication (9/29 vs. 7/21 patients) [147,148]. The choice between central and bilateral EUS-CPN remains difficult, depending on the personal skills and experience of the individual endosonographer. We have achieved good results with the central technique, which we consider easier to perform [148].

EUS-guided direct ganglia neurolysis resulted in better pain alleviation than bilateral injection [149,150]; but no randomized study has yet compared these two techniques. No difference in pain alleviation was noted between injection of 10 or 20 ml alcohol [149].

Pain reduction was more effective and the need for increased opioids was prevented in patients without radiochemotherapy compared with patients who had radiochemotherapy [151]. The benefit of repeated EUS-CPN was studied in 24 patients and results were less encouraging. The rate of successful pain relief was much lower than for the first EUS-CPN (29% vs. 67% at 1-month follow-up), and disease progression was a factor which limited the response [152].

The predictors of pain alleviation were found to be lack of ganglia visualization [153], direct invasion of celiac ganglia, and leftward diffusion of the neurolytic agent [145]. The pain was also less severe, albeit not significantly so, for tumors located in the body or tail of the pancreas, for large tumors, and for patients with severe pain at presentation [153].

Nowadays the potential immediate complications, such as hypotension, tachycardia, pain enhancement, severe bleeding, and paraplegia, are considered rare. The late side effects include diarrhea, hypotension, fever, and paraplegia [154]. Several severe side effects have been reported, e.g., permanent lower paraplegia due to spinal cord infarction [155,156], hepatic, splenic, and renal infarction [157], and lethal perforation of aorta and stomach [158].

6.5. Palliative EUS-guided treatment of jaundice

Palliative EUS-guided treatment of jaundice should be offered as an effective alternative for percutaneous transhepatic biliary drainage when ERCP fails and surgery is not indicated. One approach is transduodenal in combination with ERCP (rendez-vous technique), with reported technical success rates of 75-100% [159,160]. EUS-guided choledochoduodenostomy with transluminal stenting is successful in 75-88% of cases [161-164], while the transgastric approach has a success rate of 65-100% [165-167]. Recently, cholecysto-antrostomy has been described as an ideal alternative if the patient has duodenal strictures with or without a duodenal metal

stent and a non-dilated intrahepatic bile duct [168-169]. When duodenal stenosis is also present, double duodenal and biliary drainage by ERCP or EUS can be performed [170].

All these procedures are technically challenging and should be attempted only by very experienced endosonographers at a high-volume center for bilio-pancreatic pathology. Complications are frequent, occurring in 18-23% of cases, and are represented by pneumoperitoneum, bile peritonitis, cholangitis, bleeding, pancreatitis (in the rendez-vous approach), and stent migration (Table 2). The existing data are from single very experienced centers; further prospective multicentric results are awaited.

Author, year	No. of patients PC / total	Technical success rate	Functional success rate	Patency (days)	Complications
Song [161]	9/15 CDS	13/15	13/13	264-CDS	Pneumoperitoneum-2 Cholangitis-1 Stent migration -4
Shah[159]	70 CDS	86%(75% rendez-vous)			Pancreatitis-5, hematoma-1, bile leak-1, bacteremia -1,pneumoperitoneum-1, perforation-1
Kim[165]	9-CDS 4-HGS	12/13	11/12		Pneumoperitoneum-2 Peritonitis-2 Migration-3
Park[166]	57	55/56	49/55	132-HGS 142-CDS	Pneumoperitoneum-7 Bile peritonitis-2 Bleeding-2 Migration-4
Kim[160]	15 CDS	15/15	12/15		Acute pancreatitis-1
Park [171]	9HGS 5CDS	14/14			Migration-1
Hara[172]	18- CDS	17/18	17/17	272	Peritonitis-1 Hemobilia-1
Artifon [173]	3 CDS-metal				
Ang [174]	2 CDS-plastic				
Iwamuro [175]	7 CDS				Bile peritonitis-2 Obstruction-1
Siddiqui[176]	9CDS	8/9			Pneumoperitoneum-1 Pain-1
Belletruti [177]	4CDS 2HGS	6/7		102	

Author, year	No. of patients PC / total	Technical success rate	Functional success rate	Patency (days)	Complications
Nguyen-Tang [178]	5HGS	5/5			
Hanada[179]	4CDS	4/4			
Brauer[163]	12 -4 CDS (8pancreatic mass)	11/12			
Bories[167]	11HGS	10/11		213	Obstruction-1(plastic) Biloma-1 Cholangitis-1 Migration-1
Kahaleh[180]	13 HGS 10 CDS	21/28			Pneumoperitoneum-2 Bile leakage-1 Bleeding-1
Yamao[181]	2 CDS				
Puspok [182]	5CDS 1HGS	6/6			Acute cholecystitis-1
Mallery [183]	6CDS	5/6			
Burmester[164]	4CDS	3/4			

PC, pancreatic cancer; CDS, choledochoduodenostomy; HGS, hepaticogastrostomy

Table 2. Studies of EUS-guided biliary drainage.

7. Screening of pancreatic cancer

Multislice CT detection of pancreatic cancers less than 2 cm in diameter has sensitivity of 70-80% [184,185] and that of MRI is higher [186], but EUS can detect almost twice lesions compared to other imaging methods [184,187]. For patients with elevated CA19-9, the use of EUS detected cancer in only 0.9% of patients, with the result that the cost of detecting one pancreatic adenocarcinoma was $41,133 [188]. An initial study from the National German Familial Pancreatic Cancer Registry noted potential precursors of pancreatic cancer in 4 of 182 examinations of patients from families with familial pancreatic cancer, based on EUS and MRI, and the authors concluded that screening is not justified due to the high costs and the psychological stress to the persons concerned [189].

Screening by EUS and/or MRI is important for first-degree relatives (FDRs) of patients with PC from a familial PC kindred with at least two affected FDRs; patients with Peutz-Jeghers syndrome; and carriers of p16, BRCA2, and hereditary non-polyposis colorectal cancer (HNPCC) mutations with at least one affected FDR [190]. Another study which investigated

a high-risk population by means of EUS found a 6.8% rate of adenocarcinomas in the body and tail of the pancreas; two of the three patients had N1 tumors [186].

8. Conclusion

EUS is useful for the detection of pancreatic cancers less than 3 cm in diameter and for the staging of cases in which CT is inconclusive. EUS-FNA establishes the tumor type with high accuracy and a very low rate of complications, and it is useful for differential diagnosis. EUS-guided palliative treatments include neurolysis and therapy of jaundice, but intratumoral ablative therapy needs further evaluation. Screening in high-risk groups should take advantage of EUS evaluation.

Author details

Andrada Seicean*

Address all correspondence to: andradaseicean@yahoo.com

"Iuliu Hatieganu" University of Medicine and Pharmacy, Regional Institute of Gastroenterology and Hepatology Cluj-Napoca, Cluj-Napoca, Romania

References

[1] Ngamruengphong S, Li F, Zhou Y, Chak A, Cooper GS, Das A. EUS and survival in patients with pancreatic cancer: a population-based study. Gastrointest Endosc. 2010 Jul;72(1):78-83, 83.e1-2.

[2] Parmar AD, Sheffield KM, Han Y, Vargas GM, Guturu P, Kuo YF, Goodwin JS, Riall TS. Evaluating comparative effectiveness with observational data: Endoscopic ultrasound and survival in pancreatic cancer. Cancer. 2013; 119(21): 3861-9.

[3] Bhutani MS, Gress FG, Giovannini M, Erickson RA, Catalano MF, Chak A, Deprez PH, Faigel DO, Nguyen CC; No Endosonographic Detection of Tumor (NEST) Study.The No Endosonographic Detection of Tumor (NEST) Study: a case series of pancreatic cancers missed on endoscopic ultrasonography. Endoscopy. 2004;36(5): 385-9.

[4] Spier BJ, Johnson EA, Gopal DV, Frick T, Einstein MM, Byrne S, Koscik RL, Liou JI, Broxmeyer T, Selvaggi SM, Pfau PR. Predictors of malignancy and recommended follow-up in patients with negative endoscopic ultrasound-guided fine-needle aspiration of suspected pancreatic lesions. Can J Gastroenterol. 2009;23(4):279-86.

[5] Müller MF, Meyenberger C, Bertschinger P, Schaer R, Marincek B. Pancreatic tumors: evaluation with endoscopic US, CT, and MR imaging. Radiology. 1994;190(3):745-51.

[6] Ardengh JC, de Paulo GA, Ferrari AP. Pancreatic carcinomas smaller than 3.0 cm: endosonography (EUS) in diagnosis, staging and prediction of resectability. HPB (Oxford). 2003;5(4):226-30.

[7] Akahoshi K,Chijiiwa Y, Nakano I, Nawata H, Ogawa Y, Tanaka M, Nagai E, Tsuneyoshi M. Diagnosis and staging of pancreatic cancer by endoscopic ultrasound. Br J Radiol 1998; 71: 492- 496.

[8] Agarwal B, Krishna NB, Labundy JL, Safdar R, Akduman EI. EUS and/or EUS-guided FNA in patients with CT and/or magnetic resonance imaging findings of enlarged pancreatic head or dilated pancreatic duct with or without a dilated common bile duct. Gastrointest Endosc. 2008;68(2):237-42.

[9] Krishna NB, Mehra M, Reddy AV, Agarwal B. EUS/EUS-FNA for suspected pancreatic cancer: influence of chronic pancreatitis and clinical presentation with or without obstructive jaundice on performance characteristics. Gastrointest Endosc. 2009 ;70(1): 70-9.

[10] Ho S, Bonasera RJ, Pollack BJ, Grendell J, Feuerman M, Gress F. A single-center experience of endoscopic ultrasonography for enlarged pancreas on computed tomography. Clin Gastroenterol Hepatol. 2006 ;4(1):98-103

[11] Wang X, Gao J, Ren Y, Gu J, Du Y, Chen J, Jin Z, Zhan X, Li Z, Huang H, Lv S, Gong Y. Detection of KRAS gene mutations in endoscopic ultrasound-guided fine-needle aspiration biopsy for improving pancreatic cancer diagnosis. Am J Gastroenterol. 2011;106(12):2104-11.

[12] Krishna N, Tummala P, Reddy AV, Mehra M, Agarwal B. Dilation of both pancreatic duct and the common bile duct on computed tomography and magnetic resonance imaging scans in patients with or without obstructive jaundice. Pancreas. 2012l;41(5): 767-72.

[13] Singh S, Reddymasu S, Waheed S, Vail M, He J, Talapaneni J, Olyaee M. Endoscopic ultrasonography findings in patients with non-specific changes of the pancreas on computed tomography: a single-center experience. Dig Dis Sci. 2008 ;53(10):2799-804

[14] Rodriguez S, Faigel D. Absence of a dilated duct predicts benign disease in suspected pancreas cancer: a simple clinical rule. Dig Dis Sci. 2010;55(4):1161-6.

[15] Dufour B, Zins M, Vilgrain V, Levy P, Bernades P, Menu Y. Comparison between spiral x-ray computed tomography and endosonography in the diagnosis and staging of adenocarcinoma of the pancreas. Clinical preliminary study. Gastroenterol Clin Biol. 1997;21(2):124-30.

[16] Borbath I, Van Beers BE, Lonneux M, Schoonbroodt D, Geubel A, Gigot JF, Deprez PH. Preoperative assessement of pancreatic tumors using magnetic resonance imag-

ing, endoscopic ultrasonography, positron emission tomography and laparoscopy. Pancreatology 2005; 5(6): 553-561.

[17] Ainsworth AP, Rafaelsen SR, Wamberg PA, Durup J, Pless TK, Mortensen MB. Is there a difference in diagnostic accuracy and clinical impact between endoscopic ultrasonography and magnetic resonance cholangiopancreatography? Endoscopy. 2003 ;35(12):1029-32.

[18] Mertz HR, Sechopoulos P, Delbeke D, Leach SD. EUS, PET and CT scanning for evaluation of pancreatic adenocarcinoma. Gastrointest Endosc 2000; 52: 367-71..

[19] Schick V, Franzius C, Beyna T, Oei ML, Schnekenburger J, Weckesser M, Domschke W, Schober O, Heindel W, Pohle T, Juergens KU. Diagnostic impact of 18F-FDG PET-CT evaluating solid pancreatic lesions versus endosonography, endoscopic retrograde cholangio-pancreatography with intraductal ultrasonography and abdominal ultrasound. Eur J Nucl Med Mol Imaging. 2008 ;35(10):1775-85.

[20] Menzel J, Domschke W, Konturek JW, Gillessen A, Foerster E. Intraductal ultrasound in the pancreaticobiliary duct system. Dtsch MedWochenschr 1997;122(3): 41-49.

[21] Brand B, Pfaff T, Binmoeller KF, Sriram PV, FritscherRavens A, Knöfel WT, Jäckle S, Soehendra N. Endoscopic ultrasound for differential diagnosis of focal pancreatic lesions, confirmed by surgery. Scand J Gastroenterol 2000; 35(11): 1221-1228

[22] Lee SH, Ozden N, Pawa R, Hwangbo Y, Pleskow DK, Chuttani R, Sawhney MS. Periductal hypoechoic sign: an endosonographic finding associated with pancreatic malignancy. Gastrointest Endosc. 2010;71(2):249-55.

[23] Rösch T, Braig C, Gain T, Feuerbach S, Siewert JR, Schusdziarra V, Classen M. Staging of pancreatic and ampullary carcinoma by endoscopic ultrasonography. Comparison with conventional sonography, computed tomography, and angiography. Gastroenterology. 1992 ;102(1):188-99.

[24] Palazzo L, Roseau G, Gayet B, Vilgrain V, Belghiti J, Fékéte F, Paolaggi JA. Endoscopic ultrasonography in the diagnosis and staging of pancreatic adenocarcinoma. Results of a prospective study with comparison to ultrasonography and CT scan. Endoscopy 1993; 25(2): 143-150..

[25] Tio TL, Sie LH, Kallimanis G, Luiken GJ, Kimmings AN, Huibregtse K, Tytgat GN. Staging of ampullary and pancreatic carcinoma: comparison between endosonography and surgery. Gastrointest Endosc 1996: 44(6): 706-713.

[26] Buscail L, Pages P, Berthelemy P, Fourtanier G, Frexinos J, Escourrou J. Role of EUS in the management of pancreatic and ampullary carcinoma: a prospective study assessing resectability and prognosis. Gastrointest Endosc 1999; 50(1): 34-40.

[27] Ahmad NA, Lewis JD, Ginsberg GG, Rosato EF, Morris JB, Kochman ML. EUS in preoperative staging of pancreatic cancer. Gastrointest Endosc 2000; 52(4): 463-468.

[28] Soriano A, Castells A, Ayuso C, Ayuso JR, de Caralt MT, Ginès MA, Real MI, Gilabert R, Quintó L, Trilla A, Feu F, Montanyà X, Fernández-Cruz L, Navarro S. Preoperative staging and tumor resectability assessment of pancreatic cancer: prospective study comparing endoscopic ultrasonography, helical computed tomography, magnetic resonance imaging, and angiography. Am J Gastroenterol 2004; 99(3): 492-501.

[29] Dewitt J, Devereaux B, Chriswell M, McGreevy K, Howard T, Imperiale TF, Ciaccia D, Lane KA, Maglinte D, Kopecky K, LeBlanc J, McHenry L, Madura J, Aisen A, Cramer H, Cummings O, Sherman S. Comparison of endoscopic ultrasonography and multidetector computed tomography for detecting and staging pancreatic cancer. Ann Intern Med 2004; 141(10): 753-763.

[30] Tellez-Avila FI, Chavez-Tapia NC, López-Arce G, Franco-Guzmán AM, Sosa-Lozano LA, Alfaro-Lara R, Chan-Nuñez C, Giovannini M, Elizondo-Rivera J, Ramírez-Luna MA. Vascular invasion in pancreatic cancer: predictive values for endoscopic ultrasound and computed tomography imaging. Pancreas. 2012 ;41(4):636-8.

[31] Dewitt J, Devereaux BM, Lehman GA, Sherman S, Imperiale TF. Comparison of endoscopic ultrasound and computed tomography for the preoperative evaluation of pancreatic cancer: a systematic review. Clin Gastroenterol Hepatol 2006; 4(6): 717-725.

[32] Rösch T, Dittler HJ, Strobel K, Meining A, Schusdziarra V, Lorenz R, Allescher HD, Kassem AM, Gerhardt P, Siewert JR, Höfler H, Classen M. Endoscopic ultrasound criteria for vascular invasion in the staging of cancer of the head of the pancreas: a blind reevaluation of videotapes. Gastrointest Endosc. 2000 ;52(4):469-77.

[33] Marty O, Aubertin JM, Bouillot JL, Hernigou A, Bloch F, Petite JP. Prospective comparison of ultrasound endoscopy and computed tomography in the assessment of locoregional invasiveness of malignant ampullar and pancreatic tumors verified surgically]. Gastroenterol Clin Biol. 1995;19(2):197-203.

[34] Midwinter MJ, Beveridge CJ, Wilsdon JB, Bennett MK, Baudouin CJ, Charnley RM. Correlation between spiral computed tomography, endoscopic ultrasonography and findings at operation in pancreatic and ampullary tumours. Br J Surg. 1999;86(2): 189-93.

[35] Puli SR, Singh S, Hagedorn CH, Reddy J, Olyaee M. Diagnostic accuracy of EUS for vascular invasion in pancreatic and periampullary cancers: a meta-analysis and systematic review. Gastrointest Endosc. 2007;65(6):788-97.

[36] Kala Z, Válek V, Hlavsa J, Hana K, Vánová A. The role of CT and endoscopic ultrasound in pre-operative staging of pancreatic cancer. Eur J Radiol. 2007;62(2):166-9.

[37] Gress FG, Hawes RH, Savides TJ, Ikenberry SO, Cummings O, Kopecky K, Sherman S, Wiersema M, Lehman GA. Role of EUS in the preoperative staging of pancreatic cancer: a large single-center experience. Gastrointest Endosc. 1999;50(6):786-91.

[38] Rivadeneira DE, Pochapin M, Grobmyer SR, Lieberman MD, Christos PJ, Jacobson I, Daly JM Comparison of linear array endoscopic ultrasound and helical computed tomography for the staging of periampullary malignancies. Ann Surg Oncol. 2003;10(8):890-7.

[39] Agarwal B, Abu-Hamda E, Molke KL, Correa AM, Ho L. Endoscopic ultrasound-guided fine needle aspiration and multidetector spiral CT in the diagnosis of pancreatic cancer. Am J Gastroenterol. 2004;99(5):844-50.

[40] Morris-Stiff G, Escofet X, Barry JD, Lewis WG, Puntis MC, Roberts SA. Selective use of endoscopic ultrasound in the evaluation of carcinomas of the pancreatic head. Dig Surg. 2011;28(5-6):373-8.

[41] Sugiyama M, Hagi H, Atomi Y, Saito M. Diagnosis of portal venous invasion by pancreatobiliary carcinoma: value of endoscopic ultrasonography. Abdom Imaging. 1997;22(4):434-8.

[42] Legmann P, Vignaux O, Dousset B, Baraza AJ, Palazzo L, Dumontier I, Coste J, Louvel A, Roseau G, Couturier D, Bonnin A. Pancreatic tumors: comparison of dual-phase helical CT and endoscopic sonography. AJR Am J Roentgenol. 1998;170(5): 1315-22.

[43] Shami VM, Mahajan A, Loch MM, Stella AC, Northup PG, White GE, Brock AS, Srinivasan I, de Lange EE, Kahaleh M. Comparison between endoscopic ultrasound and magnetic resonance imaging for thestaging of pancreatic cancer. Pancreas. 2011;40(4): 567-70.

[44] Bipat S, Phoa S, vanDelden O, Bossuyt PM, Gouma DJ, Laméris JS, Stoker J. Ultrasonography, computed tomography and magnetic resonance imaging for diagnosis and determining resectability of pancreatic adenocarcinoma: a meta-analysis. J Comput Assist Tomogr 2005; 29(4):438-445.

[45] Möller K, Papanikolaou IS, Toermer T, Delicha EM, Sarbia M, Schenck U, Koch M, Al-Abadi H, Meining A, Schmidt H, Schulz HJ, Wiedenmann B,Rösch T. EUS-guided FNA of solid pancreatic masses: high yield of 2 passes with combined histologic-cytologic analysis. Gastrointest Endosc 2009; 70(1): 60-9.

[46] Iglesias-Garcia J, Dominguez-Munoz E, Lozano-Leon A, Abdulkader I, Larino-Noia J, Antunez J, Forteza J. Impact of endoscopic ultrasound-guided fine needle biopsy for diagnosis of pancreatic masses. World J Gastroenterol 2007; 13(2): 289-93.

[47] Abdelgawwad MS, Alston E, Eltoum IA. The frequency and cancer risk associated with the atypical cytologic diagnostic category in endoscopic ultrasound-guided fine-needle aspiration specimens of solid pancreaticlesions: A meta-analysis and argument for a Bethesda system for reporting cytopathology of the pancreas. Cancer Cytopathol. 2013 Jul 23.

[48] Papanikolaou IS, Adler A, Wegener K, Al-Abadi H, Dürr A, Koch M, Pohl H, Abou-Rebyeh H, Veltzke-Schlieker W, Wiedenmann B, Rösch T. Prospective pilot evalua-

tion of a new needle prototype for endoscopic ultrasonography-guided fine-needle aspiration: comparison of cytology and histology yield. Eur J Gastroenterol Hepatol 2008; 20(4): 342-8..

[49] Savides TJ. Tricks for improving EUS-FNA accuracy and maximizing cellular yield. Gastrointest Endosc 2009; 69: S130- 132.

[50] Itoi T, Itokawa F, Kurihara T, Sofuni A, Tsuchiya T, Ishii K, Tsuji S, Ikeuchi N, Kawai T, Moriyasu F.Experimental endoscopy: objective evaluation of EUS needles. Gastrointest Endosc 2009; 69 (3Pt1): 509-16.

[51] Eloubeidi MA, Jhala D, Chhieng DC, Chen VK, Eltoum I, Vickers S, Mel Wilcox C, Jhala N. Yield of endoscopic ultrasound-guided fine-needle aspiration biopsy in patients with suspected pancreatic carcinoma. Cancer 2003; 99(5): 285-92.

[52] Affolter KE, Schmidt RL, Matynia AP, Adler DG, Factor RE. Needle Size Has Only a Limited Effect on Outcomes in EUS-Guided Fine Needle Aspiration: A Systematic Review and Meta-Analysis.Dig Dis Sci. 2013;58(4):1026-34

[53] Eloubeidi MA, Varadarajulu S, Desai S, Wilcox CM. Value of repeat endoscopic ultrasound-guided fine needle aspiration for suspected pancreatic cancer. J Gastroenterol Hepatol 2008; 23(4): 567-70.

[54] Nicaud M, Hou W, Collins D, Wagh MS, Chauhan S, Draganov PV. The utility of repeat endoscopic ultrasound-guided fine needle aspiration for suspected pancreatic cancer. Gastroenterol Res Pract 2010; 2010: 268290.

[55] Tadic M, Kujundzic M, Stoos-Veic T, Kaic G, Vukelic-Markovic M. Role of repeated endoscopic ultrasound-guided fine needle aspiration in small solid pancreatic masses with previous indeterminate and negative cytological findings. Dig Dis 2008; 26(4): 377-82.

[56] Vilmann P, Săftoiu A, Hollerbach S, Skov BG, Linnemann D, Popescu CF, Wellmann A, Gorunescu F, Clementsen P, Freund U, Flemming P, Hassan H, Gheonea DI, Streba L, Ioncică AM, Streba CT. Multicenter randomized controlled trial comparing the performance of 22 gauge versus 25 gauge EUS-FNA needles in solid masses. Scand J Gastroenterol. 2013 ;48(7):877-83.

[57] Song TJ, Kim JH, Lee SS, Eum JB, Moon SH, Park do H, Seo DW, Lee SK, Jang SJ, Yun SC, Kim MH. The prospective randomized, controlled trial of endoscopic ultrasound-guided fine-needle aspiration using 22G and 19G aspiration needles for solid pancreatic or peripancreatic masses. Am J Gastroenterol 2010; 105(8): 1739-1745

[58] Songür N, Songür Y, Bırcan S, Kapucuoğlu N. Comparison of 19- and 22-gauge needles in EUS-guided fine needle aspiration in patients with mediastinal masses and lymph nodes. Turk J Gastroenterol 2011; 22(5): 472-8.

[59] Levy MJ, Wiersema MJ. EUS-guided Trucut biopsy. Gastrointest Endosc 2005; 62: 417-26.

[60] Bang JY, Hebert-Magee S, Trevino J, Ramesh J, Varadarajulu S. Randomized trial comparing the 22-gauge aspiration and 22-gauge biopsy needles for EUS-guided sampling of solid pancreatic mass lesions. Gastrointest Endosc 2012; 76(2): 321-7.

[61] Iwashita T, Nakai Y, Samarasena JB, Park do H, Zhang Z, Gu M, Lee JG, Chang KJ. High single-pass diagnostic yield of a new 25-gauge core biopsy needle for EUS-guided FNA biopsy in solid pancreatic lesions. Gastrointest Endosc 2013, 77(6): 909-15.

[62] Wani S, Early D, Kunkel J, Leathersich A, Hovis CE, Hollander TG, Kohlmeier C, Zelenka C, Azar R, Edmundowicz S, Collins B, Liu J, Hall M, Mullady D. Diagnostic yield of malignancy during EUS-guided FNA of solid lesions with and without a stylet: a prospective, single blind, randomized, controlled trial. Gastrointest Endosc 2012; 76(2): 328-35.

[63] Wani S, Gupta N, Gaddam S, Singh V, Ulusarac O, Romanas M, Bansal A, Sharma P, Olyaee MS, Rastogi A. A comparative study of endoscopic ultrasound guided fine needle aspiration with and without a stylet. Dig Dis Sci 2011; 56(8): 2409-14.

[64] Lee JK, Choi JH, Lee KH, Kim KM, Shin JU, Lee JK, Lee KT, Jang KT. A prospective, comparative trial to optimize sampling techniques in EUS-guided FNA of solid pancreatic masses. Gastrointest Endosc 2013, 77(5):745-51.

[65] Sahai AV, Paquin SC, Gariépy G. A prospective comparison of endoscopic ultrasound-guided fine needle aspiration results obtained in the same lesion, with and without the needle stylet. Endoscopy 2010; 42(11): 900-3.

[66] Bang JY, Magee SH, Ramesh J, Trevino JM, Varadarajulu S. Randomized trial comparing fanning with standard technique for endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic mass lesions. Endoscopy 2013, 45(6):445-50.

[67] Thomas T, Kaye PV, Ragunath K, Aithal GP. Efficacy, safety, and predictive factors for a positive yield of EUS-guided Trucut biopsy: a large tertiary referral center experience. Am J Gastroenterol 2009; 104(3): 584-91.

[68] LeBlanc JK, Emerson RE, Dewitt J, Symms M, Cramer HM, McHenry L, Wade CL, Wang X, Musto P, Eichelberger L, Al-Haddad M, Johnson C, Sherman S. A prospective study comparing rapid assessment of smears and ThinPrep for endoscopic ultrasound-guided fine-needle aspirates. Endoscopy 2010; 42(5): 389-94.

[69] Eloubeidi MA, Tamhane A, Jhala N, Chhieng D, Jhala D, Crowe DR, Eltoum IA. Agreement between rapid onsite and final cytologic interpretations of EUS-guided FNA specimens: implications for the endosonographer and patient management. Am J Gastroenterol 2006; 101(12): 2841-2847.

[70] Varadarajulu S, Tamhane A, Eloubeidi MA. Yield of EUS-guided FNA of pancreatic masses in the presence or the absence of chronic pancreatitis. Gastrointest Endosc. 2005; 62(5): 728-36.

[71] Ranney N, Phadnis M, Trevino J, Ramesh J, Wilcox CM, Varadarajulu S. Impact of biliary stents on EUS-guided FNA of pancreatic mass lesions. Gastrointest Endosc. 2012; 76(1): 76-83.

[72] Fisher JM, Gordon SR, Gardner TB. The impact of prior biliary stenting on the accuracy and complication rate of endoscopic ultrasound fine-needle aspiration for diagnosing pancreatic adenocarcinoma.Pancreas 2011 ; 40(1):21-4

[73] Siddiqui AA, Fein M, Kowalski TE, Loren DE, Eloubeidi MA. Comparison of the influence of plastic and fully covered metal biliary stents on the accuracy of EUS-FNA for the diagnosis of pancreatic cancer. Dig Dis Sci 2012; 57(9): 2438-45.

[74] Ardengh JC, Lopes CV, de Lim LF, de Oliveira JR, Venco F, Santo GC, Modena JL. Diagnosis of pancreatic tumors by endoscopic ultrasound-guided fine-needle aspiration. World J Gastroenterol 2007; 13(22): 3112-6.

[75] Siddiqui AA, Brown LJ, Hong SK, Draganova-Tacheva RA, Korenblit J, Loren DE, Kowalski TE, Solomides C. Relationship of pancreatic mass size and diagnostic yield of endoscopic ultrasound-guided fine needle aspiration. Dig Dis Sci 2011; 56(11): 3370-5.

[76] Puli SR, Bechtold ML, Buxbaum JL, Eloubeidi MA.How good is endoscopic ultrasound-guided fine-needle aspiration in diagnosing the correct etiology for a solid pancreatic mass?: A meta-analysis and systematic review. Pancreas. 2013;42(1): 20-6.

[77] Saftoiu A, Vilman P. Endoscopic ultrasound elastography-- a new imaging technique for the visualization of tissue elasticity distribution. J Gastrointestin Liver Dis. 2006 15(2): 161-5.

[78] Giovannini M, Thomas B, Erwan B, Christian P, Fabrice C, Benjamin E, Geneviève M, Paolo A, Pierre D, Robert Y, Walter S, Hanz S, Carl S, Christoph D, Pierre E, Jean-Luc VL, Jacques D, Peter V, Andrian S. Endoscopic ultrasound elastography for evaluation of lymph nodes and pancreaticmasses: a multicenter study. World J Gastroenterol. 2009;15(13):1587-93.

[79] Iglesias-Garcia J, Larino-Noia J, Abdulkader I, Forteza J, Dominguez-Munoz JE. EUS elastography for the characterization of solid pancreatic masses. Gastrointest Endosc. 2009 ;70(6):1101-8.

[80] Săftoiu A, Vilmann P, Gorunescu F, Gheonea DI, Gorunescu M, Ciurea T, Popescu GL, Iordache A, Hassan H, Iordache S. Neural network analysis of dynamic sequences of EUS elastography used for the differential diagnosis of chronic pancreatitis and pancreatic cancer. Gastrointest Endosc. 2008;68(6): 1086-94.

[81] Săftoiu A, Vilmann P, Gorunescu F, Janssen J, Hocke M, Larsen M, Iglesias-Garcia J, Arcidiacono P, Will U, Giovannini M, Dietrich C, Havre R, Gheorghe C, McKay C, Gheonea DI, Ciurea T; European EUS Elastography Multicentric Study Group. Accu-

racy of endoscopic ultrasound elastography used for differential diagnosis of focal-pancreatic masses: a multicenter study.Endoscopy. 2011;43(7): 596-603.

[82] Dawwas MF, Taha H, Leeds JS, Nayar MK, Oppong KW. Diagnostic accuracy of quantitative EUS elastography for discriminating malignant from benign solid pancreatic masses: a prospective, single-center study. Gastrointest Endosc. 2012 76(5): 953-61.

[83] Iglesias-Garcia J, Larino-Noia J, Abdulkader I, Forteza J, Dominguez-Munoz JE. Quantitative endoscopic ultrasound elastography: an accurate method for the differentiation of solid pancreatic masses. Gastroenterology. 2010 ;139(4):1172-80.

[84] Itokawa F, Itoi T, Sofuni A, Kurihara T, Tsuchiya T, Ishii K, Tsuji S, Ikeuchi N, Umeda J, Tanaka R, Yokoyama N, Moriyasu F, Kasuya K, Nagao T, Kamisawa T, Tsuchida A. EUS elastography combined with the strain ratio of tissue elasticity for diagnosis of solid pancreatic masses. J Gastroenterol. 2011;46(6):843-53.

[85] Hu DM, Gong TT, Zhu Q. Endoscopic ultrasound elastography for differential diagnosis of pancreatic masses: a meta-analysis.Dig Dis Sci. 2013;58(4):1125-31.

[86] Xu W, Shi J, Li X, Zeng X, Lin Y. Endoscopic ultrasound elastography for differentiation of benign and malignantpancreatic masses: a systemic review and meta-analysis. Eur J Gastroenterol Hepatol. 2013;25(2):218-24.

[87] Pei Q, Zou X, Zhang X, Chen M, Guo Y, Luo H. Diagnostic value of EUS elastography in differentiation of benign and malignant solidpancreatic masses: a meta-analysis. Pancreatology. 2012;12(5):402-8.

[88] Săftoiu A, Iordache SA, Gheonea DI, Popescu C, Maloş A, Gorunescu F, Ciurea T, Iordache A, Popescu GL, Manea CT. Combined contrast-enhanced power Doppler and real-time sonoelastography performed during EUS, used in the differential diagnosis of focal pancreatic masses (with videos). Gastrointest Endosc. 2010;72(4):739-47.

[89] Yamashita Y, Ueda K, Itonaga M, Yoshida T, Maeda H, Maekita T, Iguchi M, Tamai H, Ichinose M, Kato J. Tumor vessel depiction with contrast-enhanced endoscopic ultrasonography predicts efficacy of chemotherapy in pancreatic cancer. Pancreas. 2013;42(6):990-5.

[90] Becker D, Strobel D, Bernatik T, Hahn EG. Echo-enhanced color- and power-Doppler EUS for the discrimination between focal pancreatitis and pancreatic carcinoma. Gastrointest Endosc. 2001;53(7):784-9.

[91] Hocke M, Schmidt C, Zimmer B, Topalidis T, Dietrich CF, Stallmach A.Contrast enhanced endosonography for improving differential diagnosis between chronic pancreatitis and pancreatic cancer.Dtsch Med Wochenschr. 2008 ;133(38): 1888-92.

[92] Hocke M, Schulze E, Gottschalk P, Topalidis T, Dietrich CF. Contrast-enhanced endoscopic ultrasound in discrimination between focal pancreatitis and pancreatic cancer. World J Gastroenterol. 2006;12(2):246-50.

[93] Săftoiu A, Popescu C, Cazacu S, Dumitrescu D, Georgescu CV, Popescu M, Ciurea T, Gorunescu F. Power Doppler endoscopic ultrasonography for the differential diagnosis between pancreatic cancer and pseudotumoral chronic pancreatitis. J Ultrasound Med. 2006 ;25(3):363-72.

[94] Sakamoto H, Kitano M, Suetomi Y, Maekawa K, Takeyama Y, Kudo M. Utility of contrast-enhanced endoscopic ultrasonography for diagnosis of small pancreatic carcinomas. Ultrasound Med Biol. 2008;34(4):525-32.

[95] Seicean A, Badea R, Stan-Iuga R, Gulei I, Pop T, Pascu O. The added value of real-time harmonics contrast-enhanced endoscopic ultrasonography for the characterisation of pancreatic diseases in routine practice. J Gastrointestin Liver Dis. 2010;19(1): 99-104.

[96] Napoleon B, Alvarez-Sanchez MV, Gincoul R, Pujol B, Lefort C, Lepilliez V, Labadie M, Souquet JC, Queneau PE, Scoazec JY, Chayvialle JA, Ponchon T. Contrast-enhanced harmonic endoscopic ultrasound in solid lesions of the pancreas: results of a pilot study. Endoscopy. 2010 Jul;42(7):564-70.

[97] Fusaroli P, Spada A, Mancino MG, Caletti G.Contrast harmonic echo-endoscopic ultrasound improves accuracy in diagnosis of solid pancreatic masses. Clin Gastroenterol Hepatol. 2010;8(7):629-34.

[98] Kitano M, Kudo M, Yamao K, Takagi T, Sakamoto H, Komaki T, Kamata K, Imai H, Chiba Y, Okada M, Murakami T, Takeyama Y. Characterization of small solid tumors in the pancreas: the value of contrast-enhanced harmonic endoscopic ultrasonography. Am J Gastroenterol. 2012;107(2):303-10.

[99] Gong TT, Hu DM, Zhu Q. Contrast-enhanced EUS for differential diagnosis of pancreatic mass lesions: a meta-analysis. Gastrointest Endosc. 2012;76(2):301-9.

[100] Seicean A, Badea R, Stan-Iuga R, Mocan T, Gulei I, Pascu O. Quantitative contrast-enhanced harmonic endoscopic ultrasonography for the discrimination of solid pancreatic masses. Ultraschall Med. 2010;31(6):571-6.

[101] Gheonea DI, Streba CT, Ciurea T, Săftoiu A. Quantitative low mechanical index contrast-enhanced endoscopic ultrasound for the differential diagnosis of chronic pseudotumoral pancreatitis and pancreatic cancer. BMC Gastroenterol. 2013;13:2.

[102] Imazu H, Uchiyama Y, Matsunaga K, Ikeda K, Kakutani H, Sasaki Y, Sumiyama K, Ang TL, Omar S, Tajiri H. Contrast-enhanced harmonic EUS with novel ultrasonographic contrast (Sonazoid) in the preoperative T-staging for pancreaticobiliary malignancies. Scand J Gastroenterol. 2010;45(6):732-8.

[103] Matsubara H, Itoh A, Kawashima H, Kasugai T, Ohno E, Ishikawa T, Itoh Y, Nakamura Y, Hiramatsu T, Nakamura M, Miyahara R, Ohmiya N, Ishigami M, Katano Y, Goto H, Hirooka Y. Dynamic quantitative evaluation of contrast-enhanced endoscop-

ic ultrasonography in the diagnosis of pancreatic diseases. Pancreas. 2011;40(7): 1073-9.

[104] Hocke M, Ignee A, Dietrich CF. Advanced endosonographic diagnostic tools for discrimination of focal chronic pancreatitis and pancreatic carcinoma--elastography, contrast enhanced high mechanical index (CEHMI) and low mechanical index (CEL-MI) endosonography in direct comparison. Z Gastroenterol. 2012;50(2):199-203.

[105] Fritscher-Ravens A, Knoefel WT, Krause C, Swain CP, Brandt L, Patel K. Three-dimensional linear endoscopic ultrasound-feasibility of a novel technique applied for the detection of vessel involvement of pancreatic masses. Am J Gastroenterol. 2005;100(6):1296-302.

[106] Săftoiu A.State-of-the-art imaging techniques in endoscopic ultrasound. World J Gastroenterol. 2011; 17(6):691-6.

[107] Zhang MM, Yang H, Jin ZD, Yu JG, Cai ZY, Li ZS. Differential diagnosis of pancreatic cancer from normal tissue with digital imaging processing and pattern recognition based on a support vector machine of EUS images. Gastrointest Endosc. 2010;72(5): 978-85.

[108] Zhu M, Xu C, Yu J, Wu Y, Li C, Zhang M, Jin Z, Li Z. Differentiation of pancreatic cancer and chronic pancreatitis using computer-aideddiagnosis of endoscopic ultrasound (EUS) images: a diagnostic test. PLoS One. 2013;8(5):e63820.

[109] Das A, Nguyen CC, Li F, Li B. Digital image analysis of EUS images accurately differentiates pancreatic cancer from chronic pancreatitis and normal tissue.Gastrointest Endosc. 2008;67(6): 861-7.

[110] Kubiliun N, Ribeiro A, Fan YS, Rocha-Lima CM, Sleeman D, Merchan J, Barkin J, Levi J. EUS-FNA with rescue fluorescence in situ hybridization for the diagnosis of pancreaticcarcinoma in patients with inconclusive on-site cytopathology results. Gastrointest Endosc. 2011;74(3):541-7.

[111] Khalid A, Dewitt J, Ohori NP, Chen JH, Fasanella KE, Sanders M, McGrath KM, Nikiforova M. EUS-FNA mutational analysis in differentiating autoimmune pancreatitis and pancreatic cancer.Pancreatology. 2011;11(5):482-6.

[112] Ogura T, Yamao K, Sawaki A, Mizuno N, Hara K, Hijioka S, Niwa Y, Tajika M, Kondo S, Shimizu Y, Bhatia V, Higuchi K, Hosoda W, Yatabe Y. Clinical impact of K-ras mutation analysis in EUS-guided FNA specimens from pancreatic masses. Gastrointest Endosc. 2012 ;75(4):769-74.

[113] Fuccio L, Hassan C, Laterza L, Correale L, Pagano N, Bocus P, Fabbri C, Maimone A, Cennamo V, Repici A, Costamagna G, Bazzoli F, Larghi A. The role of K-ras gene mutation analysis in EUS-guided FNA cytology specimens for the differential diagnosis of pancreatic solid masses: a meta-analysis of prospective studies. Gastrointest Endosc. 2013;78(4):596-608.

[114] Mishra G, Zhao Y, Sweeney J, Pineau BC, Case D, Ho C, Blackstock AW, Geisinger K, Howerton R, Levine E, Shen P, Ibdah J. Determination of qualitative telomerase activity as an adjunct to the diagnosis of pancreatic adenocarcinoma by EUS-guided fine-needle aspiration. Gastrointest Endosc. 2006;63(4):648–65

[115] Endo H, Saito T, Kenjo A, Hoshino M, Terashima M, Sato T, Anazawa T, Kimura T, Tsuchiya T, Irisawa A, Ohira H, Hikichi T, Takagi T, Gotoh M. Phase I trial of preoperative intratumoral injection of immature dendritic cells and OK-432 for resectable pancreatic cancer patients. J Hepatobiliary Pancreat Sci. 2012;19(4):465-75.

[116] Seo DW. EUS-Guided Antitumor Therapy for Pancreatic Tumors. Gut Liver. 2010; 4(Suppl 1): S76–S81.

[117] Hirooka Y, Itoh A, Kawashima H, Hara K, Nonogaki K, Kasugai T, Ohno E, Ishikawa T, Matsubara H, Ishigami M, Katano Y, Ohmiya N, Niwa Y,Yamamoto K, Kaneko T, Nieda M, Yokokawa K, Goto H. A combination therapy of gemcitabine with immunotherapy for patients with inoperable locally advanced pancreatic cancer. Pancreas. 2009;38(3):e69-74.

[118] Chang KJ, Nguyen PT, Thompson JA, Kurosaki TT, Casey LR, Leung EC, Granger GA. Phase I clinical trial of allogeneic mixed lymphocyte culture (cytoimplant) delivered by endoscopic ultrasound-guided fine-needle injection in patients with advanced pancreatic carcinoma. Cancer. 2000;88(6):1325-35.

[119] Hecht JR, Bedford R, Abbruzzese JL, Lahoti S, Reid TR, Soetikno RM, Kirn DH, Freeman SM. A phase I/II trial of intratumoral endoscopic ultrasound injection of ONYX-015 with intravenous gemcitabine in unresectable pancreatic carcinoma. Clin Cancer Res. 2003;9(2):555-61.

[120] Chang KJ, Lee JG, Holcombe RF, Kuo J, Muthusamy R, Wu ML. Endoscopic ultrasound delivery of an antitumor agent to treat a case of pancreaticcancer. Nat Clin Pract Gastroenterol Hepatol. 2008;5(2):107-11.

[121] Hecht JR, Farrell JJ, Senzer N, Nemunaitis J, Rosemurgy A, Chung T, Hanna N, Chang KJ, Javle M, Posner M, Waxman I, Reid A, Erickson R, Canto M, Chak A, Blatner G, Kovacevic M, Thornton M. EUS or percutaneously guided intratumoral TNFerade biologic with 5-fluorouracil and radiotherapy for first-line treatment of locally advanced pancreatic cancer: a phase I/II study. Gastrointest Endosc. 2012;75(2): 332-8.

[122] Herman JM, Wild AT, Wang H, Tran PT, Chang KJ, Taylor GE, Donehower RC, Pawlik TM, Ziegler MA, Cai H, Savage DT, Canto MI, Klapman J, Reid T, Shah RJ, Hoffe SE, Rosemurgy A, Wolfgang CL, Laheru DA.Randomized phase III multi-institutional study of TNFerade biologic with fluorouracil and radiotherapy for locally advanced pancreatic cancer: final results. J Clin Oncol. 2013;31(7):886-94.

[123] Wakatsuki T, Irisawa A, Imamura H, Terashima M, Shibukawa G, Takagi T, Takahashi Y, Sato A, Sato M, Ikeda T, Suzuki R, Hikichi T, Obara K, Ohira H. Complete re-

sponse of anaplastic pancreatic carcinoma to paclitaxel treatment selected by chemosensitivity testing. Int J Clin Oncol. 2010;15(3):310-3.

[124] Goldberg SN, Mallery S, Gazelle GS, Brugge WR. EUSguided radiofrequency ablation in the pancreas: results in a porcine model. Gastrointest Endosc 1999; 50: 392-401

[125] Carrara S, Arcidiacono PG, Albarello L, Addis A, Enderle MD, Boemo C, Neugebauer A, Campagnol M, Doglioni C, Testoni PA. Endoscopic ultrasound-guided application of a new internally gas-cooled radiofrequency ablation probe in the liver and spleen of an animal model: a preliminary study. Endoscopy 2008; 40(4): 759-763

[126] Gaidhane M, Smith I, Ellen K, Gatesman J, Habib N, Foley P, Moskaluk C, Kahaleh M. Endoscopic Ultrasound-Guided Radiofrequency Ablation (EUS-RFA) of the Pancreas in a Porcine Model. Gastroenterol Res Pract. 2012;2012:431451.

[127] Kim HJ, Seo DW, Hassanuddin A, Kim SH, Chae HJ, Jang JW, Park do H, Lee SS, Lee SK, Kim MH. EUS-guided radiofrequency ablation of the porcine pancreas. Gastrointest Endosc. 2012;76(5):1039-43.

[128] Arcidiacono PG, Carrara S, Reni M, Petrone MC, Cappio S, Balzano G, Boemo C, Cereda S, Nicoletti R, Enderle MD, Neugebauer A, von Renteln D, Eickhoff A, Testoni A. Feasibility and safety of EUS-guided cryothermal ablation in patients with locally advanced pancreatic cancer. Gastrointest Endosc. 2012;76(6):1142-51.

[129] Di Matteo F, Martino M, Rea R, Pandolfi M, Rabitti C, Masselli GM, Silvestri S, Pacella CM, Papini E, Panzera F, Valeri S, Coppola R, Costamagna G. EUS-guided Nd:YAG laser ablation of normal pancreatic tissue: a pilot study in a pig model. Gastrointest Endosc. 2010;72(2):358-63.

[130] Yusuf TE, Matthes K, Brugge WR. EUS-guided photodynamic therapy with verteporfin for ablation of normal pancreatictissue: a pilot study in a porcine model (with video).Gastrointest Endosc. 2008;67(6):957-61.

[131] Jin Z, Du Y, Li Z, Jiang Y, Chen J, Liu Y. Endoscopic ultrasonography-guided interstitial implantation of iodine 125-seeds combined with chemotherapy in the treatment of unresectable pancreatic carcinoma: a prospective pilot study. Endoscopy. 2008;40(4):314–320.

[132] Sun S, Xu H, Xin J, Liu J, Guo Q, Li S. Endoscopic ultrasound-guided interstitial brachytherapy of unresectable pancreatic cancer: results of a pilot trial. Endoscopy. 2006;38(4):399–403.

[133] Khashab MA, Kim KJ, Tryggestad EJ, Wild AT, Roland T, Singh VK, Lennon AM, Shin EJ, Ziegler MA, Sharaiha RZ, Canto MI, Herman JM. Comparative analysis of traditional and coiled fiducials implanted during EUS forpancreatic cancer patients receiving stereotactic body radiation therapy. Gastrointest Endosc. 2012;76(5):962-71.

[134] Park WG, Yan BM, Schellenberg D, Kim J, Chang DT, Koong A, Patalano C, Van Dam J. EUS-guided gold fiducial insertion for image-guided radiation therapy of

pancreatic cancer: 50 successful cases without fluoroscopy. Gastrointest Endosc. 2010;71(3):513-8.

[135] Majumder S, Berzin TM, Mahadevan A, Pawa R, Ellsmere J, Sepe PS, Larosa SA, Pleskow DK, Chuttani R, Sawhney MS. Endoscopic ultrasound-guided pancreatic fiducial placement: how important is ideal fiducial geometry? Pancreas. 2013;42(4):692-5.

[136] Varadarajulu S, Trevino JM, Shen S, Jacob R. The use of endoscopic ultrasound-guided gold markers in image-guided radiation therapy of pancreatic cancers: a case series. Endoscopy. 2010;42(5):423-5.

[137] Sanders MK, Moser AJ, Khalid A, Fasanella KE, Zeh HJ, Burton S, McGrath K. EUS-guided fiducial placement for stereotactic body radiotherapy in locally advanced and recurrent pancreatic cancer. Gastrointest Endosc. 2010;71(7):1178-84.

[138] Soweid AM, Azar C. Endoscopic ultrasound-guided celiac plexus neurolysis. World J Gastrointest Endosc 2010; 2(6):228-31.

[139] Levy MJ, Chari ST, Wiersema MJ. Endoscopic ultrasound-guided celiac neurolysis. Gastrointest Endoscopy Clin N Am 2012; 22(2):231–247.

[140] Puli SR, Reddy JB, Bechtold ML, Antillon MR, Brugge WR. EUS-guided celiac plexus neurolysis for pain due to chronic pancreatitis or pancreatic cancer pain: a meta-analysis and systematic review. Dig Dis Sci 2009; 54(11): 2330-2337.

[141] Kaufman M, Singh G, Das S, Concha-Parra R, Erber J, Micames C, Gress F. Efficacy of endoscopic ultrasound-guided celiac plexus block and celiac plexus neurolysis for managing abdominal pain associated with chronic pancreatitis and pancreatic cancer. J Clin Gastroenterol 2010; 44(2):127-134.

[142] Wiersema MJ, Wiersema. Endosonography-guided celiac plexus neurolysis. Gastrointest Endosc 1996; 44:656-62

[143] Gunaratnam NT, Sarma AV, Norton ID, Wiersema MJ. A prospective study of EUS-guided celiac plexus neurolysis for pancreatic cancer pain. Gastrointest Endosc 2001; 54(3):316-324.

[144] Sahai AV, Lemelin V, Lam E, Paquin SC. Central vs. bilateral endoscopic ultrasound-guided celiac plexus block or neurolysis: a comparative study of short-term effectiveness. Am J Gastroenterol 2009; 104(2):326-329.

[145] Iwata K, Yasuda I, Enya M, Mukai T, Nakashima M, Doi S, Iwashita T, Tomita E, Moriwaki H. Predictive factors for pain relief after endoscopic ultrasound-guided celiac plexus neurolysis. Dig Endos 2011; 23(2):140-145.

[146] Ramirez-Luna M, Chavez-Tapia N, Franco-Guzman A, Garcia-Saenz-de Sicilia M, Tellez-Avila F. Endoscopic ultrasound-guided celiac plexus neurolysis in patients with unresectable pancreatic cancer. Rev Gastroenterol Mex 2008; 73(2):63-67.

[147] Leblanc JK, Al-Haddad M, McHenry L, Sherman S, Juan M, McGreevy K, Johnson C, Howard TJ, Lillemoe KD, DeWitt J. A prospective, randomized study of EUS-guided

celiac plexus neurolysis for pancreatic cancer: one injection or two? Gastrointest Endosc 2011; 74(6):1300-1307.

[148] Seicean A, Cainap C, Gulei I, Tantau M, Seicean R. Pain palliation by endoscopic ultrasound-guided celiac plexus neurolysis in patients with unresectable pancreatic cancer. J Gastrointestin Liver Dis 2013; 22(1):59-64

[149] Leblanc JK, Rawl S, Juan M, Johnson C, Kroenke K, McHenry L, Sherman S, McGreevy K, Al-Haddad M, Dewitt J. Endoscopic ultrasound-guided celiac plexus neurolysis in pancreatic cancer: a prospective pilot study of safety using 10 mL versus 20 mL alcohol. Diagn Ther Endosc 2013;2013:327036.

[150] Levy MJ, Topazian MD, Wiersema MJ, Clain JE, Rajan E, Wang KK, de la Mora JG, Gleeson FC, Pearson RK, Pelaez MC, Petersen BT, Vege SS, Chari ST. Initial evaluation of the efficacy and safety of endoscopic ultrasound-guided direct ganglia neurolysis and block. Am J Gastroenterol 2008; 103(1):98-103

[151] Wyse JM, Carone M, Paquin SC, Usatii M, Sahai AV. Randomized, double-blind, controlled trial of early endoscopic ultrasound-guided celiac plexus neurolysis to prevent pain progression in patients with newly diagnosed, painful, inoperable pancreatic cancer. J Clin Oncol 2011;29(26):3541-3546.

[152] McGreevy K, Hurley RW, Erdek MA, Aner MM, Li S, Cohen SP. The effectiveness of repeat celiac plexus neurolysis for pancreatic cancer: a pilot study. Pain Pract 2012; 13(2):89-95

[153] Ascunce G, Ribeiro A, Reis I, Sleeman D, Merchan J, Levi J. EUS visualization and direct celiac ganglia neurolysis predicts better pain relief in patients with pancreatic malignancy (with video). Gastrointest Endosc 2011; 73(2):267-274.

[154] Sakamoto H, Kitano M, Komaki T, Kudo M. Endoscopic ultrasound-guided neurolysis in pancreatic cancer. Pancreatology 2011; 11 Suppl 2:52-8

[155] Fujii L, Clain JE, Morris JM, Levy MJ. Anterior spinal cord infarction with permanent paralysis following endoscopic ultrasound celiac plexus neurolysis. Endoscopy 2012; 44 Suppl 2 UCTN:E265-6.

[156] Mittal MK, Rabinstein AA, Wijdicks EF. Pearls & oy-sters: Acute spinal cord infarction following endoscopic ultrasound-guided celiac plexus neurolysis. Neurology 2012; 78:e57-9.

[157] Ahmed HM, Friedman SE, Henriques HF, Berk BS. End-organ ischemia as an unforeseen complication of endoscopic-ultrasound-guided celiac plexus neurolysis. Endoscopy 2009; 41 Suppl 2:E218-9

[158] Loeve US, Mortensen MB. Lethal necrosis and perforation of the stomach and the aorta after multiple EUS-guided celiac plexus neurolysis procedures in a patient with chronic pancreatitis.Gastrointest Endosc 2012; 77(1):151-2.

[159] Shah JN, Marson F, Weilert F, Bhat YM, Nguyen-Tang T, Shaw RE, Binmoeller KF. Single-operator, single-session EUS-guided anterograde cholangiopancreatography in failed ERCP or inaccessible papilla. Gastrointest Endosc. 2012 75(1):56-64.

[160] Kim YS, Gupta K, Mallery S, Li R, Kinney T, Freeman ML Endoscopic ultrasound rendezvous for bile duct access using a transduodenal approach: cumulative experience at a single center. A case series. Endoscopy. 2010;42(6):496-502.

[161] Song TJ, Hyun YS, Lee SS, Park do H, Seo DW, Lee SK, Kim MH. Endoscopic ultrasound-guided choledochoduodenostomies with fully covered self-expandable metallic stents. World J Gastroenterol. 2012; 28;18(32):4435-40.

[162] Kahaleh M, Hernandez AJ, Tokar J, Adams RB, Shami VM, Yeaton P. Interventional EUS-guided cholangiography: evaluation of a technique in evolution. Gastrointest Endosc 2006; 64(1): 52-59

[163] Brauer BC, Chen YK, Fukami N, Shah RJ. Single-operator EUS-guided cholangiopancreatography for difficult pancreaticobiliary access (with video). Gastrointest Endosc 2009; 70(3): 471-479

[164] Burmester E, Niehaus J, Leineweber T, Huetteroth T. EUS-cholangio-drainage of the bile duct: report of 4 cases. Gastrointest Endosc. 2003;57(2):246-51.

[165] Kim TH, Kim SH, Oh HJ, Sohn YW, Lee SO.Endoscopic ultrasound-guided biliary drainage with placement of a fully covered metal stent for malignant biliary obstruction. World J Gastroenterol. 2012; 18(20):2526-32

[166] Park do H, Jang JW, Lee SS, Seo DW, Lee SK, Kim MH.EUS-guided biliary drainage with transluminal stenting after failed ERCP: predictors of adverse events and long-term results. Gastrointest Endosc. 2011; 74(6):1276-84.

[167] Bories E, Pesenti C, Caillol F, Lopes C, Giovannini M. Transgastric endoscopic ultrasonography-guided biliary drainage: results of a pilot study. Endoscopy. 2007;39(4): 287-91.

[168] Itoi T, Binmoeller K, Itokawa F, Umeda J, Tanaka R. Endoscopic ultrasonography-guided cholecystogastrostomy using a lumen-apposing metal stent as an alternative to extrahepatic bile duct drainage in pancreatic cancer with duodenal invasion.Dig Endosc. 2013;25 Suppl 2:137-41

[169] Itoi T, Itokawa F, Tsuchiya T, Tsuji S, Tonozuka R. Endoscopic ultrasound-guided choledochoantrostomy as an alternative extrahepatic bile duct drainage method in pancreatic cancer with duodenal invasion. Dig Endosc. 2013;25 Suppl 2:142-5.

[170] Tonozuka R, Itoi T, Sofuni A, Itokawa F, Moriyasu F. Endoscopic double stenting for the treatment of malignant biliary and duodenal obstruction due to pancreatic cancer. Dig Endosc. 2013;25 Suppl 2:100-8.

[171] Park do H, Koo JE, Oh J, Lee YH, Moon SH, Lee SS, Seo DW, Lee SK, Kim MH. EUS-guided biliary drainage with one-step placement of a fully covered metal stent for

malignant biliary obstruction: a prospective feasibility study. Am J Gastroenterol. 2009;104(9):2168-74.

[172] Hara K, Yamao K, Niwa Y, Sawaki A, Mizuno N, Hijioka S, Tajika M, Kawai H, Kondo S, Kobayashi Y, Matumoto K, Bhatia V, Shimizu Y, Ito A, Hirooka Y,Goto H. Prospective clinical study of EUS-guided choledochoduodenostomy for malignant lower biliary tract obstruction. Am J Gastroenterol. 2011;106(7):1239-45.

[173] Artifon EL, Takada J, Okawa L, Moura EG, Sakai P. EUS-guided choledochoduodenostomy for biliary drainage in unresectable pancreatic cancer: a case series. JOP. 2010;11(6):597-600.

[174] Ang TL, Teo EK, Fock KM. EUS-guided transduodenal biliary drainage in unresectable pancreatic cancer with obstructive jaundice. JOP. 2007 ;8(4):438-43

[175] Iwamuro M, Kawamoto H, Harada R, Kato H, Hirao K, Mizuno O, Ishida E, Ogawa T, Okada H, Yamamoto K. Combined duodenal stent placement and endoscopic ultrasonography-guided biliary drainage for malignant duodenal obstruction with biliary stricture. Dig Endosc. 2010;22(3):236-40.

[176] Siddiqui AA, Sreenarasimhaiah J, Lara LF, Harford W, Lee C, Eloubeidi MA. Endoscopic ultrasound-guided transduodenal placement of a fully covered metal stent for palliative biliary drainage in patients with malignant biliary obstruction. Surg Endosc. 2011;25(2):549-55.

[177] Belletrutti PJ, DiMaio CJ, Gerdes H, Schattner MA. Endoscopic ultrasound guided biliary drainage in patients with unapproachable ampullae due to malignant duodenal obstruction. J Gastrointest Cancer. 2011;42(3):137-42.

[178] Nguyen-Tang T, Binmoeller KF, Sanchez-Yague A, Shah JN. Endoscopic ultrasound (EUS)-guided transhepatic anterograde self-expandable metal stent (SEMS) placement across malignant biliary obstruction. Endoscopy. 2010 ;42(3):232-6.

[179] Hanada K, Iiboshi T, Ishii Y. Endoscopic ultrasound-guided choledochoduodenostomy for palliative biliary drainage in cases with inoperable pancreas head carcinoma. Dig Endosc. 2009;21 Suppl 1:S75-8.

[180] Kahaleh M, Hernandez AJ, Tokar J, Adams RB, Shami VM, Yeaton P. Interventional EUS-guided cholangiography: evaluation of a technique in evolution. Gastrointest Endosc 2006; 64: 52-59

[181] Yamao K, Sawaki A, Takahashi K, Imaoka H, Ashida R, Mizuno N. EUS-guided choledochoduodenostomy for palliative biliary drainage in case of papillary obstruction: report of 2 cases. Gastrointest Endosc. 2006;64(4):663-7.

[182] Püspök A, Lomoschitz F, Dejaco C, Hejna M, Sautner T, Gangl A. Endoscopic ultrasound guided therapy of benign and malignant biliary obstruction: a case series. Am J Gastroenterol. 2005;100(8):1743-7

[183] Mallery S, Matlock J, Freeman ML EUS-guided rendezvous drainage of obstructed biliary and pancreatic ducts: Report of 6 cases. Gastrointest Endosc. 2004; 59(1):100-7

[184] Steinberg WM, Barkin JS, Bradley EL 3rd, DiMagno E, Layer P, Canto MI, Levy MJ. Should patients with a strong family history of pancreatic cancer be screened on a periodic basis for cancer of the pancreas? Pancreas. 2009; 38(5):e137-50

[185] Vasen HF, Wasser M, van Mil A, Tollenaar RA, Konstantinovski M, Gruis NA, Bergman W, Hes FJ, Hommes DW, Offerhaus GJ, Morreau H, Bonsing BA, de Vos tot Nederveen Cappel WH. Magnetic resonance imaging surveillance detects early-stage pancreatic cancer in carriers of a p16-Leiden mutation. Gastroenterology. 2011;140(3): 850–6.

[186] Poley JW, Kluijt I, Gouma DJ, Harinck F, Wagner A, Aalfs C, van Eijck CH, Cats A, Kuipers EJ, Nio Y, Fockens P, Bruno MJ. The yield of first-time endoscopic ultrasonography in screening individuals at a high risk of developing pancreatic cancer.Am J Gastroenterol. 2009; 104(9):2175-81.

[187] Canto MI, Hruban RH, Fishman EK, Kamel IR, Schulick R, Zhang Z, Topazian M, Takahashi N, Fletcher J, Petersen G, Klein AP, Axilbund J, Griffin C, Syngal S, Saltzman JR, Mortele KJ, Lee J, Tamm E, Vikram R, Bhosale P, Margolis D, Farrell J, Goggins M; American Cancer of the Pancreas Screening (CAPS) Consortium. Frequent detection of pancreatic lesions in asymptomatic high-risk individuals. Gastroenterology. 2012 ;142(4):796-804; quiz e14-5.

[188] Zubarik R, Gordon SR, Lidofsky SD, Anderson SR, Pipas JM, Badger G, Ganguly E, Vecchio J. Screening for pancreatic cancer in a high-risk population with serum CA 19-9 and targeted EUS: a feasibility study. Gastrointest Endosc. 2011;74(1):87-95.

[189] Langer P, Kann PH, Fendrich V, Habbe N, Schneider M, Sina M, Slater EP, Heverhagen JT, Gress TM, Rothmund M, Bartsch DK. Five years of prospective screening of high-risk individuals from families with familial pancreatic cancer. Gut. 2009;58(10): 1410-8.

[190] Canto MI, Harinck F, Hruban RH, Offerhaus GJ, Poley JW, Kamel I, Nio Y, Schulick RS, Bassi C, Kluijt I, Levy MJ, Chak A, Fockens P, Goggins M, Bruno M; International Cancerof Pancreas Screening (CAPS) Consortium. International Cancer of the Pancreas Screening (CAPS) Consortium summit on the management of patients with increased risk for familial pancreatic cancer. Gut. 2013;62(3):339-47.

Chapter 6

Select Population-Based and Translational Research for Improving Pancreatic Cancer Early Detection

Shiro Urayama

Additional information is available at the end of the chapter

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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) comprises majority of pancreatic neoplasm and remains to pose an enormous challenge to patients and clinicians with the worst survival rate among all major malignancies. PDAC is the fourth leading cause overall and second leading cause of gastrointestinal cancer death in the United States. [1] It is estimated that 45,220 new cases and 38,460 deaths would result from pancreatic cancer in the United States in 2013. [2] Worldwide, there were more than 277,668 new cases and 266,029 deaths from this cancer in 2008. [3] In comparison to other major malignancies such as breast, colon, lung and prostate cancers with their respective 89, 64, 16, 99% 5-yr survival rate, PDAC at 6% is conspicuously low[2]. For PDAC, the only curative option is surgical resection, which is applicable in only 10–15% of patients due to the common discovery of late stage at diagnosis. [4] In fact, PDAC is notorious for late stage discovery as evidenced by the low percentage of localized disease at diagnosis, compared to other major malignancies: breast (61%), colon (40%), lung (16%), ovarian (19%), prostate (91%), and pancreatic cancer (7%) [5].

With the high contribution of late-stage discovery and general lack of effective medical therapy, one critical approach in reversing the poor outcome of pancreatic cancer is to develop an early detection scheme for the tumor. Despite the poor prognosis of the disease, for those who have undergone curative resection with negative margins, the 5-year survival rate is 22% in contrast to 2% for the advanced-stage with distant metastasis. [6, 7] An earlier diagnosis with tumor less than 2 cm (T1) is associated with a better 5-yr survival of 58% compared to 17% for stage IIB PDAC. [8] Ariyama, et al showed 100% survival in 79 patients with tumors less than 1 cm undergoing curative resection. [9] Also as the recent report indicates, the estimated time from the transformation to pre-metastatic growths of pancreatic cancer is

approximately 15 years [10], there is a wide potential window of opportunity to apply developing technologies in early detection of this cancer.

In this article, we will discuss the current status of the PDAC cancer detection/diagnostic modalities and ongoing research endeavors in developing early detection schemes for this devastating disease.

2. Current status of PDAC cancer detection and diagnosis — Imaging-based tests

As clinical symptoms of early stages of PDAC is commonly nonspecific and as currently available clinical markers such as CA19-9, CEA, have low sensitivity and specificity at early stage disease 11, clinicians who are suspecting the occurrence of PDAC in a patient rely heavily on diagnostic imaging tests for assessment of a potential tumor.

Over the past few decades, endoscopic ultrasound (EUS) has proven itself to be a superior imaging modality for detection of a small or early-stage pancreatic neoplasm as compared to others such as transabdominal ultrasound (US), computed tomography (CT), endoscopic retrograde cholangiopancreatography (ERCP), magnetic resonance imaging (MRI), positron emission tomography (PET) and angiography. [12, 13, 14, 15, 16, 17] Yasuda and Rosch had initially demonstrated the superiority of EUS in detection of pancreatic lesions <2 cm in diameter. [12, 18] More recently, De Witt, et al had verified the superiority of EUS as compared to multi-detector CT scan. In their study, the sensitivity of endoscopic ultrasonography (98% [95% CI=91% to 100%]) for detecting a pancreatic mass (of any size) was significantly greater than that of CT images (86% [CI=77% to 93%]; p=0. 012) [13]. In another study, Khashab, et al demonstrated that the sensitivity of EUS in detecting pancreatic tumor was greater than CT (91. 7% vs. 63. 3%; P=. 0002) and particularly for pancreatic neuroendocrine tumors (84. 2% vs. 31. 6%; P=. 001), which commonly consist of smaller pancreatic lesions. Furthermore, EUS detected 20 of 22 CT-negative tumors (91%) in this study. [14] In a retrospective study published by Klapman, et al, EUS diagnosis of pancreatic cancer was found to be highly specific with a negative predictive value (NPV) of 100%. Following the EUS examination, no work-up was required in 119/135 (88%) of patients. [15]

A challenge in imaging-based studies remains to be distinguishing pancreatic malignant lesions from chronic inflammatory changes. Bhutani, et al reviewed 20 cases of missed pancreatic cancer on EUS evaluation in a multicenter retrospective study. They found missed neoplasms in patients with chronic pancreatitis, recent episodes of acute pancreatitis, diffusely infiltrating carcinoma, or a prominent ventral/dorsal split. [16] Conventional power Doppler EUS has some utility in this regard; Sa`ftoiu, et al in a study of 42 patients showed that absence of power Doppler signals inside a suspicious pancreatic mass had a sensitivity of 93% and a specificity of 77%, with an accuracy of 88% in the diagnosis of pancreatic cancer. In the presence of peripancreatic collaterals, the sensitivity and specificity for the diagnosis of pancreatic cancer rose to 97% and 92%, respectively, with an accuracy of 95%. [17]

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Elastography is a newer EUS imaging modality used for the real-time visualization of tissue elasticity, and it demonstrates the difference in tissue stiffness between diseased and normal regions. [19, 20] Tumor is commonly stiffer than the normal surrounding tissue, and this characteristic is utilized in the determination of presence of neoplastic lesion, including pancreatic cancer. [21] Giovannini, et al tested this method for the differential diagnosis of benign and malignant lymph nodes and focal pancreatic masses in a small study of 49 patients and showed a sensitivity and specificity of 100% and 67% for the diagnosis of malignant pancreatic lesions. They concluded that this technique could be used to guide biopsy sampling for PDAC diagnosis. [22]

Contrast enhancing agents such as galactose microparticles (Levovist) and sulfur hexafluoride microparticles (SonoVue, a second-generation agent) have been applied in the diagnosis of pancreatic malignancy by assessing the differential vascular perfusion in the pancreatic mass. [23, 24] Hocke, et al reported the differentiation of inflammation versus pancreatic carcinoma based on perfusion characteristics of the microvessels. [25] By using the contrast-enhanced EUS, the sensitivity of the diagnosis of malignant pancreatic lesion with chronic inflammatory pancreatic disease increased to 91. 1% (in 51 of 56 patients) and the specificity to 93. 3% (in 28 of 30 patients) in comparison to conventional EUS sensitivity and specificity of 73. 2% and 83. 3%, respectively. Applicability of an additional modality such as the low mechanical index contrast-enhanced imaging (wide band harmonic imaging) technique has been reported in 6 patients by Dietrich, et al with good arterial, portal venous and parenchymal contrast enhancement. [26] Further study for accuracy of this particular diagnostic testing is anticipated.

2.1. EUS-guided Fine Needle Aspiration (FNA) in pancreatic cancer

Studies have shown that the accuracy of EUS-FNA is better compared to both ERCP brushings and CT-or transabdominal ultrasound-guided FNA for the PDAC diagnosis. [27, 28] EUS-FNA has reported success rates of 90–95%, with an overall sensitivity and specificity of 90% and near-100%, respectively. [29, 30, 31, 32] The main advantage of EUS-guidance is the ability to visualize and target small pancreatic masses. Lesions of 5 mm or less could be visualized and sampled, which might not have been accessible or identifiable by other imaging modalities. [33] Krishna, et al, in a review of 213 patients, found EUS-guided FNA to be highly accurate for diagnosing malignancy in patients with a focal pancreatic lesion noted on CT scan/MRI without obstructive jaundice. EUS-FNA had 97. 6% accuracy for diagnosing a malignant neoplasm, with 96. 6% sensitivity, 99. 0% specificity, 96. 2% negative predictive value, and 99. 1% positive predictive value. [34] Agarwal, et al compared 81 consecutive patients who underwent EUS, EUS-FNA and spiral CT with a multiphasic pancreatic protocol for clinical suspicion of PDAC. They showed that the accuracy of spiral CT, EUS, and EUS-FNA was 74% (n=60/81, CI 63-83%), 94% (n=76/81, CI 87-98%), and 88% (n=73/81, CI 81-96%), respectively, for detecting pancreatic cancer. In their study, absence of a focal lesion on EUS reliably excluded pancreatic cancer irrespective of clinical presentation (NPV 100% n=5/5, CI 48-100%). [35]

From a practical standpoint, tumor cell seeding of the FNA tract is rare and only a few EUS cases have been reported. Micames, et al in their study demonstrated that EUS-FNA has a

lower risk of peritoneal contamination with malignancy than CT-guided FNA (2. 2% versus 16. 3%), respectively. [36] This is a potential complication of EUS-FNA that would need to be kept in mind by clinicians when FNA sampling of a lesion is being considered. [37, 38]

3. Molecular markers & pancreatic cancer

In order to enhance the diagnostic accuracy of PDAC, molecular markers on EUS-FNA samples have been evaluated in recent years. Utilities of DNA mutations such as *k-ras* and loss of heterozygosity are being reported as potential surrogate markers of the malignancy. [39, 40] In a recent study, Takahashi, et al assessed *k-ras* point mutations in PDAC and chronic focal pancreatitis samples obtained by EUS-FNA. [41, 42, 43] The study revealed the presence of point mutations of *k-ras* in 74% of patients with PDAC compared to no mutations in chronic focal pancreatitis. In another study, Tada, et al reported a high (more than 2% of total *k-ras* gene) mutation rate in 20 of 26 cases of EUS-FNA specimens (77%) and in 12 of 19 cases of pancreatic juice (63%) in PDAC. [44] However, the presence of *k-ras* mutations in chronic pancreatitis and premalignant conditions such as intraductal papillary mucinous neoplasm as well as lack of such mutations in 20% of pancreatic cancer has limitations for using this test solely as a diagnostic tool. Other studies analyzing p53 by immunohistochemistry, [45] telomerase activity with a ribonucleoprotein enzyme, [46] and a broad panel of microsatellite allele loss markers demonstrated similar results. [47] In the presence of inconclusive EUS-FNA cytology, molecular markers could complement EUS-FNA cytology results to help establish the diagnosis of malignancy.

4. Select population-based research for early detection scheme development

4.1. Screening for pancreatic cancer in high-risk individuals

Currently, a general population-screening program for PDAC is not cost-effective because of low relative disease incidence and non-availability of simple, cheap, highly accurate non-invasive tests. The main aim of the screening is to detect clinically significant precursor lesions or early stage PDAC. However, since the overwhelming majority of premalignant lesions and small pancreatic cancers are asymptomatic, we do not yet have a routinely utilized surrogate marker to identify a subset population for screening. Consequently, as one of the approaches in investigating the genetic risks, research has focused on investigating a subset of individuals with a higher-risk for PDAC development in order to elucidate the genetic predilection. Up to 10% of pancreatic cancer patients have a familial basis and they have increased risk of developing both pancreatic and extra-pancreatic malignancies. [48, 49, 50, 51, 52] Classic categorization of high-risk patients are based on the highly associated genetic risks defined as those who are either members of a family with at least two first-degree relatives affected by

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the disease or are part of an inherited pancreatic cancer syndrome with a known genetic mutation. (Table 1)

Syndrome	Inheritance	Gene Mutation	Risk of PDAC
Peutz-Jeghers syndrome [53]	Autosomal dominant (AD)	STK11/LKB1	Standardized Incidence Ratio (SIR) = 132
Hereditary Pancreatitis [54, 55, 56]	AD	PRSS1 SPINK1	Odds ratio (OR) = 69. 9
Familial atypical multiple mole melanoma syndrome [57, 58, 59]	AD	CDKN2A	SIR=13-38
Hereditary breast-ovarian cancer syndrome [60, 61, 62, 63, 64, 65, 66]	AD	BRCA2 BRCA1	BRCA2: OR=3. 5-10-fold increased risk BRCA1: OR=2. 26 times average population
Lynch syndrome [59, 67]	AD	MLH1, MSH2, MSH6 or PMS2	SIR = up to 8. 6
Cystic fibrosis [68]	Autosomal recessive	CFTR	OR = 5. 3-6. 6

Table 1. PDAC related genetic syndromes

4.1.1. Familial pancreatic cancer

Familial pancreatic cancer (FPC) cohort is distinguished by individuals with a strong family history of PDAC-i. e. with the cancer in at least two first-degree relatives and individuals with three or more affected family members (one of whom must be a first-degree relative) – and is considered to be high-risk and a candidate for screening programs. [69, 70, 71] Currently, the genetic basis for most cases of FPC is not fully understood. However, various studies have demonstrated the presence of a germline mutation in the BRCA2 gene [61, 62, 63, 64], association of BRCA1 [72], paladin gene mutation [73] and involvement of other genes: apolipoprotein A4, CEA, keratin 19, stratifin (14-3-3σ), trefoil factor, and calcium binding protein S100 A6 [74, 75] in FPC, and more recently identification of PALB2, [76] as a pancreatic cancer susceptibility gene. These facts suggest that multiple and heterogeneous factors are likely at play for the genesis of PDAC in this subset.

Analysis of the PDAC kindred data from Johns Hopkins' National Familial Pancreas Tumor Registry (NFPTR) has demonstrated that the relative risk of PDAC in persons with two affected first-degree relatives is 6. 4% and the cumulative life-time risk is 8%-12%; in individuals with three affected first-degree relatives, the relative risk for PDAC increases to 32% and the cumulative life-time risk to 16%-32%. [77] Tersmette, et al in their analysis of the NFPTR found

an 18-fold increase in risk of PDAC, and an estimated lifetime risk of 9%-18% in the group. [78] Brune, et al in their recent article reported a higher risk of PDAC among members of FPC kindred with a younger age of onset (age < 50 years). [79] Rulyak, et al in another study found smoking as a strong risk factor in FPC kindred, particularly among males and those under age 50. This risk increases by 2. 0-3. 7 times over the inherited predisposition and lowers the age of onset by 10 years. [80] A computer-based risk assessment tool, PancPRO, has been developed and is available for calculating the risk assessment for individuals with familial pancreatic cancer (http://www4. utsouthwestern. edu/breasthealth/cagene/default. asp). [81]

4.1.2. Screening modalities & the current screening programs

Most of the screening programs have tried to use biomarkers complemented by imaging tests to identify the early lesions. As stated earlier, a commonly used marker, CA19-9, is neither specific nor sensitive independently for reliable detection of early pancreatic cancer or pancreatic precursor lesions. Kim, et al in their studies found only 0. 9% positive predictive value using a cut-off value of 37 U/mL. [82] Recently, many biomarkers have been investigated including MIC-1, CEACAM-1, SPan1, DUPAN, Alpha4GNT, and PAM4, but none is validated for routine clinical use. [83] In another approach, elevated fasting-glucose level has been shown to be a marker for early cancer in sporadic cases [84] and is currently used by the EUROPAC study in high-risk individuals with molecular analysis of pancreatic juice for the *k-ras* and *p53* mutations in addition to *p16* promoter methylation status.

Multiple international programs exist that screen for pancreatic cancer in high-risk individuals in a research setting. "Cancer of the Pancreas Screening Study" (CAPS study), led by John Hopkins University, is the largest screening program that involves 24 American Centers of Excellence. To date, three studies, CAPS 1, CAPS 2 and CAPS 3, have been completed. (Table 2)

In the CAPS 1 study, thirty-eight patients were studied; 31 (mean age, 58-yr; 42% men) from a kindred with > 3 affected with pancreatic cancer; 6 from a kindred with 2 affected relatives, and 1 was a patient with Peutz-Jeghers syndrome (PJS). Six pancreatic masses were found by EUS: 1 invasive ductal adenocarcinoma, 1 benign intraductal papillary mucinous neoplasm, 2 serous cystadenomas, and 2 nonneoplastic masses. In this study, the diagnostic yield of screening was 5. 3%. [85] In the CAPS 2 study a 10% diagnostic yield of screening for pre-invasive malignant lesions was found. [86] In this study, screening was performed using annual EUS and CT. If an abnormality was detected, ERCP was offered. Seventy-eight high-risk patients (72 from a FPC kindred, 6 PJS) and 149 control patients were studied. Of these, eight patients had confirmed pancreatic neoplasia by surgery or FNA (10% yield of screening); 6 patients had benign intraductal papillary mucinous neoplasms (IPMNs), 1 had an IPMN that progressed to invasive ductal adenocarcinoma, and 1 had high-grade pancreatic intraepithelial neoplasia (PanIN-3). The CAPS 3 study was a multicenter prospective, controlled cohort study that involved annual screening using EUS and MRCP, MRI with secretin and a panel of candidate DNA and protein markers in serum and pancreatic juice (CA19-9, macrophage inhibitory cytokine-1 (MIC-1), DNA hypermethylation, and *k-ras* gene mutations) as indicators of pancreatic neoplasm. Over 200 patients were enrolled over a three-year period. The study

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has recently been completed and the results on the detection modality comparison demonstrate that the EUS has the highest rate of detection of early neoplastic changes in up to 42. 6% of the asymptomatic high-risk group. [87]

In another study from the University of Washington, high-risk familial cohorts were screened using EUS and beginning 10 years prior to the earliest PDAC death in the family. If EUS was normal, then they were followed-up with a repeat EUS at 2-3 year intervals. In case of abnormal EUS findings, they were referred for ERCP and if abnormalities were noted, patients were offered surgical intervention. [88] Patients with abnormal EUS, but normal ERCP were offered annual EUS. Out of 75 subjects screened, 15 had abnormalities on EUS and ERCP and went to surgery. The histology revealed premalignant lesions in all: PanIN-3 in 10 cases and PanIN-2 in five. [89] This study gave a diagnostic yield of 13% (10 out of 75) for detecting PanIN-3 premalignant lesions. One patient developed unresectable pancreatic cancer while under annual surveillance.

In Europe, the European Registry for Familial Pancreatic Cancer and Hereditary Pancreatitis (EUROPAC) incorporated EUS, ERCP and molecular analysis of the pancreatic juice looking for early mutations (*p53*, *k-ras*, and *p16*), and the results are pending. A German Study (FaPaCa) enrolled 76 patients in a screening program using yearly EUS, MRCP and laboratory tests (genetic analysis of CDKN2a and BRCA2 genes, CA19-9 and CEA). Any suspicious lesion was evaluated with EUS ± FNA after 6 weeks and a close follow-up at 12 weeks. If an abnormality was detected, the patient underwent operative exploration with intraoperative ultrasound, limited pancreatic resection with frozen section, and if cancer was detected, total pancreatectomy was performed. Ten solid lesions were seen on EUS as compared to only seven detected by MRCP. Out of the seven MRCP-detected lesions, six had limited resections and the histology showed one patient with PanIN-3, one with PanIN-2, one with PanIN-1, and three were benign lesions. These results gave a diagnostic yield of 1. 3% in detecting PanIN-3. [90] A recent study from the Netherlands that used only EUS as the first screening modality in 44 high risk asymptomatic subjects showed a 7% diagnostic yield for asymptomatic cancers and a 16% diagnostic yield for premalignant lesions (IPMN-like lesions). [91]

Study	CAPS1	CAPS2	CAPS3	U of Washington	FaPaCa	Dutch Study
Diagnostic Yield*	5. 3 (2/38)	10 (8/78)	42 (92/216)	13% (10/75)	1. 3 (1/76	23 (10/44)

*Represents finding of abnormal imaging such as mass (solid, cyst) or abnormal duct

CAPS: Cancer of the Pancreas Screening Study; FaPaCa: Familial Pancreatic Cancer Study

Table 2. Results of screening programs for pancreatic cancer in high-risk groups

Questions remain regarding the cost-effectiveness of these screening modalities. Rulyak, et al reported that screening was cost-effective with an incremental cost-effectiveness ratio of $16,885/life-year saved (assuming a 20% incidence of dysplasia and a 90% sensitivity of EUS and ERCP). [92] Rubenstein, et al performed a systematic review, and created a Markov model

for 45-year-old male first-degree relatives, with findings of chronic pancreatitis on screening by EUS. They compared 4 strategies: do-nothing, prophylactic total pancreatectomy (PTP), annual surveillance by EUS, and annual surveillance with EUS and fine needle aspiration. In the do-nothing strategy, the lifetime risk of cancer was 20% and it provided the greatest remaining years of life, the lowest cost, and the greatest remaining quality-adjusted life years (QALYs). PTP provided the fewest remaining years of life and QALYs. Screening with EUS provided nearly identical results to PTP, and screening with EUS/FNA provided intermediate results between PTP and the do-nothing approach. Total pancreatectomy provided the longest life expectancy if the lifetime risk of PDAC was at least 46% and provided the most QALYs if the risk was at least 68%. [93] Further assessment of the models in other clinical scenarios with developing technology would be in order.

5. Future of pancreatic cancer screening

Current EUS screening programs have demonstrated that the endoscopic evaluation can detect premalignant lesions and early cancers in certain subsets of high-risk groups, although cost-effectiveness still remains an issue. However, as the majority of PDAC diagnosis is given to patients who develop the disease sporadically without a recognized genetic abnormality, the application of this modality for PDAC detection screening is very limited for the general adult population. In order to further delineate and expand the at-risk subset, there is a strong need for novel surrogate markers which allow identification of the group with increased PDAC risk for whom the endoscopic/imaging-based screening strategy could be applied.

5.1. Select population based research — Identification of a higher-PDAC-risk group

A practical approach for further selection of the potential screening population is to focus on selective clinical parameters that would be used to characterize the subset of the general population at increased PDAC risk. For instance, based on the epidemiological evidence, such clinical parameters include incidence of hyperglycemia or diabetes, which are being noted in 50-80% of pancreatic cancer patients [94, 95, 96, 97, 98]. Though this subset does not encompass all PDAC patients, this group includes a much larger proportion of PDAC patients whom we may select further to screen for PDAC. Similarly, patients with a history of chronic pancreatitis or obesity are reported to have increased PDAC risk during their lifetime [99, 100, 101, 102, 103, 104]. Animal studies investigating effects of diet-induced obesity in a PDAC mouse model demonstrated increased occurrence of pancreatic inflammation and accelerated pancreatic neoplastic changes, supporting the association of obesity and pancreatic inflammation and PDAC risks. [105, 106] Considering the millions of patients who are being diagnosed with diabetes, chronic pancreatitis, or obesity annually as opposed to PDAC, further refinement of screening of these patient groups is critically needed to justify developing a larger scale screening protocol in the future.

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5.2. Translational research — Application of systems biology approach

As we continue to translate the advancement of biological understanding of PDAC, we strongly anticipate that better biomarkers will become available in the near future that would identify higher-risk individuals within the general population for developing early-stage PDAC. Aside from the previously referenced reports, many genetic, epigenetic, proteomics, metabolomics, glycomics findings-utilizing systems biology approaches-are being considered for biomarker identifications for PDAC detection. In transcriptomics analysis of blood biomarkers in PDAC-associated diabetes mellitus, for example, gene expression analysis in blood from PDAC patients with new-onset diabetes versus long-term or no history of diabetes revealed a set of differentially expressed genes such as vanin-1 and matrix metalloproteinase 9, which are able to discriminate the PDAC group with sensitivity of 92% and specificity of 84%. [107] From proteomics analyses, shotgun approaches with highly accurate mass spectrometric assays demonstrated such proteins as apolipoprotein CIII [108], mannose-binding lectin 2, myosin light chain kinase 2 [109], CXC chemokine ligand 7 [110], TIMP1-ICAM1 [111], and alpha-1 antitrypsin [112] as candidate biomarkers of PDAC. These and other candidate biomarkers need to be validated with larger populations with appropriate control groups.

With the technological advancement in the mass spectrometric techniques over the recent decades and resumed interest in the cancer-associated metabolic abnormality, [113, 114] application of metabolomics in the cancer field has attracted more attention. Metabolomics allows for elucidating the complete set of metabolites or low-molecular-weight intermediates in the physiological, developmental or pathological state of the cell, tissue, organ, or organism. [115] And metabolomics study of PDAC detection biomarkers will seek identification of a set of small molecules or metabolites (or chemical intermediates) that are potential discriminators of developing PDAC and the controls. Recent reports from our group as well as others have demonstrated specific small molecules such as amino acids, bile acids, and various lipids and fatty acids as potential candidates for PDAC biomarkers. [116, 117, 118, 119] Since a metabolome represents a current physiological readout of the biochemical state in an individual's biofluid or tissue space and as the functional end-product of the varying signals from the genome and proteome, it reflects the up-to-date phenotypic state of an individual in the presence of environmental stimuli. Thus, metabolomics data potentially provides additional temporal information to cancer risks derived from gene-based PDAC risk data alone. Since many enzymes in a metabolic network determine metabolites' concentrations and nonlinear quantitative relationship from the genes to the proteome and metabolome levels exist, a metabolome cannot be easily decomposed to a specific single marker, which will designate the disease state. [120] So, in order to delineate a physiological or pathological state, multiple metabolomic features might be required for accurate depiction of such a state as a developing cancer. In addition, future studies are anticipated to incorporate further cancer systems' biological knowledge, including multi-omics-based analyses for optimal designation of PDAC biomarkers, which would be utilized in conjunction with a clinical-parameter-derived population subset for establishing the PDAC screening population. Subsequently, further validation studies for the PDAC biomarkers need to be performed.

6. Conclusion

Current imaging-based detection and diagnostic methods for PDAC is effectively providing answers to clinical questions raised for patients with signs or symptoms of suspected pancreatic lesions. However, the endoscopic/imaging-based schemes are currently limited in applications to early PDAC detection in asymptomatic patients, aside from a relatively small group of known genetically high-risk groups. There is a high demand for developing a method of selecting distinct subsets among the general population for implementing the endoscopic/imaging screening test effectively. Application of combinations of clinical risk parameters/factors with the developing molecular biomarkers from translational science brings high hopes of providing us with early PDAC detection markers, and developing effective early detection screening scheme for the patients in the near future.

Author details

Shiro Urayama

Division of Gastroenterology & Hepatology, Department of Internal Medicine, University of California, Davis, USA

References

[1] National Cancer Institute. Cancer topics: Pancreatic cancer. http://www. cancer. gov/cancertopics/types/pancreatic

[2] American Cancer Society. Cancer Facts & Figures, 2013.

[3] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008:GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-2917.

[4] Beger HG, Rau B, Gansauge F, et al. Treatment of pancreatic cancer: challenge of the facts. World J Surg 2003;27:1075.

[5] Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer Statistics, 2009. CA Cancer J Clin 2009;59:225-249.

[6] Ahmad NA, Lewis JD, Ginsberg GG, et al. Long term survival after pancreatic resection for pancreatic adenocarcinoma. Am J Gastroenterol 2001; 96:2609.

[7] Howard TJ, Krug JE, Yu J, et al. A margin-negative R0 resection accomplished with minimal postoperative complications is the surgeon's contribution to long-term survival in pancreatic cancer. J Gastrointest Surg 2006;10:1338.

[8] Egawa S, Takeda K, Fukuyama S, Motoi F, Sunamura M, Matsuno S. Clinicopathological aspects of small pancreatic cancer. Pancreas 2004; 28: 235-240.

[9] Ariyama J, Suyama M, Ogawa K, Ikari T. Screening of pancreatic neoplasms and the diagnostic rate of small pancreatic neoplasms. Nippon Rinsho 1986; 44: 1729-1734.

[10] Yachida S, Jones S, Bozic I, Antal T, Leary R, Fu B, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature 2010;467(7319):1114-1117.

[11] Ranganathan, P., Harsha, H. C. & Pandey, A. Molecular alterations in exocrine neoplasms of the pancreas. Arch Pathol Lab Med 2009;133:405-412.

[12] Yasuda, K,Mukai, H, Fujimoto, Set al. The diagnosis of pancreatic cancer by endoscopic ultrasonography. Gastrointest Endosc 1988;34(1):1-8.

[13] DeWitt, J,Devereaux, B,Chriswell, M et al. Comparison of Endoscopic Ultrasonography and Multidetector Computed Tomography for Detecting and Staging Pancreatic Cancer. Ann Int Med 2004;141(10):753-763.

[14] Khashab, M, Yong, E, Lennon, A et al. EUS is still superior to multidetector computerized tomography for detection of pancreatic neuroendocrine tumors. Gastrointest Endosc 2011;73(4):691-696.

[15] Klapman JB, Chang KJ, Lee JG, et al. Negative predictive value of endoscopic ultrasound in a large series of patients with a clinical suspicion of pancreatic cancer. Am J Gastroenterol 2005;100:2658.

[16] Bhutani MS, Gress FG, Giovannini M, et al. The No Endosonographic Detection of Tumor (NEST) Study: A case series of pancreatic cancers missed on endoscopic ultrasonography. Endoscopy 2004; 36:385.

[17] Saˇftoiu A, Popescu C, Cazacu S, et al. Power Doppler endoscopic ultrasonography for the differential diagnosis between pancreatic cancer and pseudotumoral chronic pancreatitis. J Ultrasound Med 2006;25:363.

[18] Rosch T, Lorenz R, Briag C, et al: Endoscopic ultrasound in pancreatic tumor diagnosis. Gastrointest Endosc 1991;37:347-352.

[19] Frey H. Real-time elastography. A new ultrasound procedure for the reconstruction of tissue elasticity. Radiologe 2003;43:850-855.

[20] Konofagou EE. Quo vadis elasticity imaging? Ultrasonics 2004;42:331.

[21] Saˇftoiu A, Vilmann P. Endoscopic ultrasound elastography—a new imaging technique for the visualization of tissue elasticity distribution. J Gastrointestin Liver Dis 2006;15:161.

[22] Giovannini M, Hookey LC, Bories E, et al. Endoscopic ultrasound elastography: the first step towards virtual biopsy? Preliminary results in 49 patients. Endoscopy 2006;38:344.

[23] Bhutani MS, Hoffman BJ, van Velse A, et al. Contrast enhanced endoscopic ultrasonography with galactose microparticles:SHU508A (Levovist). Endoscopy 1997;29:635.

[24] Becker D, Strobel D, Bernatik T, et al. Echoenhanced and power Doppler EUS for the discrimination between focal pancreatitis and pancreatic carcinoma. Gastrointest Endosc 2001;53:784.

[25] Hocke M, Schulze E, Gottschalk P, et al. Contrast-enhanced endoscopic ultrasound in discrimination between focal pancreatitis and pancreatic cancer. World J Gastroenterol 2006;12:246.

[26] Dietrich CF, Ignee A, Frey H. Contrast-enhanced endoscopic ultrasound with low mechanical index: a new technique. Z Gastroenterol 2005;43:1219.

[27] Røsch T, Hofrichter K, Frimberger E, et al. ERCP or EUS for tissue diagnosis of biliary strictures? A prospective comparative study. Gastrointest Endosc 2004;60:390.

[28] Brugge WR. Pancreatic fine needle aspiration: to do or not to do? J Pancreas 2004;5:282.

[29] Vilmann P, Hancke S, FW Henriksen, et al. Endosonographically-guided fine needle aspiration biopsy of malignant lesions in the upper GI tract. Endoscopy 1993;25:523.

[30] Wiersema MJ, Wiersema LM, Khusro Q, et al. Combined endosonography and fine-needle aspiration cytology in the evaluation of gastrointestinal lesions. Gastrointest Endosc 1994;40:199.

[31] Chang KJ, Katz D, Durbin TE, et al. Endoscopic ultrasound-guided fine-needle aspiration. Gastrointest Endosc 1994;40:694.

[32] Giovannini M, Seitz JF, Monges G, et al. Fine needle aspiration cytology guided by endoscopic ultrasonography: results in 141 patients. Endoscopy 1995;27:171.

[33] Fritscher-Ravens A, Izbicki JR, Sriram PV, Krause C, Knoefel WT, Topalidis T, et al. Endosonography-guided, fine-needle aspiration cytology extending the indication for organ-preserving pancreatic surgery. Am J Gastroenterol 2000;95:2255-60.

[34] Krishna, N B,LaBundy, J L, Saripalli, S et al. Diagnostic Value of EUS-FNA in Patients Suspected of Having Pancreatic Cancer With a Focal Lesion on CT Scan/MRI but Without Obstructive Jaundice. Pancreas 2009;38(6):625-630.

[35] Agarwal B, Abu-Hamda E, Molke KL, et al. Endoscopic ultrasound-guided fine needle aspiration and multidetector spiral CT in the diagnosis of pancreatic cancer. Am J Gastroenterol 2004;99: 844.

[36] Micames C, Jowell PS, White R, et al. Lower frequency of peritoneal carcinomatosis in patients with pancreatic cancer diagnosed by EUS-guided FNA versus percutaneous FNA. Gastrointest Endosc 2003;58:690.

[37] Shah JN, Fraker D, Guerry D, et al. Melanoma seeding of an EUS-guided fine needle track. Gastrointest Endosc 2004;59:923.

[38] Hirooka Y, Goto H, Itoh A, et al. Case of intraductal papillary mucinous tumor in which endosonography-guided fine-needle aspiration biopsy caused dissemination. J Gastroenterol Hepatol 2003;18:1323.

[39] Pellisé, M. ; Castells, A. ; Ginès, A. ; et al. Clinical usefulness of KRAS mutational analysis in the diagnosis of pancreatic adenocarcinoma by means of endosonography-guided fine-needle aspiration biopsy. Aliment Pharmacol Ther 2003; 17: 1299-1307.

[40] Goggins M. Molecular markers of early pancreatic cancer. J Clin Oncol 2005;10:4524.

[41] Takahashi, K. ; Yamao, K. ; Okubo, K. ; et al. Differential diagnosis of pancreatic cancer and focal pancreatitis by using EUS-guided FNA. Gastrointest Endosc 2005; 61:76-79.

[42] Maluf-Filho F, Kumar A, Gerhardt R, et al. Kras mutation analysis of fine needle aspirate under EUS guidance facilitates risk stratification of patients with pancreatic mass. J Clin Gastroenterol 2007; 41:906-910.

[43] Bournet B, Souque A, Senesse P, et al. EUS-guided fine needle-aspiration coupled to KRAS mutation assay to differentiate pancreatic cancer from pseudo-tumorous chronic pancreatitis. Endoscopy 2009;41:552-557.

[44] Tada M, Komatsu Y, Kawabe T, et al. Quantitative analysis of K-ras gene mutation in pancreatic tissue obtained by endoscopic ultrasonography-guided fine needle aspiration: clinical utility for diagnosis of pancreatic tumor. Am J Gastroenterol 2002;97:2263.

[45] Itoi T, Takei K, Sofuni A, et al. Imunohistochemical analysis of p53 and MIB-1 in tissue specimens obtained from endoscopic ultrasonongraphy-guided fine needle aspiration biopsy for the diagnosis of solid pancreatic masses. Oncol Rep 13:229–234.

[46] Mishra G, Zhao Y, Sweeney J et al. Determination of qualitative telomerase activity as an adjunct to the diagnosis of pancreatic adenocarcinoma by EUS-guided fine-needle aspiration. Gastrointest Endosc 63:648–654.

[47] Salek, C. ; Benasova, L. ; Zavoral, M. ; et al. Evaluation of clinical relevance of examining K-ras, p16 and p53 mutations along with allelic losses at 9p and 18q in EUS-guided fine needle aspiration samples of patients with chronic pancreatitis and pancreatic cancer. World J Gastroenterol 2007; 13: 3714-3720.

[48] Shi C, Hruban RH, Klein AP. Familial pancreatic cancer. Arch Pathol Lab Med 2009;133(3): 365–374.

[49] Permuth-Wey J, Egan KM. Family history is a significant risk factor for pancreatic cancer: results from a systematic review and meta-analysis. Fam Cancer 2009;8(2): 109–117.

[50] Wang W, Chen S, Brune KA, et al. PancPRO: risk assessment in individuals with a family history of pancreatic cancer. J Clin Oncol 2007;25(11):1417–1422.

[51] Klein AP, Brune KA, Petersen GM, et al. Prospective risk of pancreatic cancer in familial pancreatic cancer kindreds. Cancer Res 2004;64(7):2634–2638.

[52] Wang L, Brune KA, Visvanathan K, et al. Elevated cancer mortality in the relatives of pancreatic cancer patients. Epidemiol Biomarkers Prev 2009;18(11):2829-34.

[53] Giardiello FM, Brensinger JD, Tersmette AC, et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology 2000;119:1447-1453.

[54] Rebours V, Boutron-Ruault MC, Schnee M, et al. Risk of pancreatic adenocarcinoma in patients with hereditary pancreatitis: a national exhaustive series. Am J Gastroenterol 2008;103:111-119.

[55] Gates LK Jr, Perrault J, Whitcomb DC. Hereditary pancreatitis and the risk of pancreatic cancer. International Hereditary Pancreatitis Study Group. J Natl Cancer Inst 1997;89:442-446.

[56] Raimondi S, Lowenfels AB, Morselli-Labate AM, et al. Pancreatic cancer in chronic pancreatitis; aetiology, incidence, and early detection. Best Pract Res Clin Gastroenterol 2010;24:349-358.

[57] Goldstein AM, Fraser MC, Struewing JP, et al. Increased risk of pancreatic cancer in melanoma-prone kindreds with p16INK4 mutations. N Engl J Med 1995;333:970-974.

[58] Whelan AJ, Bartsch D, Goodfellow PJ. Brief report: a familial syndrome of pancreatic cancer and melanoma with a mutation in the CDKN2 tumor-suppressor gene. N Engl J Med 1995;333:975-977.

[59] Klein AP. Genetic susceptibility to pancreatic cancer. Mol Carcinog 2012:51:14-24.

[60] Van Asperen CJ, Brohet RM, Meijers-Heijboer EJ, et al. Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. J Med Genet 2005;42(9):711-719.

[61] Brose MS, Rebbeck TR, Calzone KA, et al. Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. J Natl Cancer Inst 2002;94(18): 1365-1372.

[62] Couch FJ, Johnson MR, Rabe KG, et al. The Prevalence of BRCA2 Mutations in Familial Pancreatic Cancer. Cancer Epidemiol Biomarkers Prev 2007 February;16(2):342–346.

[63] Goggins M, Schutte M, Lu J, et al. Germline BRCA2 gene mutations in patients with apparently sporadic pancreatic carcinomas. Cancer Res 1996;56:5360–5364.

[64] Hahn SA, Greenhalf B, Ellis I, et al. BRCA2 germline mutations in familial pancreatic carcinoma. J Natl Cancer Inst 2003 February 5;95(3):214–221.

[65] Lal G, Liu G, Schmocker B, et al. Inherited predisposition to pancreatic adenocarcinoma: role of family history and germ-line p16, BRCA1, and BRCA2 mutations. Cancer Res 2000 January 15;60:409–416.

[66] Murphy KM, Brune KA, Griffin CA, et al. Evaluation of candidate genes MAP2K4, MADH4, ACVR1B, and BRCA2 in familial pancreatic cancer: deleterious BRCA2 mutations in 17%. Cancer Res 2002;62(13):3789–3793.

[67] Kastrinos F, Mukherjee B, Tayob N, et al. Risk of pancreatic cancer in families with Lynch syndrome. JAMA 2009;302:1790-1795.

[68] McWilliams RR, Petersen GM, Rabe KG, et al. Cystic fibrosis transmembrane regular gene carrier status is a risk factor for young onset pancreatic adenocarcinoma. Gut 2005;54:661-662.

[69] Greenhalf W, Grocock C, Harcus M, Neoptolemos J. Screening of high-risk families for pancreatic cancer. Pancreatology 2009; 9: 215-222.

[70] Brand RE, Lerch MM, Rubinstein WS, et al. Advances in counseling and surveillance of patients at risk for pancreatic cancer. Gut 2007; 56: 1460-1469.

[71] Couch FJ, Johnson MR, Rabe KG, et al. The Prevalence of BRCA2 Mutations in Familial Pancreatic Cancer. Cancer Epidemiol Biomarkers Prev 2007 February;16(2):342–346.

[72] Lynch HT, Deters CA, Snyder CL, et al. BRCA1 and pancreatic cancer: pedigree findings and their causal relationships. Cancer Genet Cytogenet 2005; 158: 119-125.

[73] Pogue-Geile KL, Chen R, Bronner MP, et al. Palladin mutation causes familial pancreatic cancer and suggests a new cancer mechanism. PLoS Med 2006; 3: e516.

[74] Earl J, Yan L, Vitone LJ, Risk J, et al. Evaluation of the 4q32-34 locus in European familial pancreatic cancer. Cancer Epidemiol Biomarkers Prev 2006; 15: 1948-1955.

[75] Zervos EE, Tanner SM, Osborne DA, et al. Differential gene expression in patients genetically predisposed to pancreatic cancer. J Surg Res 2006; 135: 317-322.

[76] Jones S, Hruban RH, Kamiyama M, Borges M, et al. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. Science 2009; 324: 217.

[77] Klein AP, Brune KA, Petersen GM, et al. Prospective risk of pancreatic cancer in familial pancreatic cancer kindreds. Cancer Res 2004;64(7):2634–2638.

[78] Tersmette AC, Petersen GM, Offerhaus GJ, et al. Increased risk of incident pancreatic cancer among first-degree relatives of patients with familial pancreatic cancer. Clin Cancer Res 2001; 7: 738-744.

[79] Brune KA, Lau B, Palmisano E, et al. Importance of age of onset in pancreatic cancer kindreds. J Natl Cancer Inst 2010; 102: 119-126.

[80] Rulyak SJ, Lowenfels AB, Maisonneuve P, Brentnall TA. Risk factors for the development of pancreatic cancer in familial pancreatic cancer kindreds. Gastroenterology 2003; 124: 1292-1299.

[81] Wang W, Chen S, Brune KA, et al. PancPRO: risk assessment in individuals with a family history of pancreatic cancer. J Clin Oncol 2007;25(11):1417–1422.

[82] Kim JE, Lee KT, Lee JK, Paik SW, Rhee JC, Choi KW. Clinical usefulness of carbohydrate antigen 19-9 as a screening test for pancreatic cancer in an asymptomatic population. J Gastroenterol Hepatol 2004; 19: 182-186.

[83] Bussom S, Saif MW. Methods and rationale for the early detection of pancreatic cancer. Highlights from the "2010 ASCO Gastrointestinal Cancers Symposium". Orlando, FL, USA. January 22-24, 2010. JOP 2010; 11: 128-130.

[84] Stolzenberg-Solomon RZ, Graubard BI, Chari S, et al. Insulin, glucose, insulin resistance, and pancreatic cancer in male smokers. JAMA 2005; 294: 2872-2878.

[85] Canto MI, Goggins M, Yeo CJ, et al. Screening for pancreatic neoplasia in high-risk individuals: an EUS based approach. Clin Gastroenterol Hepatol 2004; 2: 606-621.

[86] Canto MI, Goggins M, Hruban RH, et al. Screening for early pancreatic neoplasia in high-risk individuals: a prospective controlled study. Clin Gastroenterol Hepatol 2006; 4: 766-781.

[87] Canto MI, Hruban RH, Fishman EK, Kamel IR, Schulick R, Zhang Z, et. al. Frequent detection of pancreatic lesions in asymptomatic high-risk individuals. Gastroenterology 2012;142:796-804.

[88] Kimmey MB, Bronner MP, Byrd DR, et al. Screening and surveillance for hereditary pancreatic cancer. Gastrointest Endosc 2002; 56: S82-S86.

[89] Carlson C, Greenhalf W,Brentnall TA. Screening of hereditary pancreatic cancer families, in The Pancreas: An Integrated Textbook of Basic Science, Medicine and Surgery. Second Edition. In: Berger HG,Bucher M, Kozarek R, Lerch M, Neoptolemos J, Warshaw A, Whitcomb D, Shiratori K, editors. Oxford UK: Wiley-Blackwell Publishing, 2008: 636-642.

[90] Langer P, Kann PH, Fendrich V, et al. Five years of prospective screening of high-risk individuals from families with familial pancreatic cancer. Gut 2009; 58: 1410-1418.

[91] Poley JW, Kluijt I, Gouma DJ, et al. The yield of first-time endoscopic ultrasonography in screening individuals at a high risk of developing pancreatic cancer. Am J Gastroenterol 2009; 104: 2175-2181.

[92] Rulyak SJ, Kimmey MB, Veenstra DL, et al. Costeffectiveness of pancreatic cancer screening in familial pancreatic cancer kindreds. Gastrointest Endosc 2003; 57: 23-29.

[93] Rubenstein JH, Scheiman JM, Anderson MA. A clinical and economic evaluation of endoscopic ultrasound for patients at risk for familial pancreatic adenocarcinoma. Pancreatology 2007; 7: 514-525.

[94] Chari ST, Leibson CL, Rabe KG, et al. Pancreatic cancer-associated diabetes mellitus: prevalence and temporal association with diagnosis of cancer. Gastroenterology 2008;134:95-101.

[95] Chari ST, Leibson CL, Rabe KG, et al. Probability of pancreatic cancer following diabetes: a population-based study. Gastroenterology 2005;129:504-11.

[96] Ogawa Y, Tanaka M, Inoue K, et al. A prospective pancreatographic study of the prevalence of pancreatic carcinoma in patients with diabetes mellitus. Cancer 2002;94:2344-2349.

[97] Pannala R, Basu A, Petersen GM, et al. New-onset diabetes: a potential clue to the early diagnosis of pancreatic cancer. Lancet Oncol 2009;10:88-95.

[98] Wang F, Herrington M, Larsson J, et al. The relationship between diabetes and pancreatic cancer. Molecular Cancer 2003;2:4.

[99] Lowenfels AB, Maisonneuve P, Cavallini G, et al. Pancreatitis and the risk of pancreatic cancer. N Engl J Med 1993;328:1433-1437.

[100] Arslan AA, Helzlsouer KJ, Kooperberg C, et al. Anthropometric measures, body mass index, and pancreatic cancer: a pooled analysis from the Pancreatic Cancer Cohort Consortium (PanScan). Arch Intern Med 2010;170:791-802.

[101] Li D, Morris JS, Liu J, et al. Body mass index and risk, age of onset, and survival in patients with pancreatic cancer. JAMA 2009;301:2553-2562.

[102] Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004;4:579-591.

[103] Rapp K, Schroeder J, Klenk J, et al. Obesity and incidence of cancer: a large cohort study of over 145,000 adults in Austria. Br J Cancer 2005;93:1062-1067.

[104] Larsson SC, Permert J, Hakansson N, et al. Overall obesity, abdominal adiposity, diabetes and cigarette smoking in relation to the risk of pancreatic cancer in two Swedish population-based cohorts. Br J Cancer 2005;93:1310-1315.

[105] Dawson DW, Hertzer K, Moro A, et al. High-fat, high-calorie diet promotes early pancreatic neoplasia in the conditional KrasG[12]D mouse model. Cancer Prev Res 2013;6:1064-1073.

[106] Philip B, Roland CL, Daniluk J, et al. A high-fat diet activates oncogenic Kras and COX2 to induce development of pancreatic ductal adenocarcinoma in mice. Gastroenterology 2013;145(6):1449-1458.

[107] Huang H, Dong X, Kang MX, et al. Novel blood biomarkers of pancreatic cancer-associated diabetes mellitus identified by peripheral blood-based gene expression profiles. Am J Gastroenterol 2010;105:1661-1669.

[108] Kojima K, Asmellash S, Klug CA, et al. Applying proteomic-based biomarker tools for the accurate diagnosis of pancreatic cancer. J Gastrointestinal Surg 2008;12(10): 1683-1690.

[109] Rong Y, Jin D, Hou C, et al. Proteomics analysis of serum protein profiling in pancreatic cancer patients by DIGE: upregulation of mannose-binding lectin 2 and myosin light chain kinase 2. BMC Gastroenterology 2010;10:68.

[110] Matsubara J, Honda K, Ono M, et al. Reduced plasma level of CXC chemokine ligand 7 in patients with pancreatic cancer. Cancer Epidemiol Biomarkers Prev 2011;20(1): 160-171.

[111] Pan S, Chen R, Crispin DA, et al. Protein alterations associated with pancreatic cancer and chronic pancreatitis found in human plasma using global quantitative proteomics profiling. J Proteome Res 2011;10(5):2359-2376.

[112] Bloomston M, Zhou JX, Rosemurgy AS, et al. Fibrinogen γ overexpression in pancreatic cancer identified by large-scale proteomic analysis of serum samples. Cancer Res 2006;66(5):2592-2599.

[113] Garber K. Energy boost: the Warburg effect returns in a new theory of cancer. J Natl Cancer Inst 2004;96:1805-1806.

[114] DeBerardinis R, Lum JJ, Hatzivassiliou G, et al. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metabolism 2008;7:11-20.

[115] Oliver SG. Functional genomics: Lessons from yeast. Phil Trans R Soc Long B 2002;357:17-23.

[116] Urayama S, Zou W, Brooks K, et al. Comprehensive mass spectrometry based metabolic profiling of blood plasma reveals potent discriminatory classifiers of pancreatic cancer. Rapid Commun Mass Spectrom 2010; 24(5):613-620.

[117] Bathe OF, Shaykhutdinov R, Kopciuk K, et al. Feasibility ofidentifying pancreatic cancer based on serum metabolomics. Cancer Epidemiol Biomarkers Prev 2011;20:140-147.

[118] Kobayashi T, Nishiumi S, Ikeda A, et al. A novel serum metabolomics-based diagnostic approach to pancreatic cancer. Cancer Epidemiol Biomarkers Prev 2013;22(4): 571-579.

[119] Ritchie SA, Akita H, Takemasa I, et al. Metabolic system alterations in pancreatic cancer patient serum: potential for early detection. BMC Cancer 2013;13:416.

[120] Kuile BH, Westerhoff HV. Transcriptome meets metabolome: hierarchical and metabolic regulation of the glycolytic pathway. FEBS Letts 2001;500:169-171.

Section 3

A Novel Approach to Treatment

Chapter 7

Metformin and Pancreatic Cancer Metabolism

Mary Jo Cantoria, Hitendra Patel,
Laszlo G. Boros and Emmanuelle J. Meuillet

Additional information is available at the end of the chapter

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1. Introduction

Numerous epidemiological studies have reported that metformin, a well-known and widely used anti-diabetic drug, may provide protective benefits in decreasing pancreatic cancer risk among the diabetic population. Following a brief introduction regarding metformin's history and pharmacological properties, this book chapter presents epidemiological findings showing how metformin is associated with protection against pancreatic cancer. We also introduce the anti-cancer effects of metformin through AMPK-independent and AMPK-dependent manners [1-6]. These mechanisms include its inhibitory effects on the insulin growth factor-1 (IGF-1), G protein-coupled receptor (GPCR) and mTORC1 signaling pathways [3-10]. We then discuss the metabolic effects of metformin in cancer. For example, metformin has been shown to inhibit glycolysis in various cancer cell lines [11-13]. Metformin is a known inhibitor of complex I of the electron transport chain [14-18], potentially limiting the intact oxidative respiration capabilities of the cancer cell. We also discuss in depth the anti-cancer mechanisms of action of metformin in the context of lipid metabolism as reported in numerous models. These include metformin's ability to increase fatty acid β-oxidation in adipocytes [19] and its ability to inhibit hepatic lipogenesis [2]. As shown by numerous studies [20-23], metformin also possesses anti-lipogenic properties, potentially limiting this critical metabolic pathway that confers cancer pancreatic cell survival advantage.

We provide preclinical and clinical evidences of the potential utility of metformin in pancreatic cancer. For example, a very recent report has shown tumor growth inhibition *in vitro* and *in vivo* by metformin through down-regulation of Sp (specificity protein) transcription factors and consequent down-regulation of the Sp-regulated genes.[24]. Metformin has been also shown to impair tumor development in pancreatic cancer in xenografts models [25]. Finally, we also explore the role of lipid metabolism in the context-specific ability of metformin to act as a chemopreventive/therapeutic agent. As early as 2001, it has been reported that metformin significantly impairs the formation of pancreatic lesions induced by the pancreatic carcinogen

N-nitrosobis-(2-oxopropyl)amine in hamsters fed a high fat diet [26]. We will argue that the in order for metformin to exert its anti-cancer properties, consideration of the genetic and metabolic status of the model system is critical.

We conclude this chapter by discussing our most recent findings that show how metformin inhibits glucose-derived fatty acid synthesis in the context of available acetyl-CoA and the presence of K-*ras* mutation in pancreatic cancer cells in the context of obesity, the metabolic syndrome and diabetes [21]. These results strongly suggest that metformin, being an anti-lipogenic drug, may be useful when combined with lipid lowering and chemotherapeutic agents. Finally, because up-regulation of fatty acid synthase (FAS), the enzyme that catalyzes the terminal step in palmitate synthesis, is associated with increased resistance to gemcitabine and radiation treatments in human pancreatic cancer tissues [27], we argue that the use of metformin could synergize with these treatments.

However, an important question remains on whether or not metformin really has chemopreventive and/or therapeutic use for pancreatic cancer. This chapter argues that metformin does have anti-cancer properties by examining numerous experimental studies on metformin's potential mechanisms of action along with the metabolic and genetic context by which metformin may act as an anti-cancer drug.

2. The history behind metformin

Galega officinalis (also known as the French lilac or Goat's Rue) is a plant that has been used for the treatment of *diabetes mellitus* in traditional medicine for centuries. At the end of the 19th century, guanidine compounds were discovered in *Galega* extracts (Figure 1). Shortly after, in 1918 animal studies showed that these compounds lowered blood glucose levels [28]. However, guanidines are fairly toxic. Following this discovery, some less toxic derivatives, named synthalin A and synthalin B (Figure 1), were synthesized based on the structure of galegine and used for diabetes treatment under the marketed name of Synthalin in the 1940s. The discovery of insulin overshadowed the use and further development of synthalin compounds and they were forgotten for the next several decades. When chemists found that they could also make the guanidine compounds more tolerable by bonding two guanidines together, forming a biguanide, interest in these molecules was regained and attention was focused on metformin, phenformin and buformin (Figure 1). Finally, the interest in metformin, synthesized by K. Slotta, was further renewed in the late 1950s after several reports that it could reduce blood sugar levels in people. The French physician Jean Sterne published the first clinical trial of metformin as a treatment for diabetes in 1957 [29].

3. Synthesis, structure and pharmacology of metformin

Synthesis: Metformin is synthesized by equimolar fusion of hydrochloric dimethylamine and dicyandinamide at 130-150°C for 0.5 to 2 hours [30].

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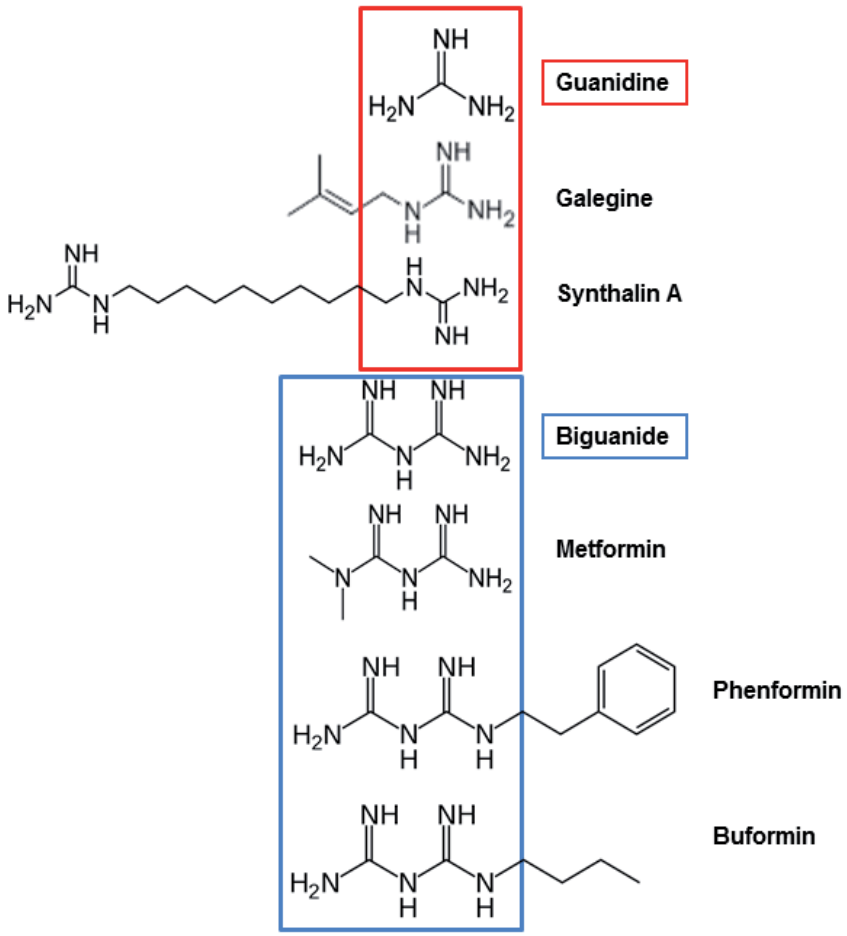

Figure 1. Structures of guanidine and biguanides.

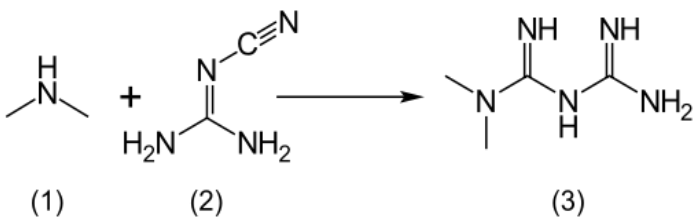

Figure 2. Synthesis of N,N′-dimethyl biguanide.

Structure: Metformin is globally charged positively and does not or poorly permeates the plasma membrane. The structure was represented in a wrong tautomeric form until it was corrected in 2005 by a group of chemists from India [31]. Metformin is given to patients as a

hydrochloride from. Several studies have demonstrated an affinity of biguanides for phospholipids at the plasma membrane [32] as well as some protein binding [33]. The interaction of the biguanide with the polar head group of phospholipids induces a diminution of the plasma membrane fluidity leading to the rigidification of the plasma membrane [32]. However, this reduction of the fluidity has not been reproduced by Wiernsperger and collaborators [33] who showed an increase in fluidity of red blood cells membranes.

Metformin has an oral bioavailability of 50-60% under fasting conditions and the peak plasma concentration is reached within a couple of hours. The plasma protein binding is negligible. Metformin is not metabolized but it is accumulated in tissues such as the liver, the kidneys, the salivary glands and the gastrointestinal tract [34]. Eighty percent of the elimination of metformin occurs by the urinary tract. The average elimination half-life in plasma is 6.2 hours. The half-life of biguanides is approximately 2 hours [34]. Interestingly, metformin is distributed to (and appears to accumulate in) red blood cells with a much longer elimination half-life: 17.6 hours.

4. Chemopreventive properties of metformin

Numerous observational studies show that metformin use, when compared against other diabetic agents such as insulin and sulfonylureas, decreases cancer risk and overall cancer mortality among the diabetic population. These protective associations have been reported across different cancer types and among various diabetic populations.

Table 1A summarizes epidemiological studies that show pancreatic cancer risk and cancer mortality associated with metformin use while Table 1B presents observational studies and clinical trials on overall cancer risk and mortality in relation to metformin use. While some studies show a reduction in pancreatic cancer [35-39] and overall cancer risk [37, 39-45] among diabetic metformin users, there are also studies that report no significant difference in cancer risk among diabetics who take metformin compared to patients who take other anti-diabetic treatments [36, 46-51]. These conflicting results may be explained by differences in the study population, the confounding factors accounted for during statistical analysis and the selected study design (e.g., cohort *versus* case-control). For example, patients who were prescribed metformin may generally have better glucose control compared to those prescribed insulin hence, the risk for future diseases such as cancer may be lower at baseline for metformin users versus diabetics treated with other modalities. As shown in Tables 1A and 1B, different studies account for different confounding factors which can largely influence the results of disease risk calculations. There is no standardized procedure for the selection of which potential confounding factors are to be included in a statistical model hence; this can lead to large variations among observational study outcomes. Overall, although these epidemiological studies are correlative in nature and hence, cannot establish causality between metformin use and cancer risk and mortality, they provide a biological basis to further explore whether metformin possesses chemopreventive and/or chemotherapeutic properties.

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5. Chemotherapeutic properties of metformin

Experimental studies show that metformin possesses anti-cancer effects in various cancer types. As the metabolic effects of metformin are discussed in the section "Metformin as an Anti-lipogenic Drug," figure 2 provides a summary of metformin's effects exclusively on cancer signaling pathways. Overall, we present metformin's effects on cancer cells as AMPK-dependent (pathways 2, 4, 5, 6 and 10) and independent (pathways 1, 3, 7- 9 and 11). Although there is an overlap between cell signaling and metabolic alterations due to metformin treatment (e.g., via ETC complex I and ATP production, AMPK/mTORC1 axis and metabolic control), metformin's anti-cancer effects can be grouped into: a) inhibition of ATP and ROS production, b) inhibition of IRS-1/Akt/mTORC1 axis, c) anti-inflammatory effects, d) cell cycle arrest and e) inhibition of general transcription factors.

First Author/ Year (reference)	Location	Design	Outcome	Comparison	Risk (95% CI)	Confounding Factors
Oliveria et al., 2007 [46]	United States	Cohort	Pancreatic cancer risk	Metformin vs no Metformin	RR: 1.26 (0.80-1.99)	Age, gender, gastrectomy, chronic pancreatitis, deep venous thrombosis, dermatomyositis/ polymyositis, alcoholism, hepatitis B/C, history of polyps
Currie et al., 2009 [47]	United Kingdom	Cohort	Progression to pancreatic cancer	Sulfonylureas vs Metformin Metformin + Sulfonylureas vs Metformin Insulin-based therapies	**HR: 4.95 (2.74-8.96)** HR: 0.38 (0.13-1.12) **HR: 4.63 (2.64-8.10)**	Age, sex, smoking status, diagnosis of a previous cancer
Li et al., 2009 [35]	United States	Case-control	Pancreatic cancer risk	Metformin	**OR: 0.38 (0.22-0.69)**	Age, sex, race smoking, alcohol, BMI, family history of cancer, diabetes duration, use of insulin
Bodmer et al., 2011 [36]	United Kingdom	Case-control	Pancreatic cancer risk	Metformin (both sexes) Metformin (females only) Sulfonylureas Insulin	OR: 0.87 (0.59-1.29) **OR: 0.43 (0.23-0.80)** **OR: 1.90 (1.32-2.74)** **OR: 2.29 (1.34-3.92)**	BMI, smoking, alcohol consumption, diabetes duration
Ferrara et al., 2011 [48]	United States	Cohort	Pancreatic cancer risk	Metformin and pioglitazone	**HR: 1.2 (1.0-1.5)**	Age, ever use of other diabetes medications, year of cohort entry, sex, race/ethnicity, income, current

First Author/ Year (reference)	Location	Design	Outcome	Comparison	Risk (95% CI)	Confounding Factors
						smoking, baseline HbA1c, diabetes duration, new diabetes diagnosis, creatinine, and congestive heart failure
Liao et al., 2011 [49]	Taiwan	Cohort	Pancreatic cancer risk	Metformin	HR: 0.85 (0.39-1.89)	Crude/ Unadjusted
Lee et al., 2011 [37]	Taiwan	Cohort	Pancreatic cancer risk	Metformin vs. potential use of other oral anti-hyperglycemic medications	**HR: 0.15 (0.03-0.79)**	Age, gender, other oral anti-hyperglycemic medication, Charlson comorbidity index score, time-dependent metformin use
Morden et al., 2011 [50]	United States	Cohort	Pancreatic cancer risk	Metformin	HR: 1.25 (0.89-1.75)	Age category, race/ ethnicity, diabetes complications, obesity diagnosis, oral estrogen use, Part D low income subsidy (a poverty indicator), 14 Charlson comorbidities, and tobacco exposure diagnosis
Ruiter et al., 2012 [38]	Netherlands	Cohort	Pancreatic cancer risk	Metformin vs. sulfonylureas	**HR: 0.73 (0.66-0.80)**	Age at first oral glucose-lowering drug (OGLD) prescription, sex, year in which the first OGLD prescription was dispensed, number of unique drugs used in the year, number of hospitalizations in the year before the start of OGLD
Sadeghi et al., 2012 [52]	United States	Cohort	Median survival in pancreatic cancer, prognostic factors of overall survival in pancreatic cancer	Metformin vs. Non-metformin Univariate Analysis: Metformin Multivariate Analysis: Metformin	15.2 months vs. 11.1 months **(P = 0.009)** **HR: 0.68 (0.52-0.89)** **HR: 0.64 (0.48-0.86)**	No significant differences in BMI, age, sex, race, diabetes duration, disease stage, tumor size, performance status, serum CA-19-9 between metformin and non-metformin group

First Author/ Year (reference)	Location	Design	Outcome	Comparison	Risk (95% CI)	Confounding Factors
Nakai et al., 2013 [51]	Japan	Cohort	Prognostic factors of overall survival in pancreatic cancer	Univariate Analysis: Biguanide Sulfonylureas Insulin Thiazolidine-dione	HR: 0.61 (0.19-1.44) **HR: 0.60 (0.39-0.88)** **HR: 0.83 (0.59-1.15)** **HR: 0.19 (0.01-0.84)**	Age (G65 or Q65 years old), sex (male or female), performance status (PS; 0Y1 or 2Y3), primary tumor size (G30 or Q30 mm), distant metastasis (yes or no), body mass index (G22 or Q22 kg/m2), chemotherapy (combination therapy with gemcitabine and S-1 vs others), DM (yes or no), insulin (yes or no), sulfonylurea (yes or no), biguanide (yes or no), thiazolidine (yes or no), hypertension (yes or no), ACEI or ARB (yes or no), Ca-blocker (yes or no), A-blocker (yes or no), and statin (yes or no)
Singh et al., 2013 [53]	Various	Meta-analysis	Pancreatic cancer risk	Metformin use	OR: 0.76 (0.57-1.03)	
Zhang et al., 2013 [39]	Various	Meta-analysis	Cancer incidence and mortality	Metformin (and in combination with other drugs) vs. non-users	SRR: Incidence **0.54 (0.35-0.83)** **Mortality 0.64 (0.48-0.86)**	

(a)

Bold type under "Risk" column indicates statistical significance using 95% confidence interval. HR, hazard ratio; OR, odds ratio, SRR, summary relative risk.

First Author/ Year	Location	Design	Outcome	Comparison	Relative Risk (95% CI)	Confounding Factors Accounted for
Evans et al., 2005 [41]	Scotland	Case-control	Cancer incidence	Metformin vs. no metformin	OR: 0.77 (0.64-0.92)	Smoking, body mass index, blood pressure, and postcode rank for material deprivation
Libby et al., 2009 [40]	Scotland	Nested case-control	Cancer deaths	Metformin vs. no metformin	HR: 0.63 (0.53-0.75)	Sex, age, BMI, A1C, deprivation, smoking, other drug use
Monami et al., 2009 [42]	Italy	Case-control	Cancer incidence	36 mo metformin vs no metformin	OR: 0.28 (0.13-0.57)	Concomitant therapies, exposure to

First Author/ Year (reference)	Location	Design	Outcome	Comparison	Risk (95% CI)	Confounding Factors
						metformin and gliclazide
Bowker et al., 2010 [44]	Canada	Cohort	Cancer death	Metformin vs. sulfonylurea	HR: 0.80 (0.65-0.98)	Age, sex and chronic disease score
Home et al., 2010 [54]	Various	Randomized control trials	Cancer incidence	Metformin vs. rosiglitazone Metformin vs. glibenclamide Metformin + sulfonylurea vs. Rosiglitazone + sulfonylurea	HR: 0.92 (0.63-1.35) HR: 0.78 (0.53-1.14) HR: 1.22 (0.86-1.74)	Not reported
Landman et al., 2010 [45]	Netherlands	Cohort	Cancer mortality	Metformin vs. no metformin	HR: 0.43 (0.23-0.80)	Smoking (yes or no), age, sex, diabetes duration, A1C, serum creatinine, BMI, blood pressure, total cholesterol–to–HDL ratio, albuminuria, insulin use, sulfonylurea use, and macrovascular complications (yes or no)
Lee et al., 2011 [37]	Taiwan	Cohort	Cancer incidence	Metformin vs. no metformin	HR: 0.12 (0.08-0.19)	age, gender, other oral anti-hyperglycemic medication usage, CCI score and dose and duration of metformin exposure
Monami et al., 2011 [43]	Italy	Case-control	Cancer incidence	Metformin vs. no metformin in patients under insulin treatment,	OR: 0.46 (0.25-0.85)	Charlson comorbidity score (CCS), glargine mean daily dose (MDD), and total MDD of insulin
Zhang et al., 2013 [39]	Various	Meta-analysis	Cancer incidence and mortality	Metformin (and in combination with other drugs) vs. non-users	SRR: Incidence 0.73 (0.64-0.83) Mortality 0.82 (0.76-0.89	

(b)

Bold type under "Risk" column indicates statistical significance using 95% confidence interval. HR, hazard ratio; OR, odds ratio, SRR, summary relative risk.

Table 1. A. Human Studies on Pancreatic Cancer Risk and Mortality with Metformin Use Among Diabetics, B. Human Studies on Overall Cancer Risk and Mortality with Metformin Use Among Diabetics

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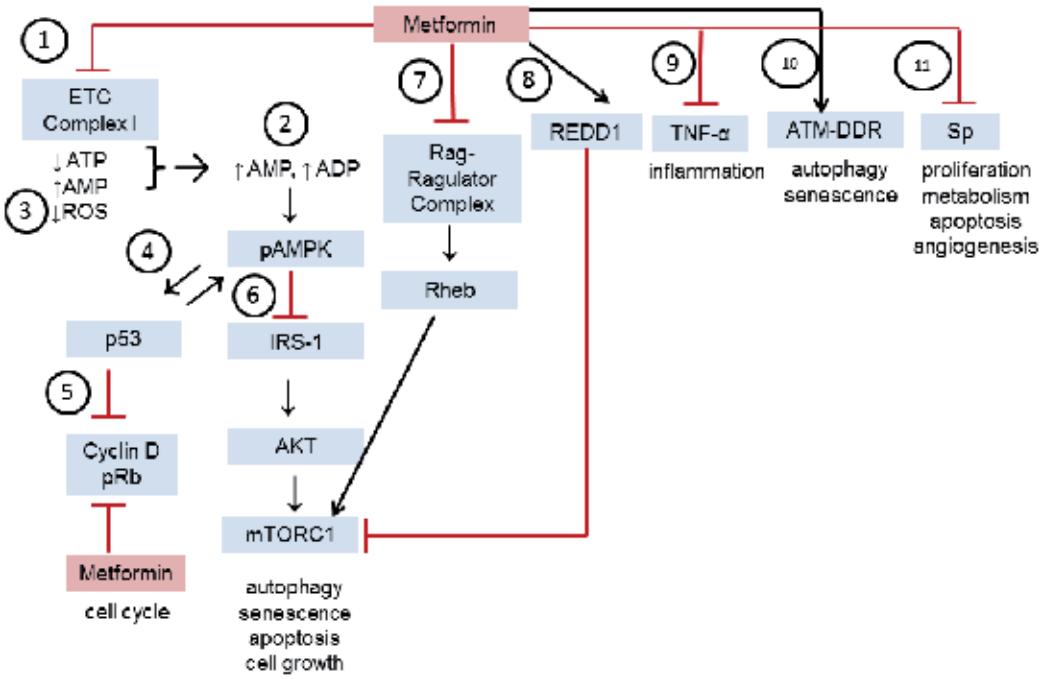

1] Metformin is a known inhibitor of complex I of the electron transport chain (ETC) [14, 15]. 2) The resulting decrease in adenosine triphosphate (ATP) production and increase in adenosine monophosphate (AMP) levels activate the kinase AMP-activated protein kinase (AMPK), a regulator of cellular energy status. Besides inhibition of energy-consuming biosynthetic processes (e.g., lipid synthesis and gluconeogenesis) and up-regulation of energy-generating catabolic metabolic pathways (e.g., β-oxidation of fatty acids and glycolysis), AMPK also signals to numerous proteins involved in cell survival, senescence, autophagy and death. For example, both high AMP and adenosine diphosphate (ADP) levels (from ETC complex I inhibition) is permissive for AMPK activation. AMP promotes AMPK phosphorylation at its catalytic α-subunit (Thr-172) by its upstream kinases liver kinase B1 (LKB1) and calcium/calmodulin-dependent protein kinase kinase-beta (CaMKKβ), its allosteric activation and prevents dephosphorylation by protein phosphatase type 2a (PP2a) and protein type 2c (PP2c) phosphatases [55-57]. ADP also protects AMPK from dephosphorylation [58]. 3) The metformin-induced decline in endogenous reactive oxygen species (ROS) levels has been implicated to be involved in cancer risk reduction owing to its ability to reduce ROS-induced DNA damage [59]. 4) AMPK has also been shown to activate the tumor suppressor protein 53 (p53) (Ser-15) in inducing cancer cell cycle arrest and senescence [60]. The reversible arrows between p53 and pAMPK indicate that p53 has been shown to increase AMPK activity which, ultimately leads to mammalian target of rapamycin (mTOR) inhibition *in vitro* [61]. 5) Metformin has been shown to cause a G0/G1 cell cycle arrest by decreasing the expression of cyclin D1 and preventing the phosphorylation of pRb and hence, its inactivation [62]. 6) Metformin-induced AMPK activation has been shown to phosphorylate insulin receptor substrate-1 (IRS-1) at Ser-794 which results in decreased recruitment of the p85 subunit of phosphoinositide-3-kinase (PI3K), thus, impairing the insulin-like growth factor (IGF)-stimulated PI3K/protein kinase B/ mammalian target of rapamycin complex 1 (PI3K/Akt/mTORC1) signaling pathway [63]. Metformin has also been shown to inhibit the crosstalk between the insulin/IGF receptor and G protein-coupled receptor (GPCR) signaling, resulting in the inhibition of mTORC1 [4, 5]. 7) Biguanides are implicated in the inhibition of the Rag-dependent mTORC1 signaling [8], by preventing the co-localization of mTORC1 with its activator Ras homolog enriched in brain (Rheb). Rags are GTPases comprised of four proteins RagA, RagB, RagC and RagD that heterodimerize to activate mTORC1 upon amino acid stimulation [10]. Rags bind to the Ragulator complex made up of mitogen-activated protein kinase scaffold protein 1 (MP1), p14 and p18 trimeric proteins, localizing mTORC1 from the perinulcear compartment (where Rheb is located) into the cytoplasm, preventing Rheb activation of mTORC1 [64]. 8) Metformin also increases the expression of the mTOR inhibitor, regulated in development and DNA damage responses (REDD1), consequently down-regulating mTOR signaling [65]. 9) In human monocytes, metformin prevents lipopolysaccharide (LPS) and oxidized low density lipoprotein (LDL)-induced tumor necrosis factor (TNF) production at micromolar concentrations [66]. 10) Activation

and phosphorylation of AMPK is dependent on the serine-threonine kinase, ataxia telangiectasia mutated (ATM), a checkpoint that responds to double-strand breaks and oxidative stress by activating the DNA damage response involving numerous downstream targets such as p53, checkpoint kinase 2 (Chk2), breast cancer 1 (BRCA1), Fanconi anemia, complementation group 2 (FANCD2), Nijmegen breakage syndrome 1 (Nbs1), p53 upregulated modulator of apoptosis (Puma) - Phorbol-12-myristate-13-acetate-induced protein 1 (Noxa) and BCL2-associated X protein (Bax) [67, 68] thus, preventing further DNA insult. 11) Metformin induces nuclear degradation and decreased expression of Sp proteins, transcription factors for genes involved in cell proliferation (cyclin D1), metabolism (FAS), apoptosis ((B-cell lymphoma 2 (bcl-2) and survivin)) and angiogenesis ((vascular endothelial growth factor (VEGF) and its receptor VEGFR1)) [24].

Figure 3. Metformin impairs signaling molecules for cancer survival.

6. Overall physiological and cellular effects of metformin in cancer models

Contrary to sulfonylureas, which act at the level of the pancreatic secretion of insulin, biguanides act at the level of sensitivity of the target tissues for insulin. Moreover, the biguanides can reduce the hyperglycemia without leading to incidental hypoglycemia. Hence, the term "anti-glycemic" agent was coined for metformin.

In the late 90s, amongst many studies published on the cellular effects of metformin, we showed that metformin is able to modulate the insulin receptor (IR) in cholesterol (chol)-treated human hepatoma cells, HepG2 [69]. In that study, we used a cellular model in which insulin sensitivity was altered by supplementing the culture medium of HepG2 cells with a derivative of CHOL, cholesteryl hemisuccinate (CHS) [70, 71]. Overall, metformin did not affect IR phosphorylation in control cells. However, metformin affected IR autophosphorylation in CHS-treated cells. At 1 and 5 min of insulin stimulation, metformin increased IR phosphorylation in these cells, restoring IR phosphorylation in CHS-treated cells towards control levels. As mentioned earlier, metformin is a charged biguanide, requiring cell surface transport protein for its influx [72] and exhibits membrane effects as well as cellular effects [73]. Pertinent to our early work, recent studies from Algire et al. [74] demonstrated that a high energy diet promotes tumor growth and that metformin decreases tumor volume only in high-energy fed animals. The authors suggest that, *"the inhibitory effect of metformin on tumor growth was restricted to animals on the high-energy diet. These results suggested that any benefits of this drug in reducing cancer aggressiveness may be restricted to a metabolically defined subset of cancer patients."* [74].

After nearly two decades of research and approval of metformin by the FDA in 1994, the target of the compound has yet to be identified. Arguably, as mentioned above, metformin is a charged biguanide, requiring cell surface transport protein for its influx [75] and exhibits membrane effects as well as cellular effects [73]. While Algire et al found that the anti-tumor effect of metformin was limited in animals on high-energy diets using *in vivo* models of lung and colorectal xenografts [76]; very recently, the work from Rozengurt and Eibl (from UCLA) demonstrates a strong tumor growth delay effect of metformin in pancreatic cancer xenograft models [5]. However, the doses used (>200mg/kg, i.p.) may not be clinically relevant. The ongoing debate on metformin dosages in animal models and human clinical trials has yet to define clearly the anti-diabetic dose *versus* the anti-cancer dose as well as a preventive *versus*

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a treatment dosage. The usual anti-diabetic dose of metformin is 500 - 1250 mg PO BID. Maximum recommended dose in patients with diabetes in 1250 mg PO BID. There seem to be some difference in data regarding prevention or actual treatment of cancer with metformin. The typical serum concentration from 1000- 2000 mg/day of metformin in diabetic patients is about 0.5 - 2 mg/L. The retrospective data so far have primarily looked at these doses and have shown that metformin prevents some cancers (including pancreatic cancer) [52, 77]. If we look at data regarding treatment of pancreatic cancer with metformin, it is different in terms of dosing. The pre-clinical data show that much higher doses are used (10-100 times the clinical used doses). If we look at data in humans using metformin for treatment of cancers, it shows some benefit at clinically used doses but it certainly is not as impressive as pre-clinical high dose metformin [78-80]. We did not find any ongoing human study using very high doses (beyond 3000 mg/day) primarily to treat cancers. Further studies need to be performed addressing this issue within the same animal model or within a similar patient population.

Finally, the fact that metformin prevents tumor development and growth in nude mice [5], supports a potential priming effect of metformin on the host potentially limiting the availability of 'onco' metabolites for which the tumor is 'addicted'. These types of studies investigating the processes of carcinogenesis, may address important gaps in current knowledge regarding the role of tumor metabolism in drug response. We strongly believe that mechanistic insight on these issues will have exceptionally high impact and potentially re-shape current paradigms about anti-metabolic drugs, pancreatic cancer treatment and personalized medicine. Indeed, we speculate that the efficacy of metformin – and possibly drugs with similar mechanism – depends on the metabolic context in which the tumor exists. This is potentially, a paradigm-changing concept as it suggests that host/tissue metabolic factors play a role in tumor conditioning and influence treatment response; a hypothesis that has not previously been considered in the clinical evaluation of metformin.

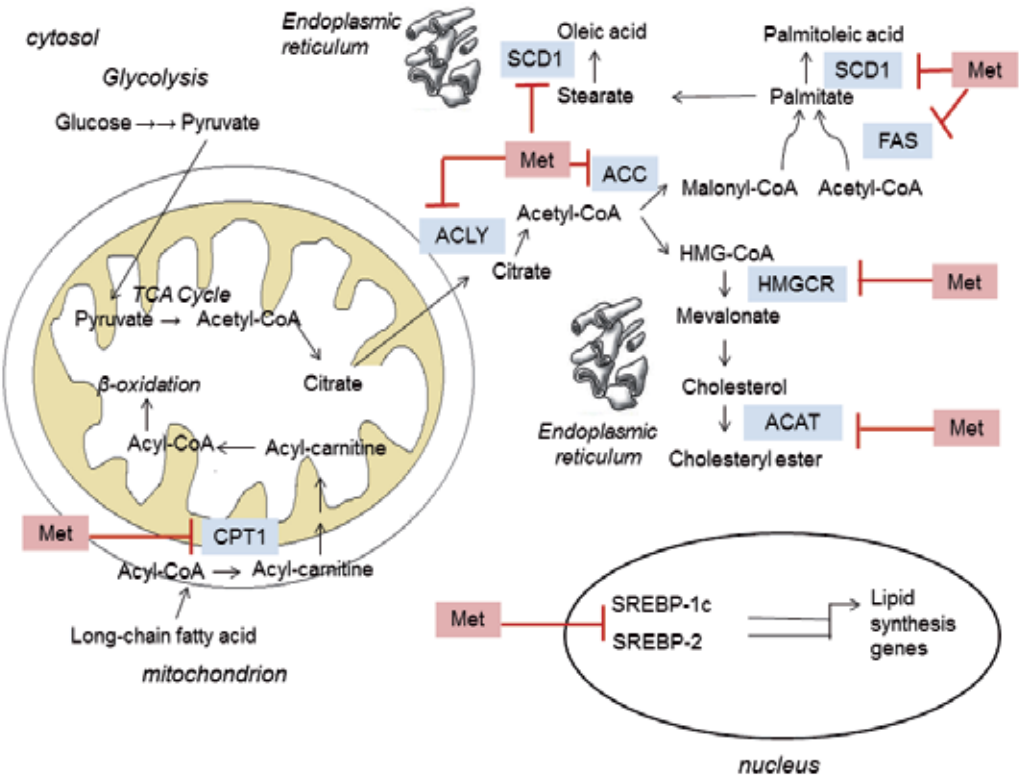
6.1. Metformin as a glucose lowering drug

Metformin works by decreasing hepatic gluconeogenesis [81], activating insulin receptor tyrosine phosphorylation [82], decreasing intestinal glucose absorption and increasing skeletal and adipose tissue glucose uptake [82]. One mechanistic study conducted in mice demonstrates that metformin (250 mg/kg/day for three consecutive days) increases the association of the glycolytic enzymes hexokinase to the mitochondria and phosphofuctokinase to F-actin in mice hearts [83]. These associations result in their activation and up-regulation in glycolysis, increasing cardiac glucose utilization which may partly explain the cardio protective effects of the drug [84, 85]. Since 1995, metformin has been a widely prescribed glucose lowering agent in the United States for type 2 diabetic and polycystic ovary syndrome patients. It is a well-tolerated drug with lactic acidosis as a reported serious side effect [86]. However, the link between lactic acidosis and metformin use has recently been questioned [87].

6.2. Metformin as an anti-lipogenic drug

Dating back to the 1920's, Otto Warburg published his observations on the metabolic aberrations of cancer cells. In the seminal paper entitled "The Metabolism of Tumors in the Body,"

Warburg and colleagues showed the absence of lactic acid accumulation in the blood of normal animals (no cancer) [88]. Whereas, animals with tumors accrued greater concentration of lactic acid in venous compared to arterial blood as well as in the tumor cavity, indicating the formation of lactic acid from glucose fermentation as blood goes through the tumor [88].



Metformin inhibits the gene expression of carnitine palmitoyltransferase 1 (CPT1), a mitochondrial enzyme that is the rate-limiting step in long-chain fatty acid β-oxidation. CPT1 catalyzes the transfer of acyl-CoA to the carnitine hydroxyl group, forming acyl-carnitine which is then transported into the mitochondrial matrix via translocase. Carnitine palmitoyltransferase 2 (CPT2) catalyzes the formation of acyl-CoA from acyl-carnitine. Acyl-CoA then undergoes β-oxidation. Metformin prevents the nuclear activation of sterol-regulatory element-binding protein 1c isoform (SREBP-1c) and SREBP-2 sterol-regulatory element-binding protein 2 isoform (SREBP-2), transcription factors that induce the expression of enzymatic genes involved in fatty acid and cholesterol synthesis, respectively. Metformin decreases the activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR) and acyl coenzyme A:cholesterol acyltransferase (ACAT). HMGCR is the rate-limiting enzyme of the mevalonate pathway, that catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) to mevalonate. The mevalonate pathway synthesizes isoprenoids and cholesterol. ACAT is an endoplasmic reticulum protein that catalyzes the formation of cholesterol esters from acyl-CoA and cholesterol. Metformin decreases the gene expression of steroyl-CoA desaturase 1 (SCD1), the enzyme responsible for desaturation of stearic acid (18:0) into oleic acid (18:1 n-9) and of palmitic acid (16:0) to palmitoleic acid (16:1 n-7). MET decreases the protein expression of FAS, acetyl-CoA carboxylase (ACC) and ATP citrate lyase (ACLY), which are enzymes involved in fatty acid synthesis.

Figure 4. Metformin inhibits key metabolic steps in lipogenesis.

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The reliance of cancer cells on glucose metabolism stems from their need to generate metabolites and reducing equivalents that are used to support crucial biosynthetic reactions that make lipids, nucleotides and amino acids. These biomolecules are rate-limiting for cell proliferation and survival. Glycolysis yields glucose-6-phosphate that enters the oxidative arm of the Pentose Phosphate Pathway (PPP). The oxidative PPP produces NADPH which, together with acetyl-CoA, fuels lipid synthesis in the cytosol. The non-oxidative branch of the PPP yields ribose-5-phosphate that is the precursor for nucleotides. In fact, as early as 1998, it has been argued that the both PPP branches (but primarily the non-oxidative branch) serve to produce ribose to sustain the increased needs of the cancer cell for DNA and RNA [89]. Fructose-6-phosphate and glyceraldehyde-3-phosphate are by-products of the glycolytic and the non-oxidative pentose phosphate pathways, providing an intimate link between glucose metabolism and nucleotide generation. Acetyl-CoA produced from the pyruvate dehydrogenase reaction enters the tricarboxylic acid (TCA) cycle in the mitochondria. Citrate can be exported from the mitochondria into the cytosol and converted back to acetyl-CoA (catalyzed by ATP citrate lyase) for lipid synthesis. Malate, an intermediate in the TCA cycle, can be converted into pyruvate by malic enzyme with the production of NADPH, a reducing equivalent that is used to generate reduced glutathione, allowing cancer cells greater tolerance to free radical-induced damage [90]. Glutaminase catalyses the hydrolysis of the amine group of glutamine to form glutamate and ammonia. Glutamate equilibrates with α-ketoglutarate via glutamate dehydrogenase. In a process termed reductive carboxylation, glutamine-derived citrate provides the acetyl-CoA for lipid synthesis and TCA cycle intermediates [91]. Hence, the glutamine addiction of cancer cells is another mechanism by which the metabolism is rewired to support biosynthesis [90, 91]. Please refer to Figure 3 for an integrated visual of cancer metabolism.

Diabetes and cancer are both metabolic diseases. It is therefore, not surprising that the mechanisms of action of metformin against type 2 diabetes and cancer include the drug's ability to alter critical metabolic circuits that lead to the normalization of blood glucose in diabetes and the impairment of biosynthetic pathways in cancer cells. For example, it is well-established that metformin is an inhibitor of complex I of the ETC. In 2000, two research groups have independently shown that dimethylbiguanide selectively blocks complex I of the ETC [14, 15]. In intact isolated hepatocytes, dimethylbiguanide has been reported to dose-dependently (0.1 to 10mM) inhibit oxygen consumption maximally at 20-30min [15]. The inhibition of respiration only occurred when glutamate-malate (complex I substrates) were used as substrates versus when succinate (complex II substrate)-rotenone or *N, N, N′, N′*-tetramethyl-1,4-phenylenediamine dihydrochloride (TMPD)-ascorbate (complex IV substrates) were added during the assessment of oxygen uptake. It is interesting to note that El-Mir and colleagues did not observe these changes when oxygen uptake experiments were performed in digitonin-permeabilized hepatocytes or in isolated liver mitochondria [15]. This is in contradiction to what Owen and others published when they showed that lower metformin concentrations of 50 and 100 μM were able to significantly decrease state 3 respiration rate in digitonin-permeabilized rat hepatoma cells [14]. Metformin has slow permeation properties across the inner mitochondrial membrane [14] and longer incubation periods (30 min in the El-Mir group versus 24-60 hours in the Owen group) may have eventually yielded comparable

results. In support of the previous findings, recent reports confirm that metformin is a specific inhibitor of the ETC complex I which leads to some impairment in mitochondrial function in human-derived non-malignant and in cancer cells [16-18, 92, 93]. This potentially limits the intact oxidative respiration capabilities of the cancer cell [16].

Besides inhibition of complex I and effects on glucose metabolism, numerous studies also show metformin-induced metabolic changes in non-cancer and cancer cells. One of the most notable effects of metformin is inhibition of lipogenesis, a metabolic pathway that is critical for a cancer cell's survival advantage. Under lipogenic conditions, surplus glucose in the cell is converted to pyruvate via glycolysis in the cytoplasm. Pyruvate is converted to acetyl-CoA and transported as citrate from the mitochondria into the cytoplasm. ATP citrate lyase (ACLY) converts citrate back to acetyl-CoA. Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA in an ATP-dependent manner. Acetyl-CoA and malonyl-CoA are then used as substrates for the production of palmitate by the seven enzymatic reactions catalyzed by FAS. In cancer, *de novo* fatty acid (FA) synthesis is up-regulated mainly for membrane production (usage of FA for phospholipids) and post-translational modification of proteins [94]. ACLY, ACC and FAS expression and activity have been shown to be up-regulated in cancers including pancreatic cancer. Thus, metabolic enzymes involved in FA synthesis have emerged as therapeutic targets in cancer [94]. The effects of MET on energy homeostasis in normal hepatocytes and breast and colon cancer cells have been characterized by the blocked activation or expression of key lipid biosynthesis enzymes such as ACC, FAS, HMGCR and enhanced expression of regulators of mitochondrial biogenesis, peroxisome proliferator-activated receptor-gamma co-activator 1 (PGC-1) [1, 74, 95].

Suppression of anabolic pathways (metformin is anti-lipogenic) is in keeping with the expected consequences of AMPK activation [1]. HMGCR may also play an important role in human malignancies. Indeed, recent transcriptional profiling demonstrated that cholesterol and lipid metabolisms are linked to cellular transformation [96]. Interestingly, high HMGCR mRNA levels correlated with poor patient prognosis and reduced survival. The levels of additional mevalonate (MVA) pathway genes were also significantly correlated with poor prognosis of breast cancer patients, suggesting the entire pathway may be deregulated in these cases [97]. It is interesting to note that the metformin-induced inhibition of respiration is blocked by the addition of palmitate in 3T3-L1 adipocytes [19]. Adipocytes treated with palmitate complexed to albumin in the presence of carnitine had comparable oxygen consumption rates when compared to control. These results indicate that the metformin-induced inhibition of respiration can be reversed by the addition of fatty acids, which led the authors to conclude that the mechanism of action of metformin may be linked to fatty acid metabolism [19]. Although indirect, this article presented a link between metformin and its effects on lipid metabolism or *vice versa*.

The normoglycemic effects of metformin has also been attributed to its ability to prevent fatty acid oxidation which decreases acetyl-CoA, ATP and reducing equivalents' availability for hepatic gluconeogenesis [98], an effect likely mediated by a reduction in the expression of the carnitine palmitoyltransferase I gene [99] and eventually, a decrease in protein expression and

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activity of the enzyme resulting in impairment in long fatty acid chain transport from the mitochondrial outer membrane into the matrix where β-oxidation takes place. Current publications also render support to the lipid-inhibitory effects of metformin. Metformin (0.2 to 1.0 mM for 16 h) has been shown to activate AMPK and decrease the mRNA, nuclear translocation and consequent activation via cleavage of the nuclear portion and the promoter activity of SREBP-1c in rat hepatoma McA-RH7777 cells [22, 23]. The mRNA and nuclear protein levels of SREBP-2 were also reduced after metformin treatment. This AMPK-mediated suppression of SREBP-1c has also been reported to prevent lipogenesis in an insulin resistant mouse model [20] and is consistent with a decrease in hepatic SREBP-1 expression in mice fed a high fat (60% lipids) diet for 10 weeks and then metformin (0.48mg% of the diet) for another six weeks [100]. Since SREBP-1c and SREBP-2 are transcription factors that promote the expression of enzymatic genes involved in fatty acid and cholesterol synthesis, respectively [101] we would expect diminished lipid synthesis as a biological endpoint of their down-regulation. In accordance, MRC5 human fetal lung fibroblasts incubated for 72 h with metformin (5×10^{-5} to 5×10^{-4} M) decreased 1-^{14}acetate incorporation into sterols, fatty acids and triglycerides compared to control, accompanied by a reduction in the activities of HMGCR and ACAT, enzymes that catalyze the formation of mevalonic acid from HMGCoA and the esterification of cholesterol, respectively [102]. Also, metformin has been shown to induce the phosphorylation (Ser-351) of the nuclear receptor TR4 via AMPK, leading to decreased TR4 transactivation and a decrease in the gene expression of its target, steroyl-CoA desaturase 1 (*SCD1*) gene expression. SCD is an enzyme that catalyzes the synthesis of monounsaturated fatty acids palmitoleic acid (16:1 n-7) and oleic acid (18:1 n-9) from saturated fatty acids obtained from *de novo* lipogenesis or from the diet [103]. SCD1 has been shown to be associated to numerous diseases including but not limited to obesity, hepatic steatosis, hypertriglyceridemia, insulin resistance, low grade inflammation and bone fractures [103].

The role of SCD1 in cancer has been gaining more attention as a potential pharmacological target in cancer interventions [104]. SCD is an endoplasmic reticulum-bound protein encoded by the *SCD1* and *SCD5* genes in humans [105]. They are highly expressed in liver and adipose tissue (SCD1) and in the brain and the pancreas (SCD5) [103]. Observational studies have reported a positive association between saturation index (18:0 to 18:1 n-9 ratio used by investigators as a marker for SCD activity) with cancer risk [106-110]. The first cDNA of human SCD published in 1994 revealed that the mRNA levels of this enzyme were elevated in tissues derived from esophageal carcinoma, colorectal cancer and hepatocellular adenoma [111]. Its protein levels are highly expressed in SV40-transformed fibroblasts compared to their wild type counterpart [112]. However, decreased transcript expression was reported in prostate cancer when compared to normal epithelium [113]. These seemingly conflicting results may reflect variation in expression depending on the tissue type or some malignancy-induced metabolic changes in lipid synthesis and/or lipid profile which would overall guarantee cancer cell survival advantage. Indeed, Moore and others speculated that the down-regulation of SCD cDNA in prostate carcinoma may be due to: a) the need of the cancer cell to increase the levels of palmitate which can be done by decreasing SCD activity, b) eliminate the SCD-induced

down-regulation of lipid membrane rafts and c) down-regulate susceptibility of tumor cells containing more unsaturated fatty acids to TNF-induced free radical attack [113]. Nevertheless, knockdown of human SCD in transformed human fibroblasts resulted in decreased oleic acid synthesis, lowered desaturation index profiles of the main polar lipids phosphatidylcholine and phosphatidylethanolamine, and decreased *de novo* synthesis of ^{14}C-labeled phospholipids, cholesterol and cholesterol esters, free fatty acids and triacylglycerols [114]. Interestingly, there was an inhibition in cellular proliferation and anchorage-independent growth in the SCD knockdown cells [114]. Other studies confirm that SCD inhibition by chemical or genetic manipulations resulted in inhibition of cancer cell proliferation and/or death [115-118]. Thus, SCD appears to be involved in modulating lipid metabolism and signaling processes crucial for cancer cell replication and anchorage-independent growth, effects are likely influenced by the effects of SCD loss on membrane integrity [114].

In support of the anti-lipogenic effects of metformin, Bhalla and others [2] have reported that metformin decreased the gene and protein expression of enzymes involved in fatty acid synthesis namely, ACC, FAS and ACLY which was accompanied by a reduction in hepatic triglycerides in a mouse model of hepatocellular cancer fed metformin at a dose of 250 mg/kg for 24-36 weeks.

Obesity is a known risk factor for cancers of the pancreas, colon and rectum, esophagus, kidney, prostate, breast, uterus and ovaries [119-123]. In order to recapitulate this condition in the preclinical setting, animal models are fed high energy (HE), high fat (HF) diets to induce the metabolic syndrome and/or obesity. In an *in vivo* model of colon carcinoma by Algire and colleagues [124], metformin (50mg/kg/day for five weeks) significantly decreased tumor volume only in mice fed the HF/HE diet. Of note, these concentrations are more likely to be physiologically relevant to what a diabetic patient would have in their system. They also found that metformin reduced the expression of SREBP-1 and one of its target enzymes, FAS, regardless of the type of diet. Interestingly, a previous study from the same group using an *in vivo* model of lung cancer back in 2008 showed that the tumor growth inhibitory impact of metformin is exclusive to the mice under the HF/HE diet [76]. These two studies suggest that metformin may retard cancer growth depending on a particular metabolic state of the organism which in this case, is the abundance of circulating lipids from the HE/HF diet.

We recently found that the *in vitro* response to metformin depends on the level of intracellular cholesterol synthesis of the tumor [125]. We were the first to demonstrate that a physiologically relevant dose of metformin impairs glucose utilization in pancreatic cancer by inhibiting FAS when cholesterol synthesis is limited. Specifically, we found that pancreatic cells that have a K-*ras* mutation and that require *de novo* fatty acid (FA) synthesis for lipids ('lipogenic cells') were unable to synthesize FA from acetyl-CoA in the presence of an inhibitor of cholesterol synthesis and metformin. Our *in vitro* model shows that a physiologically relevant dose of metformin (100 μM) using an acute treatment of 24 h decreases *de novo* lipid synthesis via the FAS pathway in pancreatic adenocarcinoma only when: a) the glucose-derived acetyl-CoA is made available for fatty acid synthesis by inhibition of cholesterol synthesis (addition of exogenous cholesterol) and b) K-*ras* mutation is present [21]. As the fatty acid and cholesterol

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synthesis pathways utilize acetyl-CoA as a common substrate [126], the addition of cholesterol (in the form of the more water-soluble cholesteryl hemisuccinate) inhibits the thiolase-catalyzed cholesterol pathway and shifts the glucose-derived acetyl CoA- towards the acetyl-CoA carboxylase-catalyzed fatty acid synthesis pathway. These effects are observed only in MiaPaCa-2-cells, which harbor the GGT(Gly) to TGT(Cys) codon 12 K-*ras* mutation. Further, non-lipogenic cancer cells harboring a K-ras^{G12C} mutation [127] with suppressed cholesterol synthesis were significantly more sensitive to the growth inhibiting effects of metformin than tumor cells containing wild-type K-*ras* with normal cholesterol synthesis. These results are consistent with expected modulation of AMPK [1] and/or mTOR [128].

Title	Recruitment	Results	Conditions	Interventions
Metformin Hydrochloride in Treating Patients With Pancreatic Cancer That Can be Removed by Surgery	Not yet recruiting	No Results Available	*Pancreatic Cancer*: Stage IA Stage IB Stage IIA Stage IIB	*Drug*: metformin hydrochloride *Other*: pharmacological study
Metformin Combined With Chemotherapy for Pancreatic Cancer	Recruiting	No Results Available	Locally Advanced Pancreatic Cancer/ Metastatic Pancreatic Cancer	*Drug*: gemcitabine, erlotinib, metformin, placebo
Metformin Plus Modified FOLFOX 6 in Metastatic Pancreatic Cancer	Recruiting	No Results Available	Acinar Cell Adenocarcinoma of the Pancreas, Duct Cell, Adenocarcinoma of the Pancreas, Recurrent Pancreatic Cancer, Stage IV Pancreatic Cancer	*Drug*: metformin hydrochloride, oxaliplatin, leucovorin calcium, fluorouracil *Other*: laboratory biomarker analysis
Combination Chemotherapy With or Without Metformin Hydrochloride in Treating Patients With Metastatic Pancreatic Cancer	Recruiting	No Results Available	Pancreatic Cancer	*Drug*: capecitabine, cisplatin, epirubicin, gemcitabine, metformin
Treatment of Patients With Advanced Pancreatic Cancer After Gemcitabine Failure	Recruiting	No Results Available	Pancreatic Adenocarcinoma Advanced or Metastatic	*Drug*: paclitaxel, metformin
Gemcitabine+Nab-paclitaxel and FOLFIRINOX and Molecular Profiling for Patients With Advanced Pancreatic Cancer	Recruiting	No Results Available	Stage IV Pancreatic Cancer	*Drug*: Gemcitabine, nab-paclitaxel, FOLFIRINOX, metformin *Genetic*: Immunohistochemistry (IHC) Analysis

Table 2. Ongoing Clinical Trials on Metformin and Pancreatic Cancer

In summary, metformin's anti-cancer properties rest on its ability to impair cancer cell lipogenesis, a critical mechanism by which cancer cells maintain their survival advantage over normal cells. Metformin is able to control lipogenesis through inhibition of the transcription factors SREBP-1 and SREBP-2, inhibition of activities and/or expression of enzymes involved in cholesterol and fatty acid synthesis. We have shown that metformin's anti-cancer role is effective in select metabolic phenotype and likely, a particulate cancer genotype. Thus, it is important to understand the metabolic context by which metformin exerts anti-cancer effects so that the correct patient population can be selected for therapeutic purposes.

7. Ongoing clinical trials on metformin as a chemotherapeutic drug for pancreatic cancer

There is considerable interest in the anti-tumor action of the commonly used anti-diabetic drug metformin for the treatment and management of patients with pancreatic cancer. Enthusiasm for metformin has been significantly strengthened by *in vitro* and *in vivo* experimental findings of potent anti-tumor activity of metformin at therapeutically safe doses. As a result, a number of early phase trials are now being conducted to assess the efficacy of metformin in combination with standard and experimental therapeutics in pancreatic cancer patients. Although there are numerous studies that show the cancer preventive and cancer therapeutic actions of metformin in preclinical models, there is a need to conduct adequately powered clinical trials on the therapeutic effects of metformin that include prognosis and survival markers. At the time that this book chapter was being written, there are six ongoing clinical trials specifically on pancreatic cancer and metformin from the ClinicalTrials.gov website (Table 2).

8. Conclusions and perspectives

Metformin is an inexpensive and well-tolerated drug and its utility as a chemopreventive and/or chemotherapeutic agent can be harnessed when we identify the drug's target/s, optimal dosage, and the correct patient sub-population who will benefit from metformin treatment. Until then, metformin remains the most widely prescribed anti-diabetic drug in the world with an unknown mechanism of action. In the era of targeted cancer therapy, one may cautiously link gene mutations and oncogenes up and down-regulation to cancer and involve metabolic phenotyping of the patient for better selection and truly personalized medicine.

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Author details

Mary Jo Cantoria[1,2], Hitendra Patel[2,3], Laszlo G. Boros[4,5] and Emmanuelle J. Meuillet[2,6*]

*Address all correspondence to: emeuillet@azcc.arizona.edu

1 Department of Nutritional Sciences, The University of Arizona, Tucson, USA

2 The University of Arizona Cancer Center, Tucson, AZ, USA

3 College of Medicine, The University of Arizona, Tucson, USA

4 SiDMAP, LLC, Los Angeles, CA, USA

5 Department of Pediatrics, Los Angeles Biomedical Research, USA

6 Institute at the Harbor-UCLA Medical Center, Torrance, CA, USA

Department of Nutritional Sciences, The University of Arizona, Tucson, USA

References

[1] Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. The Journal of clinical investigation. 2001;108(8):1167-74.

[2] Bhalla K, Hwang BJ, Dewi RE, Twaddel W, Goloubeva OG, Wong KK, et al. Metformin prevents liver tumorigenesis by inhibiting pathways driving hepatic lipogenesis. Cancer Prev Res (Phila). 2012;5(4):544-52.

[3] Sinnett-Smith J, Kisfalvi K, Kui R, Rozengurt E. Metformin inhibition of mTORC1 activation, DNA synthesis and proliferation in pancreatic cancer cells: dependence on glucose concentration and role of AMPK. Biochemical and biophysical research communications. 2013;430(1):352-7.

[4] Rozengurt E, Sinnett-Smith J, Kisfalvi K. Crosstalk between insulin/insulin-like growth factor-1 receptors and G protein-coupled receptor signaling systems: a novel target for the antidiabetic drug metformin in pancreatic cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2010;16(9):2505-11.

[5] Kisfalvi K, Eibl G, Sinnett-Smith J, Rozengurt E. Metformin disrupts crosstalk between G protein-coupled receptor and insulin receptor signaling systems and inhibits pancreatic cancer growth. Cancer research. 2009;69(16):6539-45.

[6] Karnevi E, Said K, Andersson R, Rosendahl AH. Metformin-mediated growth inhibition involves suppression of the IGF-I receptor signalling pathway in human pancreatic cancer cells. BMC cancer. 2013;13:235.

[7] Soares HP, Ni Y, Kisfalvi K, Sinnett-Smith J, Rozengurt E. Different patterns of Akt and ERK feedback activation in response to rapamycin, active-site mTOR inhibitors and metformin in pancreatic cancer cells. PloS one. 2013;8(2):e57289.

[8] Kalender A, Selvaraj A, Kim SY, Gulati P, Brule S, Viollet B, et al. Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. Cell metabolism. 2010;11(5):390-401.

[9] Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, et al. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science. 2008;320(5882):1496-501.

[10] Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell. 2010;141(2):290-303.

[11] Ben Sahra I, Laurent K, Giuliano S, Larbret F, Ponzio G, Gounon P, et al. Targeting cancer cell metabolism: the combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. Cancer research. 2010;70(6):2465-75.

[12] Cheong JH, Park ES, Liang J, Dennison JB, Tsavachidou D, Nguyen-Charles C, et al. Dual inhibition of tumor energy pathway by 2-deoxyglucose and metformin is effective against a broad spectrum of preclinical cancer models. Molecular cancer therapeutics. 2011;10(12):2350-62.

[13] Beckner ME, Gobbel GT, Abounader R, Burovic F, Agostino NR, Laterra J, et al. Glycolytic glioma cells with active glycogen synthase are sensitive to PTEN and inhibitors of PI3K and gluconeogenesis. Laboratory investigation; a journal of technical methods and pathology. 2005;85(12):1457-70.

[14] Owen MR, Doran, E., Halestrap, A. P. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex I of the mitochondrial respiratory chain. Biochem J. 2000;348:607-14.

[15] El-Mir MY, Nogueira V, Fontaine E, Averet N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. The Journal of biological chemistry. 2000;275(1):223-8.

[16] Whitaker-Menezes D, Martinez-Outschoorn UE, Flomenberg N, Birbe RC, Witkiewicz AK, Howell A, et al. Hyperactivation of oxidative mitochondrial metabolism in epithelial cancer cells in situ: visualizing the therapeutic effects of metformin in tumor tissue. Cell Cycle. 2011;10(23):4047-64.

[17] Hirsch A, Hahn D, Kempna P, Hofer G, Nuoffer JM, Mullis PE, et al. Metformin inhibits human androgen production by regulating steroidogenic enzymes HSD3B2

and CYP17A1 and complex I activity of the respiratory chain. Endocrinology. 2012;153(9):4354-66.

[18] Dykens JA, Jamieson J, Marroquin L, Nadanaciva S, Billis PA, Will Y. Biguanide-induced mitochondrial dysfunction yields increased lactate production and cytotoxicity of aerobically-poised HepG2 cells and human hepatocytes in vitro. Toxicology and applied pharmacology. 2008;233(2):203-10.

[19] Gonzalez-Barroso MM, Anedda A, Gallardo-Vara E, Redondo-Horcajo M, Rodriguez-Sanchez L, Rial E. Fatty acids revert the inhibition of respiration caused by the antidiabetic drug metformin to facilitate their mitochondrial beta-oxidation. Biochimica et biophysica acta. 2012;1817(10):1768-75.

[20] Li Y, Xu S, Mihaylova MM, Zheng B, Hou X, Jiang B, et al. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. Cell metabolism. 2011;13(4):376-88.

[21] Cantoria MJ, Boros, L. G., Meuillet, E. J. Contextual inhibition of fatty acid synthesis by metformin involves glucose-derived acetyl-CoA and cholesterol in pancreatic tumor cells. Metabolomics. 2013.

[22] Yap F, Craddock L, Yang J. Mechanism of AMPK suppression of LXR-dependent Srebp-1c transcription. International journal of biological sciences. 2011;7(5):645-50.

[23] Yang J, Craddock L, Hong S, Liu ZM. AMP-activated protein kinase suppresses LXR-dependent sterol regulatory element-binding protein-1c transcription in rat hepatoma McA-RH7777 cells. Journal of cellular biochemistry. 2009;106(3):414-26.

[24] Nair V, Pathi S, Jutooru I, Sreevalsan S, Basha R, Abdelrahim M, et al. Metformin Inhibits Pancreatic Cancer Cell and Tumor Growth and Downregulates Sp Transcription Factors. Carcinogenesis. 2013.

[25] Kisfalvi K, Moro A, Sinnett-Smith J, Eibl G, Rozengurt E. Metformin inhibits the growth of human pancreatic cancer xenografts. Pancreas. 2013;42(5):781-5.

[26] Schneider MB, Matsuzaki H, Haorah J, Ulrich A, Standop J, Ding XZ, et al. Prevention of pancreatic cancer induction in hamsters by metformin. Gastroenterology. 2001;120(5):1263-70.

[27] Yang Y, Liu H, Li Z, Zhao Z, Yip-Schneider M, Fan Q, et al. Role of fatty acid synthase in gemcitabine and radiation resistance of pancreatic cancers. International journal of biochemistry and molecular biology. 2011;2(1):89-98.

[28] Watanabe CK. Studies in the metabolic changes induced by administration of guanidine bases. Journal of Biological Chemistry. 1918;33:253-65.

[29] Sterne J. Oral hypoglycaemic agents.: London Academic Press; 1969 1969. 193-245 p.

[30] Werner EA, Bell J. CCXIV.-The preparation of methylguanidine, and of [small beta] [small beta]-dimethylguanidine by the interaction of dicyanodiamide, and methyl-

ammonium and dimethylammonium chlorides respectively. Journal of the Chemical Society, Transactions. 1922;121(0):1790-4.

[31] Bharatam PV, Patel DS, Iqbal P. Pharmacophoric Features of Biguanide Derivatives: An Electronic and Structural Analysis. Journal of Medicinal Chemistry. 2005;48(24): 7615-22.

[32] Schäfer G. On the mechanism of action of hypoglycemia-producing biguanides. A reevaluation and a molecular theory. Biochemical Pharmacology. 1976;25(18): 2005-14.

[33] Wiernsperger NF, Bailey CJ. The antihyperglycaemic effect of metformin: therapeutic and cellular mechanisms. Drugs. 1999;58 Suppl 1:31-9; discussion 75-82.

[34] Beckmann R. [Absorption, distribution in the organism and elimination of metformin]. Diabetologia. 1969;5(5):318-24.

[35] Li D, Yeung SC, Hassan MM, Konopleva M, Abbruzzese JL. Antidiabetic therapies affect risk of pancreatic cancer. Gastroenterology. 2009;137(2):482-8.

[36] Bodmer M, Becker C, Meier C, Jick SS, Meier CR. Use of antidiabetic agents and the risk of pancreatic cancer: a case-control analysis. The American journal of gastroenterology. 2012;107(4):620-6.

[37] Lee MS, Hsu CC, Wahlqvist ML, Tsai HN, Chang YH, Huang YC. Type 2 diabetes increases and metformin reduces total, colorectal, liver and pancreatic cancer incidences in Taiwanese: a representative population prospective cohort study of 800,000 individuals. BMC cancer. 2011;11:20.

[38] Ruiter R, Visser LE, van Herk-Sukel MP, Coebergh JW, Haak HR, Geelhoed-Duijvestijn PH, et al. Lower risk of cancer in patients on metformin in comparison with those on sulfonylurea derivatives: results from a large population-based follow-up study. Diabetes Care. 2012;35(1):119-24.

[39] Zhang P, Li H, Tan X, Chen L, Wang S. Association of metformin use with cancer incidence and mortality: a meta-analysis. Cancer Epidemiol. 2013;37(3):207-18.

[40] Libby G, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. Diabetes Care. 2009;32(9):1620-5.

[41] Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. BMJ. 2005;330(7503):1304-5.

[42] Monami M, Lamanna C, Balzi D, Marchionni N, Mannucci E. Sulphonylureas and cancer: a case-control study. Acta Diabetol. 2009;46(4):279-84.

[43] Monami M, Colombi C, Balzi D, Dicembrini I, Giannini S, Melani C, et al. Metformin and cancer occurrence in insulin-treated type 2 diabetic patients. Diabetes Care. 2011;34(1):129-31.

[44] Bowker SL, Yasui Y, Veugelers P, Johnson JA. Glucose-lowering agents and cancer mortality rates in type 2 diabetes: assessing effects of time-varying exposure. Diabetologia. 2010;53(8):1631-7.

[45] Landman GW, Kleefstra N, van Hateren KJ, Groenier KH, Gans RO, Bilo HJ. Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16. Diabetes Care. 2010;33(2):322-6.

[46] Oliveria SA KC, Yood MU et al. Cancer incidence among patients treated with antidiabetic pharmacotherapy. Diabetes Metabol Syndrome: Clin Res Rev. 2007;2:47-57.

[47] Currie CJ, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia. 2009;52(9):1766-77.

[48] Ferrara A, Lewis JD, Quesenberry CP, Jr., Peng T, Strom BL, Van Den Eeden SK, et al. Cohort study of pioglitazone and cancer incidence in patients with diabetes. Diabetes Care. 2011;34(4):923-9.

[49] Liao KF, Lai SW, Li CI, Chen WC. Diabetes mellitus correlates with increased risk of pancreatic cancer: a population-based cohort study in Taiwan. J Gastroenterol Hepatol. 2012;27(4):709-13.

[50] Morden NE, Liu SK, Smith J, Mackenzie TA, Skinner J, Korc M. Further exploration of the relationship between insulin glargine and incident cancer: a retrospective cohort study of older Medicare patients. Diabetes Care. 2011;34(9):1965-71.

[51] Nakai Y, Isayama H, Sasaki T, Mizuno S, Sasahira N, Kogure H, et al. Clinical outcomes of chemotherapy for diabetic and nondiabetic patients with pancreatic cancer: better prognosis with statin use in diabetic patients. Pancreas. 2013;42(2):202-8.

[52] Sadeghi N, Abbruzzese JL, Yeung SC, Hassan M, Li D. Metformin use is associated with better survival of diabetic patients with pancreatic cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2012;18(10):2905-12.

[53] Singh S, Singh PP, Singh AG, Murad MH, McWilliams RR, Chari ST. Anti-diabetic medications and risk of pancreatic cancer in patients with diabetes mellitus: a systematic review and meta-analysis. The American journal of gastroenterology. 2013;108(4):510-9; quiz 20.

[54] Home PD, Kahn SE, Jones NP, Noronha D, Beck-Nielsen H, Viberti G. Experience of malignancies with oral glucose-lowering drugs in the randomised controlled ADOPT (A Diabetes Outcome Progression Trial) and RECORD (Rosiglitazone Evaluated for Cardiovascular Outcomes and Regulation of Glycaemia in Diabetes) clinical trials. Diabetologia. 2010;53(9):1838-45.

[55] Oakhill JS, Steel R, Chen ZP, Scott JW, Ling N, Tam S, et al. AMPK is a direct adenylate charge-regulated protein kinase. Science. 2011;332(6036):1433-5.

[56] Davies SP, Helps NR, Cohen PT, Hardie DG. 5'-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase-2C alpha and native bovine protein phosphatase-2AC. FEBS Lett. 1995;377(3):421-5.

[57] Carling D, Mayer FV, Sanders MJ, Gamblin SJ. AMP-activated protein kinase: nature's energy sensor. Nat Chem Biol. 2011;7(8):512-8.

[58] Xiao B, Sanders MJ, Underwood E, Heath R, Mayer FV, Carmena D, et al. Structure of mammalian AMPK and its regulation by ADP. Nature. 2011;472(7342):230-3.

[59] Algire C, Moiseeva O, Deschenes-Simard X, Amrein L, Petruccelli L, Birman E, et al. Metformin reduces endogenous reactive oxygen species and associated DNA damage. Cancer Prev Res (Phila). 2012;5(4):536-43.

[60] Jones RG, Plas DR, Kubek S, Buzzai M, Mu J, Xu Y, et al. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Mol Cell. 2005;18(3):283-93.

[61] Feng Z, Zhang H, Levine AJ, Jin S. The coordinate regulation of the p53 and mTOR pathways in cells. Proc Natl Acad Sci U S A. 2005;102(23):8204-9.

[62] Ben Sahra I, Laurent K, Loubat A, Giorgetti-Peraldi S, Colosetti P, Auberger P, et al. The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. Oncogene. 2008;27(25):3576-86.

[63] Ning J, Clemmons DR. AMP-activated protein kinase inhibits IGF-I signaling and protein synthesis in vascular smooth muscle cells via stimulation of insulin receptor substrate 1 S794 and tuberous sclerosis 2 S1345 phosphorylation. Mol Endocrinol. 2010;24(6):1218-29.

[64] Pierotti MA, Berrino F, Gariboldi M, Melani C, Mogavero A, Negri T, et al. Targeting metabolism for cancer treatment and prevention: metformin, an old drug with multifaceted effects. Oncogene. 2013;32(12):1475-87.

[65] Ben Sahra I, Regazzetti C, Robert G, Laurent K, Le Marchand-Brustel Y, Auberger P, et al. Metformin, independent of AMPK, induces mTOR inhibition and cell-cycle arrest through REDD1. Cancer research. 2011;71(13):4366-72.

[66] Arai M, Uchiba M, Komura H, Mizuochi Y, Harada N, Okajima K. Metformin, an antidiabetic agent, suppresses the production of tumor necrosis factor and tissue factor by inhibiting early growth response factor-1 expression in human monocytes in vitro. J Pharmacol Exp Ther. 2010;334(1):206-13.

[67] Menendez JA, Cufi S, Oliveras-Ferraros C, Martin-Castillo B, Joven J, Vellon L, et al. Metformin and the ATM DNA damage response (DDR): accelerating the onset of stress-induced senescence to boost protection against cancer. Aging (Albany NY). 2011;3(11):1063-77.

[68] Lavin MF, Kozlov S. ATM activation and DNA damage response. Cell Cycle. 2007;6(8):931-42.

[69] Meuillet EJ, Wiernsperger N, Mania-Farnell B, Hubert P, Cremel G. Metformin modulates insulin receptor signaling in normal and cholesterol-treated human hepatoma cells (HepG2). European journal of pharmacology. 1999;377(2-3):241-52.

[70] Shinitzky M. An efficient method for modulation of cholesterol level in cell membranes. FEBS Lett. 1978;85(2):317-20.

[71] Meuillet EJ, Leray V, Hubert P, Leray C, Cremel G. Incorporation of exogenous lipids modulates insulin signaling in the hepatoma cell line, HepG2. Biochimica et biophysica acta. 1999;1454(1):38-48.

[72] Nies AT, Hofmann U, Resch C, Schaeffeler E, Rius M, Schwab M. Proton pump inhibitors inhibit metformin uptake by organic cation transporters (OCTs). PLoS One. 2011;6(7):e22163.

[73] Viollet B, Guigas B, Sans Garcia N, Leclerc J, Foretz M, Adreelli F. Cellular and molecular mechanisms of metformin: an overview. Clinical Science. 2012;122:253-70.

[74] Algire C, Amrein L, Zakikhani M, Panasci L, Pollak M. Metformin blocks the stimulative effect of a high-energy diet on colon carcinoma growth in vivo and is associated with reduced expression of fatty acid synthase. Endocrine Related Cancer. 2010;17(2):351-60.

[75] Nies AT, Hofmann U, Resch C, Schaeffeler E, Rius M, Schwab M. Proton Pump Inhibitors Inhibit Metformin Uptake by Organic Cation Transporters (OCTs). Plos One. 2011;6(7).

[76] Algire C, Zakikhani M, Blouin MJ, Shuai JH, Pollak M. Metformin attenuates the stimulatory effect of a high-energy diet on in vivo LLC1 carcinoma growth. Endocr Relat Cancer. 2008;15(3):833-9.

[77] Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, et al. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. Cancer Prev Res (Phila). 2010;3(11):1451-61.

[78] Cazzaniga MD, A.; Pruneri, G.; Puntoni, M.; Guerrieri-Gonzaga, A.; Dell'Orto, P.; Gentilini, O.D.; Vingiani, A.; Pagani, G.; Puccio, A.; and Bonanni, B.. The effect of metformin on apoptosis in a breast cancer presurgical trial. Cancer research. 2012;72 (24 Supplement):PD03-1.

[79] Gou S, Cui P, Li X, Shi P, Liu T, Wang C. Low concentrations of metformin selectively inhibit CD133(+) cell proliferation in pancreatic cancer and have anticancer action. PloS one. 2013;8(5):e63969.

[80] Hadad S, Iwamoto T, Jordan L, Purdie C, Bray S, Baker L, et al. Evidence for biological effects of metformin in operable breast cancer: a pre-operative, window-of-opportunity, randomized trial. Breast cancer research and treatment. 2011;128(3):783-94.

[81] Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond). 2012;122(6):253-70.

[82] Del Barco S, Vazquez-Martin A, Cufi S, Oliveras-Ferraros C, Bosch-Barrera J, Joven J, et al. Metformin: multi-faceted protection against cancer. Oncotarget. 2011;2(12): 896-917.

[83] Da Silva D, Ausina P, Alencar EM, Coelho WS, Zancan P, Sola-Penna M. Metformin reverses hexokinase and phosphofructokinase downregulation and intracellular distribution in the heart of diabetic mice. IUBMB Life. 2012;64(9):766-74.

[84] Mamputu JC, Wiernsperger NF, Renier G. Antiatherogenic properties of metformin: the experimental evidence. Diabetes Metab. 2003;29(4 Pt 2):6S71-6.

[85] Molavi B, Rassouli N, Bagwe S, Rasouli N. A review of thiazolidinediones and metformin in the treatment of type 2 diabetes with focus on cardiovascular complications. Vasc Health Risk Manag. 2007;3(6):967-73.

[86] Fitzgerald E, Mathieu S, Ball A. Metformin associated lactic acidosis. BMJ. 2009;339:b3660.

[87] Preiss DJ, Sattar N. Metformin and lactic acidosis. Metformin: framed again? BMJ. 2009;339:b5570.

[88] Warburg O, Wind F, Negelein E. The Metabolism of Tumors in the Body. J Gen Physiol. 1927;8(6):519-30.

[89] Boros LG, Lee PW, Brandes JL, Cascante M, Muscarella P, Schirmer WJ, et al. Nonoxidative pentose phosphate pathways and their direct role in ribose synthesis in tumors: is cancer a disease of cellular glucose metabolism? Med Hypotheses. 1998;50(1):55-9.

[90] Son J, Lyssiotis CA, Ying H, Wang X, Hua S, Ligorio M, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. Nature. 2013;496(7443):101-5.

[91] Hensley CT, Wasti AT, Deberardinis RJ. Glutamine and cancer: cell biology, physiology, and clinical opportunities. The Journal of clinical investigation. 2013;123(9): 3678-84.

[92] Scotland S, Saland E, Skuli N, de Toni F, Boutzen H, Micklow E, et al. Mitochondrial energetic and AKT status mediate metabolic effects and apoptosis of metformin in human leukemic cells. Leukemia. 2013.

[93] Guigas B, Detaille D, Chauvin C, Batandier C, De Oliveira F, Fontaine E, et al. Metformin inhibits mitochondrial permeability transition and cell death: a pharmacological in vitro study. Biochem J. 2004;382(Pt 3):877-84.

[94] Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer. 2007;7(10):763-77.

[95] Lee KH, Hsu EC, Guh JH, Yang HC, Wang D, Kulp SK, et al. Targeting energy metabolic and oncogenic signaling pathways in triple-negative breast cancer by a novel adenosine monophosphate-activated protein kinase (AMPK) activator. The Journal of biological chemistry. 2011;286(45):39247-58.

[96] Hirsch HA, Iliopoulos D, Joshi A, Zhang Y, Jaeger SA, Bulyk M, et al. A transcriptional signature and common gene networks link cancer with lipid metabolism and diverse human diseases. Cancer cell. 2010;17(4):348-61.

[97] Clendening JW, Pandyra A, Boutros PC, El Ghamrasni S, Khosravi F, Trentin GA, et al. Dysregulation of the mevalonate pathway promotes transformation. Proc Natl Acad Sci U S A. 2010;107(34):15051-6.

[98] Wolf HP. Possible new therapeutic approach in diabetes mellitus by inhibition of carnitine palmitoyltransferase 1 (CPT1). Horm Metab Res Suppl. 1992;26:62-7.

[99] Fulgencio JP, Kohl C, Girard J, Pegorier JP. Effect of metformin on fatty acid and glucose metabolism in freshly isolated hepatocytes and on specific gene expression in cultured hepatocytes. Biochem Pharmacol. 2001;62(4):439-46.

[100] Souza-Mello V, Gregorio BM, Cardoso-de-Lemos FS, de Carvalho L, Aguila MB, Mandarim-de-Lacerda CA. Comparative effects of telmisartan, sitagliptin and metformin alone or in combination on obesity, insulin resistance, and liver and pancreas remodelling in C57BL/6 mice fed on a very high-fat diet. Clin Sci (Lond). 2010;119(6): 239-50.

[101] Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. The Journal of clinical investigation. 2002;109(9):1125-31.

[102] Maziere JC, Maziere C, Mora L, Gardette J, Salmon S, Auclair M, et al. The antidiabetic drug metformin decreases cholesterol metabolism in cultured human fibroblasts. Atherosclerosis. 1988;71(1):27-33.

[103] Hodson L, Fielding BA. Stearoyl-CoA desaturase: rogue or innocent bystander? Prog Lipid Res. 2013;52(1):15-42.

[104] Igal RA. Stearoyl-CoA desaturase-1: a novel key player in the mechanisms of cell proliferation, programmed cell death and transformation to cancer. Carcinogenesis. 2010;31(9):1509-15.

[105] Evans H, De Tomaso T, Quail M, Rogers J, Gracey AY, Cossins AR, et al. Ancient and modern duplication events and the evolution of stearoyl-CoA desaturases in teleost fishes. Physiological genomics. 2008;35(1):18-29.

[106] Chajes V, Hulten K, Van Kappel AL, Winkvist A, Kaaks R, Hallmans G, et al. Fatty-acid composition in serum phospholipids and risk of breast cancer: an incident case-control study in Sweden. International journal of cancer Journal international du cancer. 1999;83(5):585-90.

[107] Pala V, Krogh V, Muti P, Chajes V, Riboli E, Micheli A, et al. Erythrocyte membrane fatty acids and subsequent breast cancer: a prospective Italian study. J Natl Cancer Inst. 2001;93(14):1088-95.

[108] Shannon J, King IB, Moshofsky R, Lampe JW, Gao DL, Ray RM, et al. Erythrocyte fatty acids and breast cancer risk: a case-control study in Shanghai, China. Am J Clin Nutr. 2007;85(4):1090-7.

[109] Chajes V, Thiebaut AC, Rotival M, Gauthier E, Maillard V, Boutron-Ruault MC, et al. Association between serum trans-monounsaturated fatty acids and breast cancer risk in the E3N-EPIC Study. Am J Epidemiol. 2008;167(11):1312-20.

[110] Chajes V, Joulin V, Clavel-Chapelon F. The fatty acid desaturation index of blood lipids, as a biomarker of hepatic stearoyl-CoA desaturase expression, is a predictive factor of breast cancer risk. Curr Opin Lipidol. 2011;22(1):6-10.

[111] Li J, Ding SF, Habib NA, Fermor BF, Wood CB, Gilmour RS. Partial characterization of a cDNA for human stearoyl-CoA desaturase and changes in its mRNA expression in some normal and malignant tissues. International journal of cancer Journal international du cancer. 1994;57(3):348-52.

[112] Scaglia N, Caviglia JM, Igal RA. High stearoyl-CoA desaturase protein and activity levels in simian virus 40 transformed-human lung fibroblasts. Biochimica et biophysica acta. 2005;1687(1-3):141-51.

[113] Moore S, Knudsen B, True LD, Hawley S, Etzioni R, Wade C, et al. Loss of stearoyl-CoA desaturase expression is a frequent event in prostate carcinoma. International journal of cancer Journal international du cancer. 2005;114(4):563-71.

[114] Scaglia N, Igal RA. Stearoyl-CoA desaturase is involved in the control of proliferation, anchorage-independent growth, and survival in human transformed cells. The Journal of biological chemistry. 2005;280(27):25339-49.

[115] Hess D, Igal RA. Genistein downregulates de novo lipid synthesis and impairs cell proliferation in human lung cancer cells. Exp Biol Med (Maywood). 2011;236(6): 707-13.

[116] Minville-Walz M, Pierre AS, Pichon L, Bellenger S, Fevre C, Bellenger J, et al. Inhibition of stearoyl-CoA desaturase 1 expression induces CHOP-dependent cell death in human cancer cells. PloS one. 2010;5(12):e14363.

[117] Scaglia N, Chisholm JW, Igal RA. Inhibition of stearoylCoA desaturase-1 inactivates acetyl-CoA carboxylase and impairs proliferation in cancer cells: role of AMPK. PloS one. 2009;4(8):e6812.

[118] Roongta UV, Pabalan JG, Wang X, Ryseck RP, Fargnoli J, Henley BJ, et al. Cancer cell dependence on unsaturated fatty acids implicates stearoyl-CoA desaturase as a target for cancer therapy. Mol Cancer Res. 2011;9(11):1551-61.

[119] Gukovsky I, Li N, Todoric J, Gukovskaya A, Karin M. Inflammation, autophagy, and obesity: common features in the pathogenesis of pancreatitis and pancreatic cancer. Gastroenterology. 2013;144(6):1199-209 e4.

[120] Bardou M, Barkun AN, Martel M. Obesity and colorectal cancer. Gut. 2013;62(6): 933-47.

[121] Estores D, Velanovich V. Barrett esophagus: epidemiology, pathogenesis, diagnosis, and management. Curr Probl Surg. 2013;50(5):192-226.

[122] Polednak AP. Estimating the number of U.S. incident cancers attributable to obesity and the impact on temporal trends in incidence rates for obesity-related cancers. Cancer Detect Prev. 2008;32(3):190-9.

[123] Wolin KY, Carson K, Colditz GA. Obesity and cancer. The oncologist. 2010;15(6): 556-65.

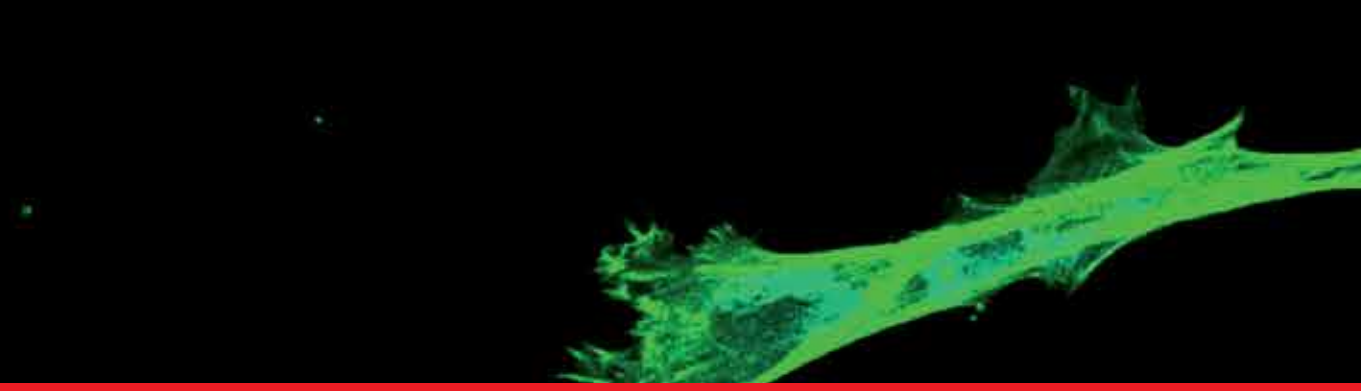
[124] Algire C, Amrein L, Zakikhani M, Panasci L, Pollak M. Metformin blocks the stimulative effect of a high-energy diet on colon carcinoma growth in vivo and is associated with reduced expression of fatty acid synthase. Endocr Relat Cancer. 2010;17(2): 351-60.

[125] Cantoria MJ, Boros LG, Meuillet EJ. Metformin Inhibits the TCA Cycle and Fatty Acid Synthesis in MIAPaCa-2 Pancreatic Cancer Cells. Pancreas. 2012;41(8):1351-.

[126] Harris DM, Li L, Chen M, Lagunero FT, Go VLW, Boros LG. Diverse mechanisms of growth inhibition by luteolin, resveratrol, and quercetin in MIA PaCa-2 cells: a comparative glucose tracer study with fatty acid synthase inhibitor C75. Metabolomics. 2012;8:201-10.

[127] Vizan P, Boros LG, Figueras A, Capella G, Mangues R, Bassilian S, et al. K-ras codon-specific mutations produce distinctive metabolic phenotypes in NIH3T3 mice [corrected] fibroblasts. Cancer research. 2005;65(13):5512-5.

[128] Laplante M, Sabatini DM. An emerging role of mTOR in lipid biosynthesis. Curr Biol. 2009;19(22):R1046-52.



Edited by Kelly McCall

This book provides the reader with an update on recent findings related to the molecular basis for the development and progression of pancreatic cancer. This book also informs the reader about the link between obesity and pancreatic cancer, and how a well-known and widely used anti-diabetic drug may also be potentially useful for the treatment of pancreatic cancer. Moreover, this book provides insight into novel approaches for improved earlier detection of pancreatic cancer.

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