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Transcriptional and Post-transcriptional Regulation

Edited by Kais Ghedira

TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION

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http://dx.doi.org/10.5772/intechopen.72023
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First published in London, United Kingdom, 2018 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Transcriptional and Post-transcriptional Regulation
Edited by Kais Ghedira

p. cm.

Print ISBN 978-1-78923-791-7

Online ISBN 978-1-78923-792-4

eBook (PDF) ISBN 978-1-83881-641-4

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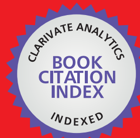

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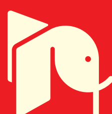

Meet the editor

Dr. Kais Ghedira is an assistant professor of Bioinformatics and a member at the Laboratory of Bioinformatics, Biomathematics and Biostatistics, Institut Pasteur de Tunis (IPT), Tunisia. He graduated in Biological Sciences in 2004 from ISBM, Monastir, Tunisia. He pursued his master degree from the Faculty of Pharmacy, Monastir, Tunisia and defended his master project on January 2007. He then participated in the "Informatics in Biology" course held at the Institut Pasteur Paris, France and obtained a diploma for this course. This course allowed him to acquire many skills in the field of bioinformatics that were valuable for his PhD degree. He started his PhD degree in Bioinformatics in September 2007 from the Faculty of Sciences, Bizerte, Tunisia, and pursued his PhD project at IPT. During his thesis, he was at BIOBASE GmbH, Germany, for several months working on predicting transcription factor binding sites (TFBSs) using several bioinformatic tools and updating existing BIOBASE databases. He is a bioinformatician with a biological background who has been published in several peer-reviewed journals with high impacts. He is involved in several national and international research projects collaborating with worldwide researchers. He is highly involved in training bioinformatics in Tunisia and Africa. He is mainly interested in gene expression and gene regulation analysis, functional genomics and integrative biology, analysis of high throughput data, comparative genomics and database, and webtool development. He is currently working on several projects related to understanding diseases/phenotypes through the analysis of gene expression, integration of multiple Omics and high throughput data, meta-analyses, and gene regulatory networks. As gene expression regulation is a fundamental process for cell living, the editor believes that researchers and the scientific community would find a variety of new ideas and hints in this book that would be helpful to them to tackle transcriptional and post-transcriptional regulations.

Contents

Preface XI

Section 1 Transcriptional and Post-transcriptional Regulation 1

Chapter 1 **Introductory Chapter: A Brief Overview of Transcriptional and Post-transcriptional Regulation 3**
Kais Ghedira

Chapter 2 **Function of the Stem Cell Transcription Factor SALL4 in Hematopoiesis 13**
Jianchang Yang

Chapter 3 **The Glucocorticoid Receptor and Certain KRÜPPEL-Like Transcription Factors have the Potential to Synergistically Stimulate Bovine Herpesvirus 1 Transcription and Reactivation from Latency 35**
Fouad S. El-mayet, Ayman S. El-Habbaa, Gabr F. El-Bagoury, Saad S.A. Sharawi, Ehab M. El-Nahas and Clinton Jones

Chapter 4 **Roles of Non-Coding RNAs in Transcriptional Regulation 55**
Loudu Srijyothi, Saravanaraman Ponne, Talukdar Prathama, Cheemala Ashok and Sudhakar Baluchamy

Chapter 5 **MicroRNAs in Bone Diseases: Progress and Prospects 77**
Hui-Yi Loh, Yuin-Yee Lau, Kok-Song Lai and Mohd Azuraidi Osman

Section 2 The Interplay Between Transcription Factors and MicroRNAs 101

Chapter 6 **Transcription Factors and MicroRNA Interplay: A New Strategy for Crop Improvement 103**
Sumit Jangra, Vrantika Chaudhary and Neelam R. Yadav

Preface

Gene expression is a complex process that is controlled at multiple cellular layers including the chromatin level through chromatin modification and remodeling, the mRNA level (transcriptional and post-transcriptional regulation) and protein level (translational regulation and post-translational degradation). It constitutes a fundamental process to diverse biological processes that occur within the cell including cell development and differentiation, the response and the adaptation to environmental stresses and others. Transcriptional and post-transcriptional regulations have been extensively studied and are the most investigated layers apart from gene regulation. This is explained by the fact that regulations at the transcriptional and post-transcriptional levels are the fundamental and the most important steps for gene regulation because biological techniques allowing for the study of transcription control are well established, accessible, and highly used by the scientific community. Transcriptional regulation involves the interaction and the specific binding of proteins called transcription factors (TFs) to regulatory elements within DNA called transcription factor binding sites (TFBSs) to control the expression of downstream genes, while the post-transcriptional regulation involves the interaction of non-coding RNAs (miRNAs) by hybridizing to target mRNAs and thereby regulating their translation and/or stability.

The book "Transcriptional and Post Transcriptional Regulation" contains six chapters.

The **first chapter** is an Introductory Chapter, where the editor introduces transcriptional and post-transcriptional regulations and gives a general overview of the contents of the book.

The **second chapter** "Function of the Stem Cell Transcription Factor SALL4 in Hematopoiesis" was written by Jianchang Yang. This chapter summarizes recent advances in the knowledge of SALL4 biology with a focus on its regulatory functions in normal and leukemic hematopoiesis.

The **third chapter** entitled "The Glucocorticoid Receptor and Certain Krüppel-Like Transcription Factors Have the Potential to Synergistically Stimulate Bovine Herpesvirus 1 Transcription and Reactivation from Latency" by Fouad S. El-mayet et al. emphasizes the effects of Krüppel-like transcription factors and glucocorticoid receptors on the reactivation of the bovine herpesvirus 1 transcription and its reactivation from latency.

The **fourth chapter** by Sudhakar Baluchamy, and co-workers entitled "Roles of Non-Coding RNAs in Transcriptional Regulation" provides an interesting depiction of non-coding RNAs (ncRNAs) and focuses mainly on their role(s) in transcriptional and post-transcriptional regulations and their relevance in cancers.

The **fifth chapter** by Lai Kok-Song and colleagues entitled "MicroRNAs in Bone Diseases: Progress and Prospects" focuses on the role of miRNAs in normal osteoblast and osteosarcoma cells. It also discusses the great potential of miRNA as a new therapeutic approach to treat human bone diseases.

The book concludes with the **sixth chapter** "Transcription Factors and MicroRNA Interplay: A New Strategy for Crop Improvement", which provides a new tip based on the relationship and the interplay between transcription factors and miRNA in different plant species.

The editor would like to thank all the authors for their contributions. The editor is also grateful to Intech Open publisher, particularly Ms. Ivana Glavic, for her assistance and patience until the publication of this book.

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Section 1

Transcriptional and Post-transcriptional Regulation

Chapter 1

Introductory Chapter: A Brief Overview of Transcriptional and Post-transcriptional Regulation

Kais Ghedira

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79753

1. Prologue

The regulation of gene expression is the process by which expression of genes is controlled (induced or repressed) at the cell level in a particular time under a particular condition. It is a fundamental process to diverse other biological processes that occur within the cell including cell development and differentiation, the response and the adaptation to environmental stresses. Gene regulation has classically been viewed as the interaction between proteins to regulatory elements located at the vicinity of the transcription start site within promoters. However, gene regulation is a more complex process that involves additional layers of control including chromatin remodeling, nucleosome positioning, histone modifications, DNA-binding regulatory proteins such as transcription factors and noncoding RNA [1–3]. Such process requires structural and chemical changes to the genetic material, binding of proteins to specific DNA elements to regulate transcription, or mechanisms that modulate translation of mRNA.

Indeed, gene expression is controlled at multiple cellular levels consisting in the chromatin level through chromatin modification and remodeling, the mRNA level (transcriptional and posttranscriptional regulation) and protein level (translation regulation and posttranslational degradation).

This introductory chapter will give a brief overview on the transcriptional and posttranscriptional regulation, list the main database resources that can be used for transcriptional and/or posttranscriptional regulation data and finally list the main tools allowing to predict TF and miRNA gene targets.

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2. Transcriptional regulation

Regulation at the transcriptional level involves proteins called transcription factors (TFs) that recognize and bind specifically to regulatory elements within the promoter regions to control the expression of a downstream gene. These TFs regulate target genes—by turning them on and off—in order to make sure that they are transcribed into mRNA within the cell at the right time and in the right amount. TFs are classified into three large families of DNA-binding domains that include:

1. Basic helix-loop-helix (bHLH) proteins found in organisms from yeast to humans and function in critical developmental processes controlling embryonic development, particularly in neurogenesis, myogenesis, heart development, and hematopoiesis [4, 5].
2. The TFs with basic leucine zipper domains [6].
3. TFs with the helix-turn-helix (HTH) domains that are involved in a wide range of functions beyond transcription regulation, including DNA repair and replication, RNA metabolism, and protein-protein interactions in diverse signaling contexts [7, 8]. This group also includes homeobox (zinc finger, HOX-like, TALE, POU, etc.) and homeodomain protein products.

High-throughput techniques including ChIP-on-chip/ChIP-seq and enhanced yeast one-hybrid have been widely employed to uncover protein-DNA interactions [9, 10] and represent convenient methods to identify and characterize the repertoire of regulatory elements that can be targeted by a protein of interest or transcription factors that can bind a DNA sequence of interest [11], respectively. Thanks to the ENCODE (Encyclopedia of DNA Elements) project aiming to build a comprehensive parts list of functional elements in the human genome including regulatory elements that control cells, such regulatory data were made available for the scientific community (https://www.encodeproject.org/; http://genome.ucsc.edu/encode/downloads.html) [12] and led to largely improve our understanding of gene regulation.

In addition to the ENCODE project, several regulatory databases have been developed for including multiple animals/plants/microorganisms regulation data. **Table 1** lists the most widely used transcriptional regulation database with a brief description, reference to original publication and current accessible website URL.

Database	Acronym	Website link	Description	References
TRANSFAC	**TRANSFAC**	http://genexplain.com/transfac/	TRANSFAC® is a maintained and curated database of eukaryotic transcription factors, their genomic binding sites, and DNA-binding profiles.	[13]
Transcription Regulatory Regions database	TRRD	http://wwwmgs.bionet.nsc.ru/mgs/gnw/trrd/	TRRD is a unique information resource, accumulating information on the structural and functional organization of transcription regulatory regions of eukaryotic genes.	[14]
Ensembl Regulation	Ensembl Regulation	https://www.ensembl.org/info/	Ensembl Regulation provides resources used for studying gene	[15]

Database	Acronym	Website link	Description	References
		genome/funcgen/index.html	expression and its regulation in human and mouse, with a focus on the transcriptional and posttranscriptional mechanisms.	
Regulatory Network Repository of Transcription Factor and microRNA Mediated Gene Regulations	RegNetwork	http://www.regnetworkweb.org/source.jsp	RegNetwork is developed based on 25 databases that provide the regulatory relationship information, annotation, and other necessary information in order to derive the regulatory relationships.	[16]
Transcriptional Regulatory Element Database	TRED	http://rulai.cshl.edu/TRED/	TRED provides good training datasets for further genome-wide cis-regulatory element prediction, assist detailed functional studies, and facilitate to decipher the gene regulatory networks.	[17]
Transcriptional Regulatory Relationships Unraveled by Sentence Based Text mining	TRRUST	http://www.grnpedia.org/trrust/	TRRUST database provides information of mode of regulation (activation or repression).	[18]
Open Regulatory Annotation database	**ORegAnno**	http://www.oreganno.org/	The Open Regulatory Annotation database (ORegAnno) is a resource for curated regulatory annotation.	[19]
PRODORIC	PRODORIC2	http://www.prodoric2.de	The PRODORIC2 database hosts one of the largest collections of DNA-binding sites for prokaryotic transcription factors.	[20]
Gene Transcription Regulation Database	GTRD	http://gtrd.biouml.org/	The most complete collection of uniformly processed ChIP-seq data to identify transcription factor binding sites for human and mouse.	[21]
Transcription factor prediction database	DBD	http://www.transcriptionfactor.org/index.cgi?Home	DBD is a database of predicted transcription factors in completely sequenced genomes.	[22]

Acronyms in bold letters denote curated databases.

Table 1. Eukaryotic and prokaryotic regulation databases.

3. Post-transcriptional regulation

A very large part of the human genome constitutes noncoding elements classified as small noncoding RNAs (sncRNAs) and long noncoding RNAs (lncRNAs). These noncoding components are receiving increased attention from researchers due to their predicted important role in posttranscriptional regulation. Small ncRNAs class includes small interfering RNAs (siRNAs), microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), endogenous small interfering RNAs (endo-siRNAs or esiRNAs), promoter associate RNAs (pRNAs), small nucleolar RNAs (snoRNAs), and sno-derived RNAs, while lncRNAs includes linc RNA, NAT, eRNA,

circ RNA, ceRNAs, PROMPTS. Both lncRNAs and sncRNAs have been identified at regulatory elements [23, 24]. Among these noncoding elements, microRNAs have been the most widely investigated since their discovery in the early 1990s, underscoring their importance in post-transcriptional gene regulation [25]. These later act as posttranscriptional regulators of their messenger RNA (mRNA) targets via mRNA degradation and/or translational repression [26]. It has been widely evidenced that miRNA-mediated downregulation is a one-way process leading to the repression of translation and/or target mRNA degradation [27–30]; however, recent studies have shown that miRNAs are able to upregulate gene expression in specific cell types and conditions with distinct transcripts and proteins [31].

Pulling down microRNA-induced silencing complexes (miRISCs) immunoprecipitation method allows researchers to collect information on microRNAs and their mRNA targets *in vivo*. Such information has been collected and stored in several public databases. **Table 2** contains the most widely used posttranscriptional regulation database with a brief description, reference to original publication and current functional website URL.

Database	Acronym	Website link	Description	References
The microRNA database	miRBase	http://www.mirbase.org/	The miRBase database is a searchable database of published miRNA sequences and annotation.	[32]
The experimentally validated microRNA-target interactions database	**miRTarBase**	http://mirtarbase.mbc.nctu.edu.tw/php/index.php	miRTarBase has accumulated miRNA-target interactions (MTIs), which are collected by manually surveying pertinent literature.	[33]
miRDB	miRDB	http://mirdb.org/miRDB/	miRDB is an online database for miRNA target prediction and functional annotations	[34]
miRNAMap	miRNAMap	http://mirnamap.mbc.nctu.edu.tw/	An online resource that stores information related to the known miRNAs in metazoan.	[35]
Vir-Mir	Vir-Mir	http://alk.ibms.sinica.edu.tw/cgi-bin/miRNA/miRNA.cgi	Contains predicted viral miRNA candidate hairpins	[36]
Virus miRNA Target	ViTA	http://vita.mbc.nctu.edu.tw/	ViTa collects virus data from miRBase and ICTV, VirGne, VBRC, etc. and provide effective annotations, including human miRNA expression, virus-infected tissues, annotation of virus, and comparisons.	[37]
miRecords	miRecords	http://c1.accurascience.com/miRecords/	miRecords is a resource for animal miRNA-target interactions.	[38]
microRNA Data Integration Portal	mirDIP	http://ophid.utoronto.ca/mirDIP/	Provides several million human microRNA-target predictions, which were collected across 30 different resources.	[39]

Acronyms in bold letters denote curated databases.

Table 2. Eukaryotic and prokaryotic posttranscriptional regulation databases.

http://dx.doi.org/10.5772/intechopen.79753

4. The interplay between TFs and miRNAs

Transcription factors (TFs) and microRNAs (miRNAs) are key regulators of gene expression. Several studies have shown that abnormal miRNA and/or TF expression can be critical for cell survival and development through targeting critical genes in the cellular system. In the last decade, several bioinformatic studies have been performed to elucidate transcriptional and posttranscriptional (mostly miRNA-mediated) regulatory interactions. Besides experimental techniques (ChIP-Seq, ChIP-ChIP, yeast two-hybrid, miRISCs), computational tools have been developed to predict the TF-gene target and/or miRNA-target interactions. **Table 3** lists some bioinformatic tools used to predict transcriptional and posttranscriptional regulation. Using such tools and/or through the integration of data collected from public databases (**Tables 1** and **2**), researchers were able to generate regulatory networks aiming to understand mechanisms involved in some phenotypes and/or diseases. Recent studies focused on the study of mixed miRNA/TF feed-forward regulatory loops (FFLs) through genome-wide transcriptional and posttranscriptional regulatory network integration to decipher the complex and interlinked cascade of events related to several diseases [46–48]. Such approaches provide the scientific community with the ability to investigate the interplay between TFs and miRNAs in a given system.

Tool/Web tool	Website link	Description	References
TF-target prediction			
TargetFinder	http://targetfinder.org/	Provides a web-based resource for finding genes that show a similar expression pattern to a group of user-selected genes.	[40]
BART: Binding analysis for regulation of transcription	http://faculty.virginia.edu/zanglab/bart/	A novel computational method and software package for predicting functional transcription factors that regulate a query gene set or associate with a query genomic profile, based on more than 6000 existing ChIP-seq datasets for over 400 factors in human or mouse.	[41]
MATCH	http://gene-regulation.com/pub/programs.html#match	Match is a weight matrix-based program for predicting transcription factor binding sites (TFBS) in DNA sequences.	[42]
MiRNA-target prediction			
RNAhybrid	https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/	RNAhybrid is a tool for finding the minimum free energy hybridization of a long and a short RNA.	[43]
TargetScan	http://www.targetscan.org/vert_72/	TargetScan predicts biological targets of miRNAs by searching for the presence of conserved 8mer, 7mer, and 6mer sites that match the seed region of each miRNA.	[44]
miRWalk	http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/	Supplying the biggest available collection of predicted and experimentally verified miRNA-target interactions with various novel and unique features.	[45]

Table 3. TF and miRNA target prediction tools.

5. Conclusion

During these last years, transcriptional and posttranscriptional regulation constituted the most important layers of gene regulation. However, a recent study by Barna group [49] has upset our understanding of gene regulation. Indeed, while researchers have believed for decades that ribosomes are identical showing no preference for translating RNA molecules into proteins, it appears that these later exhibit a preference for translating certain types of genes. One type of ribosome, for example, prefers to translate genes involved in cellular differentiation, while another specializes in genes that carry out essential metabolic duties. This study is uncovering a new layer of gene expression regulation that will have broad implications for basic science and human disease.

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References

[1] Venters BJ, Pugh BF. How eukaryotic genes are transcribed. Critical Reviews in Biochemistry and Molecular Biology. 2009;**44**:117-141

[2] Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: Insights into functions. Nature Reviews. Genetics. 2009;**10**:155-159

[3] Goodrich JA, Kugel JF. Non-coding-RNA regulators of RNA polymerase II transcription. Nature Reviews. Molecular Cell Biology. 2006;**7**:612-616

[4] Littlewood TD, Evan GI. Transcription factors 2: Helix-loop-helix. Protein Profile. 1995; **2**(6):621-702. PMID 7553065

[5] Jones S. An overview of the basic helix-loop-helix proteins. Genome Biology. 2004;**5**(6): 226. Epub 2004 May 28. Review. PubMed PMID: 15186484; PubMed Central PMCID: PMC463060

[6] Vinson C, Myakishev M, Acharya A, Mir AA, Moll JR, Bonovich M. Classification of human B-ZIP proteins based on dimerization properties. Molecular and Cellular Biology. 2002;**22**(18):6321-6335. DOI: 10.1128/MCB.22.18.6321-6335.2002. PMC 135624. PMID 12192032

[7] Wintjens R, Rooman M. Structural classification of HTH DNA-binding domains and protein-DNA interaction modes. Journal of Molecular Biology. 1996;**262**(2):294-313. DOI: 10.1006/jmbi.1996.0514. PMID 8831795

[8] Aravind L, Anantharaman V, Balaji S, Babu MM, Iyer LM. The many faces of the helix-turn-helix domain: Transcription regulation and beyond. FEMS Microbiology Reviews. 2005;**29**(2):231-262. Review. PubMed PMID: 15808743

[9] Johnson DS, Mortazavi A, Myers RM, Wold B. Genome-wide mapping of in vivo protein-DNA interactions. Science. 2007;**316**:1497-1502

[10] Reece-Hoyes JS, Barutcu AR, McCord RP, Jeong JS, Jiang L, MacWilliams A, Yang X, Salehi-Ashtiani K, Hill DE, Blackshaw S, Zhu H, Dekker J, Walhout AJM. Yeast one-hybrid assays for gene-centered human gene regulatory network mapping. Nature Methods. 2011;**8**:1050-1052

[11] Reece-Hoyes JS, Diallo A, Lajoie B, Kent A, Shrestha S, Kadreppa S, Pesyna C, Dekker J, Myers CL, Walhout AJ. Enhanced yeast one-hybrid assays for high-throughput gene-centered regulatory network mapping. Nature Methods. 2011;**8**(12):1059-1064. DOI: 10.1038/nmeth.1748. PubMed PMID: 22037705; PubMed Central PMCID: PMC3235803

[12] ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012;**489**(7414):57-74. DOI: 10.1038/nature11247. PubMed PMID: 22955616; PubMed Central PMCID: PMC3439153

[13] Wingender E. The TRANSFAC project as an example of framework technology that supports the analysis of genomic regulation. Brief Bioinform. 2008 Jul;**9**(4):326-332. DOI: 10.1093/bib/bbn016. Epub 2008 Apr 24. PubMed PMID: 18436575

[14] Kolchanov NA, Ignatieva EV, Ananko EA, Podkolodnaya OA, Stepanenko IL, Merkulova TI, Pozdnyakov MA, Podkolodny NL, Naumochkin AN, Romashchenko AG. Transcription regulatory regions database (TRRD): Its status in 2002. Nucleic Acids Research. 2002; **30**(1):312-317

[15] Zerbino DR, Johnson N, Juetteman T, Sheppard D, Wilder SP, Lavidas I, Nuhn M, Perry E, Raffaillac-Desfosses Q, Sobral D, Keefe D, Gräf S, Ahmed I, Kinsella R, Pritchard B, Brent S, Amode R, Parker A, Trevanion S, Birney E, Dunham I, Flicek P. Ensembl regulation resources. Database (Oxford). 2016 Feb 17;**2016**. pii: bav119. DOI: 10.1093/database/bav119. Print 2016. PubMed PMID: 26888907; PubMed Central PMCID: PMC4756621

[16] Liu ZP, Wu C, Miao H, Wu H. RegNetwork: An integrated database of transcriptional and post-transcriptional regulatory networks in human and mouse. Database (Oxford). 2015 Sep 30;**2015**. pii: bav095. DOI: 10.1093/database/bav095. Print 2015. PubMed PMID: 26424082; PubMed Central PMCID: PMC4589691

[17] Jiang C, Xuan Z, Zhao F, Zhang MQ. TRED: A transcriptional regulatory element database, new entries and other development. Nucleic Acids Research. 2007;**35**(Database issue):D137-D140. PubMed PMID: 17202159; PubMed Central PMCID: PMC1899102

[18] TRRUST v2: An expanded reference database of human and mouse transcriptional regulatory interactions. Nucleic Acids Research; 26 Oct, 2017

[19] Lesurf R, Cotto KC, Wang G, Griffith M, Kasaian K, Jones SJM, Montgomery SB, Griffith OL, The Open Regulatory Annotation Consortium. ORegAnno 3.0: A community-driven resource for curated regulatory annotation. Nucleic Acids Research. 2016;**44**(D1):D126-D132. DOI: 10.1093/nar/gkv1203

[20] Münch R, Hiller K, Barg H, Heldt D, Linz S, Wingender E, Jahn D. PRODORIC: Prokaryotic database of gene regulation. Nucleic Acids Research. 2003;**31**:266-269

[21] Yevshin IS, Sharipov RN, Valeev TF, Kel AE, Kolpakov FA. GTRD: A database of transcription factor binding sites identified by ChIP-seq experiments. Nucleic Acids Research. 2017;**45**(D1):D61-D67

[22] Wilson D, Charoensawan V, Kummerfeld SK, Teichmann SA. DBD—Taxonomically broad transcription factor predictions: New content and functionality. Nucleic Acids Research. 2008;**36**(suppl_1):D88-D92. DOI: 10.1093/nar/gkm964

[23] Lewis BP, Shih I-H, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell. 2003;**115**(7):787-798

[24] Brennecke J, Stark A, Russell RB, Cohen SM. Principles of microRNA-target recognition. PLoS Biology. 2005;**3**(3):e85

[25] Valinezhad Orang A, Safaralizadeh R, Kazemzadeh-Bavili M. Mechanisms of miRNA-mediated gene regulation from common downregulation to mRNA-specific upregulation. International Journal of Genomics. 2014;**2014**:970607. DOI: 10.1155/2014/970607. Epub 2014 Aug 10. Review. PubMed PMID: 25180174; PubMed Central PMCID: PMC4142390

[26] Catalanotto C, Cogoni C, Zardo G. MicroRNA in control of gene expression: An overview of nuclear functions. International Journal of Molecular Sciences. 2016;**17**(10):pii: E1712. Review. PubMed PMID: 27754357; PubMed Central PMCID: PMC5085744

[27] Zeng Y, Yi R, Cullen BR. MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**(17):9779-9784. Epub 2003 Aug 5. PubMed PMID: 12902540; PubMed Central PMCID: PMC187842

[28] Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature. 2000;**403**(6772):901-906. PubMed PMID: 10706289

[29] Llave C, Xie Z, Kasschau KD, Carrington JC. Cleavage of scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA. Science. 2002;**297**(5589):2053-2056. PubMed PMID: 12242443

[30] Lee RC, Feinbaum RL, Ambros V. The C. Elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;**75**(5):843-854. PubMed PMID: 8252621

[31] Place RF, Li LC, Pookot D, Noonan EJ, Dahiya R. MicroRNA-373 induces expression of genes with complementary promoter sequences. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**(5):1608-1613. DOI: 10.1073/pnas.0707594 105. Epub 2008 Jan 28. Erratum in: Proc Natl Acad Sci U S A. 2018 Mar 19;:. PubMed PMID: 18227514; PubMed Central PMCID: PMC2234192

[32] Kozomara A, Griffiths-Jones S. miRBase: Annotating high confidence microRNAs using deep sequencing data. Narrative. 2014;**42**:D68-D73

[33] Chou C-H, Shrestha S, Yang C-D, Chang N-W, Lin Y-L, Liao K-W, Huang W-C, Sun T-H, Tu S-J, Lee W-H, Chiew M-Y, Tai C-S, Wei T-Y, Tsai T-R, Huang H-T, Wang C-Y, Wu H-Y, Ho S-Y, Chen P-R, Chuang C-H, Hsieh P-J, Wu Y-S, Chen W-L, Li M-J, Wu Y-C, Huang X-Y, Ng FL, Buddhakosai W, Huang P-C, Lan K-C, Huang C-Y, Weng S-L, Cheng Y-N, Liang C, Hsu W-L, Huang H-D. miRTarBase update 2018: A resource for experimentally validated microRNA-target interactions. Nucleic Acids Research. 2018;**46**(D1):D296-D302. DOI: 10.1093/nar/gkx1067

[34] Wong N, Wang X. miRDB: An online resource for microRNA target prediction and functional annotations. Nucleic Acids Research. 2015;**43**(D1):D146-D152

[35] Hsu SD, Chu CH, Tsou AP, Chen SJ, Chen HC, Hsu PW, Wong YH, Chen YH, Chen GH, Huang HD. miRNAMap 2.0: Genomic maps of microRNAs in metazoan genomes. Nucleic Acids Research. 2008;**36**(Database issue):D165-D169

[36] Li SC, Shiau CK, Lin WC. Vir-Mir db: Prediction of viral microRNA candidate hairpins. Nucleic Acids Res. 2008 Jan;**36**(Database issue):D184-D189. Epub 2007 Aug 15. PubMed PMID: 17702763; PubMed Central PMCID: PMC2238904

[37] Hsu PW, Lin LZ, Hsu SD, Hsu JB, Huang HD. ViTa: Prediction of host microRNAs targets on viruses. Nucleic Acids Research. 2007;**35**(Database issue):D381-D385

[38] Xiao F, Zuo Z, Cai G, Kang S, Gao X, Li T. miRecords: An integrated resource for microRNA-target interactions. Nucleic Acids Research. 2009;**37**:D105-D110

[39] Tokar T, Pastrello C, Rossos AEM, Abovsky M, Hauschild AC, Tsay M, Lu R, Jurisica I. mirDIP 4.1-integrative database of human microRNA target predictions. Nucleic Acids Research. 2018;**46**(D1):D360-D370. DOI: 10.1093/nar/gkx1144. PubMed PMID: 29194489; PubMed Central PMCID: PMC5753284

[40] Szymon M. Kiełbasa, Nils Blüthgen, Michael Fähling, Ralf Mrowka; Targetfinder.org: A resource for systematic discovery of transcription factor target genes, Nucleic Acids Research, 38, suppl 2, 2010, W233–W238, 10.1093/nar/gkq374

[41] Wang Z, Civelek M, Miller CL, Sheffield NC, Guertin MJ, Zang C. BART: A transcription factor prediction tool with query gene sets or epigenomic profiles. Bioinformatics. 2018 Mar 28. DOI: 10.1093/bioinformatics/bty194. [Epub ahead of print] PubMed PMID: 29608647

[42] Kel AE, Goessling E, Reuter I, Cheremushkin E, Kel-Margoulis OV, Wingender E. Match (TM): A tool for searching transcription factor binding sites in DNA sequences. Nucleic Acids Research. 2003;**31**:3576-3579

[43] Marc R, Steffen P, Hoechsmann M, Giegerich R. Fast and effective prediction of microRNA/target duplexes RNA. RNA. 2004

[44] Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. Elife. 2015 Aug 12;**4**. DOI: 10.7554/eLife.05005. PubMed PMID: 26267216; PubMed Central PMCID: PMC4532895

[45] Dweep H et al. miRWalk—Database: Prediction of possible miRNA binding sites by 'walking' the genes of 3 genomes. Journal of Biomedical Informatics. 2011;**44**:839-837

[46] Friard O, Re A, Taverna D, De Bortoli M, Corá D. CircuitsDB: A database of mixed microRNA/transcription factor feed-forward regulatory circuits in human and mouse. BMC Bioinformatics. 2010;**11**:435. DOI: 10.1186/1471-2105-11-435. PubMed PMID: 20731828; PubMed Central PMCID: PMC2936401

[47] Wang H, Luo J, Liu C, Niu H, Wang J, Liu Q, Zhao Z, Xu H, Ding Y, Sun J, Zhang Q. Investigating microRNA and transcription factor co-regulatory networks in colorectal cancer. BMC Bioinformatics. 2017;**18**(1):388. DOI: 10.1186/s12859-017-1796-4. PubMed PMID: 28865443; PubMed Central PMCID: PMC5581471

[48] Nampoothiri SS, Fayaz SM, Rajanikant GK. A novel five-node feed-forward loop unravels miRNA-gene-TF regulatory relationships in ischemic stroke. Molecular Neurobiology. 2018. DOI: 10.1007/s12035-018-0963-6. [Epub ahead of print] PubMed PMID: 29524052

[49] Simsek D, Tiu GC, Flynn RA, Byeon GW, Leppek K, Xu AF, Chang HY, Barna M. The mammalian ribo-interactome reveals ribosome functional diversity and heterogeneity. Cell. 2017;**169**(6):1051-1065.e18. DOI: 10.1016/j.cell.2017.05.022. PubMed PMID: 28575669; PubMed Central PMCID: PMC5548193

Chapter 2

Function of the Stem Cell Transcription Factor SALL4 in Hematopoiesis

Jianchang Yang

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76454

Abstract

SALL4 is a zinc finger DNA-binding protein that has been well characterized in development and in embryonic stem cell (ESC) maintenance. Notably, SALL4 may be one of the few genes that are also involved in tissue stem cells in adults, and SALL4 protein expression has been correlated with the presence of stem and progenitor cell populations in various organ systems and also in human cancers. In normal hematopoiesis, SALL4 expression is restricted to the rare hematopoietic stem/progenitor cell (HSC/HPC) fractions but is rapidly silenced following lineage differentiation. In hematopoietic malignancies, however, SALL4 is persistently expressed and its expression levels are linked with deteriorated disease status. Furthermore, SALL4 activation participates in the pathogenesis of tumor initiation and disease progression. This chapter summarizes recent advances in our knowledge of SALL4 biology with a focus on its regulatory functions in normal and leukemic hematopoiesis. A better understanding of SALL4's biologic functions and mechanisms is needed to facilitate the development of advanced therapies in future.

Keywords: pluripotency, leukemogenesis, hematopoietic stem/progenitor cell, MLL-rearrangement, epigenetic, histone methylation, DNA methylation, differentiation, zinc finger domain

1. Introduction

SALL4 is one of four human homologs (*SALL-1*, *-2*, *-3*, *-4*) of the Drosophila region-specific gene *Spalt* (*sal*). In Drosophila, *sal* is a homeotic gene essential for development of posterior head and anterior tail segments. As a DNA-binding transcription factor, the SALL4 protein is characterized by multiple Cys2His2 zinc finger (C2H2-ZF) domain distributed over the entire

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protein [1–3]. In mammals, the expression of SALL4 has been primarily detected in ESCs and in adult tissue "stem-like" cells, where it mainly activates pluripotency and/or multipotency genes and suppresses differentiation-related genes, thereby modulating the cell "stemness" in development and in tissue generation [4–8]. In humans, heterozygous SALL4 mutation has been linked to Okihiro syndrome, Holt-Oram syndrome, acro-renal-ocular syndrome, and IVIC syndrome, all characterized by multiple organ malformations [9–11]. While normally downregulated or no longer expressed in fully differentiated somatic cells, abnormal reactivation of SALL4 in adult cells may lead to malignancy. To date, aberrant SALL4 expression has been detected in over 10 types of human solid tumors and in several common types of leukemias, and SALL4 has been considered a useful biomarker for these diseases [7, 8, 12, 13]. In addition, studies suggest that SALL4 may be a therapeutic target in treating human leukemias [12, 13]. For these reasons, it will be important to understand how SALL4, a critical pluripotency factor, exerts its effects in different cell contexts, and how we can effectively translate our knowledge gains into treatment breakthroughs in future.

2. SALL4 roles in stem cells and development

2.1. The roles of SALL4 in ESC property maintenance and embryonic development

SALL4 has been one of the most studied transcriptional regulators in ESC self-renewal and pluripotency maintenance. It has been reported that in human ESCs, a well-controlled SALL4/OCT4 transcription regulatory loop balances proper expression dosage of SALL4 and OCT4; and reduction of SALL4, like OCT4, results in re-specification of ESCs to the trophoblast lineage [14–17]. In mouse ESC studies, chromatin immunoprecipitation coupled to microarray hybridization (ChIP-on-chip) revealed that SALL4 binds to about twice as many gene promoters as NANOG and binds about four times more genes than OCT4; and the three factors were found to form heteromeric protein complex in regulating stem cell pluripotency. Further, SALL4 binds many genes that are regulated by chromatin-based epigenetic events mediated by cohesin complex, polycomb-repressive complexes 1 and 2 (PRC1 and PRC2), and bivalent domains [18, 19]. Thus, SALL4 plays a diverse role in regulating stem cell pluripotency (see **Figure 1**).

In early embryonic development, SALL4 expression in mouse is detected at as early as the two cell stage. At the blastocyst stage, SALL4 expression becomes enriched in the inner cell mass (ICM) and the trophectoderm [17, 20–22]. Reduction of SALL4 in oocytes and ESCs results in early embryo defects, and disruption of both *Sall4* alleles causes embryonic lethality during peri-implantation [23–25]. SALL4 is also expressed in extraembryonic endoderm (XEN) cells, where it participates in cell fate decision by simultaneously activating pluripotency-maintaining factors and silencing endoderm lineage-associated factors such as GATA6, GATA4, and SOX17 [26, 27]. During subsequent stages, heterozygous disruption of *Sall4* allele leads to multi-organ malformations including limb and heart defects, which model human disease [25]. It has been reported that TBX5, a gene encoding a T-box transcription factor, regulates SALL4 expression in the developing forelimb and heart, and interacts with SALL4 to synergistically regulate downstream gene expression [24, 25, 28].

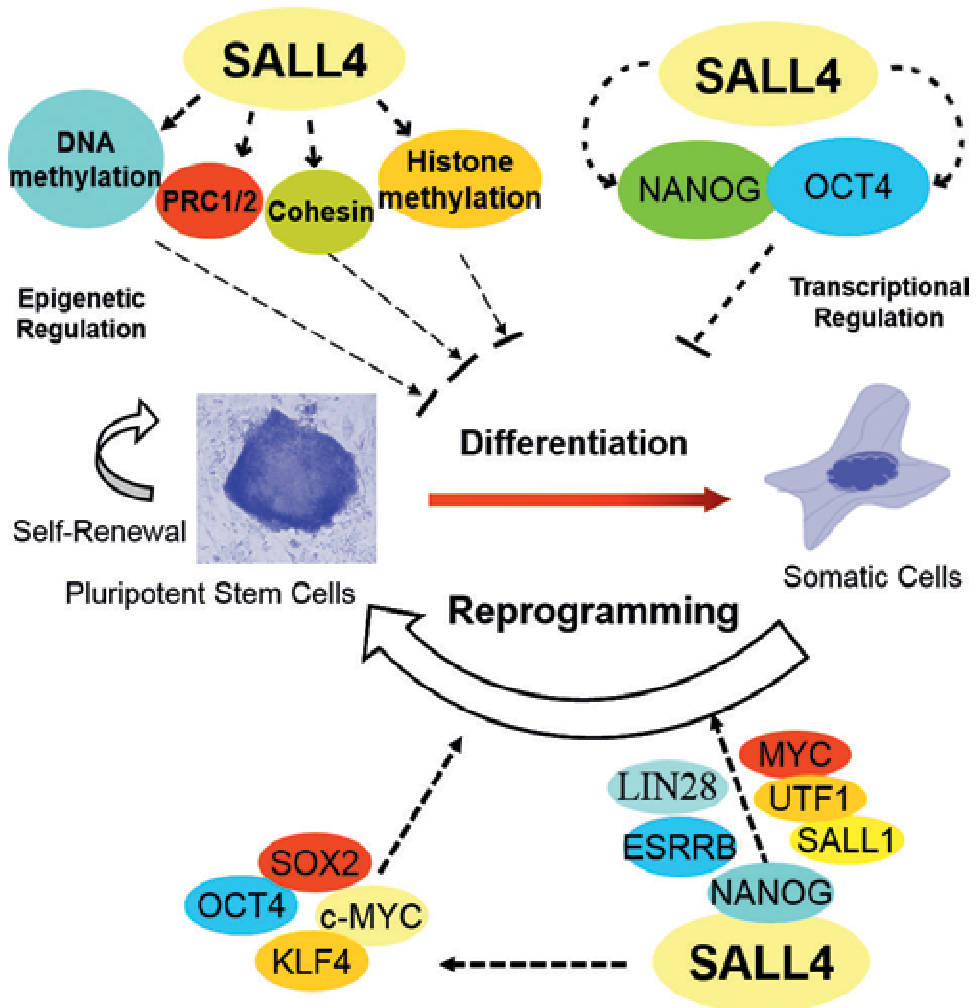

Figure 1. SALL4 plays a variety of regulatory functions in maintaining and/or reprogramming cells to pluripotency. PRC: polycomb-repressive complexes.

2.2. SALL4 is a potent regulator in reprogramming somatic cells to pluripotency

Decreased SALL4 expression in ESCs has been shown to downregulate the expression levels of Oct4, Sox2, Klf4, and c-Myc (OSKM), the four proteins capable of reprogramming murine somatic cells to an induced pluripotent state [18, 29]. Consistently, knockdown of SALL4 in fibroblasts decreased the efficiency of induced pluripotent stem cell (iPSC) generation, while overexpression of SALL4 significantly increased iPSC generation [30, 31]. In a recent study by Shu et al., the GATA family members GATA4 and GATA6 have been found to substitute for OCT4 in mouse somatic reprogramming, and SALL4 is identified as a major target gene of the GATA members [32]. In another study by Buganim et al., ectopic expression of SALL4, NANOG, ESRRB, and LIN28 in mouse fibroblasts generated high-quality iPSCs more

efficiently than the combination of OSKM [33]. Similarly, Mansour et al., showed that the combined overexpression of SALL4 with stem cell factors SALL1, UTF1, NANOG and MYC also replaced exogenous OSK expression and generated chimaera formation- competent iPSC clones [34]. Together, these studies suggest that SALL4 not only plays a role in ESC property maintenance, but its overexpression also drives reprogramming of somatic cells toward a stem cell-like fate (see **Figure 1**).

2.3. SALL4 regulates distinct transcriptional networks in ESCs and XEN cells

SALL4 appears to be unique among the core ESC pluripotency regulators because it is also expressed in non-ESC stem cell fractions where Oct4 and/or Nanog are silenced. These include XEN cells, mesodermal progenitor cells [35], embryonic cardiac progenitor cells [36], fetal liver stem/progenitor cells [27], and adult stem cells such as bone marrow HSCs/HPCs [37]. In these cells, SALL4 regulates downstream networks in a cell type-specific manner. Genome-wide promoter binding assays in murine ESCs and XEN cells revealed that SALL4 regulates disparate gene sets in these cells, and down-regulation of SALL4 targets in the respective cell types induced differentiation [26]. Also consistent with the previous report [18], Sall4, Oct4, Sox2, and Nanog in murine ESCs formed a crucial interconnected autoregulatory network. In XEN cells however, SALL4 regulates the key XEN lineage-associated genes Gata4, Gata6, Sox7, and Sox17 (see **Figure 2**). Moreover, transcription assays revealed that SALL4 regulates

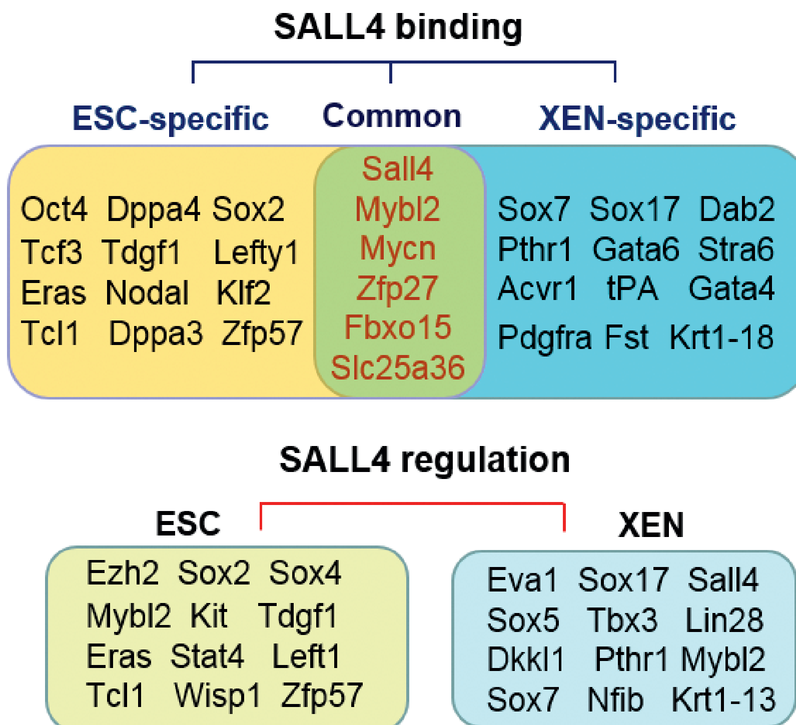

Figure 2. SALL4 binds and regulates distinct target genes in ESCs and XEN cells. Shown are examples of such genes in each cell types. Figure modified from Ref. [26].

the expression of more than half of its binding genes in ESCs, but downregulation of SALL4 did not result in similar expression changes in the majority of these genes in XEN cells [26].

3. Functions of SALL4 and its regulated networks in normal hematopoiesis

3.1. The SALL4 isoforms are robust simulators for HSC/HPC *ex vivo* expansion

In humans and mice, the SALL4 proteins exist in at least three isoforms termed A, B and C, with SALL4A (full length) and SALL4B (lacks a portion of exon2 sequence) being the most studied [38–40]. To date, the function of SALL4C isoform (exon2 sequence spliced out) has not been well characterized. In the human blood system, the cellular expressions of SALL4 isoforms have been originally investigated by immunofluorescence staining and qRT-PCR assays, which revealed that both A and B isoforms are highly expressed in bone marrow CD34+CD38− HSCs, downregulated in CD34+CD38+ HPCs, and absent in CD34− differentiated lineage cells. Similarly, the SALL4 -A and -B isoforms in mouse bone marrows were found selectively expressed in the nuclei of Lin-Sca1+cKit+ (LSK) HSCs. The functions of SALL4 in the self-renewal of HSCs/HPCs have been explored. We and others reported that the SALL4 isoforms are robust stimulators for CD34+ (or CD133+) HSCs/HPCs *ex vivo* expansion, and the SALL4-mediated cell expansion was associated with enhanced cell engraftment and long-term repopulation capacity in transplanted mice [40–44]. In mouse model studies, forced overexpression of the SALL4 isoforms in bone marrow LSK cells likewise leads to sustained cell proliferation, as well as enhanced marrow-repopulating potential *in vivo* [39]. By transcripts assays, the increased HSC/HPC growth was found associated with upregulation of important HSC regulatory genes including HOXB4, NOTCH1, BMI1, RUNX1, CMYC, MEIS1 and NF-YA [39]. Further, in a myeloid progenitor cell line (32D cell) study, overexpression of the SALL4 isoforms blocked granulocyte-colony stimulating factor (G-CSF)-induced granulocytic differentiation, and permitted expansion of undifferentiated cells in the presence of defined cytokines [39, 40]. Thus, the SALL4 isoforms stimulate HSC/HPC proliferation by activating important self-renewal regulators and simultaneously inhibiting cellular differentiation. These studies provide a new avenue for investigating mechanisms of SALL4-regulated HSC/HPC self-renewal and potentially achieving clinically significant expansion of transplantable human HSCs.

3.2. ChIP-on-chip and gene expression assays identified important target genes that are regulated by SALL4

In their study of SALL4 regulated networks in normal hematopoiesis, Gao et al. have sorted human bone marrow and cord blood CD34+ cells, and performed ChIP-on-chip together with gene expression assays . This investigation identified that CD34, RUNX1, HOXA9, and PTEN are SALL4-directed target genes in these cells. In particular, HOXA9 was characterized as a major SALL4 target in normal hematopoiesis . In another study, the polycomb complex protein BMI-1 as a critical SALL4 downstream target has been documented [45]. Chromatin

immunoprecipitation coupled with quantitative PCR (ChIP-qPCR) in the 32D myeloid progenitor cells reveals that SALL4 binds to a specific region of *Bmi-1* gene promoter, and heterozygous disruption of *Sall4* allele significantly reduced BMI-1 expression in bone marrow cells. Further, in transgenic mice that constitutively overexpress human SALL4B, there is upregulated expression of BMI-1, whose levels increase in the progression from normal to preleukemic (myelodysplastic syndrome [MDS]) and leukemic (acute myeloid leukemia [AML]) stages [45].

3.3. SALL4 roles in normal HSC/HPC capacity maintenance

In human CD34+ cell studies, a shRNA-mediated SALL4 knockdown resulted in decreased *in vitro* myeloid-colony-forming ability and impaired *in vivo* engraftment. Further, loss of either SALL4 or its downstream target HOXA9 expression in CD34+ cells shared a similar phenotype. These findings indicate that the role of SALL4 and HOXA9 in normal hematopoiesis is to maintain the HSPCs in an undifferentiated stage with self-renewal capacity [37]. Very recently, the roles of SALL4 in normal hematopoiesis have been further explored using conditional gene targeting approaches in mice [46]. Unexpectedly, wild type $Sall4^{f/f}$/$CreER^{T2}$ mice treated with tamoxifen or vav-Cre-mediated (hematopoietic-specific) $Sall4^{-/-}$ mice were all healthy and displayed no significant hematopoietic defects, which contrasts to previous findings from human CD34+ cell studies. Reasons for this discrepancy have not been fully addressed. However, it has been speculated that SALL4 may have a redundant role during homeostasis, which can be compensated by other *Sall* gene family members, or *pretreatment* of gene knockdown may not truly reflect the actual performance of gene functions *in vitro* or *in vivo*. On the other hand, some genes may exert aberrant functions only when cells encounter transplantation or replicative stress (see review [47]), and some vav/Cre knockout models may demonstrate hematopoietic defects at late stages [48]. Therefore, it might be necessary to perform serial transplantation and/or stress induction (such as 5-fluorouracil injury) assays with SALL4-deficient cells to fully clarify SALL4 effect and mechanisms in normal HSC capacity maintenance.

4. Functions of SALL4 and its regulated networks in leukemia

4.1. SALL4 is aberrantly expressed in human leukemias

SALL4 is absent in most adult tissues and SALL4 expression in bone marrow is restricted to the rare CD34+ HSCs/HPCs. However, aberrant expression of SALL4 has been detected in various human solid tumors as well as different types of leukemias [49–57]. In patients with MDS, a group of preleukemic hematologic disorders, a high level of SALL4 expression is detected and correlated with high-risk patients with poor survival [58, 59]. In AML cases, our group and others have reported that SALL4 mRNA or proteins are aberrantly expressed in various AML subtypes (ranging from M1 to M5, the French-American-British [FAB] classification), and SALL4 expression is involved in chromosomal instability and associated with disease status and drug treatments [59–65]. SALL4 expression is found significantly higher in AML patients with complex karyotype (equal to or more than three aberrant karyotypes) than that in MDS patients with normal karyotype [63]. In chemotherapy cases, it has been reported that SALL4 has the highest expression level in de novo AML patients which then decreases

in partial remission (PR), and then even lower in complete remission (CR) [61, 62]. Further, SALL4 was found to decrease throughout the treatment process in the drug responsive group but increase in drug resistant group [62]. In other leukemia cases, aberrant SALL4 expression has been reported in ALK positive anaplastic large cell lymphoma (ALK^{+} ALCL) [66], B cell acute lymphocytic leukemia (B-ALL), most prominently in B-ALL patients with TEL-AML1 translocation, which is the most common genetic abnormality in pediatric B-ALL [67, 68]. SALL4 expression is also detected in precursor B-cell (but not T-cell) lymphoblastic leukemia/lymphomas [61]. In addition, SALL4 expression has been detected in patient samples from blastic stage of chronic myeloid leukemia (CML), as opposed to the chronic phase, and in samples from CML patients who have achieved complete remission or those who have tyrosine kinase inhibitor resistance [61, 69, 70].

4.2. Role of SALL4 in transgenic model and in MLL-rearranged leukemia

Given the detection of aberrant SALL4 expression in leukemia patients, our research group has previously investigated transgenic mice that overexpress either human SALL4A or SALL4B. Interestingly, all the *SALL4B* mice developed MDS-like features at 2 months of age, and nine of them (53%) progressed to AML. In contrast, the SALL4A mice did not exhibit leukemia formation during the test period [59]. These studies suggest that SALL4B, but not SALL4A, has oncogenic activity in inducing leukemogenesis. In mechanism studies, the SALL4 isoforms were found to bind β-catenin protein, and these factors synergistically enhanced the Wnt/β-catenin signaling pathway. As expected, the expression levels of cyclin-D1 and c-Myc, the two known targets of the Wnt/β-catenin pathway, were both increased in the *SALL4B* mice bone marrow cells. Interestingly, in a recent study, transgenic activation of the SALL4 target β-catenin in osteoblasts, the HSC/HPC niche, also induced MDS and AML development. Notably, these β-catenin mutated mice were anemic at as early as 2 weeks and died before 6 weeks of age, indicating a severe driving event in leukemogenesis [71]. Further in-depth studies are therefore needed to elucidate whether SALL4B in transgenic mice potentially induces leukemogenesis via activating β-catenin in the osteoblastic niche.

Recently, our group explored SALL4 functions in leukemia pathogenesis induced by MLL-AF9, one of the most common mixed lineage leukemia (MLL)-rearranged (MLL-r) oncoproteins found in leukemia patients which is associated with very poor prognosis [72–76]. A previous study showed that SALL4 physically interacts with the MLL wild type protein in regulating HOXA9 expression [77]. In this study, our data revealed that loss of SALL4 in MLL-AF9-transformed bone marrow cells largely disrupted their clonogenic ability in methylcellulose-based medium and in liquid culture, induced markable apoptosis and cell cycle arrest at G1. Consistently, conditional disruption of both *Sall4* alleles in transplanted mice completely blocked leukemia initiation and significantly attenuated pre-existing disease progression [46]. Therefore, these studies suggest that SALL4 is an essential transcriptional regulator in MLL-r leukemogenesis.

4.3. SALL4 regulated pathways in leukemia

Our research group has previously conducted ChIP-on-chip assays with a promyelocytic leukemic cell line NB4 [78]. Analysis of the SALL4-bound genes revealed the most prominent pathways involving WNT/β-catenin, apoptosis, NOTCH signaling, the polycomb complex

protein BMI-1, PTEN, and nuclear factor-kB (see **Figure 3**). When the cells were treated with a SALL4-specific shRNA vector, the expression levels of proapoptotic genes TNF, TP53, PTEN, CARD9, CARD11, ATF3, and LTA were upregulated. In contrast, the expression levels of anti-apoptotic genes such as BCL2, BMI-1, DAD1, TEGT, BIRC7, and BIRC4 (XIAP) are downregulated. In line with the expression studies, reduction of SALL4 also diminished tumorigenicity of leukemic cells in immunodeficient mice. Further, the SALL4 knockdown-induced apoptosis was reversed by ectopic expression BMI-1. In a separate study, SALL4 knockdown in combination with a BCL-2 inhibitor also synergistically increased apoptosis in AML cells. Other studies have reported that SALL4 recruits the nucleosome remodeling and histone deacetylation (NuRD/HDAC) repressive complex to the promoter of *PTEN* and decrease its gene expression [79], while conversely, a SALL4-derived peptide blocking this protein-protein interaction resulted in notable leukemic cell death, and this effect was reversed by treatment of a PTEN inhibitor [80]. In AML differentiation studies, SALL4 expression has also been

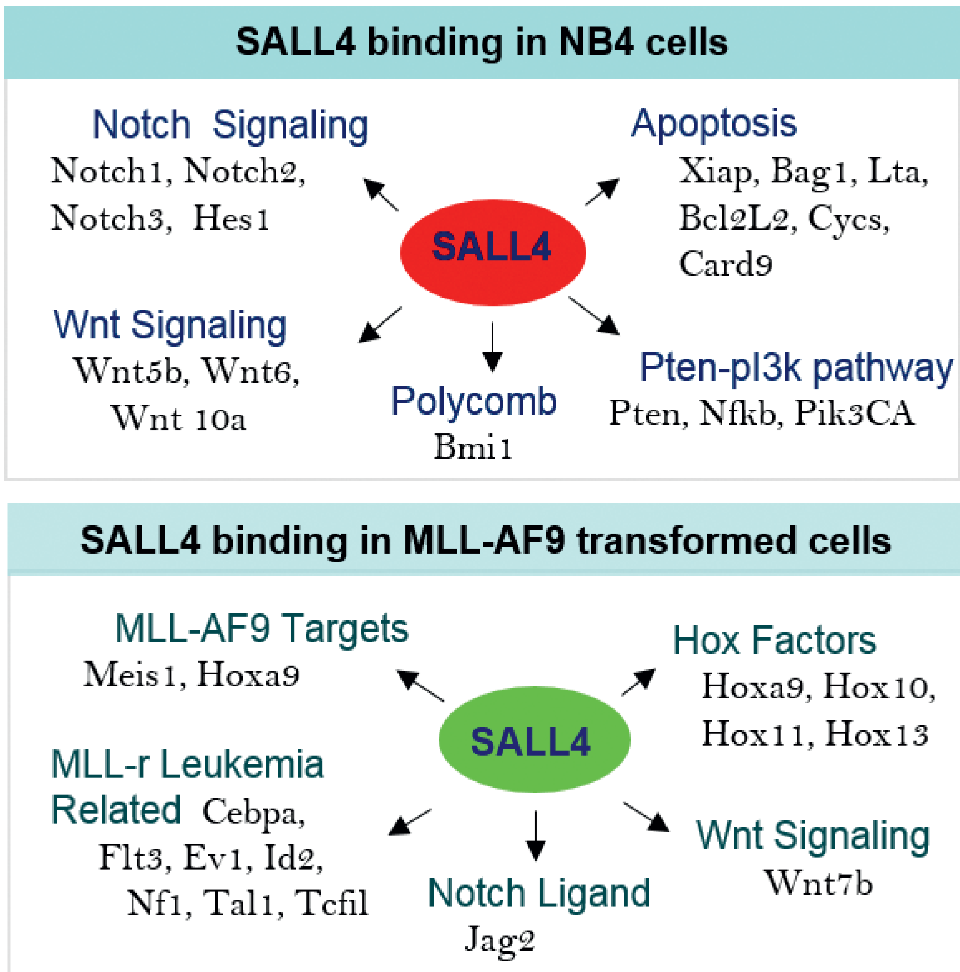

Figure 3. Key signaling pathways bound by SALL4 in NB4 acute promyelocytic and MLL-AF9 transformed leukemic cells.

reported to block all-trans retinoic acid (ATRA)-induced myeloid differentiation in ATRA-sensitive and -resistant AML cells. Further, inhibition of SALL4 and its interacting epigenetic factor LSD1 synergistically promoted ATRA-induced cell differentiation and growth arrest. In mechanistic studies, SALL4 and LSD1 have been found to co-occupy on the ATRA targets *RARβ*, *ID2*, and *CYP26* gene promoters, and cooperatively regulate their expression [81–82].

Recently, our research group also conducted ChIP assays with sequencing (ChIP-Seq) assays with MLL-AF9 transformed murine leukemic cells. This study revealed that SALL4 binds to the key MLL-AF9 target genes *Meis1*, *Hoxa9*; MLL-r leukemia related genes *Cebpα*, *Id2*, *Elf1*, *Evl*, *Flt3*, *Nf1*, *Tal1*, *Tcf7l1*, *Nkx2–3*; the Hox factors *Hoxa-9*, *−10*, *−11*, *−13*; the Notch ligand *Jag2*, and Wnt/β-catenin regulator *Wnt7b* (see **Figure 3** and [46]). mRNA microarrays assays following early *Sall4* deletion identified multiple upregulated genes including cell cycle inhibitors *Cdkn1a (p21)*, *Trp53inp1*; HSC/HPC colony-forming repressor *Slfn2*; and hematopoietic differentiation markers *Col5a1*, *Fyb*, *Irf8* and *Pira6*. In contrast, the TGFβ family genes, *Tgfβ2*, *Tgfβ3*, *Tgfβr3*, and the genes related to chemo-resistance or leukemia aggressiveness, such as *Thbs1*, *Tgm2*, and Ambp were downregulated [46, 83]. In comparison with the mRNA expression data, not many of the ChIP-Seq-identified SALL4 targets were associated with early expression changes. This limited overlap has been considered to be related to the length of time of SALL4 inactivation, the presence of other co-regulators in play, and/or the relatively lower number of genes identified in relevant assays. More detailed studies would help to address these issues.

4.4. SALL4 regulates different downstream networks in normal and leukemic cells

In the SALL4-binding genes identified in NB4 leukemia and those in normal CD34+ cells, less than 20% of the targets were found commonly bound by SALL4. This limited overlap mirrors the findings from ESC and XEN cell promoter binding studies, and further indicates that SALL4 functions in a manner specific to cell type or cell context (see **Figure 4**). Particularly, downregulation of SALL4 expression seems to have an opposite effect on genes involved apoptosis. For example, in leukemic cells, when SALL4 was downregulated along with the apoptotic phenotype, the expression levels of proapoptosis genes TRO

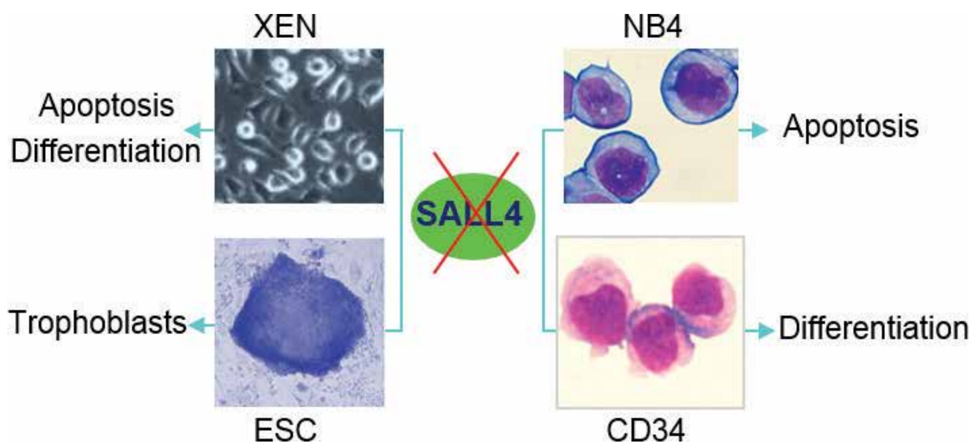

Figure 4. SALL4 functions in a manner specific to cell type or cell context. Shown are main effects following SALL4 knockdown in indicated cell types.

and ABL1 increased, and the expression of anti-apoptosis gene BCL2 decreased. While in CD34+ cells, there was no notable apoptosis with SALL4 knockdown, and the expression of BCL2 increased whereas the expression of TRO and ABL1 decreased. This differential regulatory effect by SALL4 should be helpful in developing SALL4-targeted anti-leukemia strategies to spare normal blood cells.

5. Epigenetic mechanisms involved in SALL4's regulatory functions

5.1. SALL4 interacts with a variety of epigenetic factors to regulate downstream gene expression

So far the reported SALL4-interacting epigenetic factors (see **Figure 5**) include: DNA methyltransferases DNMT-1, -3A, -3B, -3 L, methyl-CpG-binding domain 2 protein (MBD2) [84]; NuRD complex that contains histone deacetylases HDAC1/2 [79]; H3K4 methyltransferase MLL1 [77]; H3K79 methyltransferase DOT1L [46]; H3K36 methyltransferase Wolf-Hirschhorn syndrome candidate 1 (WHSC1) [85, 86]; and lysine-specific histone demethylase LSD1/KDM1A [46, 81, 87]. All of these are critical regulators in normal blood development and are frequent targets for dysregulation in hematological malignancies [88–90], and clinical epigenetic remedies inhibiting such epigenetic factors have been shown effective in treating leukemia [91–93]. In fact, in MLL-AF9-mediated mouse AML studies, genetic disruption of either SALL4, DNMT1, LSD1, or DOT1L likewise blocked leukemia initiation and delayed disease progression *in vivo* [94–96].

By interacting with specific epigenetic factors, SALL4 expression can affect DNA methylation and histone methylation/acetylation status at genes that control hematopoietic differentiation,

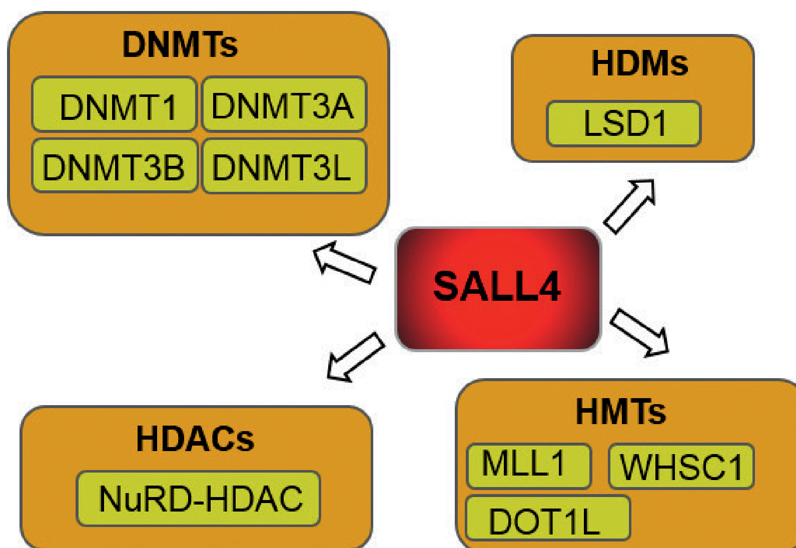

Figure 5. The SALL4-associated epigenetic factors. DNMTs: DNA methyltransferases. HDACs: histone deacetylases. HDMs: histone demethylases. HMTs: histone methyltransferases.

apoptosis, tumor induction or suppression. For example, in NB4 AML cells that were transduced with a lentiviral SALL4 vector, there was an overall increased percentage of DNA methylation at various CpG sites of tumor suppression gene *PTEN*, which co-relates with a downregulated gene transcription [84]. In mouse bone marrow LSK cells, overexpression of SALL4 also induced increased percentage of methylation at the CpG sites of early B-cell factor 1 (*Ebf1*) promoter, as well as the *Sall4* gene promoter itself, which facilitates an undifferentiated cellular status [84]. Similarly, the SALL4 overexpression levels significantly affected LSD1 binding and altered H3K4me2 levels at the promoter regions of tumor necrosis factor (*Tnf*) and differentiation-related genes *EBF1*, *GATA1*, *RARβ*, *ID2*, and *CYP26*, which are associated with relevantly altered gene transcription levels [81, 87]. Also, while SALL4 interacts with the NuRD/HDAC1/2 complex to silence *PTEN* promoter via reduced acetylation of histone H3 at its binding sites, the SALL4-derived peptide blocks this interaction and leads to reactivated PTEN expression. Additionally, in the 32D myeloid progenitor cells following lentiviral SALL4 transduction, the H3K4me3 and H3K79me2/3 levels at *Bmi1* promoter regions were increased [45]. In MLL-AF9 leukemia studies, the expression levels of SALL4 also affected LSD1 and Dot1l binding and relevant H3K4me3 and H3K79me3 amounts at the promoter regions of *Meis1* and multiple HOX family genes in bone marrow cells [46, 77, 79].

5.2. SALL4 regulated epigenetic modification programs are cell type-dependent

Consistent with the findings from SALL4 genome-wide promoter binding and relevant expression assays, SALL4-regulated epigenetic modification programs are also strictly dependent on the cellular context. As reported, SALL4-bound genomic loci in murine ESCs are largely enriched for the activating marker H3K4me3, which indicates an association of SALL4 with non-repressed genes. In XEN cells, however, SALL4-binding loci displayed significantly less H3K4me3 enrichment. Instead, most of these regions are either accompanied with H3K27me3 or lacking both H3K4me3 and H3K27me3, the "epi-markers" frequently associated with gene repression [26]. In our MLL-AF9 leukemia model studies, SALL4 has been shown to recruit DOT1L and LSD1 to *Meis1* and HOX family gene promoters and modulate their H3K79me2/3 and H3K4me3 levels [46]. The previously demonstrated SALL4-MLL interaction may contribute to the observed HEK4me3 changes. However, in some non-MLL-r human AMLs, the DOT1L-regulated H3K79 methylation may not play a role, and it has been reported that administration of DOT1l inhibitors sensitized chemotherapy in MLL-r but not in non-MLL-r AML cells [97]. Further, the DOT1L recruitment to MLL-AF9 has been associated with the level of leukemic transformation [98–100]. Therefore, one may anticipate that SALL4 differentially interacts with individual epigenetic factors to exerting a disease/subtype–dependent regulatory effect. This concept, if proven true, should further facilitate the development of SALL4-based disease subtype–specific anti-leukemia strategies.

6. Conclusions

Abnormal expression of SALL4 has been frequently detected in different types of human leukemias and associated with disease status and drug treatments. On the other hand, proper manipulation of SALL4 expression might be useful in achieving clinically significant

expansion of transplantable human HSCs. Therefore, understanding how SALL4 mechanisms maintain normal HSCs/HPCs vs. leukemic cells will facilitate development of newer, more efficient therapies in clinic.

Acknowledgements

This work was partially supported by American Cancer Society Research Scholar Grant RSG-12-216-01-LIB (to J.Y.).

Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

Acronyms and abbreviations

ESC	embryonic stem cell
HSPs/HPCs	hematopoietic stem/progenitor cells
C2H2-ZF	Cys2His2 zinc finger
PRC	polycomb-repressive complexes
ChIP-on-chip	chromatin immunoprecipitation followed by microarray hybridization
ICM	inner cell mass
XEN	extraembryonic endoderm
iPSC	induced pluripotent stem cell
LSK	lineage- Sca-1+ c-kit+
G-CSF	granulocyte-colony stimulating factor
MDS	myelodysplastic syndrome
AML	acute myeloid leukemia
FAB	the French-American-British classification
ALK+ ALCL	ALK positive anaplastic large cell lymphoma
B-ALL	B cell acute lymphocytic leukemia
CML	chronic myeloid leukemia
MLL-r	mixed lineage leukemia (MLL)-rearranged

NuRD/HDAC	nucleosome remodeling and histone deacetylation
ATRA	all-trans retinoic acid
ChIP-Seq	ChIP assays with sequencing
MBD2	methyl-CpG-binding domain 2 protein
WHSC1	Wolf-Hirschhorn syndrome candidate 1
Ebf1	early B-cell factor 1

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References

[1] Kohlhase J, Schuh R, Dowe G, Kuhnlein RP, Jackle H, Schroeder B, et al. Isolation, characterization, and organ-specific expression of two novel human zinc finger genes related to the drosophila gene Spalt. Genomics. 1996;**38**(3):291-298

[2] Eildermann K, Aeckerle N, Debowski K, Godmann M, Christiansen H, Heistermann M, et al. Developmental expression of the pluripotency factor Sal-like protein 4 in the monkey, human and mouse testis: Restriction to premeiotic germ cells. Cells, Tissues, Organs. 2012;**196**(3):206-220. PubMed PMID: 22572102

[3] Sweetman D, Munsterberg A. The vertebrate Spalt genes in development and disease. Developmental Biology. 2006;**293**(2):285-293. PubMed PMID: 16545361

[4] Yang J, Liao W, Ma Y. Role of SALL4 in hematopoiesis. Current Opinion in Hematology. 2012;**19**(4):287-291

[5] Kohlhase J, Heinrich M, Schubert L, Liebers M, Kispert A, Laccone F, et al. Okihiro syndrome is caused by SALL4 mutations. Human Molecular Genetics. 2002;**11**(23):2979-2987. PubMed PMID: 12393809

[6] Kohlhase J. SALL4-related disorders. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mefford HC, et al., editors. Gene Reviews. Seattle, WA; 1993. GeneReviews® [Internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1373/

[7] Tatetsu H, Kong NR, Chong G, Amabile G, Tenen DG, Chai L. SALL4, the missing link between stem cells, development and cancer. Gene. 2016;**584**(2):111-119

[8] Xiong J. SALL4: Engine of cell Stemness. Current Gene Therapy. 2014;**14**(5):400-411. PubMed PMID: 25174577

[9] Al-Baradie R, Yamada K, St Hilaire C, Chan WM, Andrews C, McIntosh N, et al. Duane radial ray syndrome (Okihiro syndrome) maps to 20q13 and results from mutations in SALL4, a new member of the SAL family. American Journal of Human Genetics. 2002;**71**(5):1195-1199

[10] Kohlhase J, Schubert L, Liebers M, Rauch A, Becker K, Mohammed SN, et al. Mutations at the SALL4 locus on chromosome 20 result in a range of clinically overlapping phenotypes, including Okihiro syndrome, Holt-Oram syndrome, acro-renal-ocular syndrome, and patients previously reported to represent thalidomide embryopathy. Journal of Medical Genetics. 2003;**40**(7):473-478. PubMed PMID: 12843316; PubMed Central PMCID: PMCPMC1735528

[11] Paradisi I, Arias S. IVIC syndrome is caused by a c.2607delA mutation in the SALL4 locus. American Journal of Medical Genetics Part A. 2007;**143**(4):326-332. PubMed PMID: 17256792

[12] Zhang X, Yuan X, Zhu W, Qian H, Xu W. SALL4: An emerging cancer biomarker and target. Cancer Letters. 2015;**357**(1):55-62. PubMed PMID: 25444934

[13] Wang F, Zhao W, Kong N, Cui W, Chai L. The next new target in leukemia: The embryonic stem cell gene SALL4. Mol Cell Oncol. 2014;**1**(4):e969169. PubMed PMID: 25977939; PubMed Central PMCID: PMCPMC4428154

[14] Zhang J, Tam WL, Tong GQ, Wu Q, Chan HY, Soh BS, et al. Sall4 modulates embryonic stem cell pluripotency and early embryonic development by the transcriptional regulation of Pou5f1. Nature Cell Biology. 2006;**8**(10):1114-1123

[15] Yang J, Gao C, Chai L, Ma Y. A novel SALL4/OCT4 transcriptional feedback network for pluripotency of embryonic stem cells. PLoS One. 2010;**5**(5):e10766. PubMed PMID: 20505821; PubMed Central PMCID: PMC2874005

[16] Nosi U, Lanner F, Huang T, Cox B. Overexpression of trophoblast stem cell-enriched microRNAs promotes trophoblast fate in embryonic stem cells. Cell Reports. 2017;**19**(6):1101-1109. PubMed PMID: 28494860

[17] Miller A, Gharbi S, Etienne-Dumeau C, Nishinakamura R, Hendrich B. Transcriptional control by Sall4 in blastocysts facilitates lineage commitment of inner cell mass cells. bioRxiv. 2017

[18] Yang J, Chai L, Fowles TC, Alipio Z, Xu D, Fink LM, et al. Genome-wide analysis reveals Sall4 to be a major regulator of pluripotency in murine-embryonic stem cells. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**(50):19756-19761. PubMed PMID: 19060217; PubMed Central PMCID: PMC2604985

[19] Abboud N, Moore-Morris T, Hiriart E, Yang H, Bezerra H, Gualazzi MG, et al. A cohesin-OCT4 complex mediates Sox enhancers to prime an early embryonic lineage. Nature Communications. 2015;**6**:6749. PubMed PMID: 25851587; PubMed Central PMCID: PMCPMC5531045

[20] Xu K, Chen X, Yang H, Xu Y, He Y, Wang C, et al. Maternal Sall4 is indispensable for epigenetic maturation of mouse oocytes. The Journal of Biological Chemistry. 2017;**292**(5): 1798-1807. PubMed PMID: 28031467; PubMed Central PMCID: PMCPMC5290953

[21] Sakaki-Yumoto M, Kobayashi C, Sato A, Fujimura S, Matsumoto Y, Takasato M, et al. The murine homolog of SALL4, a causative gene in Okihiro syndrome, is essential for embryonic stem cell proliferation, and cooperates with Sall1 in anorectal, heart, brain and kidney development. Development. 2006;**133**(15):3005-3013. PubMed PMID: 16790473

[22] Elling U, Klasen C, Eisenberger T, Anlag K, Treier M. Murine inner cell mass-derived lineages depend on Sall4 function. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(44):16319-16324. PubMed PMID: 17060609; PubMed Central PMCID: PMCPMC1637580

[23] Warren M, Wang W, Spiden S, Chen-Murchie D, Tannahill D, Steel KP, et al. A Sall4 mutant mouse model useful for studying the role of Sall4 in early embryonic development and organogenesis. Genesis. 2007;**45**(1):51-58. PubMed PMID: 17216607; PubMed Central PMCID: PMCPMC2593393

[24] Harvey SA, Logan MP. Sall4 acts downstream of tbx5 and is required for pectoral fin outgrowth. Development. 2006;**133**(6):1165-1173. PubMed PMID: 16501170

[25] Koshiba-Takeuchi K, Takeuchi JK, Arruda EP, Kathiriya IS, Mo R, Hui CC, et al. Cooperative and antagonistic interactions between Sall4 and Tbx5 pattern the mouse limb and heart. Nature Genetics. 2006;**38**(2):175-183. PubMed PMID: 16380715

[26] Lim CY, Tam WL, Zhang J, Ang HS, Jia H, Lipovich L, et al. Sall4 regulates distinct transcription circuitries in different blastocyst-derived stem cell lineages. Cell Stem Cell. 2008;**3**(5):543-554

[27] Oikawa T, Kamiya A, Kakinuma S, Zeniya M, Nishinakamura R, Tajiri H, et al. Sall4 regulates cell fate decision in fetal hepatic stem/progenitor cells. Gastroenterology. 2009;**136**(3):1000-1011. PubMed PMID: 19185577

[28] Bohm J, Heinritz W, Craig A, Vujic M, Ekman-Joelsson BM, Kohlhase J, et al. Functional analysis of the novel TBX5 c.1333delC mutation resulting in an extended TBX5 protein. BMC Medical Genetics. 2008;**9**:88. PubMed PMID: 18828908; PubMed Central PMCID: PMCPMC2567295

[29] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;**126**(4):663-676. PubMed PMID: 16904174

[30] Tsubooka N, Ichisaka T, Okita K, Takahashi K, Nakagawa M, Yamanaka S. Roles of Sall4 in the generation of pluripotent stem cells from blastocysts and fibroblasts. Genes to Cells. 2009;**14**(6):683-694. PubMed PMID: 19476507

[31] Wong CC, Gaspar-Maia A, Ramalho-Santos M, Reijo Pera RA. High-efficiency stem cell fusion-mediated assay reveals Sall4 as an enhancer of reprogramming. PLoS One. 2008;**3**(4):e1955. PubMed PMID: 18414659; PubMed Central PMCID: PMCPMC2278370

[32] Shu J, Zhang K, Zhang M, Yao A, Shao S, Du F, et al. GATA family members as inducers for cellular reprogramming to pluripotency. Cell Research. 2015;**25**(2):169-180. PubMed PMID: 25591928; PubMed Central PMCID: PMCPMC4650575

[33] Buganim Y, Markoulaki S, van Wietmarschen N, Hoke H, Wu T, Ganz K, et al. The developmental potential of iPSCs is greatly influenced by reprogramming factor selection. Cell Stem Cell. 2014;**15**(3):295-309. PubMed PMID: 25192464; PubMed Central PMCID: PMCPMC4170792

[34] Mansour AA, Gafni O, Weinberger L, Zviran A, Ayyash M, Rais Y, et al. The H3K27 demethylase Utx regulates somatic and germ cell epigenetic reprogramming. Nature. 2012;**488**(7411):409-413. PubMed PMID: 22801502

[35] Pacini S, Carnicelli V, Trombi L, Montali M, Fazzi R, Lazzarini E, et al. Constitutive expression of pluripotency-associated genes in mesodermal progenitor cells (MPCs). PLoS One. 2010;**5**(3):e9861. PubMed PMID: 20360837; PubMed Central PMCID: PMCPMC2845604

[36] Jia G, Preussner J, Guenther S, Yuan X, Yekelchyk M, Kuenne C, et al. Single cell RNA-seq and ATAC-seq indicate critical roles of Isl1 and Nkx2-5 for cardiac progenitor cell transition states and lineage settlement. bioRxiv. 2017

[37] Gao C, Kong NR, Li A, Tatetu H, Ueno S, Yang Y, et al. SALL4 is a key transcription regulator in normal human hematopoiesis. Transfusion. 2013;**53**(5):1037-1049. PubMed PMID: 22934838; PubMed Central PMCID: PMC3653586

[38] Rao S, Zhen S, Roumiantsev S, McDonald LT, Yuan GC, Orkin SH. Differential roles of Sall4 isoforms in embryonic stem cell pluripotency. Molecular and Cellular Biology. 2010;**30**(22):5364-5380. PubMed PMID: 20837710; PubMed Central PMCID: PMCPMC 2976381

[39] Yang J, Aguila JR, Alipio Z, Lai R, Fink LM, Ma Y. Enhanced self-renewal of hematopoietic stem/progenitor cells mediated by the stem cell gene Sall4. Journal of Hematology & Oncology. 2011;**4**:38. PubMed PMID: 21943195; PubMed Central PMCID: PMC3184628

[40] Aguila JR, Liao W, Yang J, Avila C, Hagag N, Senzel L, et al. SALL4 is a robust stimulator for the expansion of hematopoietic stem cells. Blood. 2011;**118**(3):576-585. PubMed PMID: 21602528; PubMed Central PMCID: PMC3142902

[41] Liao W, Aguila JR, Yao Y, Yang J, Zieve G, Jiang Y, et al. Enhancing bone marrow regeneration by SALL4 protein. Journal of Hematology & Oncology. 2013;**6**:84. PubMed PMID: 24283261; PubMed Central PMCID: PMCPMC3882884

[42] Mossahebi-Mohammadi M, Atashi A, Kaviani S, Soleimani M. Efficient expansion of SALL4-transduced umbilical cord blood derived CD133+hematopoietic stem cells. Acta Medica Iranica. 2017;**55**(5):290-296. PubMed PMID: 28724268

[43] Tatetsu H, Wang F, Gao C, Ueno S, Tian X, Armant M, et al. SALL4 is a key factor in HDAC inhibitor mediated ex vivo expansion of human peripheral blood mobilized stem/progenitor CD34+CD90+ cells. Blood. 2014;**124**(21):1566

[44] Akhavan Rahnama M, Movassaghpour AA, Soleimani M, Atashi A, Anbarlou A, Shams AK. MicroRNA-15b target Sall4 and diminish in vitro UCB-derived HSCs expansion. EXCLI Journal. 2015;**14**:601-610. PubMed PMID: 26648817; PubMed Central PMCID: PMCPMC4669904

[45] Yang J, Chai L, Liu F, Fink LM, Lin P, Silberstein LE, et al. Bmi-1 is a target gene for SALL4 in hematopoietic and leukemic cells. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(25):10494-10499. PubMed PMID: 17557835; PubMed Central PMCID: PMC1965541

[46] Yang L, Liu L, Gao H, Pinnamaneni JP, Sanagasetti D, Singh VP, et al. The stem cell factor SALL4 is an essential transcriptional regulator in mixed lineage leukemia-rearranged leukemogenesis. Journal of Hematology & Oncology. 2017;**10**(1):159

[47] Rossi L, Lin KK, Boles NC, Yang L, King KY, Jeong M, et al. Less is more: Unveiling the functional core of hematopoietic stem cells through knockout mice. Cell Stem Cell. 2012;**11**(3):302-317. PubMed PMID: 22958929; PubMed Central PMCID: PMCPMC3461270

[48] Damnernsawad A, Kong G, Wen Z, Liu Y, Rajagopalan A, You X, et al. Kras is required for adult hematopoiesis. Stem Cells. 2016;**34**(7):1859-1871. PubMed PMID: 26972179; PubMed Central PMCID: PMCPMC5358545

[49] Oikawa T, Kamiya A, Zeniya M, Chikada H, Hyuck AD, Yamazaki Y, et al. Sal-like protein 4 (SALL4), a stem cell biomarker in liver cancers. Hepatology. 2013;**57**(4):1469-1483. PubMed PMID: 23175232

[50] Ushiku T, Shinozaki A, Shibahara J, Iwasaki Y, Tateishi Y, Funata N, et al. SALL4 represents fetal gut differentiation of gastric cancer, and is diagnostically useful in distinguishing hepatoid gastric carcinoma from hepatocellular carcinoma. The American Journal of Surgical Pathology. 2010;**34**(4):533-540. PubMed PMID: 20182341

[51] Kobayashi D, Kuribayashi K, Tanaka M, Watanabe N. Overexpression of SALL4 in lung cancer and its importance in cell proliferation. Oncology Reports. 2011;**26**(4):965-970. PubMed PMID: 21725617

[52] Kobayashi D, Kuribayshi K, Tanaka M, Watanabe N. SALL4 is essential for cancer cell proliferation and is overexpressed at early clinical stages in breast cancer. International Journal of Oncology. 2011;**38**(4):933-939. PubMed PMID: 21274508

[53] Ardalan Khales S, Abbaszadegan MR, Abdollahi A, Raeisossadati R, Tousi MF, Forghanifard MM. SALL4 as a new biomarker for early colorectal cancers. Journal of Cancer Research and Clinical Oncology. 2014. PubMed PMID: 25156818

[54] Zhang L, Yan Y, Jiang Y, Cui Y, Zou Y, Qian J, et al. The expression of SALL4 in patients with gliomas: High level of SALL4 expression is correlated with poor outcome. Journal of Neuro-Oncology. 2015;**121**(2):261-268. PubMed PMID: 25359397

[55] Miettinen M, Wang Z, McCue PA, Sarlomo-Rikala M, Rys J, Biernat W, et al. SALL4 expression in germ cell and non-germ cell Tumors: A systematic Immunohistochemical

study of 3215 cases. The American Journal of Surgical Pathology. 2014;**38**(3):410-420. PubMed PMID: 24525512

[56] Mei K, Liu A, Allan RW, Wang P, Lane Z, Abel TW, et al. Diagnostic utility of SALL4 in primary germ cell tumors of the central nervous system: A study of 77 cases. Modern Pathology. 2009;**22**(12):1628-1636. PubMed PMID: 19820689

[57] Cao D, Guo S, Allan RW, Molberg KH, Peng Y. SALL4 is a novel sensitive and specific marker of ovarian primitive germ cell tumors and is particularly useful in distinguishing yolk sac tumor from clear cell carcinoma. The American Journal of Surgical Pathology. 2009;**33**(6):894-904. PubMed PMID: 19295406

[58] Wang F, Guo Y, Chen Q, Yang Z, Ning N, Zhang Y, et al. Stem cell factor SALL4, a potential prognostic marker for myelodysplastic syndromes. Journal of Hematology & Oncology. 2013;**6**(1):73. PubMed PMID: 24283704; PubMed Central PMCID: PMC3856454

[59] Ma Y, Cui W, Yang J, Qu J, Di C, Amin HM, et al. SALL4, a novel oncogene, is constitutively expressed in human acute myeloid leukemia (AML) and induces AML in transgenic mice. Blood. 2006;**108**:2726-2735

[60] Abo-Elwafa H, Aziz S, Salah M, Sedek O. The SALL4 gene in acute leukemias. The Egyptian Journal of Haematology. 2015;**40**(3):121-129

[61] Shen Q, Liu S, Hu J, Chen S, Yang L, Li B, et al. The differential expression pattern of the BMI-1, SALL4 and ABCA3 genes in myeloid leukemia. Cancer Cell International. 2012;**12**(1):42. PubMed PMID: 23067006; PubMed Central PMCID: PMCPMC3538712

[62] Jeong HW, Cui W, Yang Y, Lu J, He J, Li A, et al. SALL4, a stem cell factor, affects the side population by regulation of the ATP-binding cassette drug transport genes. PLoS One. 2011;**6**(4):e18372. PubMed PMID: 21526180; PubMed Central PMCID: PMC3079717

[63] Wang F, Gao C, Lu J, Tatetsu H, Williams DA, Muller LU, et al. Leukemic survival factor SALL4 contributes to defective DNA damage repair. Oncogene. 2016;**35**(47):6087-6095. PubMed PMID: 27132514; PubMed Central PMCID: PMCPMC5093088

[64] Chen Q, Qian J, Lin J, Yang J, Li Y, Wang CZ, et al. Expression of SALL4 gene in patients with acute and chronic myeloid leukemia. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2013;**21**(2):315-319. PubMed PMID: 23628023

[65] Milanovich S, Peterson J, Allred J, Stelloh C, Rajasekaran K, Fisher J, et al. Sall4 overexpression blocks murine hematopoiesis in a dose-dependent manner. Experimental Hematology. 2015;**43**(1):53-64 e1-8. PubMed PMID: 25246269; PubMed Central PMCID: PMCPMC4268405

[66] Wang P, Zhang JD, Wu F, Ye X, Sharon D, Hitt M, et al. The expression and oncogenic effects of the embryonic stem cell marker SALL4 in ALK-positive anaplastic large cell lymphoma. Cellular Signalling. 2012;**24**(10):1955-1963. PubMed PMID: 22743134

[67] Ueno S, Lu J, He J, Li A, Zhang X, Ritz J, et al. Aberrant expression of SALL4 in acute B cell lymphoblastic leukemia: Mechanism, function, and implication for a potential novel

therapeutic target. Experimental Hematology. 2014;**42**(4):307-316 e8. PubMed PMID: 24463278; PubMed Central PMCID: PMC4135469

[68] Cui W, Kong NR, Ma Y, Amin HM, Lai R, Chai L. Differential expression of the novel oncogene, SALL4, in lymphoma, plasma cell myeloma, and acute lymphoblastic leukemia. Modern Pathology. 2006;**19**(12):1585-1592. PubMed PMID: 16998462

[69] Lu J, Ma Y, Kong N, Alipio Z, Gao C, Krause DS, et al. Dissecting the role of SALL4, a newly identified stem cell factor, in chronic myelogenous leukemia. Leukemia. 2011;**25**(7):1211-1213. PubMed PMID: 21468036; PubMed Central PMCID: PMC3675449

[70] Hupfeld T, Chapuy B, Schrader V, Beutler M, Veltkamp C, Koch R, et al. Tyrosinekinase inhibition facilitates cooperation of transcription factor SALL4 and ABC transporter A3 towards intrinsic CML cell drug resistance. British Journal of Haematology. 2013;**161**(2):204-213. PubMed PMID: 23432194

[71] Kode A, Manavalan JS, Mosialou I, Bhagat G, Rathinam CV, Luo N, et al. Leukaemogenesis induced by an activating beta-catenin mutation in osteoblasts. Nature. 2014;**506**(7487): 240-244. PubMed PMID: 24429522; PubMed Central PMCID: PMCPMC4116754

[72] Prange KHM, Mandoli A, Kuznetsova T, Wang SY, Sotoca AM, Marneth AE, et al. MLL-AF9 and MLL-AF4 on cofusion proteins bind a distinct enhancer repertoire and target the RUNX1 program in 11q23 acute myeloid leukemia. Oncogene. 2017;**36**(23):3346-3356. PubMed PMID: 28114278; PubMed Central PMCID: PMCPMC547456r5

[73] Winters AC, Bernt KM. MLL-rearranged leukemias—An update on science and clinical approaches. Frontiers in Pediatrics. 2017;**5**:4. PubMed PMID: 28232907; PubMed Central PMCID: PMCPMCr5299633

[74] Zhu N, Chen M, Eng R, DeJong J, Sinha AU, Rahnamay NF, et al. MLL-AF9- and HOXA9-mediated acute myeloid leukemia stem cell self-renewal requires JMJD1C. The Journal of Clinical Investigation. 2016;**126**(3):997-1011. PubMed PMID: 26878175

[75] Marschalek R. MLL leukemia and future treatment strategies. Archiv der Pharmazie (Weinheim). 2015;**348**(4):221-228. PubMed PMID: 25740345

[76] de Boer J, Walf-Vorderwulbecke V, Williams O. In focus: MLL-rearranged leukemia. Leukemia. 2013;**27**(6):1224-1228. PubMed PMID: 23515098

[77] Li A, Yang Y, Gao C, Lu J, Jeong HW, Liu BH, et al. A SALL4/MLL/HOXA9 pathway in murine and human myeloid leukemogenesis. The Journal of Clinical Investigation. 2013;**123**(10):4195-4207. PubMed PMID: 24051379; PubMed Central PMCID: PMC3r784519

[78] Yang J, Chai L, Gao C, Fowles TC, Alipio Z, Dang H, et al. SALL4 is a key regulator of survival and apoptosis in human leukemic cells. Blood. 2008;**112**(3):805-813

[79] Lu J, Jeong HW, Kong N, Yang Y, Carroll J, Luo HR, et al. Stem cell factor SALL4 represses the transcriptions of PTEN and SALL1 through an epigenetic repressor complex. PLoS One. 2009;**4**(5):e5577. PubMed PMID: 19440552; PubMed Central PMCID: PMC2679146

[80] Gao C, Dimitrov T, Yong KJ, Tatetsu H, Jeong HW, Luo HR, et al. Targeting transcription factor SALL4 in acute myeloid leukemia by interrupting its interaction with an epigenetic complex. Blood. 2013;**121**(8):1413-1421. PubMed PMID: 23287862; PubMed Central PMCID: PMC3578956

[81] Liu L, Liu L, Leung E, Cooney AJ, Chen C, Rosengart TK, et al. Knockdown of SALL4 enhances all-trans retinoic acid-induced cellular differentiation in acute myeloid leukemia cells. The Journal of Biological Chemistry. 2015;**290**(17):10599-10609. PubMed PMID: 25737450

[82] Gao C, Kong NR, Chai L. The role of stem cell factor SALL4 in leukemogenesis. Critical Reviews in Oncogenesis. 2011;**16**(1-2):117-127. PubMed PMID: 22150312; PubMed Central PMCID: PMCPMC3238789

[83] Zhang W, Xia X, Reisenauer MR, Rieg T, Lang F, Kuhl D, et al. Aldosterone-induced Sgk1 relieves Dot1a-Af9-mediated transcriptional repression of epithelial Na+ channel alpha. The Journal of Clinical Investigation. 2007;**117**(3):773-783

[84] Yang J, Corsello TR, Ma Y. Stem cell gene SALL4 suppresses transcription through recruitment of DNA methyltransferases. The Journal of Biological Chemistry. 2012;**287**(3): 1996-2005. PubMed PMID: 22128185; PubMed Central PMCID: PMC3265879

[85] Campos-Sanchez E, Deleyto-Seldas N, Dominguez V, Carrillo-de-Santa-Pau E, Ura K, Rocha PP, et al. Wolf-Hirschhorn syndrome candidate 1 is necessary for correct hematopoietic and B cell development. Cell Reports. 2017;**19**(8):1586-1601. PubMed PMID: 28538178; PubMed Central PMCID: PMCPMC5510986

[86] Nimura K, Ura K, Shiratori H, Ikawa M, Okabe M, Schwartz RJ, et al. A histone H3 lysine 36 trimethyltransferase links Nkx2-5 to Wolf-Hirschhorn syndrome. Nature. 2009;**460**(7252):287-291. PubMed PMID: 19483677

[87] Liu L, Souto J, Liao W, Jiang Y, Li Y, Nishinakamura R, et al. Histone lysine-specific demethylase 1 (LSD1) protein is involved in Sal-like protein 4 (SALL4)-mediated transcriptional repression in hematopoietic stem cells. The Journal of Biological Chemistry. 2013;**288**(48):34719-34728

[88] Rice KL, Hormaeche I, Licht JD. Epigenetic regulation of normal and malignant hematopoiesis. Oncogene. 2007;**26**(47):6697-6714. PubMed PMID: 17934479

[89] Goyama S, Kitamura T. Epigenetics in normal and malignant hematopoiesis: An overview and update 2017. Cancer Science. 2017;**108**(4):553-562. PubMed PMID: 28100030; PubMed Central PMCID: PMCPMC5406607

[90] Ding LW, Sun QY, Tan KT, Chien W, Mayakonda A, Yeoh AEJ, et al. Mutational landscape of pediatric acute lymphoblastic leukemia. Cancer Research. 2017;**77**(2):390-400. PubMed PMID: 27872090; PubMed Central PMCID: PMCPMC5243866

[91] Wouters BJ, Delwel R. Epigenetics and approaches to targeted epigenetic therapy in acute myeloid leukemia. Blood. 2016;**127**(1):42-52. PubMed PMID: 26660432

[92] Gallipoli P, Giotopoulos G, Huntly BJ. Epigenetic regulators as promising therapeutic targets in acute myeloid leukemia. Therapeutic Advances in Hematology. 2015;**6**(3): 103-119. PubMed PMID: 26137202; PubMed Central PMCID: PMCPMC4480521

[93] Bernt KM, Armstrong SA. Targeting epigenetic programs in MLL-rearranged leukemias. Hematology. 2011;**2011**:354-360. PubMed PMID: 22160057

[94] Trowbridge JJ, Sinha AU, Zhu N, Li M, Armstrong SA, Orkin SH. Haploinsufficiency of Dnmt1 impairs leukemia stem cell function through derepression of bivalent chromatin domains. Genes & Development. 2012;**26**(4):344-349. PubMed PMID: 22345515; PubMed Central PMCID: PMCPMC3289882

[95] Kuntimaddi A, Achille NJ, Thorpe J, Lokken AA, Singh R, Hemenway CS, et al. Degree of recruitment of DOT1L to MLL-AF9 defines level of H3K79 di- and tri-methylation on target genes and transformation potential. Cell Reports. 2015;**11**(5):808-820. PubMed PMID: 25921540; PubMed Central PMCID: PMCPMC4426023

[96] Harris WJ, Huang X, Lynch JT, Spencer GJ, Hitchin JR, Li Y, et al. The histone demethylase KDM1A sustains the oncogenic potential of MLL-AF9 leukemia stem cells. Cancer Cell. 2012;**21**(4):473-487. PubMed PMID: 22464800

[97] Liu W, Deng L, Song Y, Redell M. DOT1L inhibition sensitizes MLL-rearranged AML to chemotherapy. PLoS One. 2014;**9**(5):e98270. PubMed PMID: 24858818; PubMed Central PMCID: PMCPMC4032273

[98] Bernt KM, Zhu N, Sinha AU, Vempati S, Faber J, Krivtsov AV, et al. MLL-rearranged leukemia is dependent on aberrant H3K79 methylation by DOT1L. Cancer Cell. 2011;**20**(1):66-78. PubMed PMID: 21741597; PubMed Central PMCID: PMCPMC3329803

[99] Bernt KM, Armstrong SA. A role for DOT1L in MLL-rearranged leukemias. Epigenomics. 2011;**3**(6):667-670. PubMed PMID: 22126283

[100] Chen CW, Armstrong SA. Targeting DOT1L and HOX gene expression in MLL-rearranged leukemia and beyond. Experimental Hematology. 2015;**43**(8):673-684. PubMed PMID: 26118503; PubMed Central PMCID: PMCPMC4540610

The Glucocorticoid Receptor and Certain KRÜPPEL-Like Transcription Factors have the Potential to Synergistically Stimulate Bovine Herpesvirus 1 Transcription and Reactivation from Latency

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75451

Abstract

Bovine herpesvirus 1 (BoHV-1), an important bovine pathogen, establishes life-long latency in sensory neurons within trigeminal ganglia (TG). Stress, as mimicked by the synthetic corticosteroid dexamethasone, consistently induces reactivation in calves latently infected with BoHV-1. Dexamethasone induces expression of several transcription factors in TG neurons during early stages of reactivation, including Krüppel-like transcription factors (KLF): KLF4, KLF6, KLF15, and promyelocytic leukemia zinc finger. Furthermore, the glucocorticoid receptor (GR) is consistently detected in TG neurons expressing viral regulatory proteins during reactivation from latency. The viral immediate early transcription unit 1 (IEtu1) promoter that drives expression of two viral transcription factors (bICP0 and bICP4) contains two GR response elements (GREs) and is stimulated by DEX. KLF15 and the GR form a feed forward transcription loop that synergistically stimulates productive infection and IEtu1 promoter activity. New studies demonstrate the GR and KLF6 synergistically stimulate productive infection and IEtu1 promoter activity if the GREs are intact. Furthermore, the GR and KLF6 interact with wild-type GREs within the IEtu1 promoter, but not GRE mutants. These studies suggest that certain KLF family members and the GR can convert a silent viral genome in latently infected neurons into an actively transcribing genome during reactivation from latency.

Keywords: bovine herpesvirus 1, immediate early transcription, reactivation from latency, sensory neurons, glucocorticoid receptors, Krüppel-like transcription factors

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1. Introduction

Bovine herpesvirus 1 (BoHV-1), an alpha-herpesvirinae subfamily member, is an important bovine pathogen that causes conjunctivitis and/or upper respiratory tract disease [1–3]. BoHV-1 is a significant cofactor in the polymicrobial disease referred to as bovine respiratory disease complex (BRDC), which is the most important disease of cattle. BoHV-1 infection impairs cell-mediated immunity, CD8+ T cell recognition of infected cells, and induces apoptosis in CD4+ T cells [4]. Viral proteins, VP8, bICP0 and bICP27, inhibit interferon dependent transcription [4–8]. Infection also erodes mucosal surfaces of the upper respiratory tract, which promotes establishment of the bacterial pathogen, *Mannheimia haemolytica (MH)* in the lower respiratory tract [9]. BoHV-1 productive infection increases neutrophil adhesion and activation [10], thus amplifying the pathogenic potential of *MH*. *MH*, a gram negative bacterium, exists as normal flora within the upper respiratory tract of healthy ruminants [11]. Stress and/or co-infections disrupt this commensal relationship; consequently *MH* becomes the predominant organism that causes life-threatening bronchopneumonia in BRDC cases [9]. BRDC is the most important disease in cattle because it costs the US cattle industry more than one billion dollars in losses each year [9, 12, 13]. A BoHV-1 entry protein is a BRDC susceptibility gene for Holstein calves [14] confirming BoHV-1 is a significant BRDC cofactor.

Like most alpha-herpesvirinae subfamily members, including human herpes simplex virus 1 (HSV-1) and HSV2, BoHV-1 initiates acute infection on mucosal surfaces [1–3]. High levels of infectious virus are produced; consequently BoHV-1, HSV-1, or HSV-2, spread to the peripheral nervous system via cell-to-cell spread. Latency is subsequently established in sensory neurons, but periodically reactivates from latency, and thus is widespread in cattle throughout the world. Reactivation of the virus from the latent state is initiated by external stimuli (e.g. stress and immunosuppression). During reactivation, viral gene expression is stimulated and infectious virus is produced and transported back to mucosal surfaces. The ability of alpha-herpesvirinae subfamily members to reactivate from the latent state is critical for virus transmission. Regulation of the complex virus host interactions controlling the latency-reactivation cycle is not well understood, which hinders developing therapeutic strategies that prevent reactivation from latency.

BoHV-1 is an excellent model to study these events because the natural host can be used and the synthetic corticosteroid dexamethasone (DEX) consistently initiates reactivation from latency in infected calves [2]. We have used experimentally infected calves treated with DEX to initiate reactivation from latency in order to identify virus-host interactions important for the latency-reactivation cycle. These studies identified host cellular factors and pathways that may be crucial for latency maintenance [15] and reactivation [16]. The following discussion focuses on the mechanisms by which BoHV-1 "escapes" a latent infection following a stressful stimulus and subsequently successfully reactivates from latency. Certain steps during BoHV-1 reactivation from latency are likely to be similar during reactivation of latency of other alpha-herpesvirinae subfamily members.

http://dx.doi.org/10.5772/intechopen.75451

2. Acute infection leads to a life-long latent infection in sensory neurons

Acute infection of calves induces programmed cell death, inflammation and high levels of virus production [1–3]. BoHV-1 genes are expressed in three distinct phases during acute infection or productive infection of cultured cells: immediate early (IE), early (E), or late (L) [1–3]. IE gene expression is specifically stimulated by viral protein 16 (VP16), a tegument protein. IE transcription unit 1 (IEtu1) encodes two transcriptional regulatory proteins, BoHV-1 infected cell protein 0 (bICP0) and bICP4, because a single IE transcript is differentially spliced and subsequently translated into bICP0 or bICP4 (**Figure 1**). The bICP0 protein is also translated from an E mRNA (E2.6) because a separate E promoter drives expression of the bICP0 E transcript.

During acute infection of calves, infectious virus particles enter the peripheral nervous system via cell–cell spread. If infection is initiated within the oral, nasal, or ocular cavity, the primary site for latency is sensory neurons located in trigeminal ganglia (TG) [1–3]. Viral gene expression and infectious virus are detected in TG from 2 to 6 days after infection. Lytic cycle viral gene expression is then extinguished, a significant number of infected neurons survive, and these neurons harbor viral genomes, which is operationally defined as the establishment of latency. Abundant expression of the BoHV-1 encoded latency related (LR) gene occurs in latently infected neurons, but infectious virus is not detected (maintenance of latency) [1–3]. LR-RNA is anti-sense to and overlaps the BoHV-1 infected cell protein 0 (bICP0) gene. The LR gene has two open reading frames (ORF1 and ORF2), and two reading frames lacking an initiating methionine (RF-B and RF-C). In addition, the LR gene encodes two micro-RNAs that interfere with bICP0 expression in transfected cells [17]. A LR mutant virus strain with three stop codons at the N-terminus of ORF2 exhibits diminished clinical symptoms, and reduced virus shedding from

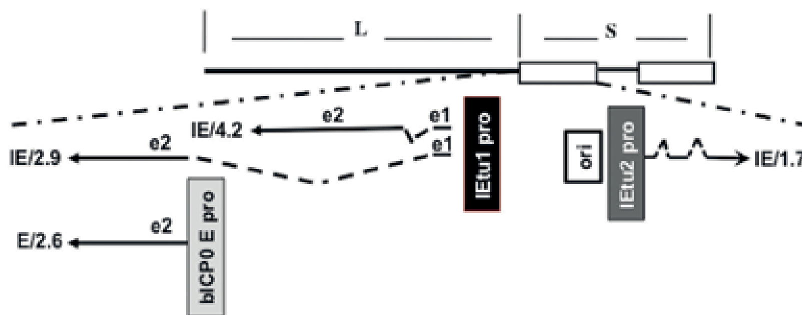

Figure 1. Location of IE transcripts and promoters actively expressed during productive infection. The mRNA IE/4.2 encodes the bICP4 protein and IE/2.9 encodes the bICP0 protein [58, 59, 72]. A single IE promoter activates expression of IE/4.2 and IE/2.9 and is designated IEtu1 (black rectangle). E/2.6 is the early transcript that encodes bICP0 and an early promoter activates expression of this transcript (bICP0 E pro; gray rectangle). All bICP0 protein-coding sequences are contained in Exon 2 (e2). The origin of replication (ORI) separates IEtu1 from IEtu2. The IEtu2 promoter (IEtu2 pro) regulates expression of the IE1.7 mRNA that is translated into the bICP22 protein. Solid lines in the transcript position map represent exons (e1, e2, or e3) and dashed lines denote introns.

the eye, TG, or tonsils of infected calves [1–3]. ORF1, ORF2, and RF-C are expressed when bovine cells are infected with wild-type or the LR-rescued virus, but these proteins have reduced or no expression following infection with the LR mutant virus [1–3]. Wild-type (wt) BoHV-1, but not the LR mutant virus, reactivates from latency after treatment with the synthetic corticosteroid DEX. The anti-apoptosis activity of ORF2 is predicted to increase the survival of infected neurons and thus would be important for the latency-reactivation cycle [1–3].

Recent studies demonstrated that during latency, the canonical Wnt/β-catenin signaling is active and ORF2 appears to be important for maintaining this important signaling pathway [15, 18]. Although dysregulation of the Wnt/β-catenin signaling is frequently associated with many types of cancer [19, 20], this signaling pathway has the potential to promote the establishment and maintenance of latency in sensory neurons because it enhances cell survival, axonal growth, and directs axons to their proper synaptic targets [21–25].

3. Stress-induced reactivation from latency

Increased corticosteroid levels, due to increased stress, correlates with increasing the incidence of BoHV-1 reactivation from latency [1–3]. DEX can also stimulate productive infection [26], and initiate reactivation from latency in calves or rabbits latently infected with BoHV-1 [1–3]. Six hours after DEX treatment lytic cycle viral RNA expression is detected in neurons of latently infected calves [27, 28]. Certain lytic cycle viral proteins, bICP0 and VP16 for example, are readily detected in TG neurons within hours after DEX treatment [29, 30]. The glucocorticoid receptor (GR) and mineralocorticoid receptor (MR), which are present in subpopulations of sensory neurons [31, 32], are activated by interacting with corticosteroids. The GR is frequently detected in TG neurons that express bICP0 or VP16 [31, 32]. IEtu1 promoter activity is stimulated by the GR and the synthetic corticosteroid DEX because there are two consensus GREs in the promoter [26] suggesting this promoter is activated by the GR and/or MR following stressful stimuli. Since the IEtu1 promoter drives expression of two viral transcriptional regulatory proteins (bICP0 and bICP4; **Figure 1**), activation of this promoter may stimulate productive infection in certain latently infected neurons.

DEX treatment of latently infected calves induces apoptosis of T cells that persist in TG after infection [27]. T cells also persist in TG of humans or mice latently infected with HSV-1 and may promote maintenance of latency [33–37]. Within 3 h after DEX treatment, 11 cellular genes are induced more than ten fold in TG [16]. Pentraxin 3, a regulator of innate immunity and neurodegeneration, is stimulated 35–63 fold at 3 or 6 h after DEX treatment. Furthermore, expression of a soluble Wnt antagonist, Dickkopf-1 is induced more than 10 fold [15, 16]. Dickkopf-1 is responsible for stress-induced neuronal death [38, 39] indicating there is a correlation between disrupting the Wnt signaling pathway and activation of lytic cycle viral gene expression during reactivation. Two transcription factors, promyelocytic leukemia zinc finger (PLZF) and Slug are induced more than 15-fold 3 h after DEX treatment. PLZF or Slug stimulates BoHV-1 productive infection 20-fold or 5-fold respectively, and Slug stimulates the late glycoprotein C promoter more than 10-fold. Additional DEX induced transcription factors, SPDEF (Sam-pointed domain containing Ets transcription factor), Kruppel-like transcription factor 15 (KLF15), KLF4, KLF6, and GATA6, stimulate productive infection and certain key viral promoters.

The finding that four KLF family members (KLF4, KLF6, KLF15, and PLZF) are stimulated during DEX induced reactivation from latency is intriguing because KLF family members resemble the SP1 transcription factor family and both family of transcription factors interact with guanine-cytosine (GC) rich motifs, reviewed in [40, 41]. Genomes of alpha-herpesvirinae subfamily members, including BoHV-1, are GC rich and many viral promoters contain Sp1 consensus binding sites as well as other GC rich motifs [40]. In fact, HSV-1 gene expression is activated by Sp1 [42]. HSV-1 and probably BoHV-1 genomes exist as silent chromatin during latency, [43]: however, HSV-1 DNA is associated with unstable chromatin during productive infection [44–46]. Regardless of the stimulus that initiates reactivation from latency, silent viral heterochromatin must be converted into an actively transcribing template for reactivation from latency to be successful suggesting cellular transcription factors initially stimulate lytic cycle viral gene expression.

To test whether the GR and certain stress-induced transcription factors can cooperate to stimulate viral transcription, the IEtu1 promoter and BoHV-1 DNA fragments (less than 400 bp) containing potential GR and KLF binding sites were identified and examined for transcriptional activation by stress-induced transcription factors. The rational for testing intergenic regions of the BoHV-1 genome is the viral genome contains more than 100 putative GRE binding sites [26] and a subset of GREs in cellular chromatin can activate transcription from greater than 5 kb to the nearest promoter [47]. KLF15 cooperated with the GR to stimulate the IEtu1 promoter activity and productive infection [48]. Furthermore, intergenic regions within the unique long 52 gene (UL-52; component of DNA primase/helicase complex), bICP4, IEtu2 that expresses the regulatory protein (bICP22), and unique short region were stimulated by KLF15 and the GR. In contrast to KLF15, the other stress-induced transcription factors only have a modest effect on IEtu1 promoter activity. The GR and KLF15 interact with sequences within wild-type IEtu1 GREs and UL-52 fragment, but not GRE mutants. Co-immunoprecipitation studies indicated that KLF15 and the GR are stably associated with each other. Interestingly, the GR and KLF15 can synergistically regulate gene expression by a feed-forward transcription loop [49–51]. Hallmarks of a feed-forward loop are a primary factor (GR in this example) induces expression of a second factor, KLF15 [16, 49–54], and the two factors synergistically activate expression of genes in a specific pathway. Adipogenesis [55] and amino acid metabolizing enzymes are also synergistically regulated by the GR and KLF15 [50, 51]. In summary, these studies suggest that activation of BoHV-1 gene expression during DEX induced reactivation from latency is, in part, regulated by a feed-forward transcription loop containing the GR and KLF15.

4. The GR and KLF6 cooperate to stimulate productive infection

To test whether KLF6 and the activated GR have a cooperative effect on productive infection, a mouse neuroblastoma cell line (Neuro-2A) was cotransfected with gCblue genomic DNA and KLF6 and/or the GR. The gCblue virus grows to similar titers as the wt parental virus and expresses the Lac Z gene from the gC locus during productive infection (**Figure 2A**). Neuro-2A cells were used for these studies because they have neuronal like properties [56], can be readily transfected, and are semi-permissive for BoHV-1 [57]. Neuro-2A cells were transfected with gCblue DNA instead of infecting cells because VP16 and other viral regulatory proteins in the virion particle can diminish the stimulatory effects of DEX on productive infection (data not shown). KLF6 and the GR plus DEX treatment increased the number of β-Gal+ Neuro-2A cells

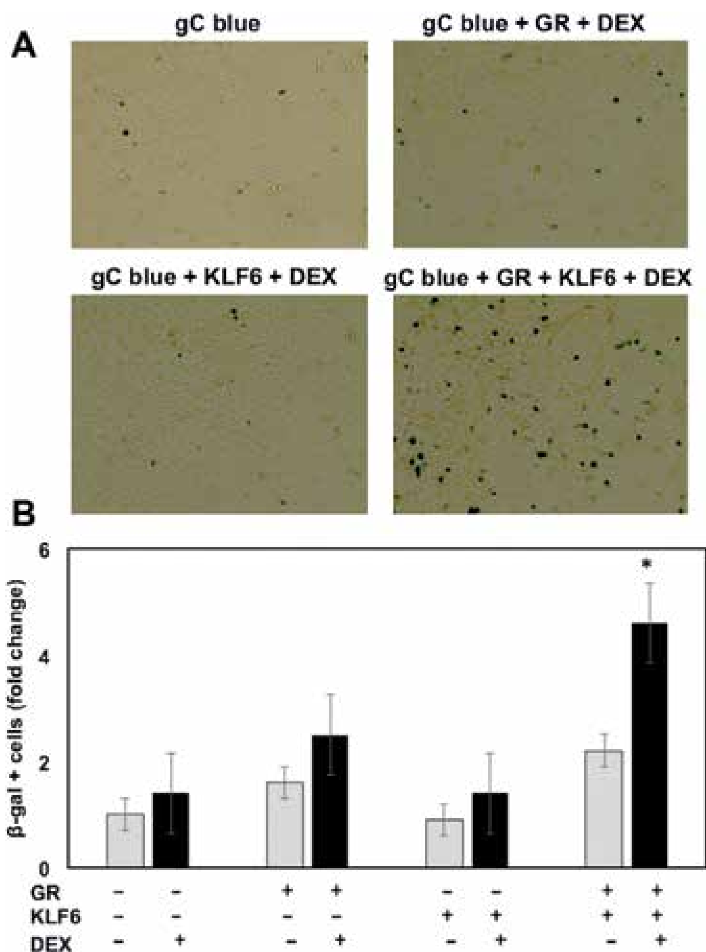

Figure 2. KLF6 and the GR cooperate to stimulate productive infection. Neuro-2A cells were transfected with 2 ug BoHV-1 gCblue genomic DNA and where indicated a plasmid that expresses the mouse GR protein (1.0 ug DNA) and/or KLF6 (0.5 ug DNA) using Lipofectamine 3000 (catalog no. L3000075; Invitrogen). A mouse GR expression vector was obtained from Dr. Joseph Cidlowski, NIH and the KLF6 expression vector was obtained from Bin Guo (North Dakota State University). Neuro-2A cells were grown in Eagle's minimal essential medium (EMEM) supplemented with 10% FCS, penicillin (10 U/ml), and streptomycin (100 μg/ml). The BoHV-1 mutant containing the β-Gal gene in place of the viral gC gene was obtained from S. Chowdury (LSU School of Veterinary Medicine) (gCblue virus) and stocks of this virus grown in bovine kidney cells (CRIB). The gCblue virus grows to similar titers as the wt parental virus and expresses the Lac Z gene. Procedures for preparing genomic DNA were described previously [73]. To maintain the same amount of DNA in each sample, empty vector was included in samples. Cells were incubated with stripped fetal calf serum 24 h after transfection and then treated with water soluble DEX (10 μM; Sigma, D2915). At 40 h after transfection, cells were fixed and stained for counting the number of β-Gal+ cells as previously described [48]. Representative cultures stained for Lac Z expression are shown in (**Panel A**). The value for the control (gCblue virus DNA treated with PBS after transfection) was set at 1. The results from DEX treated cultures were compared to the control and are an average of three independent studies (**Panel B**). The asterisk denotes a significant difference between the control and samples transfected with the GR or KLF6 and treated with DEX ($P < 0.05$) using the student's T test.

more than 4-fold, which was significantly higher than GR + DEX and the GR or KLF6 alone (**Figure 2A** and **B**). Cotransfection of gCblue and the GR + KLF6 stimulated productive infection 2-fold even when cultures were not treated with DEX, which was similar to the effects observed when gCblue genomic DNA was cotransfected with the GR and DEX treatment.

5. KLF6 and GR synergistically trans-activates the IEtu1 promoter

Transient transfection studies were performed in Neuro-2A cells to test whether KLF6 and the GR synergistically trans-activate the IEtu1 promoter because this promoter contains two consensus GR binding sites (**Figure 3A**) required for DEX mediated transactivation [26]. The

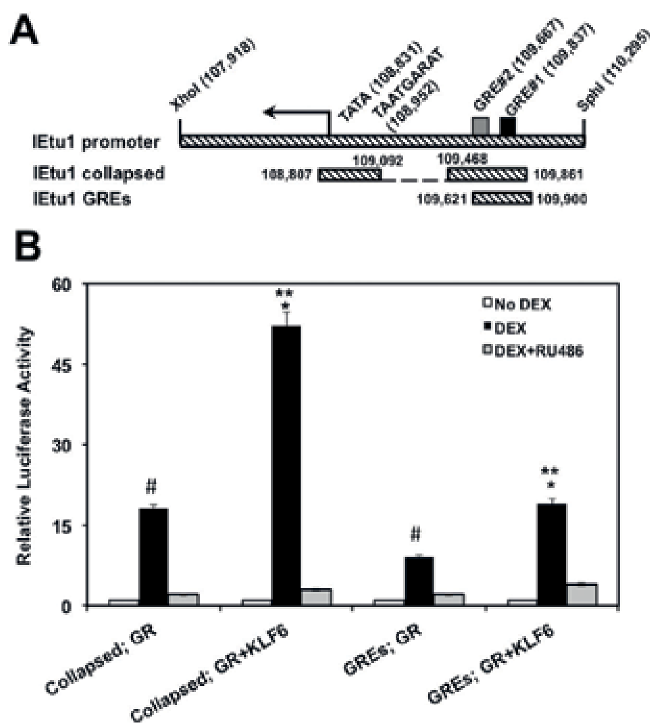

Figure 3. KLF6 and the GR cooperatively transactivate the IEtu1 promoter. **Panel A:** The full length IEtu1 promoter was cloned as an XhoI-SphI restriction site. Start site of transcription (arrow), TATA box, binding site for VP16/Oct1 complex is denoted as TAATGARAT [74], and location of GRE#1 and GRE#2 (black and grey rectangles) are shown. Numbers are genomic coordinates of the first nucleotide of each respective motif or restriction enzyme site. GenScript synthesized the IEtu1 collapsed promoter construct and genomic coordinates are included: this fragment is inserted at KpnI and HindIII restriction sites of pGL3-Basic Vector. A 280 bp fragment (IEtu1 GREs) was cloned into the pGL3-Promoter Vector at unique KpnI and XhoI restriction sites [48]. **Panel B:** Neuro-2A cells were transfected with 0.5 ug DNA of the IEtu1 collapsed promoter (Collapsed) or IEtu1 GREs plasmid (GREs) and where indicated a plasmid that expresses the mouse GR protein (1.0 ug DNA) and/or KLF6 (0.5 ug DNA). To maintain equal plasmid amounts in the transfection mixtures, the empty expression vector was added as needed. Designated cultures were treated with water-soluble DEX (10 uM; Sigma) or DEX + RU486 (10 uM; Sigma) at 24 h after transfection. At 48 h after transfection, cells were harvested, and protein lysate subjected to dual-luciferase assay using a commercially available kit (E1910; Promega). Luminescence was measured by using a GloMax 20/20 luminometer (E5331; Promega). All transfections contained a plasmid encoding *Renilla* luciferase under the control of a minimal herpesvirus thymidine kinase (TK) promoter (0.050 ug DNA) as an internal control. Promoter activity in the empty luciferase vector (pGL3-Promoter Vector) was normalized to a value of 1 and fold activation for other samples presented. The results are the average of three independent experiments and error bars denote the standard error. A single asterisk denotes a significant difference ($P < 0.05$) between the IEtu1 collapsed or IEtu1 GREs when cotransfected with GR and KLF6 plus DEX treatment when compared to promoter activity of the respective promoter construct cotransfected with GR plus DEX treatment. Two asterisks denote a significant difference ($P < 0.05$) between the IEtu1 collapsed or IEtu1 GREs when cotransfected with GR and KLF6 and DEX treatment versus the same study conducted but treated with DEX+ RU486 or no DEX. A (#) denotes a significant difference between IEtu1 collapsed or IEtu1 GREs cotransfected with the GR and treated with DEX when compared to the same luciferase reporter cotransfected with GR and treated with DEX+ RU486 or no DEX. Statistical analysis was performed using the Student *t* test.

IEtu1 promoter drives IE expression of bICP0 and bICP4, the most important viral transcriptional regulatory proteins encoded by BoHV-1 [58–60] (**Figure 1**). The IEtu1 collapsed promoter construct (**Figure 3A**) was initially used to test whether sequences adjacent to the GREs were trans-activated by KLF6 and the GR. The full-length IEtu1 promoter construct contains extensive sequences downstream from the start site of transcription and has sequences between the TATA box and the GREs that are important for KLF trans-activation [16]: consequently the collapsed IEtu1 collapsed promoter construct was used for these studies. We have consistently found that the GR+ DEX stimulated promoter activity more than 15 fold and GR + KLF6 + DEX stimulated promoter activity more than 50 fold (**Figure 3B**). RU486 antagonizes corticosteroid/GR signaling [61, 62] and as expected RU486 significantly reduced the ability of KLF6 and GR to transactivate the IEtu1 collapsed construct.

A 280 bp fragment containing both GREs within the IEtu1 promoter and flanking sequences was cloned upstream of the minimal SV40 early promoter and designated IEtu1 GREs [48] (**Figure 3A**). This construct was examined for its ability to be activated by KLF6 and the GRE as a comparison to the IEtu1 collapsed promoter construct. KLF6 and the GR consistently stimulated the IEtu1 GREs construct approximately 16-fold whereas the GR + DEX stimulated this construct only 6-fold (**Figure 3B**). RU486 also significantly reduced the ability of KLF6 and GR to transactivate the IEtu1 GREs construct. Although the IEtu1 collapsed construct was trans-activated more by the GR + KLF6+ DEX relative to the IEtu1 GREs construct, the overall trends were similar.

6. The GREs are necessary for transactivation by the GR and KLF6

To identify sequences in the IEtu1 GREs that mediate transactivation by KLF6 and the GR, constructs containing site-specific mutations in GRE#1, GRE#2, and KLF like binding sites were compared to the wt IEtu1 GREs (**Figure 4A–C**) [48]. Mutagenesis of GRE1 (ΔGRE1) or both GREs and the KLF binding sites (Δ2xGREΔKLF) significantly reduced cooperative activation by KLF6 and the GR when DEX was added to the cultures (**Figure 4D**). Mutagenesis of the 2 putative KLF sites (ΔKLF) had no effect on trans-activation by KLF6 and the GR when cultures were treated with DEX. As previously reported [48] and shown in **Figure 4D**, the effect of DEX and the GR was significantly reduced when GRE#1 (ΔGRE1) was mutated and abolished when both GREs and putative KLF sites (Δ2xGREΔKLF) were mutated. In summary, mutagenesis of the GRE#1 significantly reduced synergistic transactivation by KLF6 and the GR when cultures were treated with DEX.

7. KLF6 and the GR interact with sequences located in the IEtu1 GREs

To test whether KLF6 and the GR interact with sequences located in the IEtu1 GREs, chromatin immuno-precipitation (ChIP) studies were performed in Neuro-2A cells. Cells were transfected with the promoter construct containing the IEtu1 GREs followed by treatment with Vehicle

or DEX. As shown in **Figure 5A**, ChIP studies demonstrated that the GR and KLF6 occupied the GRE region of the IEtu1 GREs (lanes 2–4). No specific PCR product was amplified from ChIPs of cells transfected with the IEtu1 GREs from IPs using the control IgG (IgG C Panel) or cells transfected with the Δ2XGREΔKLF construct (**Figure 5C** and **D**). Treatment with DEX had little effect on the levels of GR bound to IEtu1 GRE sequences (**Figure 5A** and **B**); however, we detected an increase in KLF6 bound to the IEtu1 GREs when cotransfected with KLF6 and GR in the absence of DEX when compared to DEX treatment. At least three reasons may have led to this unexpected result. First, we suggest that low levels of corticosteroids in media containing

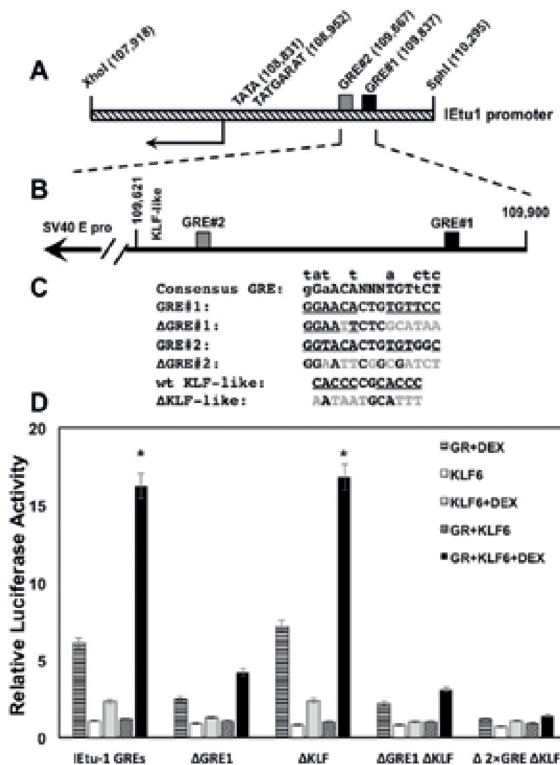

Figure 4. Identification of sequences in the IEtu1 GREs that are responsive to KLF6 and the GR. **Panel A:** Schematic of IEtu1 promoter and location of TATA box, TAATGARAT motif, and the two GREs. Numbers denote the genomic location of the first nucleotide of each motif. **Panel B:** Schematic of 280 bp fragment that contains the IEtu1 GREs and putative KLF-binding sites. **Panel C:** Nucleotide sequence of motifs in the IEtu1 GREs and mutations that were prepared. Mutations in GRE#1 and GRE#2 were previously described and were shown to disrupt trans-activation by the GR in transient transfection studies [26, 48]. **Panel D:** Neuro-2A cells were transfected with the designated luciferase plasmid (0.5 ug DNA) and where indicated a plasmid that expresses the mouse GR protein (1.0 ug DNA) and/or KLF6 (0.5 ug DNA). To maintain the same amount of DNA in each sample, empty vector was included in certain samples. Cultures were then treated with 2% "stripped" fetal calf serum and then water soluble DEX (10 uM; Sigma) at 24 h after transfection. At 48 h after transfection, cells were harvested, and protein lysate was subjected to dual-luciferase assay as described in **Figure 3B**. The level of promoter activity in the empty luciferase vector (pGL3-Promoter Vector) was normalized to a value of 1 and the fold activation values for other samples are presented. The results are the average of three independent experiments and error bars denote the standard error. The asterisks denote a significant difference ($P < 0.05$) between IEtu1 GREs (wt) and the ΔKLF mutant when compared to the other mutants (ΔGRE1, ΔGRE1ΔKLF and Δ2XGREΔKLF) after cotransfection with GR + KLF6 and treated with DEX, as determined by the Student *t* test.

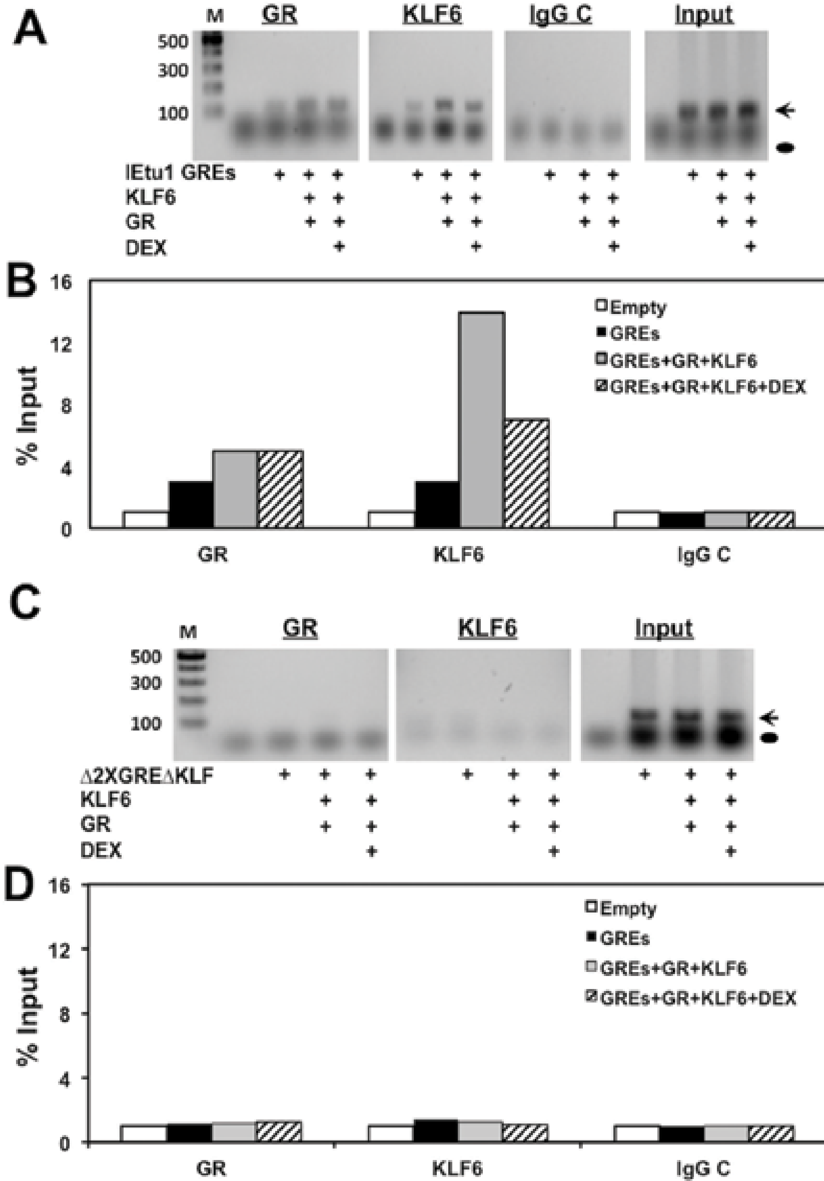

Figure 5. Interaction between GR and KLF6 with IEtu1 GREs. Neuro-2A cells were cotransfected with the IEtu1 GREs construct (**Panel A**; 4 ug DNA) or Δ2XGREΔKLF fragment (**Panel C**; 4 ug DNA), KLF6 expression plasmid (1.5 ug DNA) and/or the GR plasmid (2 ug DNA). Empty vector was added to maintain the same concentration of DNA in each transfection assay. Designated cultures were treated with DEX (10 uM; Sigma) 4 h before cells were harvested. ChIP studies were performed as previously described in Neuro-2A cells [48]. Polymerase chain reaction (PCR) was performed using primers that amplify the IEtu1 GREs and Δ2XGREΔKLF: forward primer is 5′- CCCACTTTTGCCTGTGTG-3′ and reverse primer is 5′-TTTTCCTCCTCCTTCCCC-3′. These primers yield a product of 107 base pairs. Input was 10% of the total DNA: protein complexes that used for IP and then PCR performed using PCR primers described in the materials and methods. Arrows denote the specific PCR product, 107 bp for IEtu1 GREs or for Δ2XGREΔKLF, and the circle denotes the position of primer dimers. Estimation of the level of binding to wild-type IEtu1 GREs sequences (**Panel B**) or Δ2XGREΔKLF (**Panel D**) is shown. The results are representative of three independent studies.

http://dx.doi.org/10.5772/intechopen.75451

2% stripped fetal bovine serum may be a reason why the GR was associated with the IEtu1 GREs in the absence of DEX. Secondly, independent studies concluded that the GR can be associated with GREs in the absence of corticosteroids [61, 63]. Thirdly, treatment of cells with DEX reduces GR levels and the availability of GR to bind to DNA [26, 64]. All input samples (whole lysate prior to IP) yielded the specific 107 bp PCR product except Neuro-2A cells not transfected with the IEtu1 GREs construct (**Figure 5A**, Input panel, lane 1). In summary, the GR and KLF6 were specifically recruited to IEtu1 GRE sequences when the GREs were intact.

8. The GR does not stably interact with KLF6

Co-immunoprecipitation (co-IP) studies were used to test whether GR and KLF6 physically interact. Neuro-2A cells were cotransfected with plasmids that express KLF6 and the GR. Following IP with the GR antibody, we were unable to detect KLF6 in the immunoprecipitate regardless of DEX treatment (**Figure 6**). As expected, both proteins were detected in whole cell lysate (input). Furthermore, the GR was detected in the immunoprecipitate after IP was performed with the GR antibody. When KLF6 was used to IP whole cell lysate, the GR was not detected in the immunoprecipitate (data not shown). The same experimental conditions revealed that KLF15 and the GR were stably associated in transfected Neuro-2A cells [48]. In summary, co-IP studies suggested KLF6 and the GR were not stably associated with each other.

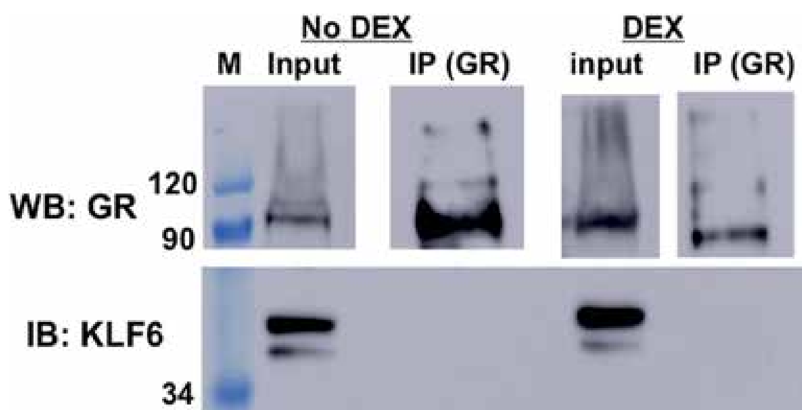

Figure 6. The GR does not physically interact with KLF6. Neuro-2A cells were grown to confluence on 100 mm dishes. Cells were cotransfected with plasmids that express KLF6 (1.5 μg) and the GR (2 μg). Cultures were treated with DEX (10 μM) in 2% stripped serum medium for 4 h before harvesting of transfected cells and other cultures were not treated DEX. Whole cell lysate was prepared with RIPA lysis buffer with 1× Protease Inhibitor cocktail (Thermo-scientific, cat. No: 78430) and protein concentration quantified. Protein extracts (500 μg) were combined with anti-GR (Cell Signaling; 3660) and /or anti-KLF6 (5 μg) antibodies (Thermo Fisher Scientific, 39–6900) and reactions were incubated for overnight at 4°C on rotator. Co-IP and Western blot studies were performed as described previously [48]. The secondary donkey anti-rabbit antibody (NA9340V) was purchased from GE Healthcare and secondary sheep anti-mouse antibody was purchased from GE Healthcare. Following immunoprecipitation with the GR antibody, KLF6 was not detected in the immune-precipitate by western blotting in samples treated with or without DEX. Input lanes are (whole cell lysate) used as positive controls for expression of the both proteins. Molecular weight markers (lane M) are shown to the left of the panels.

9. Discussion and summary

In this study, we provided evidence that KLF6 and the GR synergistically stimulate productive infection and IEtu1 promoter activity. The IEtu1 promoter must be activated for productive infection because it encodes two viral transcriptional regulators, bICP0 and bICP4 (**Figure 7A**)

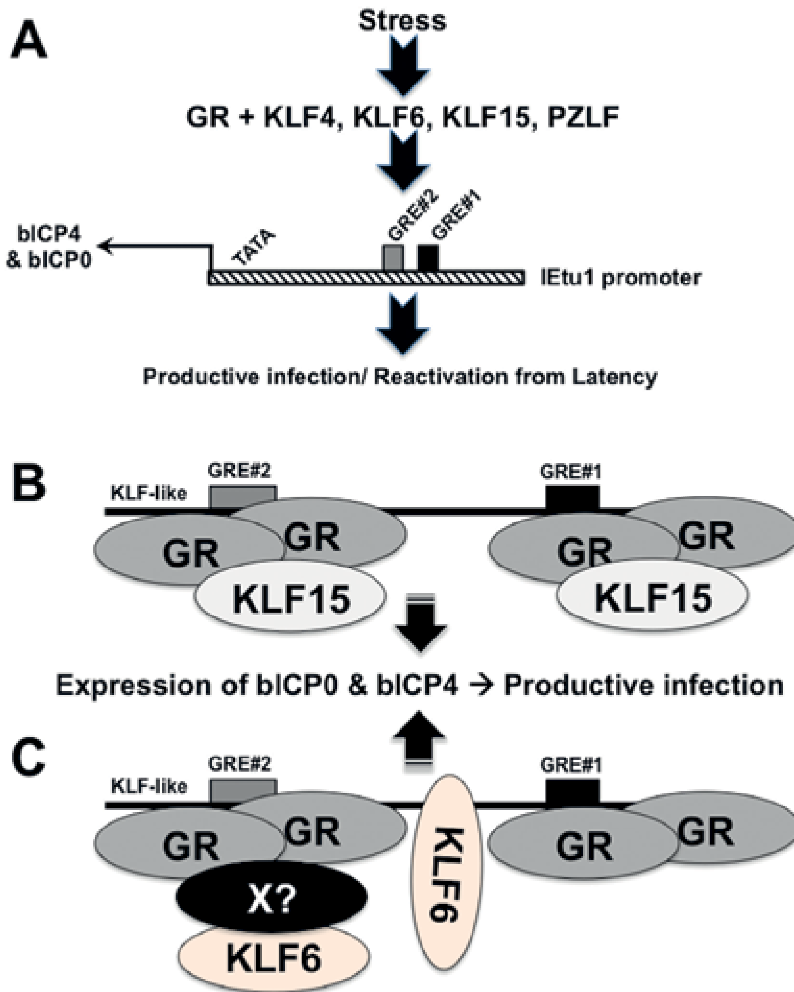

Figure 7. The GR and certain KLF family members stimulate BoHV-1 replication and IEtu1 promoter activity. **Panel A:** Stress activates the GR, which in turn stimulates expression of four stress-induced KLF family members in TG neurons [16]. Recent studies demonstrated that stress, as mimicked by DEX plus the GR, activates IEtu1 promoter activity because two GREs are located in the promoter [26]. KLF6 and KLF15 cooperate with the GR to activate IEtu1 promoter activity. Stress mediated activation of the IEtu1 promoter is crucial for productive infection because this promoter drives expression of two viral regulatory proteins (bIC0 and bICP4). **Panel B:** KLF15 stably interacts with the GR: consequently, this complex synergistically stimulates IEtu1 promoter activity by binding to the GREs [48]. **Panel C:** KLF6 and the GR cooperate to stimulate expression of IEtu1 promoter activity and productive infection. In contrast, to KLF15, KLF6 did not stably interact with the GR. Consequently, we propose that a KLF6 indirectly interacts with the GR via an unknown GR coactivator (X) or binding of the GR to a GRE promotes KLF6 interactions with sequences between GRE#1 and GRE#2. This schematic does not suggest that the interactions occur at independent GREs within the IEtu1 promoter; it merely suggests that these are the two likely mechanisms by which KLF6 cooperates with the GR to stimulate IEtu1 promoter activity.

[2]. During reactivation from latency, stress, as mimicked by the synthetic corticosteroid DEX, activates the GR and induces expression of several KLF family members (KLF4, KLF6, KLF15, and PLZF) [16]. A previous study demonstrated that KLF15, but not KLF4, and the GR synergistically stimulate IEtu1 promoter activity [48]. In contrast to KLF6, KLF15 stably interacts with the GR to establish a feed-forward transcriptional loop [48, 51, 53, 65, 66] (**Figure 7B**). Although KLF6 and KLF15 can both positively regulate promoter activity, they also can repress transcription in a promoter-specific manner [67, 68]. One study concluded there is a synergistic effect between the GR and transcriptional factors that recognize CACCC motifs [69], a known KLF6 binding site [70, 71]. There are no CACCC motifs on the positive strand of the IEtu1 GREs fragment; however, there are 2 CACCC motifs on the negative strand (KLF-1 like; **Figure 7B**). When these motifs were mutated (ΔKLF mutant), there was no difference in KLF6 and GR mediated trans-activation suggesting there may be KLF binding sites located between GRE#2 and GRE#1.

Relative to GRE#2, mutating GRE#1 was more important for GR mediated trans-activation [26, 48]. To ablate DEX induction of the IEtu1 promoter or the IEtu1 GREs, both GREs must be mutated [26, 48]. This is consistent with the results demonstrating there are cooperative effects between KLF15 [48] or KLF6 and the GR. ChIP results demonstrated that mutagenesis of both GREs interfered with KLF6 binding to sequences spanning the IEtu1 GREs, suggesting: 1) an unknown GR or KLF6 coactivator functions as a bridge between the GR and KLF6, which allows interactions between these two transcription factors (**Figure 7C**; left scenario at GRE#2), or 2) GR interactions with GRE#1 and/or GRE#2 influence adjacent sequences that are necessary for KLF6 to bind DNA **Figure 7B**; right scenario at GRE#1). Since KLF family members can bind to several GC or CA rich motifs, it is difficult to predict which sequences adjacent to GRE#1 or GRE#2 are important for interacting with KLF6 and/or KLF15.

The BoHV-1 genome contains approximately 100 putative GREs [26]. We identified 13 intergenic regions in the viral genome that contain at least 2 putative GREs and potential KLF binding sites within 400 base pairs. KLF15 and the GR significantly transactivate fragments present in unique long (UL)-52, bICP4, IEtu2, and Us fragments when DEX was added to cultures [48]. In contrast, KLF6 and the GR were unable to transactivate these intergenic fragments in the presence or absence of DEX (data not shown) confirming KLF15 has novel properties relative to KLF6.

KLF4, KLF6, and KLF15 expression are induced in TG neurons of calves that are latently infected with BoHV-1 during early stages of DEX induced reactivation from latency [16]. Cellular, not viral encoded, transcription factors are predicted to be crucial for initiating viral transcription during initial stages of reactivation from latency because lytic cycle viral gene expression is not readily detected in TG of latently infected calves [29, 30]. Thus, activation of the IEtu1 promoter by the GR and DEX-induced transcription factors, KLF6 and KLF15 for example, may be sufficient to trigger lytic cycle viral gene expression in a subset of latently infected neurons following a stressful stimulus, as shown in **Figure 7B** and **C**.

Acknowledgements

This research was supported by grants from the USDA-NIFA Competitive Grants Program (13-01041 and 16-09370), funds derived from the Sitlington Endowment, and support from the Oklahoma Center for Respiratory and Infectious Diseases (National Institutes of Health

Centers for Biomedical Research Excellence Grant # P20GM103648). Research reported in this publication was also partially supported by the National Institute Of Neurological Disorders And Stroke of the National Institutes of Health under Award Number R21NS102290. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Fouad S. El-mayet was supported by a fellowship from the Egyptian Ministry of Higher Education, Mission Sector (JS-3541).

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References

[1] Jones C. Bovine herpes virus 1 (BHV-1) and herpes simplex virus type 1 (HSV-1) promote survival of latently infected sensory neurons, in part by inhibiting apoptosis. Journal of Cell Death. 2013;**6**:1-16

[2] Jones C. Reactivation from latency by alpha-herpesvirinae submfamily members: A stressful situation. Current Topics in Virology. 2014;**12**:99-118

[3] Jones, C. Latency of bovine Herpesvirus 1 (BoHV-1) in Sensory Neurons, Herpesviridae. In: Joseph O, editor. Rijeka: InTech; 2016. DOI: 10.5772/63750

[4] Jones C. Regulation of innate immune responses by bovine herpesvirus 1 and infected cell protein 0. Virus. 2009;**1**:255-275

[5] Henderson G, Zhang Y, Jones C. The bovine herpesvirus 1 gene encoding infected cell protein 0 (bICP0) can inhibit interferon-dependent transcription in the absence of other viral genes. The Journal of General Virology. 2005;**86**:2697-2702

[6] Saira K, Zhou Y, Jones C. The infected cell protein 0 encoded by bovine herpesvirus 1 (bICP0) induces degradation of interferon response factor 3 (IRF3), and consequently inhibits beta interferon promoter activity. Journal of Virology. 2007;**81**:3077-3086

[7] Saira K, Jones C. The infected cell protein 0 encoded by bovine herpesvirus 1 (bICP0) associates with interferon regulatory factor 7 (IRF7), and consequently inhibits beta interferon promoter activity. Journal of Virology. 2009;**83**:3977-3981

[8] Afroz S, Brownlie R, Fodjec M, van Drunen Littel-van den Hurk S. VP8, the major tegument protein of bovine herpesvirus 1, interacts with cellular STAT1 and inhibits interferon beta signaling. Journal of Virology. 2016;**90**:4889-4904

[9] Highlander SK. Molecular genetic analysis of virulence in *Mannheimia (Pasteurella) haemolytica*. Frontiers in Bioscience. 2001:D1128-D1150

[10] Rivera-Rivas JJ, Kisiela D, Czuprynski CJ. Bovine herpesvirus type 1 infection of bovine bronchial epithelial cells increases neutrophil adhesion and activation. Veterinary Immunology and Immunopathology. 2009;**131**:167-176

[11] Frank GH, editor. Bacteria as etiologic agents in bovine respiratory disease. College Station, TX: Texas A&M University Press; 1984

[12] Hodgson PD, Aich P, Manuja A, Hokamp K, Roche FM, Brinkman FSL, Potter A, Babiuk LA, Griebel PJ. Effect of stress on viral-bacterial synergy in bovine respiratoryt disease: novel mechanisms to regulate inflammation. Comparative and Functional Genomics. 2005;**6**: 244-250

[13] Jones C, Chowdhury S. Bovine herpesvirus type 1 (BHV-1) is an important cofactor in the bovine respiratory disease complex. In: Broderson VLCB, editor. Veterinary Clinics of North America, Food Animal Practice, Bovine Respiratory Disease, vol 26. New York, NY: Elsevier; 2010. pp. 303-321

[14] Neibergs HL, Seabury CM, Wojtowicz AJ, Wang Z, Scraggs E, Kiser JN, Neupane M, Womack JE, Van Eenennaam A, Hagevortm GR, Lehenbauer TW, Aly S, Davis J, Taylor JF, the Bovine Respiratory Disease Complex Coordinatefd Agricultural Research Team. Susceptibility loci revealed for bovine respiratory disease complex in pre-weaned holstein calves. BMC Genomics.2014;**15:**1-19

[15] Liu Y, Hancock M, Workman A, Doster A, Jones C. Beta-catenin, a transcription factor activated by canonical Wnt signaling, is expressed in sensory neurons of calves latently infected with bovine herpesvirus 1. Journal of Virology. 2016;**90**:3148-3159

[16] Workman A, Eudy J, Smith L, Frizzo da Silva L, Sinani D, Bricker H, Cook E, Doster A, Jones C. Cellular transcription factors induced in trigeminal ganglia during dexamethasone-induced reactivation from latency stimulate bovine herpesvirus 1 productive infection and certain viral promoters. Journal of Virology. 2012;**86**:2459-2473

[17] Jaber T, Workman A, Jones C. Small noncoding RNAs encoded within the bovine herpesvirus 1 latency-related gene can reduce steady-state levels of infected cell protein 0 (bICP0). Journal of Virology. 2010;**84**:6297-6307

[18] Zhu L, Workman A, Jones C. A potential role for a beta-catenin coactivator (high mobility group AT-hook 1 protein) during the latency-reactivation cycle of bovine herpesvirus 1. Journal of Virology. 2017;**91**:e02132-e20136

[19] Clevers H, Nusse R. Wnt/B-catenin signaling and disease. Cell. 2012;**149**:1192-1205

[20] Polakis P. Wnt signaling in cancer. In: Nusse R, He X, van Amerongen R, editor. Cold Spring Harbor Perspectives in Biology. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2012

[21] Salinas PC. Wnt signaling in the vertebrate central nervous system: From axon guidance to synaptic function. Cold Spring Harbor Perspectives in Biology. 2012;**4**:a008003

[22] Purro SA, Galli S, Salinas PC. Dysfunction of Wnt signaling and synaptic disassembly in neurodegenerative diseases. Journal of Molecular Cell Biology. 2014;**6**:75-80

[23] Bhardwaji D, Nager M, Camats J, David M, Benguira A, dopazo A, Canti C, Herreros J. Chemokines induce axon outgrowth downstream of hepatocyte growth factor and TCF/beta-catenin signaling. Frontiers in Cellular Neuroscience. 2013;**7**:1-10

[24] Murase S, Mosser E, Schuman EM. Depolarization drives beta-catenin into neuronal spines promoting changes in synaptic structure and function. Neuron. 2002;**35**:91-105

[25] Bamji SX, Rico B, Kimes N, Reichardt LF. BDNF mobilizes synaptic vesicles and enhances synaptic vesicles and enhances synapse formation by disrupting cadherin-beta-catenin interactions. The Journal of Cell Biology. 2006;**174**:289-299

[26] Kook ICH, Meyer F, Hoffmann F, Jones C. Bovine herpesvirus 1 productive infection and the immediate early transcription unit 1 are stimulated by the synthetic corticosteroid dexamethasone. Virology. 2015;**484**:377-385

[27] Winkler MT, Doster A, Sur JH, Jones C. Analysis of bovine trigeminal ganglia following infection with bovine herpesvirus 1. Veterinary Microbiology. 2002;**86**:139-155

[28] Winkler MT, Doster A, Jones C. Persistence and reactivation of bovine herpesvirus 1 in the tonsil of latently infected calves. Journal of Virology. 2000;**74**:5337-5346

[29] Frizzo da Silva L, Kook I, Doster A, Jones C. Bovine herpesvirus 1 regulatory proteins, bICP0 and VP16, are readily detected in trigeminal ganglionic neurons expressing the glucocorticoid receptor during the early stages of reactivation from latency. Journal of Virology. 2013;**87**:11214-11222

[30] Kook I, Doster A, Jones C. Bovine herpesvirus 1 regulatory proteins are detected in trigeminal ganglionic neurons during the early stages of stress-induced escape from latency. Journal of Neurovirology. 2015;**21**:585-591

[31] DeLeon M, Covenas R, Chadi G, Narvaez JA, Fuxe K, Cintra A. Subpopulations of primary sensory neurons show coexistence of neuropeptides and glucocorticoid receptors in the rat spinal and trigeminal gnaglia. Brain Research. 1994;**14**:338-342

[32] Arriza JL, Simerly RB, Swanson LW, Evans RM. The neuronal mieralocorticoid receptor as a mediator of glucocorticoid response. Neuron. 1988;**1**:887-900

[33] Liu T, Khanna KM, Chen X, Fink DJ, Hendricks RL. CD8(+) T cells can block herpes simplex virus type 1 (HSV-1) reactivation from latency in sensory neurons [In Process Citation]. The Journal of Experimental Medicine. 2000;**191**:1459-1466

[34] Liu T, Khanna KM, Carriere BN, Hendricks RL. Gamma interferon can prevent herpes simplex virus type 1 reactivation from latency in sensory neurons. Journal of Virology. 2001;**75**:11178-11184

http://dx.doi.org/10.5772/intechopen.75451

[35] Khanna KM, Bonneau RH, Kinchington PR, Hendricks RL. Herpes simplex virus-specific memory CD8+ T cells are selectively activated and retained in latently infected sensory ganglia. Immunity. 2003;**18**:593-603

[36] Prbhakaran K, Sheridan BS, Kinchington PR, Khanna KM, Decman V, Lathrop K, Hendricks RL. Sensory neurons regulate the effector functions of CD8+ T cells in controlling HSV-1 latency ex vivo. Immunity. 2005;**23**:515-523

[37] Knickelbein JE, Khanna KM, Yee MB, Baty CJ, Kinchington PR, Hendricks RL. Noncytotoxic lytic granule-mediated CD8+ T cell inhibition of HSV-1 reactivation from neuronal latency. Science. 2008;**322**:268-272

[38] Matrisciano F, Buscetti CL, Bucci D, Orlando R, Caruso A, Molinaro G, Cappuccion I, Riozzi B, Gradini R, Motolese M, Caraci F, Copani A, Scaccianoce S, Melchiorri D, Bruno V, Battaglia G, Nicoletti F. Induction of the Wnt antagonist Dickkopf-1 is involved in stress-induced hippocampal damage. PLoS One. 2011;**6**:e16447

[39] Moors M, Bose R, Johansson-Haque K, Edoff K, Okret S, Ceccatelli S. Dickkopf mediates glucocorticoid-induced changes in human neural progenitor cell proliferation and differentiation. Toxicological Sciences. 2012;**125**:488-495

[40] Kaczynski J, Cook T, Urrutia R. Sp1- and Kruppel-like transcription factors. Genome Biology. 2003;**4**:206.201-206.208

[41] Bieker JJ. Kruppel-like factors: three fingers in many pies. The Journal of Biological Chemistry. 2001;**276**:34355-34358

[42] Jones KA, Tjian R. Sp1 binds to promoter sequences and activates herpes simples virus 'immediate-early' gene transcription in vitro. Nature. 1985;**317**:179-182

[43] Knipe DM, Cliffe A. Chromatin control of herpes simplex virus lytic and latent infection. Nature Reviews. Microbiology. 2008;**6**:211-221

[44] Lacasse JL, Schang LM. Herpes simplex virus 1 DNA is in unstable nucleosomes throughout the lytic infection cycle, and the instability of the nucleosomes is independent of DNA replication. Journal of Virology. 2012;**86**:112870-111300

[45] Lacasse JL, Schang LM. During lytic infection, herpes simplex virus type 1 DNA is in complexes with the properties of unstable nucleosomes. Journal of Virology. 2010;**84**:1920-1933

[46] Rock D, Lokensgard J, Lewis T, Kutish G. Characterization of dexamethasone-induced reactivation of latent bovine herpesvirus 1. Journal of Virology. 1992;**66**:2484-2490

[47] Welten PJAJE, Bosch DS, de Jonge RT, Balog J, van der Maarel SM, de Kloet ER, Datson NA. A genome-wide signature of glucocorticoid receptor binding in neuronal PC12 cells. BMC Neuroscience. 2012;**13**:118-125

[48] El-Mayet FS, Sawant L, Thungunutla P, Jones C. Combinatorial effects of the glucocorticoid receptor and Krüppel-like transcription factor 15 on bovine herpesvirus 1 transcription and productive infection. Journal of Virology. 2017;**91**(91):e00904-00917

[49] Mangan S, Alon U. Structure and function of the feed-forward loop network motif. Proceedings of the National Academy of Sciences of the United States of America. 2003; **100**:11980-11985

[50] Sasse S, Zuo Z, Kadiyala V, Zhang L, Pufall MA, Jain MK, Phang TL, Stormo GD, Gerber AN. Response element composition governs correlations between binding site affinity and transcription in glucocorticoid receptor feed-forward loops. The Journal of Biological Chemistry. 2015;**290**:19756-19769

[51] Sasse S, Mailoux CM, Barczak AJ, Wang Q, Altonsy MO, Jain MK, Haldar SM, Gerber AN. The glucocorticoid receptor and KLF15 regulate gene expression dynamics and integrate signals through feed-forward circuitry. Molecular Cell Biology. 2013;**33**:2104-2115

[52] Alon U. Network motifs: theory and experimental approaches. Nature Reviews. Genetics. 2007;**8**:450-461

[53] Masuno K, Haldar SM, Jeyaraji D, Mailoux C, Huang X, Panettieri RA, Jain MK, Gerber AN. Expression profiling identifies Klf15 as a glucocorticoid target that regulates airway hyperresponsiveness. American Journal of Respiratory Cell and Molecular Biology. 2011;**10**:1165

[54] Sinani D, Cordes E, Workman A, Thunuguntia P, Jones C. Stress induced cellular transcription factors expressed in trigeminal ganglionic neurons stimulate the herpes simplex virus type 1 (HSV-1) infected cell protein 0 (ICP0) promoter. Journal of Virology. 2013; **87**:1183-1192

[55] Asada M, Rauch A, Shimizu H, Maruyama H, Miaki S, Shigamori M, Kawasome H, Ishiyama H, Tuckermann J, Asahara H. DNA-binding dependent glucocorticoid receptor activity promotes adipogenesis via Kruppel-like factor 15 gene expression. Laboratory Investigation. 2011;**91**:203-215

[56] Tremblay R, Sikorska M, Sandhu JK, Lanthier P, Ribecco-Lutkiewicz M, Bani-Yaghoub M. Differentiation of mouse Neuro-2A cells into dopamine neurons. Journal of Neuroscience Methods. 2010;**186**:60-67

[57] Thunuguntla P, El-mayet FS, Jones C. Bovine herpesvirus 1 can efficiently infect the human (SH-SY5Y) but not the mouse neuroblastoma cell line (Neuro-2A). Virus Research. 2017;**232**:1-5

[58] Wirth UV, Fraefel C, Vogt B, Vlcek C, Paces V, Schwyzer M. Immediate-early RNA 2.9 and early RNA 2.6 of bovine herpesvirus 1 are 3′ coterminal and encode a putative zinc finger transactivator protein. Journal of Virology. 1992;**66**:2763-2772

[59] Wirth UV, Vogt B, Schwyzer M. The three major immediate-early transcripts of bovine herpesvirus 1 arise from two divergent and spliced transcription units. Journal of Virology. 1991;**65**:195-205

[60] Fraefel C, Zeng J, Choffat Y, Engels M, Schwyzer M, Ackermann M. Identification and zinc dependence of the bovine herpesvirus 1 transactivator protein BICP0. Journal of Virology. 1994;**68**:3154-3162

[61] Pandit S, Geissler W, Harris G, Sitlani A. Allosteric effects of dexamethasone and RU486 on glucocorticoid receptor-DNA interactions. The Journal of Biological Chemistry. 2002;**277**:1538-1543

[62] Schulz M, Eggert M, Baniahmad A, Dostert A, Heinzel T, Renkatz R. RU486-induced glococorticoid receptor agonism is controlled by the receptor N terminus and by corepressor binding. The Journal of Biological Chemistry. 2002;**277**:26238-26243

[63] Schmitt J, Stunnenberg HG. The glucocorticoid receptor hormone binding domain mediates transcriptional activation in vitro in the absence of ligand. Nucleic Acids Research. 1993;**21**:2673-2681

[64] Nishimura K, Nonomura N, Satoh E, Harada Y, Nakayama M, Tokizane T, Fuki T, Ono Y, Inoue H, Shin M, Tsujimoto Y, Takayama H, Aozasa K, Okuyama A. Potential mechansim for the effects of dexamethasone on growth of androgen-independent prostate cancer. Journal of the National Cancer Institute. 2014;**93**:1739-1746

[65] Takeda K, Yahagi N, Aita Y, Murayama Y, Sawada Y, Piao X, Toya N, Oya Y, Shikama A, Takarada A, Masuda Y, Nishi M, Kuobota M, Izumida Y, Yamamoto T, Sekiya M, Matsuzaka T, Nakagawa Y, Urayama O, Kawakami Y, Iizuka Y, Gotoda T, Itaka K, Kataoka K, Nagai R, Kadowaki T, yamada N, Lin Y, Jain MK, Shimano H. KLF15 enables switching between lipogenesis aand gluconeogenesis during fasting. Cell Reports. 2016;**16**:2373-2386

[66] Yamamoto J, Ikeda Y, Iguchi H, Fujino T, Tanaka T, Asaba H, Iwasaki S, Ioka RX, Kaneko IW, Magoori K, Takahashi S, Mori T, Sakaue H, Kodama T, Yanagisawa M, Yamamoto TT, Ito S, Sakai J. A Kruppel-like factor KLF15 contributes fasting-induced transcriptional activation of mitochondrial acetyl-CoA synthtase gene AceCS2. The Journal of Biological Chemistry. 2004;**279**:16954-16962

[67] Otteson DC, Lai H, Liu Y, Zack DJ. Zinc-finger domains of the transcriptional repressor KLF15 binds multiple sites in rhodopsin and IRBP promoters including the CRS-1 and G-rich elements. BMC Molecular Biology. 2005;**6**:1-16. DOI: 10.1186/1471-2199-1186-1115

[68] Calderon MR, Verway M, An B-S, DiFeo A, Bismar TA, Ann DK, Martignetti JA, Shalom-Barak T, White JH. Ligand-dependent corepressor (LCoR) recruitment by Kruppel-like factor 6 (KLF6) regulates expression of the cyclin dependent kinase inhibitor CDKN1A gene. The Journal of Biological Chemistry. 2012;**287**:8662-8674

[69] Strahle U, Schmid W, Schutz G. Synergistic action of the glucocorticoid receptor with transcription factors. The EMBO Journal. 1988;**7**:3389-3395

[70] Chiambaretta F, Blanchon L, Rabier B, Kao WW-Y, Liu JJ, Dastuge B, Rigal D, Sapin V. Regulation of corneal keratin-12 gene expression by the human Kruppel-like transcription factor 6. Investigative ophtalmology and Visual. Science. 2002;**43**:3422-3429

[71] Koritschoner N, Bocco JL, Panzetta-Dutari GM, Dumar CI, Flury A, Patrito LC. A novel human zinc finger protein that interacts with the core promoter element of a TATA box-less gene. The Journal of Biological Chemistry. 1997;**272**:9573-9580

[72] Wirth UV, Gunkel K, Engels M, Schwyzer M. Spatial and temporal distribution of bovine herpesvirus 1 transcripts. Journal of Virology. 1989;**63**:4882-4889

[73] Inman M, Lovato L, Doster A, Jones C. A mutation in the latency-related gene of bovine herpesvirus 1 leads to impaired ocular shedding in acutely infected calves. Journal of Virology. 2001;**75**:8507-8515

[74] Misra V, Bratanich AC, Carpenter D, O'Hare P. Protein and DNA elements involved in transactivation of the promoter of the bovine herpesvirus (BHV) 1 IE-1 transcription unit by the BHV alpha gene trans-inducing factor. Journal of Virology. 1994;**68**:4898-4909

Chapter 4

Roles of Non-Coding RNAs in Transcriptional Regulation

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76125

Abstract

Non-coding RNAs (ncRNAs) are functional RNA molecules that are transcribed from mammalian genome but lack protein coding capacity. Nearly 80% of the human genome constitutes non-coding elements such as small non-coding RNAs, sncRNAs (miRNA, piRNA, SiRNA, SnRNA) and long non-coding RNAs, lncRNAs (linc RNA, NAT, eRNA, circ RNA, ceRNAs, PROMPTS). These ncRNAs have been extensively studied and are known to mediate the regulation of gene expression. In recent decades, lncRNAs have emerged as pivotal molecules that participate in the post-transcriptional regulation by acting as a signal, guide, scaffold and decoy molecules in addition to their role(s) in transcription. ncRNAs are known to play critical roles in defining DNA methylation patterns, imprinting as well as chromatin remodeling, thus having a substantial effect in epigenetic signaling. The expression of lncRNAs is regulated in a tissue specific and developmental stage specific manner and their mis-regulation is often associated with tumorigenesis. Henceforth, this chapter focuses mainly on the role(s) of ncRNAs in transcriptional and post-transcriptional regulation and their relevance in cancers.

Keywords: lncRNAs, miRNAs, DNA methylation, epigenetic signaling, transcriptional regulation, cancer

1. Introduction

1.1. The incredible RNA molecules

According to “RNA world hypothesis”, early life was started with RNA molecules. Later with time, storage of information evolved to more stable DNA and RNA which emerged as a

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messenger of stored information thereby completing the central dogma of life. Though 80% of the human genome is transcribed into RNA, majority of RNA lacks protein coding potential and referred as "non-coding RNA" (ncRNA). Further, genome sequencing technologies have revealed that the mammalian transcriptome is much more complex and their transcription is regulated by developmental stages [1]. The continuing discovery of new classes of regulatory ncRNAs suggests that RNA has continued to evolve along with proteins and DNA.

ncRNAs are divided into two major groups based on an arbitrary threshold of 200 nucleotides (nt) namely short ncRNAs (sncRNA) and long ncRNAs (lncRNAs) (**Figure 1**). sncRNAs include functional RNAs such as t-RNAs, r-RNAs and snRNAs which are involved in transcriptional and translational regulation. In addition to these conventional RNAs, short ncRNAs also include different regulatory RNAs such as microRNAs (miRNAs) [2, 3], small interfering RNAs (siRNAs) and P-element-induced wimpy testis (PIWI) interacting RNAs (piRNAs) [4], all of which regulate gene expression. In contrast to sncRNAs, the lncRNAs are a group of large, heterogeneous ncRNAs of unknown function. Similar to coding RNA transcripts, lncRNAs contains epigenetic marks indicating their ability to express differentially [5] and the presence of introns in lncRNAs emphasizes the existence of splice variants. These lncRNAs exist in both polyadenylated and non-polyadenylated forms and hence are termed "bimorphic" [6]. LncRNAs include many different types of RNA and exhibit a wide range of secondary and tertiary structures compared to the coding transcriptome. Some pseudogenes and

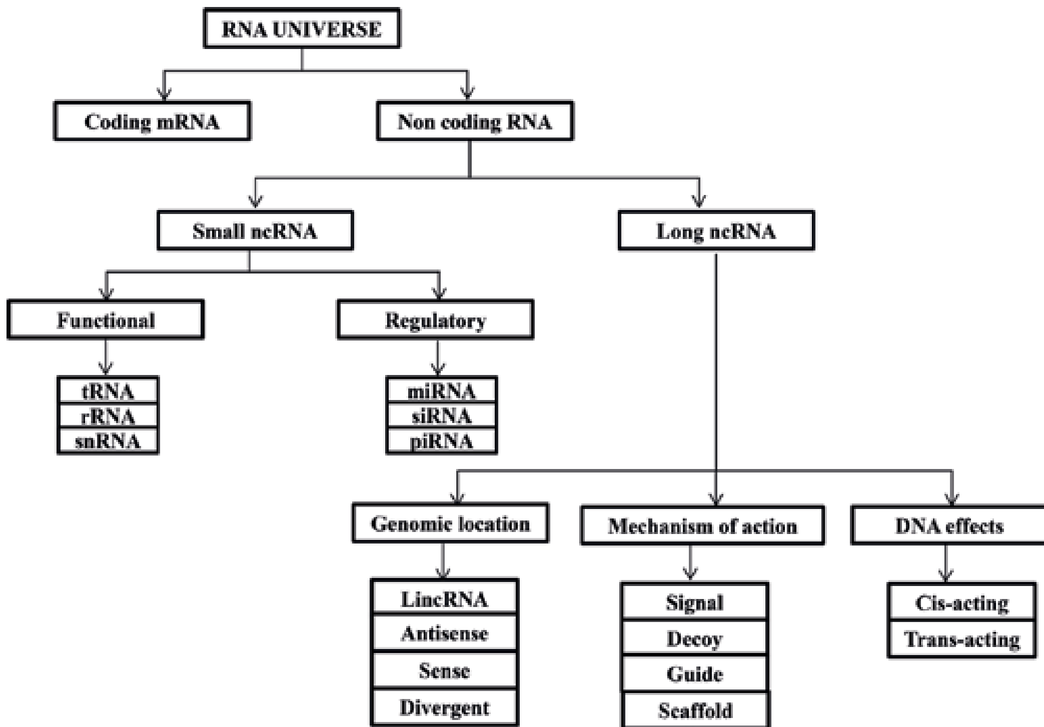

Figure 1. Classification of non-coding RNA (ncRNA).

copies of coding genes harboring mutations render lncRNAs non-coding [7]. Many lncRNAs are known to overlap coding genes [8]. A lncRNA might encompass either the entire gene or only a part of it and these lncRNA may originate from either the sense or antisense strand [9, 10]. The lncRNAs were termed based on their mechanism of action, such as intergenic (lincRNA), natural antisense transcripts (NATs), enhancer RNA (eRNA), circular RNA (circRNA), promoter associated long RNA (pRNA), etc. LncRNAs act at different levels of gene expression to exhibit diverse cellular functions. This functional diversity reflects the versatility of ncRNA and its interaction with a large number of substrates in a highly specific manner. Moreover, the expression of ncRNA is dynamic and can be rapidly up-regulated or down-regulated during developmental stages or differentiation without being translated [11]. Henceforth, in this chapter, we will discuss mainly on the gene regulatory roles of lncRNAs and miRNAs in distinct cellular functions and developmental regulation.

2. The small non-coding RNAs (sncRNAs)

The sncRNAs are extensively studied in the last decade and have been associated with RNA interference (RNAi) pathways, which lead to silencing of specific genes and protection of the cell or genome against viruses, mobile repetitive DNA sequences, retro-elements and transposons [12].

2.1. miRNAs and siRNAs

Both the siRNAs and miRNAs are 20–30 nucleotides long and generated from sense and antisense DNA strands, pseudogenes and inverted repeats. These molecules induce mRNA degradation or translational repression, which in turn result in the alteration of gene expression. About 60% of translated protein coding genes are negatively regulated by miRNAs [13]. Some transcripts are regulated by a single miRNA, while others are regulated by more than one miRNAs [14]. In addition to the transcriptional gene regulation, miRNAs play important roles in pivotal biological processes such as cell proliferation, cell differentiation, development, and cell death [15–18].

2.2. miRNA and siRNA biogenesis and mechanism of action

The process of miRNA biogenesis is quite characteristic for the ncRNAs subclass. Based on cellular requirement, the primary miRNA transcript (pri-miRNA) is first transcribed from the DNA by RNA polymerase II and characterized by one or many stem-loop hairpins which encompass the functional mature miRNA in their stem. In animals, the first step occurs in nucleus, in which the pri-miRNA upon recognition by two nuclear enzymes, Drosha and DGCR8 is processed into dsRNA molecule containing one or more hairpins of approximately 70 nucleotides long, which are called as precursor miRNAs (pre-miRNAs). Then they are exported to the cytoplasm by the nuclear export protein exportin-5 [19]. In cytoplasm, the pre-miRNA is recognized and processed by the RNase III enzyme, Dicer which removes the hairpin loop resulting in 20–23 nt dsRNA (miRNA-miRNA*) molecule. In case of siRNAs, the small RNA

duplex molecules produced by the action of Dicer, creates a RNA duplexes with 2-nt overhangs at their 3′ ends and phosphate groups at their 5′ ends [19]. Only one of the two strands of dsRNA acts as a guide strand and directs gene-silencing while, the other strand incorporates into the RNA-induced silencing complex (RISC) containing the Argonaute proteins (Ago1/2) and the GW182, where the anti-guide or passenger strand is degraded resulting in 20–23 nt mature miRNA (**Figure 2**). The siRNAs are recognized by Argonaute protein 2 (Ago2) [18, 20], and the selection of the different Ago proteins are based on the small interfering RNA duplex structure. Generally, siRNAs that are perfect duplexes in terms of base pairing are loaded into Ago2, whereas duplexes presenting mismatches as in the case of miRNAs, are driven by Argonaute 1 (Ago1) [21, 22]. When the complementarity between the miRNA bound to Ago1 and the target m-RNA is high, miRNA tailing and 3′–5′ trimming occurs. The discrimination between Ago1 and Ago2 depends on the action of Hen1; an enzyme that adds the 2′-*O*-methyl group at the 3′ ends of small RNAs bound to Ago2, but not those bound to Ago1 [23]. This methyl group is known to block tailing and trimming of the miRNA. The RISC complex then targets the mRNA transcript based on sequence complementarity between the

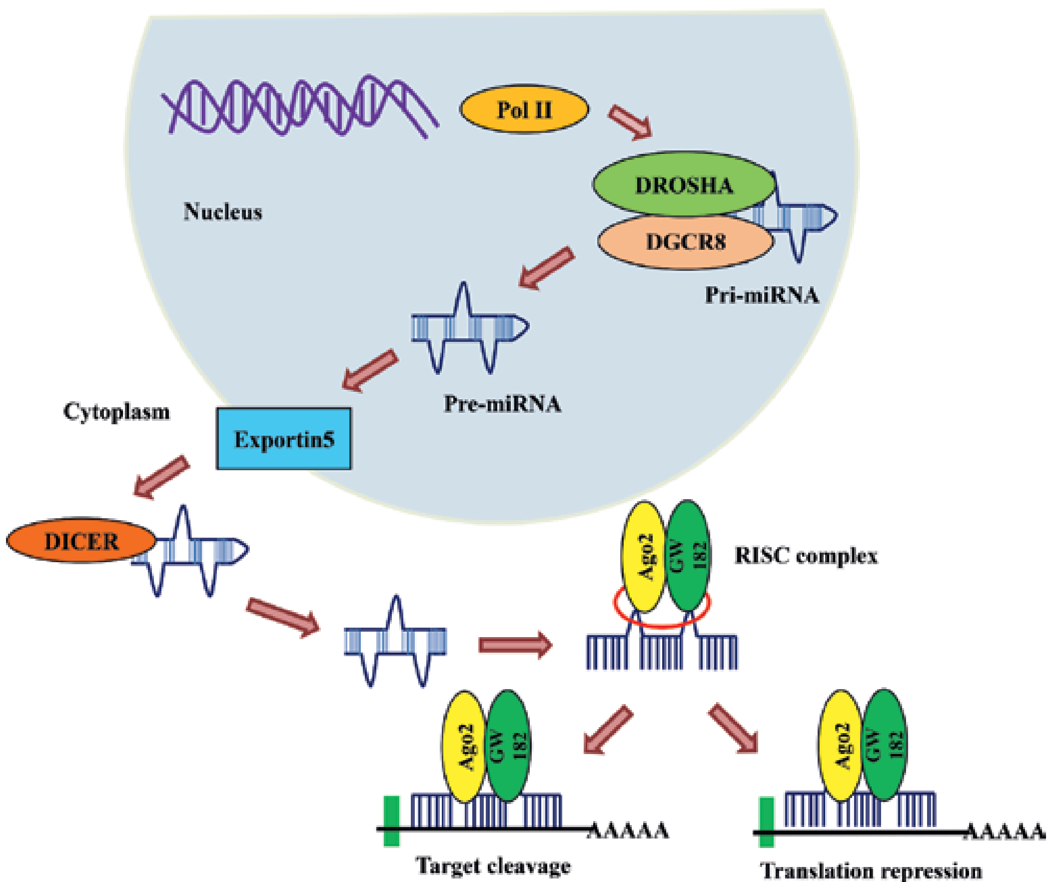

Figure 2. Biogenesis of miRNA and its mechanism of action (modified from Hrdlickova B *et al*. [18]).

miRNA sequence and nucleotides in the 3′ untranslated regions (3′ UTR) of the target mRNAs [24]. The binding of the RISC complex to its target leads to direct Ago-mediated cleavage of the target and causes mRNA degradation if the homology between miRNA and its target mRNA is extensive or to deadenylation followed by translation prevention if the homology between the miRNA and its target is less extensive [20, 25]. Efficient targeting requires continuous base pairing of the miRNA seed region (which is a stretch of 6–8 nucleotides of the mature miRNA) with its target mRNA [25, 26]. Unlike miRNA, siRNA base pairs perfectly and induce mRNA cleavage only in a single specific target. Initially, it has been showed that miRNAs mainly target the 3′ UTRs of mRNAs [20], but recently, it was found that miRNA target sites also been located in the 5′ UTRs and even in coding regions of some of the target mRNAs [20, 27]. For example, mir-148 targets on the coding regions of DNMT3B.

2.3. Role of miRNA in cancer and diseases

miRNAs have been shown to be involved in several human diseases including cancer, neurodegenerative, cardiovascular and autoimmune diseases [14]. Differential expression of specific miRNA will result in the up-regulation or down-regulation of their targets leading to the deregulation of cellular pathways.

In human diseases, expression of miRNAs could be differentially regulated by:

i. Altered functions of the enzymes involved in the miRNA biogenesis pathway. For example, DiGeorge syndrome results due to haploinsufficiency of DGCR8 [18].

ii. Transcriptional repression of miRNAs by promoter hypermethylation [28]. For example, the miR-200 family is involved in the control of the epithelial-mesenchymal transition (EMT) [18].

iii. Genetic alterations in miRNA genes or in their regulatory motifs which can have deleterious consequences [29]. The deletion of chromosome 13q14 in chronic lymphocytic leukemia (CLL) patients is the best studied example in which the deleted area contains the miR-15a and miR-16-1 genes that target the anti-apoptotic/pro-survival gene BCL-2 (B-cell lymphoma 2) and thus deletion of this region contributes to the greater survival of cancerous cells [18].

3. The long non-coding RNAs (lncRNAs)

LncRNAs are defined as a heterogeneous group of transcripts that are >200 nucleotides (nt) in length. These lncRNAs do not exhibit coding potential [30–32] and are transcribed from DNA. These lncRNAs can be intergenic, exonic, in enhancer regions or in the regions distal to protein-coding genes [11, 33]. Like mRNAs, lncRNAs are transcribed by RNA polymerase II (RNA PolII) and undergo post-transcriptional processing such as alternative splicing, 5′ capping, polyadenylation and RNA editing and also carry single nucleotide polymorphisms (SNPs) [31, 34].

In comparison to protein coding RNAs, lncRNAs have few, but longer exons [30, 35]. Other characteristics of lncRNAs include: (i) well conserved lncRNA promoter regions between vertebrates; (ii) unique promoters, DNA-binding motifs and preferred transcription factors (TFs), (iii) less conserved lncRNA exons between species and (iv) tissue specific expression profiles [5, 31, 36–38]. Compared with protein coding genes, only 11–29% of lncRNAs are ubiquitously expressed in all tissues and they are expressed at very minimum levels [31, 39]. Computational analysis of RNA-Seq data has suggested that lncRNA transcription is independent and influence the transcription of neighboring protein coding genes [31, 38]. The origin of lncRNAs is still under debate. A recent study [40], has reported that more than two-thirds of mature lncRNA transcripts contain transposable elements (TEs). This observation has led to the postulation that the majority of lncRNAs might have arisen via insertion of TEs [41].

3.1. Classification of lncRNAs

LncRNAs have been classified based on their: (i) genomic location, (ii) mechanism of action, and (iii) effects on DNA sequences.

3.1.1. Classification of lncRNAs based on genomic location

LncRNAs could be classified into four broad categories based on their relative position to the nearest protein coding genes (**Figure 3**). The first class is the "long intergenic non-coding RNAs" (lincRNAs) which is the largest group of lncRNAs and these genes do not overlap or lie in close proximity to protein coding genes [5, 42]. The second most prevalent class of lncRNA is the "antisense lncRNA" that is transcribed from the antisense strand and are overlapping. Based on their overlap, the antisense lncRNAs are subdivided into two:(i) "intronic antisense lncRNAs" where the lncRNA transcript falls completely within the boundaries of an opposing intron, and (ii) "natural antisense transcripts" (NATs) which partially overlaps around the promoter or at the terminator site of the coding gene [43, 44]. The third class of lncRNAs comprises the "sense lncRNA" transcripts which can be "sense intronic or "sense overlapping." Such transcripts are located on the same strand and transcribed in the same direction as a protein coding gene. The fourth class of lncRNAs is the "bidirectional lncRNAs" or "divergent lncRNAs." These transcripts are located on the antisense strand and have their transcription start site (TSS) close to the TSS of the protein-coding gene, but are transcribed in the opposite direction [45–47].

3.1.2. Classification of lncRNAs based on their mechanism of action

LncRNAs can interact with DNA, RNA as well as proteins. LncRNAs have been implicated mainly in post-transcriptional gene regulation by controlling processes like protein synthesis, RNA sequestration, RNA transport and have been shown to control transcriptional gene silencing via epigenetic regulation and chromatin remodeling [48, 49]. LncRNAs are divided into four archetypes based on their molecular mechanism (**Figure 4**) [18]. LncRNAs that belongs to the "signaling archetype" acts as a molecular signal for a particular biological condition and may activate or silence the genes depending on the stimulus (**Figure 4A**).

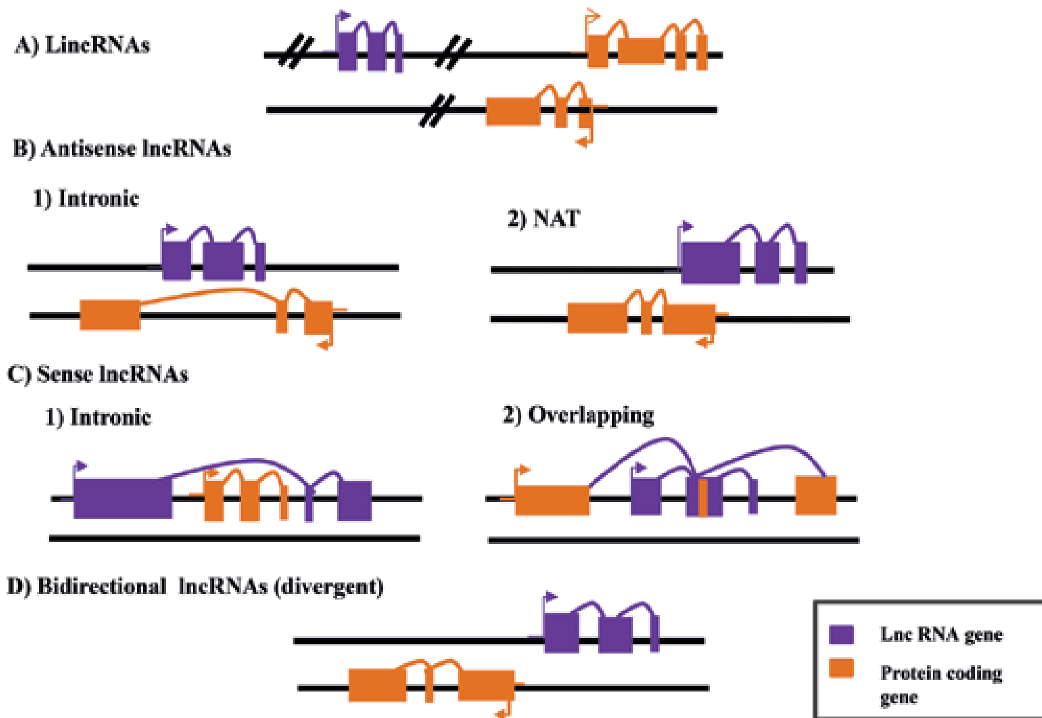

Figure 3. Classification of lncRNAs based on position relative to the nearest protein coding gene (modified from Hrdlickova B *et al*. [18]).

Some of the examples of lncRNAs displaying the signaling archetype are lncRNAs involved in embryonic development (HOTAIR and HOTTIP), DNA damage response (lincRNA-p21 and PANDA), stress responses (COLDAIR and COOLAIR), etc. [18]. The second category is the "decoy archetype" where the lncRNAs act as decoys that bind to and interfere with the function of other RNAs or proteins. They act by competing with their sequences or structures for binding and are considered to be negative regulators (**Figure 4B**). For example, PANDA binds to the transcription factor NF-YA and prevents the activation of NF-YA induced pro-apoptotic targets [18]. The "guide archetype" is the third class, in which the lncRNAs binds to specific proteins and transport them to the specific targets. The interaction may be direct (between lncRNA-protein complex and the DNA) or indirect (between lncRNA-protein and protein-DNA complexes) (**Figure 4C**). These lncRNAs may interact as activators or repressors with neighboring (cis-acting) or distant (trans-acting) genes. Examples of lncRNAs employing this mechanism are HOTAIR, lincRNAp21, Xist, COLDAIR and Jpx (just proximal to XIST). The fourth archetype is "scaffold archetype" (**Figure 4D**), where the lncRNAs act by bringing the bound proteins into a complex or in spatial proximity. Examples of this lncRNAs are ANRIL (antisense ncRNA in the INK4 locus) which functions as a scaffold for the chromatin remodeling complexes PRC1 and PRC2, HOTAIR (scaffold for PRC2 binding it to the LSD1 complex) and TERC (telomerase RNA component) that scaffolds the telomerase complex [18].

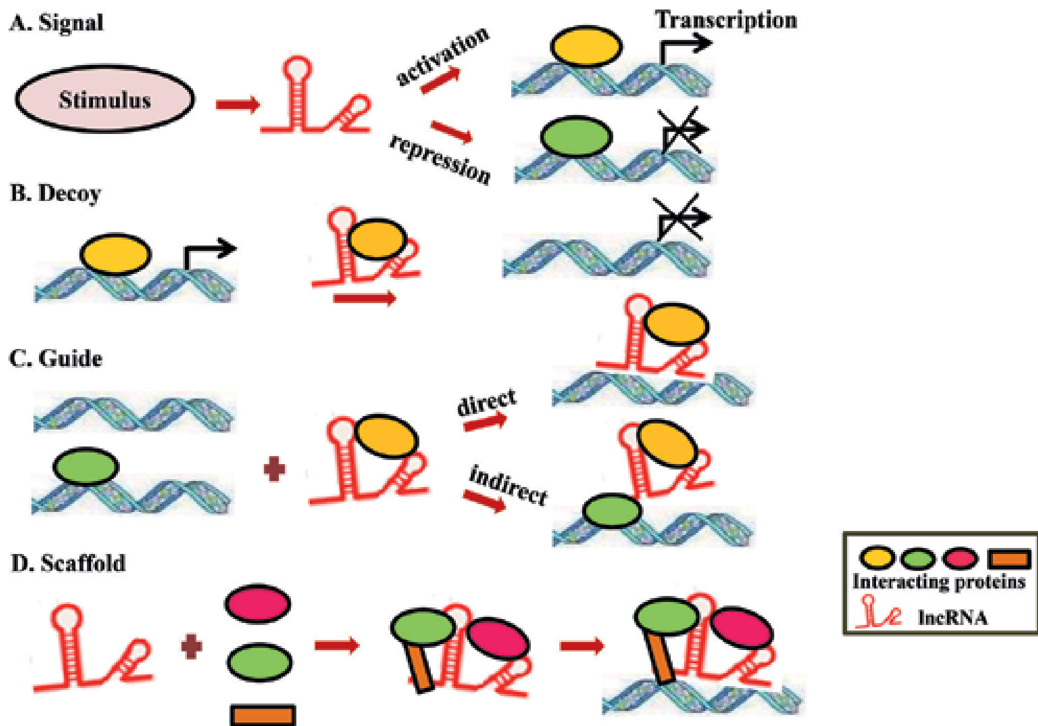

Figure 4. Classification of lncRNAs based on the mechanism of action.

3.1.3. Classification of lncRNAs based on their effects on DNA sequence

LncRNAs could be divided into "cis-acting" and "trans-acting" based on the effects exerted on DNA sequences. The effects of cis-acting lncRNAs are restricted to genes in close genomic proximity (usually the genes in the chromosome from which they are transcribed from), whereas trans-acting lncRNAs affect distant genes (the genes on other chromosomes) [50]. The action of both cis and trans lncRNAs is locus specific and in both cases, the lncRNA binds epigenetic modifiers through a specific sequence or structure and targets them to promoter regions to regulate the expression of respective genes. For example, HOTTIP and HOTAIR lncRNAs [51]. The major example of general cis-regulation is induction of X inactivation by the Xist lncRNA in female mammals. Xist is expressed from one of the two X chromosomes and induces silencing of the whole chromosome [50]. Example of trans-regulation is the B2 lncRNA that binds to RNA PolII and inhibits phosphorylation of its carboxy-terminal domain (CTD), thus affecting RNA polymerase reaction [50].

4. Gene regulation by lncRNAs

LncRNAs have diverse regulatory functions and might regulate gene expression by modulating chromatin remodeling, cis and trans gene expression, gene transcription, post-transcriptional

regulation, translation, protein trafficking and cellular signaling [33, 34]. Growing number of evidences implicate lncRNAs in transcriptional gene regulation, thereby suggesting a significant role(s) for lncRNAs in such tightly regulated process [52, 53]. The mechanisms of transcriptional and post-transcriptional regulation by lncRNAs is discussed below.

4.1. Transcriptional regulation

Regulation of transcription is considered to be an interplay of transcription factors (TFs) and chromatin modifying factors at the gene promoters. LncRNAs modulate gene expression by specifically associating with other molecules; DNA, RNA and protein, either at the promoters or at the enhancers of their target genes. LncRNAs regulate transcription by various mechanisms and some are shown below.

4.1.1. Enhancer RNAs

Enhancer RNAs (eRNAs) are a category of lncRNAs derived from enhancer regions of genes, which interact with DNA to upregulate gene transcription through two possible mechanisms such as enhancer-promoter looping and tracking of transcriptional machinery [54]. While studying the enhancers activated by calcium signaling in mouse neurons, Kim et al. for the first time, identified a eRNA of about 2 kb transcribed bidirectionally from active enhancers. The expression of this eRNA correlated with the activity of the enhancer region [55, 56], which suggests that eRNAs contribute to enhancer function and influence the transcription of genes.

4.1.2. Activating ncRNAs

Activating ncRNAs are a class of lncRNAs which are transcribed from independent loci, but not from enhancers and have a transcriptional activation function [57, 58]. Activating ncRNAs specifically activate the transcription of neighboring coding genes in an RNA-dependent fashion, and require the activity of the coding gene promoter [58]. These activating ncRNAs are functionally similar to eRNAs. However, in contrast to eRNAs, activating ncRNAs are spliced, polyadenylated stable transcripts. Gene activation mediated by the activating ncRNAs requires a change in chromosomal conformation to bring the activating ncRNAs locus close to the promoter of its target gene [59]. A number of activating ncRNAs have been shown to be associated with the mediator complex which is involved in bridging promoters with enhancers; and depletion of this complex inhibits looping between the activating ncRNAs locus and its target gene. Thus, eRNA and activating ncRNAs function by interacting with the same set of molecules, forming a scaffold for a protein complex that bridges the enhancer-like element and the promoter of a coding gene (**Figure 5**) [60].

4.1.3. Transcriptional regulation by recruitment of chromatin modifiers

As discussed earlier in this chapter, lncRNAs mediate epigenetic changes by DNA methylation, histone modification and by recruiting chromatin remodeling complexes to specific genomic loci mainly to the promoter regions and causes repression or activation of the target genes. It

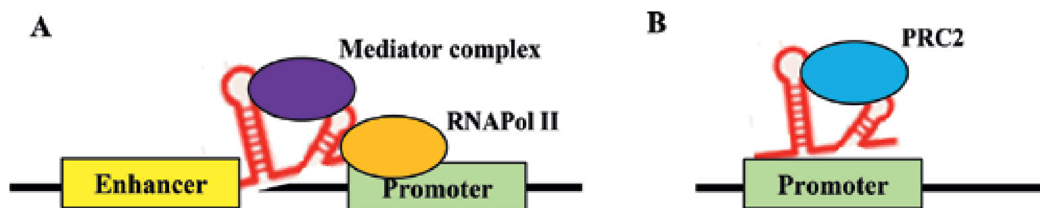

Figure 5. Models of transcriptional regulation. (A) Bridging scaffold model: lncRNAs (red line) transcribed from enhancer-like non-coding genes are required to recruit the mediator complex. (B) Tethered scaffold model: lncRNA (red line) recognizes specific DNA motifs and recruits histone modifying enzymes.

was found that the lncRNA might serve two functions. (i) lncRNAs act as a bridging scaffold and binds to a protein or protein complex to facilitate chromatin conformational changes [61]. (ii) lncRNAs act as tethered scaffold that targets chromatin modifying enzymes to specific DNA motifs (**Figure 5**). For example, the lncRNA HOTAIR (Hox transcript antisense RNA) acts as an epigenetic-protein scaffold and possess multiple binding domains for distinct proteins. At the 3′ end, HOTAIR contributes to the demethylation of H3K4 by interacting with lysine-specific histone demethylase 1A (LSD1), restrictive element 1-silencing transcription factor (REST), and REST corepressor1. At the 5′ end, *HOTAIR* originated from the *HOXC* locus and causes transcriptional gene silencing across 40 kb of the *HOXD* locus in *trans* by inducing a repressive chromatin state, by recruitment of the Polycomb chromatin remodeling complex PRC2 and reinforcing H3K27 methylation [34, 62].

4.1.4. Genomic imprinting and X-chromosome inactivation

Genomic imprinting is the phenomenon of epigenetic silencing of an allele inherited from either of the parents [63]. Imprinting Control Regions (ICRs) are short stretches of DNA that play a critical role in imprinting of multiple genes [64]. Interestingly, it has been observed that the imprinted regions show significant association with ncRNAs, which mediate the silencing by diverse mechanisms like chromatin remodeling and enhancer competition [65]. X chromosome inactivation is a process mediated by the long ncRNA- *Xist,* in which one copy of the X chromosome in females is inactivated. From the *Xist* locus, a small internal non-coding transcript *RepA* recruits PRC2 to silence one X chromosome [61]; whereas PRC2 is formed from the remaining active X chromosome by the antisense transcript *Tsix*. However, an alternative mechanism is described by another study in which *Xist* and *Tsix* anneal to form an RNA duplex that is processed by Dicer to generate small interfering RNAs (siRNAs) which are required for the repressive chromatin modifications on the inactive X chromosome [1].

4.2. Post-transcriptional regulation

At post-transcriptional level, lncRNAs regulate by acting as competing endogenous RNAs that regulate microRNA levels which in turn modulate mRNA levels by altering mRNA stability, mRNA decay, and translation [66].

4.2.1. LncRNA as a source of miRNA

Most pri-miRNAs are generally greater than 1 kb in length [67]; and therefore may be regarded as a form of lncRNA. There are two major sources of pri-miRNAs in the genome: (i) pri-miRNAs that are embedded within another gene and whose expression is usually but not always linked to the expression of the parent transcript, and (ii) pri-miRNAs that are transcribed independently from miRNA genes which contain promoters that regulate their transcription mainly by RNA polymerase II (RNA PolII) in a manner similar to mRNA [66]. Approximately 50% of miRNAs are produced from non-coding transcripts [68]; however, with miRNAs embedded in coding genes many miRNAs are also located within introns of non-coding genes (**Figure 6**) [66]. Such a genomic organization suggests that the host lncRNA does not simply act as a pri-miRNA but may have other additional roles encoded by the exons. For example, DLEU2 is the host gene of the tumor suppressor miRNA, miR-15a/16.1 cluster located within its third intron [66].

4.2.2. LncRNA as a negative regulator of miRNA

miRNAs are known to act as negative regulators of gene expression. Transcripts are targeted through binding of a short 6–8 nt seed sequence within the miRNA to a miRNA response element (MRE) in the 3′ UTR regions of targets. Computational predictions based on miRNA seed sequences found that many lncRNAs contain miRNA binding sites. This raises an interesting possibility that many lncRNAs function to regulate gene expression by sequestering miRNAs, thus limiting their concentration within the cell and thereby reducing the pool of available miRNA in the cell. In this way, the lncRNA acts as a negative regulator of miRNA function and thereby a positive regulator of gene expression. This is known as the "competing endogenous RNA (ceRNA)" hypothesis (**Figure 7**) [69, 70]. For example, the intergenic lincRNA-ROR, which inhibits miR-145 in pluripotent embryonic stem cells [66]. Competitive endogenous RNAs (ceRNAs) are lncRNAs that sequester miRNAs and inhibit miRNA functions and have two structurally distinct forms such as linear and circular. Non-circular or linear lncRNAs are single-stranded molecules that bind to miRNAs and regulate gene expression by promoting it to degradation [71]. Circular RNAs (circRNAs) are a type of ring-forming lncRNA that form

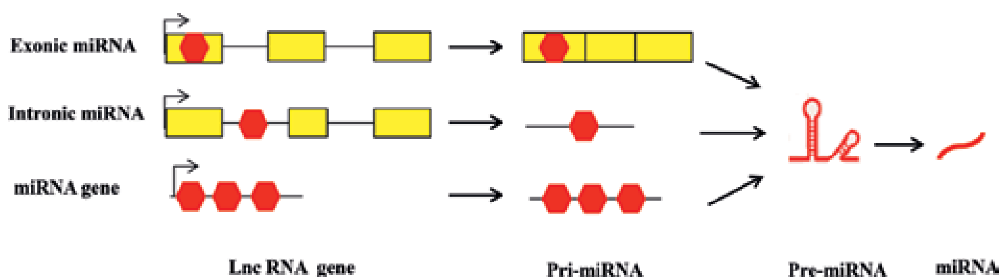

Figure 6. LncRNA as a source of miRNA. LncRNA genes contain embedded miRNA sequences (red hexagonal boxes) which may be located within an exon (orange box) or an intron (line) or occur in clusters within the genome. Though the sources are different, the pathways converge at the level of pre-miRNA structure which produce miRNA (modified from Dykes IM *et al*. [66]).

by linking the 3′ and 5′ ends with a back splicing covalent bond [72, 73]. In addition, lncRNAs can facilitate the inhibition of mRNA translation or decay by partial base pairing with the 3′ UTR sequences through their Alu elements in Staufen-mediated manner [74]. A non-coding transcript that shares a high degree of homology with a coding gene is likely to share many of its MREs and therefore pseudogenes are considered as good candidates to act as ceRNAs [7, 75, 76]. Example of such lncRNA include a pseudogene homologous to the gene encoding tumor suppressor phosphatase and tensin homolog (PTEN), which contains multiple MREs with in the 3′ UTR shared with the coding gene [76].

4.2.3. LncRNA-mediated and miRNA-independent mRNA degradation

In addition to regulating gene expression through interaction with miRNAs, some lncRNAs directly targets mRNA for degradation. For example, Staufen 1 (STAU1) is a protein that recognizes a specific motif in the 3′ UTR of mRNAs and mediates their degradation by nonsense mediated mRNA decay (NMD) [77]. STAU1 binds to a double-stranded RNA motif within the 3′ UTR of the mRNA encoding ADP-ribosylation factor 1 (ARF1), where it forms a stem loop structure. However, some mRNAs targeted by Staufen-mediated decay, lacks the stem loop structure and contains only a single stranded binding site within the 3′ UTR, e.g., serpin peptidase inhibitor-clade E member1 (SERPINE1). Interestingly, such mRNAs are targeted by a lncRNA carrying a complementary single stranded binding site and imperfect binding of lncRNA to the mRNA create a double-stranded RNA binding motif for STAU1. This class of lncRNAs are called as half STAU1 binding site RNAs [74].

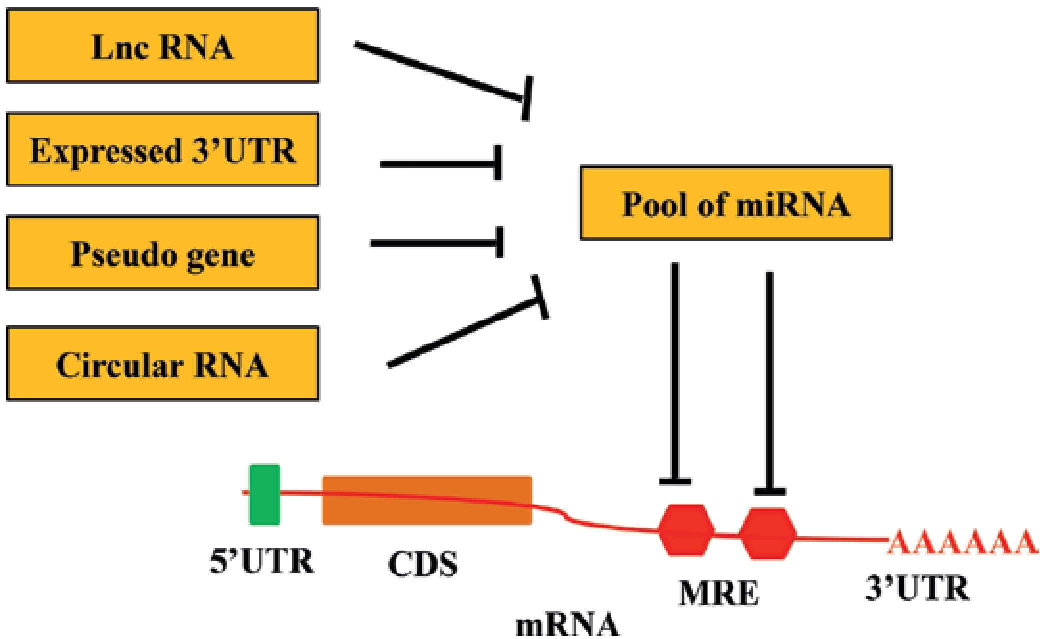

Figure 7. The ceRNA hypothesis. miRNA binds to identical MREs (hexagonal) which are usually present in a number of ncRNA species such as pseudogenes, circRNAs and other forms of lncRNAs and independently transcribed mRNA 3′UTRs. All of these RNAs compete for a limited pool of miRNA, thus positively regulating gene expression.

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5. Roles of LncRNA in diseases

5.1. LncRNAs and aging

Aging is a complex physiological phenomenon with a progressive decline in functional capacities and environmental adaptations. The expression of lncRNAs is known to be affected during aging process and in turn, many lncRNAs govern major senescent pathways and senescence-associated secretory phenotype [78–80]. In human fibroblasts, senescence-associated lncRNA-SAL-RNA1 delays senescence and reduced levels of this lncRNA enhances senescence traits such as enlarged morphology, increased p53 levels and positive β-galactosidase activity [81]. Another example is the lncRNA MIR31HG, which is upregulated in oncogene-induced senescence, and its knockdown promotes a strong tumor-suppressor p16-dependent senescence phenotype [82].

5.2. LncRNAs in cancer and other diseases

Altered lncRNA function is identified as one of the causes for the dysregulation of gene expression which leads to several human diseases including cancer. One such lncRNA is MALAT1 also known as NEAT2, (nuclear-enriched abundant transcript2) which was identified as a predictive biomarker for metastasis development in lung cancer [83, 84]. It acts by inducing the expression of metastasis-associated genes [85]; and recently it was shown that in vitro metastasis of EBC-1 cells (human lung cancer cells) can be inhibited by antisense oligonucleotides directed to MALAT1 [85, 86]. Another example is lncRNA HOTAIR that interacts with PRC2 and alters chromatin to a metastasis-promoting state [87]; and causes cancers such as breast, colon, colorectal, gastrointestinal, pancreatic and liver cancer [88–91]. The lncRNAs αHIF (antisense to hypoxia inducible factor α (HIFα)) and tie-1AS (tyrosine kinase containing immunoglobulin and epidermal growth factor homology domain-1 antisense) are known to induce angiogenesis [42, 92]. PCGEM1 (prostate-specific transcript 1), UCA1 (urothelial cancer associated 1), SPRY4-IT1 (SPRY4 intronic transcript 1) and PANDA are involved in suppressing apoptosis [93–95]. LncRNAs also have roles in other diseases like neurogenetic Angelman syndrome and Beckwith-Wiedemann syndrome (BWS) [96]. LncRNAs have also been associated with cardiovascular diseases and other neurological disorders such as BACE1-AS or BC200 in Alzheimer disease, HAR1 (human accelerated region 1 lncRNA) in Huntington disease and ATXN8OS (Ataxin8 opposite strand lncRNA) in spinocerebellar ataxia type 8 [96–98].

6. Conclusion

The highly diverse biological functions of lncRNAs reflect the versatility of RNA molecules in the cell. Studies on different classes of ncRNAs, their biogenesis and functional overlaps suggest their complexity and their ability to operate as an integrated and regulated network. In this chapter, we have highlighted different mechanisms of regulation of gene expression by lncRNAs at transcriptional and post-transcriptional level by their ability to interact with

enhancers, promoters, chromatin-modifying complexes and miRNAs. Due to environmental exposures, genetic mutations and other causes, deregulation of lncRNAs are associated with various human diseases such as cancer, neurological disorders like Alzheimer's disease, cardiovascular diseases, and autoimmune diseases. This chapter along with recent evidences emphasizes the significance of lncRNA as novel therapeutic targets in aging and aging-related human diseases.

7. Future perspectives

Mounting evidences suggest significant roles of ncRNAs in physiological and pathological processes, which have expanded our basic understanding of gene expression. However, on the other hand, we have also realized the increasing complexity in the structure and organization of genome and gene networks. Recently, our laboratory identified a novel non-coding RNA of DNMT3B variant (DNMT3B9) from leukemic cell lines and the exact roles in hematopoiesis study is underway. This chapter recommends future research on the structural motifs and gene regulatory network of ncRNAs and their stability and degradation process, which we believe will expand the horizons of ncRNAs biology to establish potential diagnostic and therapeutic strategies in this field. Another challenging avenue is to explore the mechanisms underlying the functions of ncRNAs, which still remain elusive. Also, studies on the interplay between various ncRNAs might shed light on the usage of ncRNAs as potential biomarkers for early detection and improve the treatment of various diseases including cancer. With increasing discovery of ncRNAs and advancing technologies, ncRNA based therapies would be an effective health-care strategy.

Acknowledgements

LS and TP thank Pondicherry University for their doctoral research fellowship. SP acknowledges DST-SERB-NPDF (2739) for providing post-doctoral research assistance and CA is a recipient of CSIR-UGC fellowship for doctoral research. Prof. SB thank DST-SERB, INDIA (SB/EMEQ-038/2013) for financial support.

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References

[1] Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: Insights into functions. Nature Reviews Genetics. 2009 Mar;**10**(3):155

[2] Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell. 2004 Jan;**116**(2):281-297

[3] Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. Nature Cell Biology. 2009 Mar;**11**(3):228

[4] Siomi MC, Sato K, Pezic D, Aravin AA. PIWI-interacting small RNAs: The vanguard of genome defence. Nature Reviews Molecular Cell Biology. 2011 Apr;**12**(4):246

[5] Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, Cabili MN. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature. 2009 Mar;**458**(7235):223

[6] Hangauer MJ, Vaughn IW, McManus MT. Pervasive transcription of the human genome produces thousands of previously unidentified long intergenic noncoding RNAs. PLoS Genetics. 2013 Jun;**9**(6):e1003569

[7] Milligan MJ, Lipovich L. Pseudogene-derived lncRNAs: Emerging regulators of gene expression. Frontiers in Genetics. 2015 Feb;**5**:476

[8] Chen J, Sun M, Kent WJ, Huang X, Xie H, Wang W, Zhou G, Shi RZ, Rowley JD. Over 20% of human transcripts might form sense–antisense pairs. Nucleic Acids Research. 2004 Jan;**32**(16):4812-4820

[9] Pandey RR, Mondal T, Mohammad F, Enroth S, Redrup L, Komorowski J, Nagano T, Mancini-DiNardo D, Kanduri C. Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. Molecular Cell. 2008 Oct;**32**(2):232-246

[10] Pastori C, Peschansky VJ, Barbouth D, Mehta A, Silva JP, Wahlestedt C. Comprehensive analysis of the transcriptional landscape of the human FMR1 gene reveals two new long noncoding RNAs differentially expressed in Fragile X syndrome and Fragile X-associated tremor/ataxia syndrome. Human Genetics. 2014 Jan;**133**(1):59-67

[11] Geisler S, Coller J. RNA in unexpected places: Long non-coding RNA functions in diverse cellular contexts. Nature Reviews Molecular Cell Biology. 2013 Nov;**14**(11):699

[12] Moazed D. Small RNAs in transcriptional gene silencing and genome defence. Nature. 2009 Jan;**457**(7228):413

[13] Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005 Jan;**120**(1):15-20

[14] Esteller M. Non-coding RNAs in human disease. Nature Reviews Genetics. 2011 Dec;**12**(12):861

[15] Jovanovic M, Hengartner MO. miRNAs and apoptosis: RNAs to die for. Oncogene. 2006 Oct;**25**(46):6176

[16] Büssing I, Slack FJ, Großhans H. Let-7 microRNAs in development, stem cells and cancer. Trends in Molecular Medicine. 2008 Sep;**14**(9):400-409

[17] Schickel R, Boyerinas B, Park SM, Peter ME. MicroRNAs: Key players in the immune system, differentiation, tumorigenesis and cell death. Oncogene. 2008 Oct;**27**(45):5959

[18] Hrdlickova B, de Almeida RC, Borek Z, Withoff S. Genetic variation in the non-coding genome: Involvement of micro-RNAs and long non-coding RNAs in disease. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2014 Oct;**1842**(10):1910-1922

[19] Sun W, Julie Li YS, Huang HD, Shyy JY, Chien S. microRNA: A master regulator of cellular processes for bioengineering systems. Annual Review of Biomedical Engineering. 2010 Aug;**12**:1-27

[20] Pasquinelli AE. MicroRNAs and their targets: Recognition, regulation and an emerging reciprocal relationship. Nature Reviews Genetics. 2012 Apr;**13**(4):271

[21] Förstemann K, Horwich MD, Wee L, Tomari Y, Zamore PD. Drosophila microRNAs are sorted into functionally distinct argonaute complexes after production by dicer-1. Cell. 2007 Jul;**130**(2):287-297

[22] Tomari Y, Du T, Zamore PD. Sorting of Drosophila small silencing RNAs. Cell. 2007 Jul;**130**(2):299-308

[23] Ameres SL, Horwich MD, Hung JH, Xu J, Ghildiyal M, Weng Z, Zamore PD. Target RNA–directed trimming and tailing of small silencing RNAs. Science. 2010 Jun;**328**(5985):1534-1539

[24] Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nature Reviews Genetics. 2010 Sep;**11**(9):597

[25] Bartel DP. MicroRNAs: Target recognition and regulatory functions. Cell. 2009 Jan;**136**(2):215-233

[26] Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. The 21-nucleotide let-7 RNA regulates developmental timing in caenorhabditis elegans. Nature. 2000 Feb;**403**(6772):901

[27] Wang WX, Wilfred BR, Xie K, Jennings MH, Hu Y, Stromberg AJ, Nelson P. Individual microRNAs (miRNAs) display distinct mRNA targeting "rules". RNA Biology. 2010 May;**7**(3):373-380

[28] Lopez-Serra P, Esteller M. DNA methylation-associated silencing of tumor-suppressor microRNAs in cancer. Oncogene. 2012 Mar;**31**(13):1609

[29] Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L. Frequent deletions and down-regulation of micro-RNA

genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proceedings of the National Academy of Sciences. 2002 Nov;**99**(24):15524-15529

[30] Harrow J, Frankish A, Gonzalez JM, Tapanari E, Diekhans M, Kokocinski F, Aken BL, Barrell D, Zadissa A, Searle S, Barnes I. Gencode: The reference human genome annotation for the Encode project. Genome Research. 2012 Sep;**22**(9):1760-1774

[31] Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J. The Gencode v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. Genome Research. 2012 Sep;**22**(9):1775-1789

[32] Guttman M, Russell P, Ingolia NT, Weissman JS, Lander ES. Ribosome profiling provides evidence that large noncoding RNAs do not encode proteins. Cell. 2013 Jul;**154**(1):240-251

[33] Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Molecular Cell. 2011 Sep;**43**(6):904-914

[34] Karlsson O, Baccarelli AA. Environmental health and long non-coding RNAs. Current Environmental Health Reports. 2016 Sep;**3**(3):178-187

[35] Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, Rinn JL. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes & Development. 2011 Sep;**25**(18):1915-1927

[36] Novikova IV, Hennelly SP, Sanbonmatsu KY. Sizing up long non-coding RNAs: Do lncRNAs have secondary and tertiary structure? BioArchitecture. 2012 Nov;**2**(6):189-199

[37] Alam T, Medvedeva YA, Jia H, Brown JB, Lipovich L, Bajic VB. Promoter analysis reveals globally differential regulation of human long non-coding RNA and protein-coding genes. PLoS One. 2014 Oct;**9**(10):e109443

[38] Popadin K, Gutierrez-Arcelus M, Dermitzakis ET, Antonarakis SE. Genetic and epigenetic regulation of human lincRNA gene expression. The American Journal of Human Genetics. 2013 Dec;**93**(6):1015-1026

[39] Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F, Xue C. Landscape of transcription in human cells. Nature. 2012 Sep;**489**(7414):101

[40] Kapusta A, Kronenberg Z, Lynch VJ, Zhuo X, Ramsay L, Bourque G, Yandell M, Feschotte C. Transposable elements are major contributors to the origin, diversification, and regulation of vertebrate long noncoding RNAs. PLoS Genetics. 2013 Apr;**9**(4):e1003470

[41] Nekrutenko A, Li WH. Transposable elements are found in a large number of human protein-coding genes. Trends in Genetics. 2001 Nov;**17**(11):619-621

[42] Thrash-Bingham CA, Tartof KD. aHIF: A natural antisense transcript overexpressed in human renal cancer and during hypoxia. Journal of the National Cancer Institute. 1999 Jan;**91**(2):143-151

[43] Lapidot M, Pilpel Y. Genome-wide natural antisense transcription: Coupling its regulation to its different regulatory mechanisms. EMBO Reports. 2006 Dec;**7**(12):1216-1222

[44] Faghihi MA, Wahlestedt C. Regulatory roles of natural antisense transcripts. Nature Reviews Molecular Cell Biology. 2009 Sep;**10**(9):637

[45] Chen J, Sun M, Hurst LD, Carmichael GG, Rowley JD. Genome-wide analysis of coordinate expression and evolution of human cis-encoded sense-antisense transcripts. Trends in Genetics. 2005 Jun;**21**(6):326-329

[46] Khalil AM, Faghihi MA, Modarresi F, Brothers SP, Wahlestedt C. A novel RNA transcript with antiapoptotic function is silenced in fragile X syndrome. PLoS One. 2008 Jan;**3**(1):e1486

[47] Rapicavoli NA, Poth EM, Zhu H, Blackshaw S. The long noncoding RNA Six3OS acts in trans to regulate retinal development by modulating Six3 activity. Neural Development. 2011 Dec;**6**(1):32

[48] Whitehead J, Pandey GK, Kanduri C. Regulation of the mammalian epigenome by long noncoding RNAs. Biochimica et Biophysica Acta (BBA)-General Subjects. 2009 Sep; **1790**(9):936-947

[49] Bernstein E, Allis CD. RNA meets chromatin. Genes & Development. 2005 Jul;**19**(14): 1635-1655

[50] Kornienko AE, Guenzl PM, Barlow DP, Pauler FM. Gene regulation by the act of long non-coding RNA transcription. BMC Biology. 2013 Dec;**11**(1):59

[51] Minks J, Baldry SE, Yang C, Cotton AM, Brown CJ. XIST-induced silencing of flanking genes is achieved by additive action of repeat a monomers in human somatic cells. Epigenetics & Chromatin. 2013 Dec;**6**(1):23

[52] Noble D. Physiology is rocking the foundations of evolutionary biology. Experimental Physiology. 2013 Aug;**98**(8):1235-1243

[53] Mattick JS, Taft RJ, Faulkner GJ. A global view of genomic information–moving beyond the gene and the master regulator. Trends in Genetics. 2010 Jan;**26**(1):21-28

[54] Li W, Notani D, Rosenfeld MG. Enhancers as non-coding RNA transcription units: Recent insights and future perspectives. Nature Reviews Genetics. 2016 Apr;**17**(4):207

[55] Kim TK, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, Harmin DA, Laptewicz M, Barbara-Haley K, Kuersten S, Markenscoff-Papadimitriou E. Widespread transcription at neuronal activity-regulated enhancers. Nature. 2010 May;**465**(7295):182

[56] Andersson R, Gebhard C, Miguel-Escalada I, Hoof I, Bornholdt J, Boyd M, Chen Y, Zhao X, Schmidl C, Suzuki T, Ntini E. An atlas of active enhancers across human cell types and tissues. Nature. 2014 Mar;**507**(7493):455

[57] Ørom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Bussotti G, Lai F, Zytnicki M, Notredame C, Huang Q, Guigo R. Long noncoding RNAs with enhancer-like function in human cells. Cell. 2010 Oct;**143**(1):46-58

[58] Yang Y, Su Z, Song X, Liang B, Zeng F, Chang X, Huang D. Enhancer RNA-driven looping enhances the transcription of the long noncoding RNA DHRS4-AS1, a controller of the DHRS4 gene cluster. Scientific Reports. 2016 Feb;**6**:20961

[59] Lai F, Orom UA, Cesaroni M, Beringer M, Taatjes DJ, Blobel GA, Shiekhattar R. Activating RNAs associate with mediator to enhance chromatin architecture and transcription. Nature. 2013 Feb;**494**(7438):497

[60] Malik S, Roeder RG. The metazoan mediator co-activator complex as an integrative hub for transcriptional regulation. Nature Reviews Genetics. 2010 Nov;**11**(11):761

[61] Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY. Long noncoding RNA as modular scaffold of histone modification complexes. Science. 2010 Aug;**329**(5992):689-693

[62] Luco RF, Pan Q, Tominaga K, Blencowe BJ, Pereira-Smith OM, Misteli T. Regulation of alternative splicing by histone modifications. Science. 2010 Feb;**327**(5968):996-1000

[63] Wood AJ, Oakey RJ. Genomic imprinting in mammals: Emerging themes and established theories. PLoS Genetics. 2006 Nov;**2**(11):e147

[64] Bartolomei MS. Genomic imprinting: Employing and avoiding epigenetic processes. Genes & Development. 2009 Sep;**23**(18):2124-2133

[65] Wan LB, Bartolomei MS. Regulation of imprinting in clusters: Noncoding RNAs versus insulators. Advances in Genetics. 2008 Jan;**61**:207-223

[66] Dykes IM, Emanueli C. Transcriptional and post-transcriptional gene regulation by long non-coding RNA. Genomics, Proteomics & Bioinformatics. 2017 Jun;**15**(3):177-186

[67] Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. Nature Reviews Molecular Cell Biology. 2009 Feb;**10**(2):126

[68] Saini HK, Griffiths-Jones S, Enright AJ. Genomic analysis of human microRNA transcripts. Proceedings of the National Academy of Sciences. 2007 Nov;**104**(45):17719-17724

[69] Thomas M, Lieberman J, Lal A. Desperately seeking microRNA targets. Nature Structural and Molecular Biology. 2010 Oct;**17**(10):1169

[70] Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: The Rosetta Stone of a hidden RNA language? Cell. 2011 Aug;**146**(3):353-358

[71] Dempsey JL, Cui JY. Long non-coding RNAs: A novel paradigm for toxicology. Toxicological Sciences. 2016 Nov;**155**(1):3-21

[72] Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanan M, Evantal N, Memczak S, Rajewsky N, Kadener S. circRNA biogenesis competes with pre-mRNA splicing. Molecular Cell. 2014 Oct;**56**(1):55-66

[73] Ebbesen KK, Kjems J, Hansen TB. Circular RNAs: Identification, biogenesis and function. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms. 2016 Jan; **1859**(1):163-168

[74] Gong C, Maquat LE. LncRNAs transactivate STAU1-mediated mRNA decay by duplexing with 3′ UTRs via Alu elements. Nature. 2011 Feb;**470**(7333):284

[75] An Y, Furber KL, Ji S. Pseudogenes regulate parental gene expression via ceRNA network. Journal of Cellular and Molecular Medicine. 2017 Jan;**21**(1):185-192

[76] Poliseno L, Salmena L, Zhang J, Carver B, Haveman WJ, Pandolfi PP. A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. Nature. 2010 Jun;**465**(7301):1033

[77] Park E, Maquat LE. Staufen-mediated mRNA decay. Wiley Interdisciplinary Reviews: RNA. 2013 Jul;**4**(4):423-435

[78] Abdelmohsen K, Gorospe M. Noncoding RNA control of cellular senescence. Wiley Interdisciplinary Reviews: RNA. 2015 Nov;**6**(6):615-629

[79] Costa MC, Leitão AL, Enguita FJ. Noncoding transcriptional landscape in human aging. In: Long Non-coding RNAs in Human Disease. Cham: Springer; 2015. pp. 177-202

[80] Grammatikakis I, Panda AC, Abdelmohsen K, Gorospe M. Long noncoding RNAs (lncRNAs) and the molecular hallmarks of aging. Aging (Albany NY). 2014 Dec;**6**(12):992

[81] Abdelmohsen K, Panda A, Kang MJ, Xu J, Selimyan R, Yoon JH, Martindale JL, De S, Wood WH, Becker KG, Gorospe M. Senescence-associated lncRNAs: Senescence-associated long noncoding RNAs. Aging Cell. 2013 Oct;**12**(5):890-900

[82] Montes M, Nielsen MM, Maglieri G, Jacobsen A, Højfeldt J, Agrawal-Singh S, Hansen K, Helin K, Van De Werken HJ, Pedersen JS, Lund AH. The lncRNA MIR31HG regulates p16 INK4A expression to modulate senescence. Nature Communications. 2015 Apr;**6**:6967

[83] Ji P, Diederichs S, Wang W, Böing S, Metzger R, Schneider PM, Tidow N, Brandt B, Buerger H, Bulk E, Thomas M. MALAT-1, a novel noncoding RNA, and thymosin β4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene. 2003 Sep;**22**(39):8031

[84] Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, Freier SM, Bennett CF, Sharma A, Bubulya PA, Blencowe BJ. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. Molecular Cell. 2010 Sep;**39**(6):925-938

[85] Gutschner T, Hämmerle M, Eißmann M, Hsu J, Kim Y, Hung G, Revenko A, Arun G, Stentrup M, Groß M, Zörnig M. The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. Cancer Research. 2013 Feb;**73**(3):1180-1189

[86] Gutschner T, Hämmerle M, Diederichs S. MALAT1—A paradigm for long noncoding RNA function in cancer. Journal of Molecular Medicine. 2013 Jul;**91**(7):791-801

[87] Eißmann M, Gutschner T, Hämmerle M, Günther S, Caudron-Herger M, Groß M, Schirmacher P, Rippe K, Braun T, Zörnig M, Diederichs S. Loss of the abundant nuclear

non-coding RNA MALAT1 is compatible with life and development. RNA Biology. 2012 Aug;**9**(8):1076-1087

[88] Kim K, Jutooru I, Chadalapaka G, Johnson G, Frank J, Burghardt R, Kim S, Safe S. HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. Oncogene. 2013 Mar;**32**(13):1616

[89] Kogo R, Shimamura T, Mimori K, Kawahara K, Imoto S, Sudo T, Tanaka F, Shibata K, Suzuki A, Komune S, Miyano S. Long non-coding RNA HOTAIR regulates Polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Research. 2011 Oct;**71**(20):6320-6326

[90] Niinuma T, Suzuki H, Nojima M, Nosho K, Yamamoto H, Takamaru H, Yamamoto E, Maruyama R, Nobuoka T, Miyazaki Y, Nishida T. Upregulation of miR-196a and HOTAIR drive malignant character in gastrointestinal stromal tumors. Cancer Research. 2012 Mar;**72**(5):1126-1136

[91] Yang Z, Zhou L, Wu LM, Lai MC, Xie HY, Zhang F, Zheng SS. Overexpression of long non-coding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. Annals of Surgical Oncology. 2011 May;**18**(5):1243-1250

[92] Li K, Blum Y, Verma A, Liu Z, Pramanik K, Leigh NR, Chun CZ, Samant GV, Zhao B, Garnaas MK, Horswill MA. A noncoding antisense RNA in tie-1 locus regulates tie-1 function in vivo. Blood. 2010 Jan;**115**(1):133-139

[93] Fu X, Ravindranath L, Tran N, Petrovics G, Srivastava S. Regulation of apoptosis by a prostate-specific and prostate cancer-associated noncoding gene, PCGEM1. DNA and Cell Biology. 2006 Mar;**25**(3):135-141

[94] Tsang WP, Wong TW, Cheung AH, Kwok TT. Induction of drug resistance and transformation in human cancer cells by the noncoding RNA CUDR. RNA. 2007 Jun;**13**(6):890-898

[95] Khaitan D, Dinger ME, Mazar J, Crawford J, Smith MA, Mattick JS, Perera RJ. The melanoma-upregulated long noncoding RNA SPRY4-IT1 modulates apoptosis and invasion. Cancer Research. 2011 Jun;**71**(11):3852-3862

[96] Batista PJ, Chang HY. Long noncoding RNAs: Cellular address codes in development and disease. Cell. 2013 Mar;**152**(6):1298-1307

[97] Johnson R, Richter N, Jauch R, Gaughwin PM, Zuccato C, Cattaneo E, Stanton LW. Human accelerated region 1 noncoding RNA is repressed by REST in Huntington's disease. Physiological Genomics. 2010 Feb;**41**(3):269-274

[98] Daughters RS, Tuttle DL, Gao W, Ikeda Y, Moseley ML, Ebner TJ, Swanson MS, Ranum LP. RNA gain-of-function in spinocerebellar ataxia type 8. PLoS Genetics. 2009 Aug;**5**(8):e1000600

MicroRNAs in Bone Diseases: Progress and Prospects

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79275

Abstract

With 19–25 nucleotides long, microRNAs (miRNAs) are small noncoding RNA molecules which play crucial roles in major cellular functions such as cell cycle control, apoptosis, metabolism, cell proliferation, and cell differentiation. Changes in the expression of miRNAs can cause significant effects to normal and aberrant cells. The dysregulation of miRNAs has been implicated in various human diseases such as brain tumor, osteoarthritis, schizophrenia, and breast cancer. Generally, miRNAs negatively regulate gene expression by binding to their specific mRNAs, thereby blocking their translation of the mRNAs. However, a few studies have reported that miRNAs could also upregulate the translation of certain proteins. This shows the important roles of miRNAs in various cell functions. This chapter will focus on the role of miRNAs in normal osteoblast and osteosarcoma cells. In addition, the great potential of miRNA as a new therapeutic approach to treat human bone diseases will also be discussed.

Keywords: microRNAs, bone diseases, osteoblasts, osteoclasts, bone homeostasis, gene regulation

1. Introduction

MicroRNAs (miRNAs) are short (19–25 nucleotides) single-stranded noncoding RNA molecules that regulate protein expression by complementary binding to mRNA targets with the aid of RNA-induced silencing complex (RISC) [1]. When miRNAs pair perfectly with mRNA targets, mRNAs degradation will occur. Translational repression of gene will happen when miRNAs bind partially complementary to mRNA targets [2]. Since the discovery of the first miRNA, lin-4 in *Caenorhabditis elegans* in 1993, thousands of miRNAs have been identified in animals and plants [3]. These miRNAs play crucial roles in biological processes such

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as cell growth, cell formation and differentiation, apoptosis, and cell metabolism. MiRNAs also regulate bone cells such as osteoblasts, osteoclasts, and osteocytes, which function in the mechanism of bone modeling and bone remodeling [4]. Osteoblasts play important roles in bone formation and osteoclasts function in bone resorption, whereas osteocytes regulate osteoblasts and osteoclasts activities by controlling signaling pathways [4] (**Figure 1**). Expression of many miRNAs has been found to be upregulated or downregulated in bone cancer cells compared to normal bone cells. Some of these miRNAs act as oncogenes such as miR-27, which promote the migration and invasion ability in the osteosarcoma [5]. Some other miRNAs act as tumor repressor genes such as miR-192 and miR-215, which play major roles in cell cycle arrest in cancer cells [6]. Dysregulation of miRNA expression by specific translation regulation such as DNA methylation, which leads to miRNA silencing, has been associated with bone diseases such as osteoporosis, osteogenesis imperfecta, and osteoarthritis [4, 7]. Therefore, understanding the roles of miRNAs in bone cells will provide the opportunity to develop miRNA-based therapy for bone diseases. In this chapter, we highlight the roles of various miRNAs that involve in the formation, resorption, and maintenance of bone in various bone diseases.

2. Regulatory role of microRNAs in normal bone growth and maintenance

Bone is a dynamic tissue that undergoes constant processes of modeling and remodeling throughout life. Bone modeling is the process where bones resculpture or rechange its overall size and shape as an adaptive mechanism against physiological processes or biomechanical influences, customizing or adjusting the skeleton toward the actions in which it encounters [8]. Bones may widen, change axis, or alter curvature by an independent action of osteoblasts and osteoclasts in response to biomechanical forces [9]. Bone modeling aids in the prevention of damage or injury to the bone [10] and regulates growth phase such as facilitating the increase in a child's skull size to accommodate the bigger brain as a child grows and undergoes marked change in the facial features of a child to that as an adult [11].

Meanwhile, bone remodeling is a sequential process, which involves the removal of the old bone (bone resorption) and the deposition of new bone (bone formation) [10, 12]. This process is ultimately important for the maintenance of the bone's strength and integrity by modulating the reshaping or replacement of bone during growth, preventing the accumulation of bone microdamage and regulating mineral homeostasis [8]. Bone remodeling is a lifelong, bone turnover [13] that is tightly regulated by two main population of bone cells: the bone-resorbing osteoclasts of hematopoietic lineage and the bone-forming osteoblasts of mesenchymal lineage [14, 15]. This tightly coupled process requires synchronized activities, balanced by both of these effector cells [8].

MicroRNAs serve as positive and/or negative regulators for various musculoskeletal signaling pathways or mechanisms by regulating bone biology such as in osteoblastic or osteoclastic differentiation, in accordance with the orchestrated balance between bone resorption and

bone formation. MiRNAs are known to be involved in the osteoclast-mediated bone resorption by regulating macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL)-induced signaling pathways, which involved in the commitment of osteoclasts from osteoclast progenitors [16].

2.1. MicroRNA roles in normal bone resorption

The receptor activator of NF-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) signaling pathway are the principle operating pathways that regulate osteoclast differentiation and activation in bone remodeling [16]. These signaling pathways are tightly regulated by microRNAs. Hence, the involvement of miRNAs in the process of osteoclasts differentiation is crucial for normal bone resorption.

MiR-31 has been identified to be significantly upregulated in mice bone marrow cells under RANKL-induced osteoclast formation. The suppression of miR-31 by specific antagomirs under receptor activator of NF-κB ligand (RANKL) treatment decreased the number of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated osteoblastic cells and ring-shaped mature osteoclastic cells. Additionally, less efficient resorption of synthetic calcium phosphate matrix and impaired actin ring formation for the development bone resorption sealing zone were also reported following the miR-31 antagomir transfection. In this situation of impaired osteoclastogenesis, the Ras homolog gene family member A (RhoA), which is the target of miR-31, was upregulated. Interestingly, treatment with RhoA inhibitor, coenzyme C_3 was able to rescue the decrease in number of ring-shaped TRAP-positive multinucleated cells and potentially revert the osteoclastogenesis impairment [17].

During the late stage of osteoclastogenesis, the osteoclasts undergo apoptosis to allow the reversal phase of bone remodeling, which allows the transition of bone resorption to bone formation [8]. At this stage, there is a significant upregulation of miR-26a under RANKL stimulation. Treatment with an miR-26a mimic in preosteoclast cells (pre-OCs) significantly inhibited the formation of osteoclast, peripheral actin ring, and resorption pit, whereas treatment with miR-26a inhibitor dramatically reversed these observations. The study proposed that miR-26a suppressed osteoclasts formation in the late stage of bone remodeling by targeting connective tissue growth factor/CCN family 2 (CTGF/CCN2), which plays an important role in promoting osteoclast formation via upregulation of dendritic cell-specific transmembrane protein (DC-STAMP) [18].

Another miRNA, miR-21 has been shown to be upregulated by RANKL-induced osteoclastogenesis in mouse osteoclast precursor cells' bone marrow-derived macrophages (BMMs). MiR-21 downregulates the expression of programmed cell death 4 (PDCD4), which is a repressor for c-Fos. The activated c-Fos, an important transcription factor for osteoclastogenesis, allows RANKL to induce nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) mRNA expression and stimulates osteoclast-specific markers such as tartrate-resistant acid phosphatase (TRAP) and cathepsin K. The silencing of miR-21 by transduction of BMMs with antisense oligonucleotides of miR-21 inserted in a lentiviral vector increased the expression of PDCD4 and impaired the RANKL-induced osteoclastogenesis [19].

In another study, the overexpression of miR-148a was observed during M-CSF and RANKL-stimulated osteoclast differentiation in CD14+ peripheral blood mononuclear cell (PBMCs). The overexpression of miR-148a induced the formation of osteoclast, whereas suppression of miR-148a showed an opposite outcome. *In vivo* study using ovariectomized (OVX) mice that undergoes intravenous injection with specific miR-148a silencing antagomir showed reduction in bone resorption and increase in bone mass density (BMD). Furthermore, osteoclasts number and the levels of osteoclast activity markers such as tryptophan-regulated attenuation protein (TRAP) and nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) mRNA in bone tissue were also decreased following antagomiR-148a treatment. This finding shows that decreased miR-148a levels impaired bone resorption through suppression of osteoclast activity. MiR-148a performs its regulatory role by targeting 3′UTR of V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB), a negative regulator of osteoclastogenesis and resulted in an inhibited expression of MAFB protein [20]. Additionally, MAFB serves as a negative regulator in RANKL-induced osteoclastogenesis by interfering the DNA binding capability of the three major transcription factors; NFATc1, c-Fos, and MITF in osteoclast differentiation [21].

The relative expression of miR-340 was downregulated upon M-CSF and RANKL-induced osteoblast differentiation in BMMs. It has been reported that the overexpression of miR-340 inhibits osteoclast differentiation and reduced the number of osteoclasts cells by targeting 3′UTR of microphthalmia-associated transcription factor (MITF), a transcription factor involved in osteoclast differentiation, leading to the reduced level of MITF mRNA and protein. MITF knockdown will inhibit TRAP, calcitonin receptor, V-ATPase d2, and cathepsin K expression, and thus, suggested that miR-340 may suppress osteoclast differentiation by targeting MITF [22].

2.2. MicroRNAs in normal bone formation

Canonical wnt/β-catenin signaling pathway is a major pathway required for the commitment of mesenchymal stem cells into osteoblast lineage [15]. The stabilization of β-catenin is important for the expression of wnt-responsive gene [16]. The miR-29 family is one of the well-known miRNA families that regulate osteoblast function, which plays a key role in the positive regulation of osteoblast differentiation by targeting several wnt-signaling pathway inhibitors. The expression of miR-29a is induced by canonical wnt signaling during osteoblast differentiation and has been shown to target dikkopf-1 (Dkk1), Kringle domain-containing transmembrane protein (Kremen2), and secreted frizzled related protein 2 (sFRP2), which acts as inhibitors of wnt receptor complex [23]. Moreover, miR-29b was also found to target several other inhibitors of bone formation such as histone deacetylase 4 (HDAC4), transforming growth factor beta 3 (TGFβ3), activin receptor type-2A (AcvR2A), beta-catenin-interacting protein 1 (CTNNBIP1), and dual specific phosphatase 2 (DUSP2) by binding to their mRNA 3′UTR [24]. Furthermore, the expression level of miR-29 is low during the early phase of osteoblastogenesis and increases at late phase, as miR-29 targets α1 and α2(I)collagen, α1(III)collagen, fibrillin 1, and osteonectin, which are important for the formation of collagen fibril matrix secreted by osteoblasts, and thus allowed for collagen matrix deposition before subsequent mineralization in bone formation process [25]. Therefore, miR-29 family is important in the promotion of osteoblastogenesis by repressing the inhibitors of osteogenesis and in the meanwhile plays crucial regulatory role in the attenuation of collagen synthesis in mineralized bone.

On the other hand, bone morphogenetic protein (BMP)-signaling pathway is crucial for the differentiation of myoblastic cell lines into osteoblast lineage and bone formation [16]. MiR-133 and miR-135 are downregulated in BMP-2-induced osteoblastic differentiation of C2C12 pluripotent mesenchymal cell line. MiR-133 is a negative regulator of Runt-related transcription factor 2 (Runx2), a transcription factor required for osteoblast differentiation, while miR-135 represses the osteoblastic differentiation of C2C12 cells by acting toward mothers against decapentaplegic homolog 5 (Smad5), an intracellular Runx2 co-receptor. Hence, downregulation of miR-133 and miR-135 will increase the expression of Runx2 and Smad5, promoting the BMP-2-induced osteoblast differentiation. Moreover, the overexpression of these miRNAs will suppress the expression of BMP-induced osteoblast-specific protein markers such as alkaline phosphatase (ALP), osteocalcin, and homeobox A10 (HOXA10) [26]. Another miRNA, miR-20 has been shown to involve in the transformation of osteoblast from human MSCs by downregulating the expression of silencing peroxisome proliferator-activated receptor γ (PPARγ), bone morphogenetic protein and activin membrane-bound inhibitor (Bambi), and cysteine-rich transmembrane BMP regulator 1 (Crim1) and therefore, activated the BMP-2/Runx2 signaling [27].

Another report showed that miR-2861 expression was elevated in primary mice osteoblasts. Overexpression of miR-2861 in mice bone marrow stromal cells (BMSCs) has been reported to promote BMP2-induced osteoblast differentiation. Conversely, the inhibition of miR-2861 expression results in the decrease in osteoblast differentiation. *In vivo* knockdown of miR-2861 in OVX mice resulted in enhanced decrement of bone volume and bone formation rate. Furthermore, histone deacetylase 5 (HDAC5) has been identified as the direct target of miR-2861. HDAC5 deacetylates Runx2 and allow the deacetylated Runx2 to undergo SMAD specific E3 ubiquitin protein ligase 1 (Smurf1)-mediated degradation, decreasing the rate of osteoblast differentiation. Therefore, the abundance of acetylated Runx2 will increase upon HDAC5 suppression by miR-2861 and promote osteoblast differentiation [28]. MiR-3960 is generated

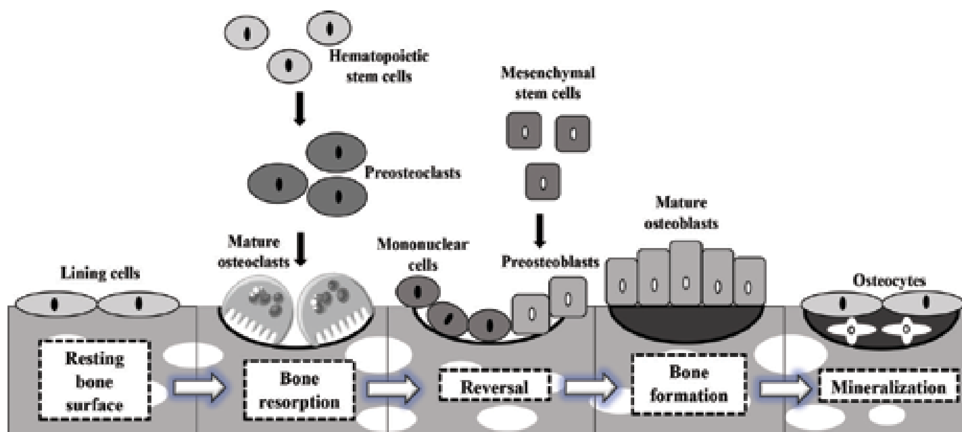

Figure 1. The process of bone remodeling begins with the recruitment of osteoclast progenitor cells to the site of bone remodeling, followed by osteoclast progenitor cells differentiation into mature osteoclasts. Reversal phase allows the transition from bone resorption phase to bone formation phase. In bone formation phase, mesenchymal stem cells differentiate into mature osteoblast and secrete collagenous components for bone formation. Bone remodeling process is completed after the mineralization of collagen fibril matrix and subsequent transformation of osteoblasts into osteocytes.

from the same genetic locus as the miR-2861 due to the transcription from the same primary microRNA (pri-miRNA). MiR-3960 was found to directly target homeobox A2 (HOXA2), a negative regulator of Runx2. Hence, the miR-3960-mediated suppression of HOXA2 by miR-3960 will increase Runx2 expression and osteoblast differentiation [29]. The summary of the MicroRNAs involved in the regulation of normal bone development is shown in **Table 1**.

MicroRNAs	**Target gene or protein encoded**	**Associated event**	**Reference**
MicroRNAs associated with bone resorption			
MiR-31	RhoA	Promotes osteoclast differentiation by targeting RhoA	[17]
MiR-26a	CTGF/CCN2	Inhibits osteoclast differentiation by targeting CTGF/CCN2	[18]
MiR-21	PDCD4	Promotes osteoclast differentiation by targeting PDCD4	[19]
MiR-148a	MAFB	Promotes osteoclast differentiation by targeting MAFB	[20]
MiR-340	MITF	Inhibits osteoclast differentiation by targeting MITF	[22]
MicroRNAs associated with bone formation			
MiR-29	COL1A1, COL3A1, fibrillin 1, osteonectin	Downregulated during the early phase of bone formation and upregulated during the late phase by targets COL1A1, COL3A1, fibrillin 1, and osteonectin to allow the formation of collagen fibril matrix	[25]
MiR-29a	Dkk1, Kremen2, sFRP2	Promotes osteoblasts differentiation by targeting Dkk1, Kremen2, sFRP2 inhibitors of wnt signaling pathway	[23]
MiR-29b	HDAC4, TGFβ3, AcvR2A, CTNNBIP1, DUSP2	Promotes osteoblasts differentiation by inhibitors of bone formation such as HDAC4, TGFβ3, AcvR2A, CTNNBIP1, and DUSP2	[24]
MiR-133	Runx2	Inhibits osteoblast differentiation by targeting Runx2	[26]
MiR-135	Smad5	Inhibits osteoblast differentiation by targeting Smad5	[26]
MiR-20	PPARγ, Bambi, Crim1	Promotes osteoblast differentiation by targeting PPARγ, Bambi and Crim1	[27]
MiR-2861	HDAC5	Promotes osteoblasts differentiation by targeting HDAC5, which represses Runx2	[28]
MiR-3960	Hoxa5	Promotes osteoblasts differentiation by targeting Hoxa5, which represses Runx2	[29]

Table 1. MicroRNAs involved in the regulation of normal bone development.

3. MicroRNAs' expression in various bone diseases

Dysregulation of miRNAs affects critical pathways and biological processes, which lead to various bone diseases. MiRNA profiling studies have revealed that miRNA expression patterns are specific to various types of bone diseases, and it reflects the developmental lineage and pathway that lead to the diseases.

3.1. Benign bone tumor: giant cell tumor

Giant cell tumor of bone (GCTB) is an aggressive benign tumor that is able to metastasize, and up to 6% of GCTB patients grow pulmonary metastases (metastatic spread via blood or lymphatics) [30]. GCTBs are characterized by the presence of numerous multinucleated osteoclast-like giant cells distributed among mononuclear stromal cells [31]. GCTBs are also characterized by extensive bone resorption, which results in regional pain and bone destruction, mostly occurring in distal femur, proximal tibia, distal radius, and sacrum [32, 33]. Histologically, GCTBs can be classified into three main types, which are osteoclast-like multinucleated giant cells, monocytic round cells, and spindle-like stromal cells [34]. Current treatments of GCTBs are ranging from intralesional curettage to wide resection [33]. Since the cause of GCTBs is extensive bone resorption by aggressive lytic process, the repression of osteoclastogenesis becomes a potential approach to cure GCTBs [30].

A study reveals that treatments with miR-16-5p mimic repressed RANKL-induced osteoclastogenesis in GCTBs. However, the formation of RANKL-induced osteoclast was enhanced with miR-16-5p inhibitor. Furthermore, the osteoclastogenesis-related genes like cathepsin K (CK), tartrate-resistant acidic phosphatase (TRAP), and matrix metallopeptidase 9 (MMP9) were also upregulated by miR-16-5p inhibitor. This finding shows that miR-16-5p inhibits osteoclastogenesis; hence, it has the potential to be used as a therapeutic target to control the excessive bone resorption in GCTBs [30]. Another study by Wang et al. found that miR-106b is another microRNA that target RANKL to inhibit osteoclastogenesis and osteolysis in GCTBs [32].

Parathyroid hormone 1 receptor (PTH1R) is a transmembrane receptor that binds to G proteins. The activation of pathways that promote osteoclastogenesis in osteoblasts is induced when PTH binds to parathyroid hormone 1 receptor (PTH1R). Wu et al. reported that miR-125b directly targets the 3′UTR of PTH1R. Overexpression of tumor suppressor miR-125b inhibits the osteoclastogenesis and also PTH1R downstream target such as RANKL and IL-8 [35]. The downregulation of miR-125b in GCTBs revealed that it suppressed the cell growth and proliferation in GCTBs.

3.2. Bone remodeling abnormality: osteoporosis

Osteoporosis is a multifactorial bone disorder characterized by low bone mass, impaired bone quality, and a more susceptibility to fracture [36]. The recent global statistics from the International Osteoporosis Foundation reported that 1 in 3 women and 1 in 5 men above the

age of 50 will suffer from osteoporotic fractures in their lifetime [37]. The primary osteoporosis is generally arising due to the postmenopausal deficiency or loss of sex hormones such as estrogen, while the secondary osteoporosis is due to the presence of underlying diseases and medication of treatments with glucocorticoids, hyperthyroidism, diabetes mellitus, and gastrointestinal disorders [37, 38]. The bone mineral density peaks during adolescence stage of puberty, which then maintained throughout an individual middle age for some decades and subsequently begins to loss upon aging. Bone tissue undergoes continuous process of resorption and formation throughout in an individual lifetime. Osteoporosis occurs when bone resorption rate exceeds the bone formation rate, resulting in a net loss of bone [39]. Studies revealed that osteoporosis incidences may be linked to bone mass-related genetic determinants including low-density lipoprotein receptor-related protein 5 (LRP5), osteoprotegerin (OPG), sclerostin (SOST), estrogen receptor 1, and the receptor activator of RANK/NF-κB signaling pathway [40].

Receptor activator of nuclear factor kappa-B ligand (RANKL) binds to the receptor activator of nuclear factor κB (RANK) that is present on the surface of the osteoclast mononuclear precursor cells and facilitates the formation of fully differentiated osteoclasts [41]. The expression of mirR-503 is significantly reduced in progenitors of osteoclasts-CD14+ peripheral blood mononuclear cells (PBMCs) of postmenopausal osteoporosis patients compared to healthy postmenopausal controls. The overexpression of miR-503 in human PBMCs had dramatically inhibited RANKL-induced osteoclast differentiation in PBMCs of postmenopausal osteoporosis samples. *In vivo* transfection of miR-503 silencing antagomir into a postmenopausal-stimulated ovariectomized (OVX) mice resulted in the increase in RANK protein expression, an increase of bone resorption rate, a decrease in bone mass, and an aggravation of bone loss. Contrastingly, the transfection of the OVX mice with miR-503 overexpressing pre-miR-503 leads to the decrease in RANK protein expression and thus a decrease in bone resorption and an increase in bone mass. Therefore, it is suggested that the low miR-503 expression in postmenopausal osteoporosis patients will promote RANKL-induced osteoclastogenesis, and consequently, bone resorption rate will increase leading to net bone loss [42].

MiR-221 expression is downregulated in postmenopausal osteoporotic bone samples compared to nonosteoporotic bones. In BMP-2-induced osteoblastogenesis, the overexpression of miR-221 resulted in reduced expression of key osteoblast markers, including osteocalcin (OC), alkaline phosphatase (ALP), and collagen type Iα 1 (COL1A1), whereas knockdown of miR-221 promoted the activity of OC, ALP, and COL1A1 [43]. The later study identified Runx2 as a potential target of miR-221. Therefore, this provided an evidence that miR-221 serves as the negative regulator of osteoblast differentiation and contributes to the osteoporosis pathogenesis through the regulation of Runx2 action [44].

Tumor necrosis factor α (TNF-α) inhibits MSC osteogenic differentiation and bone formation in estrogen deficiency-induced osteoporosis with a poorly understood mechanism. A study conducted by Yang et al. showed that the expression of miR-21 is dramatically downregulated in mesenchymal stem cells (MSCs), and this downregulation is due to the suppression by TNF-α during the osteogenesis of MSCs. Moreover, miR-21 has been proved to stimulate the osteoblast differentiation of MSCs by targeting protein sprouty homolog 1 (Spry1), a negative regulator of osteoblast differentiation from MSCs. The later study also demonstrated that the overexpression of miR-21 is able to partially rescue the osteogenic impairment induced by

TNF-α in MSCs. Furthermore, *in vivo* treatment with anti-TNF-α in OVX mice has increased bone formation by upregulating miR-21 expression, suppressing Spry1 expression and remediating the inflammatory conditions. Thus, this study indicated that TNF-α impairs osteoblastic bone formation by suppressing miR-21 expression in estrogen deficiency-induced osteoporosis [45].

A study conducted by Wang et al. showed that glucocorticoid-treated mice experienced low bone mass density (BMD) and bone mass content (BMC). Glucocorticoid treatment also significantly resulted in the decrease of bone matrix COL1A1 expression, an increase in dickkopf-1 (Dkk-1) expression and a reduction in miR-29a expression [46]. MiR-29a plays important role in osteoblast differentiation and bone homeostasis by regulating the expression of Wnt inhibitor Dkk-1 [23]. *In vivo* miR-29a precursor treatment was able to reduce the glucocorticoid-stimulated BMD and BMC, attenuate glucocorticoid-induced loss of trabecular bone volume fraction, decrease the porosity of cortical bone, and rescue the adverse effect of glucocorticoid on peak load of bone tissue. The treatment with miR-29a inhibitor, however, provided opposite effects [46]. Thus, miR-29a is important in protection against glucocorticoid-induced osteopenia, which may lead to osteoporosis by regulating the activity of Wnt signaling and Dkk-1 in osteoblast differentiation and bone mineralization [23, 47].

3.3. Bone collagen matrix retardation: osteogenesis imperfecta

Osteogenesis imperfecta (OI) is a heterogeneous group of inherited connective tissue disorder that occurs in about 1 in 10,000 to 20,000 live births [47]. OI is characterized with clinical features such as susceptibility to bone fractures due to low bone mass, reduced bone strength, or quality and bone deformity [48]. In addition, blue sclerae, short stature, dentinogenesis imperfecta (DI), and hearing loss are other clinical manifestations of OI [49]. The pathogenesis of OI involves the most prevalent autosomal dominant mutation of COL1A1 and COL1A2 genes encoding the alpha1 and alpha2 chains of type I procollagen [50]. Type I procollagen is the major bone structural protein, and therefore, the mutation of COL1A1 and COL1A2 genes may have direct link with serious defects or abnormalities including deformities of collagen primary structure, insufficient bone collagen quantity, deviated posttranslational modification, folding, intracellular transport or matrix incorporation, and bone mineralization. Recessive OI is caused by defects in genes that encode for protein products, which interact with type I collagen [51]. There are four well characterized types (I, II, III, and IV) of COL1A1/COL1A2-linked OI based on different clinical and genetic presentations [52].

Wang et al. performed the preliminary screening of more than 100 bone-related miRNAs in serum of 22 OI patients. The results showed that three miRNAs (miR-26a, miR-30e, and miR-21) were upregulated and eight miRNAs (miR-34c, miR-29a, miR-29b, miR-489, miR-133a, miR-145, miR-210, and miR-1297) were downregulated in OI patients compared to healthy controls. MiR-29a has a universal lower level in the patient group, whereas miR-26a had a universal upper level. This discovery of altered expression of bone-related miRNAs in OI patients' serum profile may become promising miRNA biomarkers for the diagnosis of OI. Although this study did not verify on the relationships of these differentially expressed miRNAs and their potential target genes, the previous studies have showed that these miRNAs may target a range of gene involved in osteogenic signaling pathways such as BMP, Wnt, RANKL, and TGFβ/activin [53].

MiR-29b has been shown to modulate osteoblast differentiation by downregulating the activity of COL1A1, COL5A3, and COL4A2 and attenuate the collagen protein accumulation during the mineralization phase of bone formation [24]. Kaneto et al. performed a sequence analysis on the coding region and intron/exon junctions of COL1A1 and COL1A2 genes in five independent patients with type I and type III OI. The sequence analysis has identified eight novel mutations, which may contribute to OI phenotype. Interestingly, Kaneto et al. also determined that the expression levels of COL1A1 and miR-29b are reduced in both type I and type III OI patients. Therefore, it is speculated that miR-29b expression is not an essential for sustaining osteoblastogenesis [54].

3.4. Enlarged, weak bone deformation: Paget's disease of bone

Paget's disease of bone (PDB) is a localized disorder of highly exaggerated bone turnover characterized by excessive bone resorption action by osteoclasts within pagetic lesions, followed by an increase in disorganized new bone formation by osteoblasts [55]. This action will eventually result in marrow fibrosis, highly vascular, weak, enlarged, and disorganized bone deformation [55, 56]. The highly fibrous woven bone with reduced mechanical strength and disorganized structural integrity tends to increase the risk of bone deformity and fracture [56]. Frequently, PDB patients are elderly aged more than 50 years and tend to slightly predominate in males [57]. Mutations in genes encoding for the components that modulate the RANK/NF-κB signaling pathway are most likely to contribute to the development of PDB. These genes are sequestosome 1 gene (SQSTM1), tumor necrosis factor receptor superfamily member IIA (TNFRSF11A), valosine-containing protein (VCP), and tumor necrosis factor receptor superfamily member IIB (TNFRSF11B) [55].

However, the regulatory roles of miRNAs in PDB remain unknown. Bianciardi et al. performed a serum miRNA expression profile in peripheral blood mononuclear cells (PBMCs) from 20 PDB patients. The results showed that 22 miRNAs were significantly upregulated with a fold change above three (miR-31, miR-32, miR-124a, miR-132, miR-182, miR-221, miR-339, miR-345, miR-410, miR-451, miR-485.3p) or between 2 and 3 (miR-19a, miR-30b, miR-30c, miR-27a, miR-125a, miR-146a, miR-148a, miR-200c, miR-223, miR-301, miR-365) when compared to non-pagetic controls. Among the 22 miRNAs, these 14 miRNAs (miR-19a, miR-miR-27a, miR-30c, miR-32, miR-125a, miR-132, miR-200c, miR-221, miR-223, miR-301, miR-345, miR-365, miR-410, and miR-485-3p) showed significantly higher expression in patients that experienced Q16STM1 mutation [58].

4. Current status and perspectives of microRNA in bone cancer diagnosis and therapy

In 2010, the first microRNA-targeting drug—miravirsen (SPC3649), a locked nucleic acid (LNAs) ribonucleotides antagomir that targets miR-122 had entered clinical trial and is currently in phase II clinical trial to treat chronic hepatitis C (HCV+) patients (ClinicalTrials.gov Identifier: NCT02508090) [59]. The occurrence of the first miRNA-based clinical trial had led to the insight that miRNAs can serve as promising therapeutic tools and perhaps as the next

magic bullet. The side effects arise from current conventional treatments of bone cancer that also lead to the path of translating the bone cancer miRNA-based therapeutic approaches from the benchworks to the clinical settings.

MRX34, a miRNA mimic encapsulated by liposomal nanoparticle developed by Mirna Therapeutics, appeared as the first miRNA mimic that had reached phase 1 clinical study in year 2013 for the treatment of primary liver cancer and other malignancies including multiple myeloma through functional restoration of endogenous miR-34a as an oncosuppressor (ClinicalTrials.gov Identifier: NCT01829971) [60]. MiR-34a is often suppressed or showed to reduce expression in various cancer types, coupled with the loss of p53 function that transcriptionally control its expression [61]. There are a wide varieties of oncogenes such as cyclin-dependent kinase (CDK) 4/6, Wnt 1/3, B-cell lymphoma 2 (BCL2), MYC, cyclin D1 (CCND1), CD44, and histone deacetylase 1 (HDAC1) that are responsible for unregulated cell cycle progression and proliferation, anti-apoptosis, metastasis, chemoresistance, cancer cell self-renewal, and oncogenic transcription, which can be downregulated by miR-34a [62, 63]. In a phase I clinical trial, adult patients with advanced solid tumors refractory to standard conventional treatment were given a standard 3 + 3 dose escalation trial by which MRX34 was infused to the patients twice a week (BIW) for a period of 3 weeks in a four-week-cycle. The phase 1 results showed that MRX34 has a tolerable toxicity or safety profile and supportive evidence of anti-tumor activity in a subset of patients with refractory advanced solid tumors. The patients generally experienced mild adverse effects such as fever, fatigue, back pain, nausea, anorexia, diarrhea, and vomiting after the treatment [64].

To date, there has been no available miRNA-based diagnostic tests or treatments for bone cancers' management. However, miR-34 anti-tumor activity had been demonstrated in numerous cancer types including bone cancer and multiple myeloma, and therefore, providing a fascinating insight into the introduction of miR-34a mimic for the treatment of bone cancers.

The expression of tumor suppressive mir-34 and miR-122 are downregulated in osteosarcoma cells contrasting to healthy normal cells. Xiao et al. has introduced miRNA response elements (MREs) of miR 34 and miR 122 in osteosarcoma cells through the employment of adenovirus to enable the selective expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). This study reported that the adenovirus (Ad) TRAIL-34-122 resulted in higher apoptotic and cytotoxicity levels in the osteosarcoma cells, compared to the normal cells by selectively expressing TRAIL in miR-34 and miR-122 modulated fashion. The following *in vivo* study in BALB/c nude mice further indicated that Ad-TRAIL-34-122 is able to reduce osteosarcoma xenografts' growth without causing significant liver toxicity [65]. Additionally, Gaur et al. reported that chitosan nanoparticle-mediated delivery of miR-34a mimic preserves bone integrity and reduces tumor growth in a tumor established, intrafemoral nude mice model that represented prostate cancer bone metastasis [66].

Furthermore, Martino et al. has evaluated the activity of synthetic miR-34a in multiple myeloma cells. This study demonstrated that transfection with miR-34a mimic tends to inactivate the early expression of prosurvival and proliferative kinases Erk-2 and Akt. The reduced expression of Erk-2 and Akt is followed by the downregulation of caspase-6/3 expression, which can next induce apoptosis in multiple myeloma cells. Martino et al. subsequently tested the efficiency of miR-34a mimic delivery by encapsulating the mimic in stable nucleic acid

lipid particles (SNALPs). SNALP-encapsulated miR-34a mimic is highly efficient with its anti-tumor activity in both multiple myeloma cells and in *in vivo* SCID mice bearing human multiple myeloma xenografts by showing reduced expression of miR-34a target notch 1 homolog (NOTCH1) and the absence of cytotoxicity effect [67].

5. Future prospects of microRNAs in the treatment of bone disorders and its potential

Although the publication of research findings on microRNAs in bone disorders are still limited, the fast-growing list of literatures indicates the significance of miRNAs in the regulation of bone biology and bone disorders. This has led to the advancement of research to explore potential relevance of miRNAs as diagnostic biomarkers and therapeutics. In this section, the potential of miRNAs as the biomarkers and therapeutic agents will be focused on cancer-related bone disorder (osteosarcoma) and noncancer-related bone disorder (osteoporosis).

5.1. MicroRNAs as diagnostic biomarkers

The comprehensive expression profile of key microRNAs in different bone disorders has the potential to increase the accuracy of the prognosis and diagnosis of bone disorders in combination with other conventional diagnostic approaches.

Hu et al. reported that a total of 268 miRNAs were dramatically dysregulated between human osteosarcoma cell line, MG-63, and human osteoblast HOB cell line. Five miRNAs (miR-9, miR-99, miR-195, miR-148a, and miR-181a) were validated to be overexpressed and four of these miRNAs (miR-143, miR-145, miR-335, and miR-539) were validated to be downregulated in the human OS MG-63 cell lines compared to osteoblast HOB cell lines. The bioinformatics analysis showed that the target genes of these nine miRNAs are associated with multiple cancer-related events including cell proliferation, differentiation, cell cycle, apoptosis, signaling, migration, and invasion [68].

Another study by Jones et al. using pretreatment biopsy samples from conventional (osteoblastic/fibroblastic) osteosarcoma patients and control samples of healthy bone tissue showed that 34 miRNAs were significantly dysregulated with 11 having higher expression and 23 having lower expression among the osteosarcoma group. MiR-181a and miR-181b were the most upregulated miRNAs in osteosarcoma group while miR-29b, miR-451, and miR-16 were among the most downregulated. The miRNA signature profile in the sample of metastatic osteosarcoma group compared to nonmetastatic osteosarcoma group showed that higher expression of miR-27a and miR-181c* was found in patients with metastatic tumor. Additionally, higher expression of miR-451 and miR-15b was associated with chemosensitive patients compared to chemoresistant samples. *In vitro* and *in vivo* functional validation in osteosarcoma cell lines confirmed the tumor suppressive role of miR-16 and the pro-metastatic role of miR-27a. The analysis of target genes of these miRNAs indicated that these miRNAs may target several known osteosarcoma-related genes that regulate transcription, cell cycle control, and cancer signaling pathways [69].

Li et al. identified potential miRNA biomarkers for the early diagnosis and relapse prediction of osteosarcoma by developing a serum-based miRNA profile. All the putative miRNAs were verified through RT-qPCR, and the expression of seven miRNAs (miR-106a-5p, miR-16-5p, miR-20a-5p, miR-425-5p, miR-451a, miR-25-3p, and miR-139-5p) was found to be downregulated in the serum of OS patients compared to the healthy control. These miRNAs are also correlated with other type of cancer pathogeneses such as lung carcinoma, colorectal carcinoma, breast carcinoma, nasopharyngeal carcinoma, etc. [70].

Yuan et al. demonstrated that miR-21 expression was significantly higher in serum from osteosarcoma patients compared to healthy controls as measured by RT-qPCR. The high expression of miR-21 is associated with aggressive Enneking tumor staging, neoadjuvant chemotherapeutic resistance, and reduced overall survival rate [71]. Previous studies indicated that miR-21 has influences on the cell proliferation, cell cycle progression, tumor metastatic behavior, and susceptibility to chemotherapeutic treatment [43, 72–74]. These tumor-promoting behaviors of miR-21 was due to its targeting regulatory roles on a vast number of tumor suppressive genes such as phosphatase and tensin homolog protein (PTEN) [72], myristoylated alanine-rich protein kinase C substrate protein (MARCKS) [43], programmed cell death 4 protein (PDCD4) [73], and cell division cycle 25 homolog A protein (CDC25A) [74].

Dong et al. showed that expression of miR-223 was significantly reduced in the serum of osteosarcoma patients and osteosarcoma cell lines compared to healthy controls as measured by RT-qPCR. Osteosarcoma patients with lower expression of serum miR-223 tend to have distant metastasis, more advanced clinical stages, and shorter survival time [75]. Furthermore, it has been demonstrated that miR-223 may play an important role in the regulation of epithelial cell transforming sequence 2 (Ect2) signaling, an important pathway for osteosarcoma pathogenesis in terms of cell cycle progression, proliferation, recurrence, and poor chemotherapeutic responses [76].

Lian et al. performed TaqMan low-density array (TLDA) and RT-qPCR on plasma samples derived from osteosarcoma patients before surgery, patients after 1 month of surgery and healthy individuals. The results showed that four plasma miRNAs (miR-195-5p, miR-199a-3p, miR-320a, and miR-374a-5p) were significantly upregulated in the presurgical osteosarcoma patients. The expression level of these four plasma miRNAs were decreased after surgical removal of the tumors, suggesting the potential of these miRNAs as the biomarkers for osteosarcoma. Additionally, circulating miR-195-5p and miR-199a-3p were correlated with metastasis status whereas miR-199a-3p and miR-320a were correlated with histological subtype [77]. Besides, it has been discovered that miR-195-5p involved in the inhibition of osteosarcoma cell migration and invasion by targeting fatty acid synthase (FASN) [78], while miR-199a-3p regulated the p53 signaling pathway and inhibits osteosarcoma cell growth, migration, and induce apoptosis [79, 80].

5.2. MicroRNAs as therapeutic agents or targets

Growing lists of *in vitro* and *in vivo* studies on the regulatory roles of microRNAs in bone disorders, which conducted by various research teams, have supported miRNAs as the potential therapeutics candidates. However, specific, efficient, and safe delivery of miRNA to its target

sites is crucial for the translation of miRNA-based therapeutics strategies. Effective delivery systems in various bone disorder models had been observed by the application of biomaterial constructs, viral vectors, nanoparticles, and polymers with the potential to restore the normal functions of bone homeostasis and carcinogenesis.

The expression of miR-199a-3p, which may inhibit tumor cell growth, is reduced in osteosarcoma cells. Zhang et al. developed a lipid-modified dextran-based polymeric nanoparticle platform for encapsulation of miR-199a-3p and another potent tumor suppressive miRNA, let-7a, and transfected into osteosarcoma cells lines, KHOS and U-2OS. Western blot analysis and 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay showed that dextran nanoparticles loaded with miRNAs could efficiently downregulate the expression of mechanistic target of rapamycin (mTOR) and Met proteins and effectively inhibit the growth and proliferation of osteosarcoma cells [81].

MiR-143 expression is downregulated in 143B human osteosarcoma cell line, an osteosarcoma cell line with high metastatic tendency to the lung. Osaki et al. inoculated the 143B osteosarcoma cells transfected with firefly luciferase gene (143B-luc) into athymic mice in order to develop a primary tumor and spontaneous lung metastasis. Then, systemic administration of miR-143 mimic and miR-negative control 1 (NC1) mixed with atelocollagen was performed on the osteosarcoma mice model to study the therapeutic potential of miR-143 against spontaneous lung metastasis of osteosarcoma. After 1 week, the luciferase signal was detected only at the right knee primary lesion where 143B-luc cells were inoculated. After 2 weeks, one out of four mice administered with miR-NC1 was detected with luciferase signal at the pulmonary area indicating lung metastasis, whereas no luciferase signal was observed in mice injected with miR-143 mimic. After days 19–20, two out of 10 mice injected with the miR-NC1 control died due to lung metastasis. At third week, six of the eight live mice administered with miR-NC1 control were identified with lung metastasis, while contrastingly, only two out of 10 mice injected with miR-143 mimic displayed lung metastasis. Furthermore, the tumor weight and the expression of proliferative cell nuclear antigen in primary tumor showed no significant difference between both groups (miR-143 mimic and miR-NC1 control). Therefore, all these data showed that miR-143 mimic suppresses lung metastasis from a primary tumor but did not have effect on the primary tumor cell proliferation. Additionally, it is speculated that the downregulation of miR-143 may promote lung metastasis of human osteosarcoma cells by promoting MMP-13 upregulation [82]. Shimbo et al. introduced synthetic miR-143 into MSC cells and increased the amount of exosome-formed miR-143 in the conditioned medium. The transfection of 143B osteosarcoma cell lines with extracellular miR-143 in the conditioned medium from MSCs (exosome-formed miR-143) reduced the migration ability of osteosarcoma cells compared to the control. In addition, Shimbo et al. also showed that the transfection efficiency of exosome-formed miR-143 was less than that attained with the lipofection. Nevertheless, migration assay performed on the 143B osteosarcoma cells showed that the inhibitory effect on cell migration was similar between exosome and lipofection method [83].

Jiang et al. constructed lentiviral vectors overexpressing and silencing miR-126. Both of the miR 126 overexpressing and silencing lentiviral vectors were then transfected into MG63 and U-2 OS osteosarcoma cell lines. This study aimed to determine the interlink between cisplatin (DDP) and methotrexate (MTX) osteosarcoma chemotherapeutic drugs and miR-126 on the effect to

inhibit osteosarcoma cell proliferation and apoptosis. The results showed that DDP and MTX induce apoptosis and inhibit the cell cycle of osteosarcoma cell lines at a greater efficiency in miR-126 overexpressing manner. Nonetheless, DDP and MTX did not significantly impact the apoptosis and cell proliferation in the miR-126 silenced group. On that account, it is suggested that miR-126 may strengthen the sensitivity of osteosarcoma cell to DDP and MTX. However, the regulatory mechanisms behind this process still remain to be discovered [84].

Cai et al. developed polyurethane (PU) nanomicelles drug carrier modified with Asp8 acidic peptide (Asp8-PU-anti-miR214) for targeted delivery of anti-miR-214. Polyurethane (PU) is a linear polymer composed of organic units molecularly linked by carbamate (urethane) group [85]. Besides, it is known that PU tends to have high compatibility in living system by not being toxic or reactive and have high mechanical flexibility [85–88]. The highly negatively charged peptide Asp8 has also been accounted as an excellent targeting tool of bone resorption area [89]. In this study, miR-214 was chosen due to its regulatory role in bone remodeling by which the elevated expression *in vivo* was associated with reduced bone formation in aged patients. This is due to the direct targeting action of miR-214 on activating transcription factor 4 (ATF4), which enable the inhibition of osteoblast activity [90]. Apart from that, miR-214 also modulates osteoclast differentiation by targeting the PTEN-PI3k-Akt pathway [91]. Asp8-PU-anti-miR214 delivery system to osteoclasts at the bone resorption surface of ovariectomized (OVX) osteoporosis mice model was able to improve the bone microarchitecture, increased bone mass, and decreased osteoclast number. Above and beyond, a number of osteoclast-related genes including tryptophan RNA-binding attenuation protein (TRAP) and cathepsin K (CTSK) were successfully downregulated by the anti-miR-214. Interestingly, Asp8-PU-anti-miR214 was also proven as a potential drug delivery candidate that does not overt toxicity or elicit an immune response. Therefore, Asp8-PU serves as a potential bone-resorption surface-targeting delivery system for the treatment of postmenopausal osteoporosis and osteoclast-stimulated bone disorders [85].

Zhang et al. designed a hyperbranched polymer (HP) and miR-26a (HP/miRNA) nanosized polyplexes, which were encapsulated in biodegradable microspheres to overcome problems with uncontrolled release and achieved the controllable two-stage delivery strategy (microspheres and polyplexes). Microspheres attach to cell-free nanofibrous polymer 3D scaffolds to prevent off-target effects of the miRNA delivery. The 3D scaffolds were implanted into osteoporotic mice model, and the results showed that this technology was able to regenerate critical-sized bone with low cytotoxicity effect by targeting glycogen synthase kinase 3 beta (Gsk-3β) to activate the osteoblastic activity of endogenous stem cells [92].

Overexpression of miR-140* and miR-214 was detected in bone marrow-derived MSCs isolated from ovariectomized rats (OVX-BMSCs). Li et al. demonstrated that engineered OVX-BMSCs expressing the hybrid baculovirus-mediated miRNA sponges can continuously antagonize cellular miR-140* and miR-214 levels *in vitro*. At the same time, the attenuation of miR-140* and miR-214 expression can also efficiently support the osteogenesis of OVX-BMSCs and intensify the capability of OVX-BMSCs to suppress osteoclast maturation. Remarkably, the osteoinductive effect of suppressing miR-214 was more potent compared to miR-140* suppression. This study also discovered that the allotransplantation of miR-214 sponges-expressing OVX-BMSCs in osteoporotic rat models with a femoral metaphysis found with critical-size bone defect was able to improve the likelihood of bone healing, remodeling,

and bone quality at 4 weeks postimplantation. Moreover, co-expression of bone morphogenic protein 2 (BMP2) and miR-214 sponges in OVX-BMSCs can synergistically enhance the bone formation and healing in osteoporotic rats [93].

6. Challenges

Although recent studies reveal that microRNA has the potential to become diagnostic biomarker and effective therapeutic agents for bone diseases, there are still challenges for developing miRNA-based treatment. Since each miRNA may regulate many different mRNA targets and the expression of target genes might be controlled by different miRNAs, it became an obstacle to identify all targets and miRNAs involved in bone diseases [94]. Moreover, miRNAs are cancer type specific, they may perform as oncogene or tumor suppressor in different cell types, and thus result in off-target effects of miRNAs [95]. Garzon et al. reveals that miR-29 mimics serve as anticancer agents and regulate in bone growth; meanwhile, they target several tumorigenesis pathways like proliferation (CDK6), methylation (DNMT1, DNMT3a and b), and apoptosis (MCL-1) [96].

Currently, one of the major challenges facing by the researches is the mechanism of *in vivo* delivery. There are lots of mechanical and biological barriers to cope with for success transferring of miRNA into the target genes. The first barrier is the abnormal tumor vessels in leaky structure, which cause the poor blood perfusion and affect the delivery of naked miRNA. In addition, the extracellular matrix is very complex, consisting of tumor-associated macrophages and monocytes, which can trap miRNA in capsule and have the ability to hinder the miRNA to target the cancer cells. MiRNA is also susceptible to nucleases such as serum RNase A-type nucleases, which break phosphodiester bonds between nucleotides [97]. Furthermore, the small-sized miRNA is easily filtered by kidney and cleared in the blood circulation [98]. Hence, the instability of miRNAs needs to be overcome in order for the miRNAs to reach the target genes. Even if miRNAs are successfully transferred into the target tissue, the uptake of miRNAs into the cells is not guaranteed. The miRNA oligonucleotides consist of negative charges, and it prevents them from passing through the plasma membranes of the target cells [96]. Strategy to improve endosomal escape should also be taken in consideration since the endocytosis mechanism that capsulated miRNA causing degradation might be happened [97].

Besides delivery considerations, the autoimmunological pathways are necessary to be emphasized. MiRNAs are recognized as foreign particles by immune system in the body, which will trigger the adaptive or innate immune responses causing unpredictable toxicities [99]. Chen et al. reported that miRNA duplexes can trigger toll-like receptors (TLRs) to secrete the inflammatory cytokines and type I interferons. Activation of TLRs 3, 7, and 8 by single- or double-stranded RNAs promotes innate and adaptive immune systems and also prepare the surrounding immune cells, for instance, natural killer cells, dendritic cells, monocytes, B cells, etc., to increase the sensitivity to RNA stimulation [97]. The immune responses toward the miRNA still required further studies.

Numerous findings of miRNA are based on *in vitro* studies using cell lines and are not fully validated in *in vivo*. In addition, the major methods used to measure miRNA levels are

http://dx.doi.org/10.5772/intechopen.79275

quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) and microarray analysis, and there is no standardized techniques to measure the miRNA expression levels [38]. Further techniques need to be optimized for better miRNA detection and analysis.

Despite there are many challenges, the potentials of miRNA as a diagnostic tool and treatment for bone diseases look promising. We believe that in the next few years, researches will be able to develop efficient delivery methods of the miRNA to its specific target site with minimum or no side effect.

7. Conclusions

As a conclusion, microRNA plays important roles in bone development and maintenance. MiRNA dysregulation leads to the pathogenesis of various bone diseases. Nowadays, miRNAs are being excavated as new directions for diagnostic biomarkers and drug targets to cure bone diseases. However, there are still many limitations and barriers for the development of miRNA-based biomarkers and therapeutics. Further investigations are needed to understand the miRNA gene regulation in bone and to overcome the challenges faced in miRNA delivery systems. MiRNA studies not only provide new eras of basic bone biology researches, but also contribute to new diagnostic and therapeutic methods into clinical practice to various bone diseases.

Acknowledgements

The authors thank Fundamental Research Grant Scheme (FRGS/1/2015/ST03/UPM/02/5) from Ministry of Higher Education, Malaysia for the support.

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References

[1] Nugent M. MicroRNA function and dysregulation in bone tumors: The evidence to date. Cancer Management and Research. 2014;**6**(1):15-25

[2] Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? Nature Reviews. Genetics. 2008;**9**: 102-114

[3] Paranjape T, Slack FJ, Weidhaas JB. MicroRNAs: Tools for cancer diagnostics. Gut. 2009; **58**(11):1546-1554

[4] Moore BT, Xiao P. MiRNAs in bone diseases. MicroRNA. 2013;**2**(1):20-31

[5] Lin T, Ma QP, Zhang YF, Zhang HF, Yan JP, Gao CH. MicroRNA-27a functions as an oncogene in human osteosarcoma by targeting CCNG1. Oncology Letters. 2018;**15**(1): 1067-1071

[6] Braun CJ, Zhang X, Savelyeva I, Wolff S, Moll UM, Schepeler T, et al. p53-responsive microRNAs 192 and 215 are capable of inducing cell cycle arrest. Cancer Research. 2008; **68**(24):10094-10104

[7] Sun MG, Zhou XY, Chen LL, Huang SS, Leung V, Wu N, et al. The regulatory roles of microRNAs in bone remodeling and perspectives as biomarkers in osteoporosis. BioMed Research International. 2016;**2016**:1652417

[8] Clarke B. Normal bone anatomy and physiology. Clinical Journal of the American Society of Nephrology. 2008;**3**(Suppl 3):131-139

[9] Pathria MN, Chung CB, Resnick DL. Acute and stress-related injuries of bone and cartilage: Pertinent anatomy, basic biomechanics, and imaging perspective. Radiology. 2016; **280**(1):21-38

[10] Martin TJ, Seeman E. Bone remodelling: Its local regulation and the emergence of bone fragility. Best Practice & Research. Clinical Endocrinology & Metabolism. 2008;**22**(5): 701-722

[11] Martin RB, Burr DB, Sharkey NA, Fyhrie DP. Growth, modelling and remodelling of bone. In: Martin RB, Burr DB, Sharkey NA, Fyhrie DP, editors. Skeletal Tissue Mechanics. 2nd ed. New York: Springer; 2015. pp. 95-173

[12] Raggatt LJ, Partridge NC. Cellular and molecular mechanisms of bone remodeling. The Journal of Biological Chemistry. 2010;**285**(33):25103-25108

[13] Langdahl B, Ferrari S, Dempster DW. Bone modeling and remodeling: Potential as therapeutic targets for the treatment of osteoporosis. Therapeutic Advances in Musculoskeletal Disease. 2016;**8**(6):225-235

[14] Feng X, McDonald JM. Disorders of bone remodelling. Annual Review of Pathology. 2011;**6**:121-145

[15] Eriksen EF. Cellular mechanisms of bone remodeling. Reviews in Endocrine & Metabolic Disorders. 2010;**11**(4):219-227

[16] Proff P, Römer P. The molecular mechanism behind bone remodelling: A review. Clinical Oral Investigations. 2009;**13**(4):355-362

[17] Mizoguchi F, Murakami Y, Saito T, Miyasaka N, Kohsaka H. MiR-31 controls osteoclast formation and bone resorption by targeting RhoA. Arthritis Research & Therapy. 2013;**15**(5):R102

[18] Kim KS, Kim JH, Kim IY, Lee JW, Seong SM, Park YW, et al. MicroRNA-26a regulates RANKL-induced osteoclast formation. Molecules and Cells. 2015;**38**(1):75-80

[19] Sugatani T, Vacher J, Hruska KA. A microRNA expression signature of osteoclastogenesis. Blood. 2012;**117**(13):3648-3657

[20] Cheng P, Chen C, He HB, Hu R, Zhou HD, Xie H, et al. MiR-148a regulates osteoclastogenesis by targeting V-maf musculoaponeurotic fibrosarcoma oncogene homolog B. Journal of Bone and Mineral Research. 2013;**28**(5):1180-1190

[21] Kim KS, Kim JH, Lee JW, Jin HM, Kook H, Kim KK, et al. MafB negatively regulates RANKL-mediated osteoclast differentiation. Blood. 2007;**109**(8):3253-3259

[22] Zhao HY, Zhang J, Shao HY, Liu JW, Jin MR, Chen JP, et al. MiRNA-340 inhibits osteoclast differentiation via repression of MITF. Bioscience Reports. 2017;**37**(4):BSR20170302

[23] Kapinas K, Kessler C, Ricks T, Gronowicz G, Delany AM. MiR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop. The Journal of Biological Chemistry. 2010;**285**(33):25221-25231

[24] Li ZY, Hassan MQ, Jafferji M, Aqeilan RI, Garzon R, Croce CM, et al. Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. The Journal of Biological Chemistry. 2009;**284**(23):15676-15684

[25] Kapinas K, Kessler CB, Delany AM. MiR-29 suppression of osteonectin in osteoblasts: Regulation during differentiation and by canonical Wnt tumour. Journal of Cellular Biochemistry. 2010;**108**(1):216-224

[26] Li ZY, Hassan MQ, Volinia S, van Wijnen AJ, Stein JL, Croce CM, et al. A microRNA signature for a BMP2-induced osteoblast lineage commitment program. Proceedings of the National Academy of Sciences. 2008;**105**(37):13906-13911

[27] Zhang JF, Fu WM, He ML, Xie WD, Lv Q, Wan G, et al. MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling. RNA Biology. 2011;**8**(5):829-838

[28] Li H, Xie H, Liu W, Hu R, Huang B, Tan YF, et al. A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. The Journal of Clinical Investigation. 2009;**119**(12):3666-3677

[29] Hu R, Liu W, Li H, Yang L, Chen C, Xia ZY, et al. A Runx2/miR-3960/miR-2861 regulatory feedback loop during mouse osteoblast differentiation. The Journal of Biological Chemistry. 2011;**286**(14):12328-12339

[30] Sang S, Zhang ZC, Qin S, Li CW, Dong Y. MicroRNA-16-5p inhibits osteoclastogenesis in giant cell tumor of bone. BioMed Research International. 2017;**2017**:1-6

[31] Cowan RW, Singh G. Giant cell tumor of bone: A basic science perspective. Bone. 2013;**52**(1):238-246

[32] Wang T, Yin HB, Wang J, Li ZX, Wei HF, Liu Z, Wu ZP, Yan WJ, et al. MicroRNA-106b inhibits osteoclastogenesis and osteolysis by targeting RANKL in giant cell tumor of bone. Oncotarget. 2015;**6**(22):18980-18996

[33] Sobti A, Agrawal P, Agarwala S, Agarwal M. Giant cell tumor of bone—An overview. Archives of Bone and Joint Surgery. 2016;**4**(1):2-9

[34] Zhang J, Xiao XJ, Liu J. The role of circulating miRNAs in multiple myeloma. Science China. Life Sciences. 2015;**58**(12):1262-1269

[35] Wu PF, Liang JY, Yu F, Zhou ZB, Tang JY, Li KH. MiR-125b inhibits stromal cell proliferation in giant cell tumor of bone by targeting parathyroid hormone 1 receptor. Iranian Journal of Basic Medical Sciences. 2015;**18**:705-709

[36] Johnell O, Kanis J. Epidemiology of osteoporotic fractures. Osteoporosis International. 2005;**16**(Suppl 2):6-10

[37] Sozen T, Ozisik L, Basaran NC. An overview and management of osteoporosis. European Journal of Rheumatology. 2017;**4**(1):46-56

[38] Mirza F, Canalis E. Management of endocrine disease: Secondary osteoporosis: Pathophysiology and management. European Journal of Endocrinology. 2015;**173**(3): R131-R151

[39] Zaheer S, LeBoff MS. Osteoporosis: Prevention and treatment. [Updated 2016 Aug 3]. In: De Groot LJ, Chrousos G, Dungan K, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279073/

[40] Ji MX, Yu Q. Primary osteoporosis in postmenopausal women. Chronic Diseases and Translational Medicine. 2015;**1**(1):9-13

[41] Lian JB, Stein GS, Van Wijnen AJ, Stein JL, Hassan MQ, Gaur T, et al. MicroRNA control of bone formation and homeostasis. Nature Reviews. Endocrinology. 2012;**8**(4):212-227

[42] Chen C, Cheng P, Xie H, Zhou HD, Wu XP, Liao EY, et al. MiR-503 regulates osteoclastogenesis via targeting RANK. Journal of Bone and Mineral Research. 2014;**29**(2):338-347

[43] Li T, Li D, Sha JJ, Sun P, Huang YR. MicroRNA-21 directly targets MARCKS and promotes apoptosis resistance and invasion in prostate cancer cells. Biochemical and Biophysical Research Communications. 2009;**383**(3):280-285

[44] Zhang YQ, Gao YL, Cai LJ, Li FN, Lou Y, Xu N, et al. MicroRNA-221 is involved in the regulation of osteoporosis through regulates RUNX2 protein expression and osteoblast differentiation. American Journal of Translational Research. 2017;**9**(1):126-135

[45] Yang N, Wang G, Hu CH, Shi YY, Liao L, Shi ST, et al. Tumor necrosis factor α suppresses the mesenchymal stem cell osteogenesis promoter miR-21 in estrogen deficiency-induced osteoporosis. Journal of Bone and Mineral Research. 2013;**28**(3):559-573

[46] Wang FS, Chung PC, Lin CL, Chen MW, Ke HJ, Chang YH, et al. MicroRNA-29a protects against glucocorticoid-induced bone loss and fragility in rats by orchestrating bone acquisition and resorption. Arthritis and Rheumatism. 2013;**65**(6):1530-1540

[47] Monti E, Mottes M, Fraschini P, Brunelli PC, Forlino A, Giacomo V, et al. Current and emerging treatments for the management of osteogenesis imperfecta. Therapeutics and Clinical Risk Management. 2010;**6**:367-381

[48] Shaker JL, Albert C, Fritz J, Harris G. Recent developments in osteogenesis imperfecta. F1000Research. 2015;**4**:2-11

[49] Abukabbos H, Al-Sineedi F. Clinical manifestations and dental management of dentinogenesis imperfecta associated with osteogenesis imperfecta: Case report. Saudi Dental Journal. 2013;**25**(4):159-165

[50] Marini JC, Forlino A, Cabral WA, Barnes AM, Antonio JDS, Milgrom S, et al. Consortium for osteogenesis imperfecta mutations in the helical domain of type I collagen: Regions rich in lethal mutations align with collagen binding sites for integrins and proteoglycans. Human Mutation. 2007;**28**:209-221

[51] Forlino A, Cabral WA, Barnes AM, Marini JC. New perspectives on osteogenesis imperfecta. Nature Reviews. Endocrinology. 2011;**7**(9):540-557

[52] Van Dijk FS, Sillence DO. Osteogenesis imperfecta: Clinical diagnosis, nomenclature and severity assessment. American Journal of Medical Genetics Part A. 2014;**164**(6):1470-1481

[53] Wang ZQ, Lu YQ, Zhang XM, Ren XZ, Wang YZ, Li ZL, et al. Serum microRNA is a promising biomarker for osteogenesis imperfecta. Intractable & Rare Diseases Research. 2012;**1**(2):81-85

[54] Kaneto CM, Lima PSP, Zanette DL, Prata KL, Pina Neto JM, de Paula FJ, et al. COL1A1 and miR-29b show lower expression levels during osteoblast differentiation of bone marrow stromal cells from Osteogenesis Imperfecta patients. BMC Medical Genetics. 2014;**15**(1):45

[55] Sabharwal R, Gupta S, Sepolia S, Panigrahi R, Mohanty S, Subudhi SK, et al. An insight in to paget's disease of bone. Nigerian Journal of Surgery: Official Publication of the Nigerian Surgical Research Society. 2014;**20**(1):9-15

[56] Ralston SH, Layfield R. Pathogenesis of paget disease of bone. Calcified Tissue International. 2012;**91**(2):97-113

[57] Roodman G, Windle J. Science in medicine-paget disease of bone. The Journal of Clinical Investigation. 2005;**115**(2):200-207

[58] Bianciardi S, Merlotti D, Sebastiani G, Valentini M, Gonnelli S, Caffarelli C, et al. MicroRNA expression profiling in paget's disease of bone. Bone Abstracts. 2016;**5**:452

[59] Gebert LFR, Rebhan MAE, Crivelli SEM, Denzler R, Stoffel M, Hall J. Miravirsen (SPC3649) can inhibit the biogenesis of miR-122. Nucleic Acids Research. 2014;**42**(1):609-621

[60] Shah MY, Ferrajoli A, Sood AK, Lopez-Berestein G, Calin GA. microRNA therapeutics in cancer—An emerging concept. eBioMedicine. 2016;**12**:34-42

[61] Slabáková E, Culig Z, Remšík J, Souček K. Alternative mechanisms of MiR-34a regulation in cancer. Cell Death & Disease. 2017;**8**(10):1-10

[62] Bouchie A. First microRNA mimic enters clinic. Nature Biotechnology. 2013;**31**(7):577-577

[63] Bader AG. MiR-34—A microRNA replacement therapy is headed to the clinic. Frontiers in Genetics. 2012;**3**(120):1-9

[64] Beg MS, Brenner AJ, Sachdev J, Borad M, Kang YK, Stoudemire J, et al. Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. Investigational New Drugs. 2017;**35**(2):180-188

[65] Xiao F, Chen JW, Lian CJ, Han PC, Zhang CY. Tumor necrosis factor-related apoptosis-inducing ligand induces cytotoxicity specific to osteosarcoma by microRNA response elements. Molecular Medicine Reports. 2015;**11**(1):739-745

[66] Gaur S, Wen YF, Song JH, Parikh NU, Mangala LS, Blessing AM, et al. Chitosan nanoparticle-mediated delivery of miRNA-34a decreases prostate tumor growth in the bone and its expression induces non-canonical autophagy. Oncotarget. 2015;**6**(30):29161-29177

[67] Di Martino MT, Campani V, Misso G, Gallo Cantafio ME, Gullà A, Foresta U, et al. In vivo activity of MiR-34a mimics delivered by stable nucleic acid lipid particles (SNALPs) against multiple myeloma. PLoS One. 2014;**9**(2):1-10

[68] Hu H, Zhang Y, Cai XH, Huang JF, Cai L. Changes in microRNA expression in the MG-63 osteosarcoma cell line compared with osteoblasts. Oncology Letters. 2012;**4**(5):1037-1042

[69] Jones KB, Salah Z, Sara DM, Galasso M, Gaudio E, Nuovo GJ, et al. MicroRNA signatures associate with pathogenesis and progression of osteosarcoma. Cancer research. 2013;**72**(7):1865-1877

[70] Li H, Zhang K, Liu LH, Ouyang Y, Guo HB, Zhang H, et al. MicroRNA screening identifies circulating microRNAs as potential biomarkers for osteosarcoma. Oncology Letters. 2015;**10**(3):1662-1668

[71] Yuan J, Chen L, Chen X, Sun W, Zhou X. Identification of serum microRNA-21 as a biomarker for chemosensitivity and prognosis in human osteosarcoma. The Journal of International Medical Research. 2012;**40**(6):2090-2097

[72] Lou YH, Yang XS, Wang FL, Cui ZM, Huang Y. MicroRNA-21 promotes the cell proliferation, invasion and migration abilities in ovarian epithelial carcinomas through inhibiting the expression of PTEN protein. International Journal of Molecular Medicine. 2010;**26**:819-827

[73] Fassan M, Pizzi M, Giacomelli L, Mescoli C, Ludwig K, Pucciarelli S, et al. PDCD4 nuclear loss inversely correlates with miR-21 levels in colon carcinogenesis. Virchows Archiv. 2011;**458**(4):413-419

[74] Wang P, Zou FD, Zhang XD, Li H, Dulak A, Robert J, et al. MiR21 negatively regulates CDC25A and cell cylce progression in colon cancer cells. Cancer Research. 2009;**69**(20):8157-8165

[75] Dong JB, Liu YL, Liao WS, Liu R, Shi P, Wang LM. MiRNA-223 is a potential diagnostic and prognostic marker for osteosarcoma. Journal of Bone Oncology. 2016;**5**(2):74-79

[76] Xu JL, Yao Q, Hou Y, Xu M, Liu S, Yang LQ, et al. MiR-223/Ect2/p21 signaling regulates osteosarcoma cell cycle progression and proliferation. Biomedicine & Pharmacotherapy. 2013;**67**(5):381-386

[77] Lian F, Cui Y, Zhou CL, Gao KW, Wu LW. Identification of a plasma four-microRNA panel as potential noninvasive biomarker for osteosarcoma. PLoS One. 2015;**10**(3):1-12

[78] Mao JH, Zhou RP, Peng AF, Liu ZL, Huang SH, Long XH, et al. MicroRNA-195 suppresses osteosarcoma cell invasion and migration in vitro by targeting FASN. Oncology Letters. 2012;**4**(5):1125-1129

[79] Tian Y, Zhang YZ, Chen W. MicroRNA-199a-3p and microRNA-34a regulate apoptosis in human osteosarcoma cells. Bioscience Reports. 2014;**34**(4). DOI: 10.1042/BSR20140084

[80] Duan ZF, Choy E, Harmon D, Liu XZ, Susa M, Mankin H, et al. MicroRNA-199a-3p is down regulated in human osteosarcoma and regulates cell proliferation and migration. Molecular Cancer Therapeutics. 2011;**10**(8):1337-1345

[81] Zhang LL, Iyer AK, Yang XQ, Kobayashi E, Guo YQ, Mankin H, et al. Polymeric nanoparticle-based delivery of microRNA-199a-3p inhibits proliferation and growth of osteosarcoma cells. International Journal of Nanomedicine. 2015;**10**:2913-2924

[82] Osaki M, Takeshita F, Sugimoto Y, Kosaka N, Yamamoto Y, Yoshioka Y, et al. MicroRNA-143 regulates human osteosarcoma metastasis by regulating matrix metalloprotease-13 expression. Molecular Therapy. 2011;**19**(6):1123-1130

[83] Shimbo K, Miyaki S, Ishitobi H, Kato Y, Kubo T, Shimose S, et al. Exosome-formed synthetic microRNA-143 is transferred to osteosarcoma cells and inhibits their migration. Biochemical and Biophysical Research Communications. 2014;**445**(2):381-387

[84] Jiang LD, He AY, He XJ, Tao C. MicroRNA-126 enhances the sensitivity of osteosarcoma cells to cisplatin and methotrexate. Oncology Letters. 2015;**10**(6):3769-3778

[85] Cai MX, Yang L, Zhang SF, Liu JF, Sun Y, Wang XG. A bone-resorption surface-targeting nanoparticle to deliver anti-miR214 for osteoporosis therapy. International Journal of Nanomedicine. 2017;**12**:7469-7482

[86] Yu SJ, Ding JX, He CL, Cao Y, Xu WG, Chen XS. Disulfide cross-linked polyurethane micelles as a reduction-t riggered drug delivery system for cancer therapy. Advanced Healthcare Materials. 2014;**3**(5):752-760

[87] Gencturk A, Kahraman E, Güngör S, Ozhan G, Ozsoy Y, Sarac AS. Polyurethane/hydroxypropyl cellulose electrospun nanofiber mats as potential transdermal drug delivery

system: Characterization studies and in vitro assays. Artificial Cells, Nanomedicine, and Biotechnology. 2017;**45**(3):655-664

[88] Akduman C, Ozgüney I, Kumbasar EP. Preparation and characterization of naproxen-loaded electrospun thermoplastic polyurethane nanofibers as a drug delivery system. Materials Science & Engineering. C, Materials for Biological Applications. 2016;**64**:383-390

[89] Carinci F. Restoration of incisor area using one-piece implants: Evaluation of crestal bone resorption. Dental Research Journal. 2012;**9**(Suppl 2):S151-S154

[90] Wang XG, Guo BS, Li Q, Peng J, Yang ZJ, Wang AY, et al. MiR-214 targets ATF4 to inhibit bone formation. Nature Medicine. 2013;**19**(1):93-100

[91] Zhao CY, Sun WJ, Zhang PF, Ling SK, Li YH, Zhao DS, et al. MiR-214 promotes osteoclastogenesis by targeting Pten/PI3k/Akt pathway. RNA Biology. 2015;**12**(3):343-353

[92] Zhang XJ, Li Y, Chen YE, Chen JH, Ma PX. Cell-free 3D scaffold with two-stage delivery of miRNA-26a to regenerate critical-sized bone defects. Nature Communications. 2016;**7**:1-15

[93] Li KC, Chang YH, Yeh CL, Hu YC. Healing of osteoporotic bone defects by baculovirus-engineered bone marrow-derived MSCs expressing MicroRNA sponges. Biomaterials. 2016;**74**:155-166

[94] Kumar RMR, Boro A, Fuchs B. Involvement and clinical aspects of microRNA in osteosarcoma. International Journal of Molecular Sciences. 2016;**17**(6):1-5

[95] Sampson VB, Yoo SM, Kumar A, Vetter NS, Kolb EA. MicroRNAs and potential targets in osteosarcoma: Review. Frontiers in Pediatrics. 2015;**3**(August):1-9

[96] Garzon R, Marcucci G, Croce CM. Targeting microRNAs in cancer: Rationale, strategies and challenges. Nature Reviews. Drug Discovery. 2010;**9**(10):775-789

[97] Chen YC, Gao DY, Huang L. In vivo delivery of miRNAs for cancer therapy: Challenges and strategies. Advanced Drug Delivery Reviews. 2015;**81**:128-141

[98] Sand M, Gambichler T, Sand D, Skrygan M, Altmeyer P, Bechara FG. MicroRNAs and the skin: Tiny players in the body's largest organ. Journal of Dermatological Science. 2009;**53**(3):169-175

[99] Greco SJ, Munoz JL, Rameshwar P. MicroRNA cancer therapeutics and the challenge of drug delivery. In: Singh SR, Rameshwar P, editors. MicroRNA in Development and in the Progression of Cancer. New York: Springer; 2014. pp. 349-358

Section 2

The Interplay Between Transcription Factors and MicroRNAs

Chapter 6

Transcription Factors and MicroRNA Interplay: A New Strategy for Crop Improvement

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75942

Abstract

MicroRNAs (miRNAs) and transcription factors are master regulators of the cellular system. Plant genomes contain thousands of protein-coding and non-coding RNA genes; which are differentially expressed in different tissues at different times during growth and development. Complex regulatory networks that are controlled by transcription factors and microRNAs, which coordinate gene expression. Transcription factors, the key regulators of plant growth and development, are the targets of the miRNAs families. The combinatorial regulation of transcription factors and miRNAs guides the appropriate implementation of biological events and developmental processes. The resources on the regulatory cascades of transcription factors and miRNAs are available in the context of human diseases, but these resources are meager in case of plant diseases. On the other hand, it is also important to understand the cellular and physiological events needed to operate the miRNAs networks. The relationship between transcription factors and miRNA in different plant species described in this chapter will be of great interest to plant scientists, providing better insights into the mechanism of action and interactions among transcription factors (TFs) and miRNA networks culminating in improving key agronomic traits for crop improvement to meet the future global food demands.

Keywords: transcription factors, microRNA, regulatory network, interplay, gene expression

1. Introduction

In the recent years, various regulatory complex networks have been identified in plants [1]. Identification of these networks has led to better understanding gene regulation at

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transcriptional and post-transcriptional level. In this chapter, we will be emphasizing on interplay of TFs and miRNAs as a major regulatory mechanism during and after mRNA synthesis. TFs interact with enhancers at transcriptional level to regulate gene expression and have been well recognized in the last decade [2]. This is also supported by the discovery of diverse family of TFs playing various roles in plants [3]. Post-transcriptional gene regulation involving small non-coding RNAs called miRNAs has also been discovered a few decades ago. These miRNAs are involved in the regulation of various genes in animal and plant system by upregulating and downregulating mRNAs [4]. With the escalating gene regulating complexity, it is fascinating to monitor and recognize a vibrant connection among small non-coding RNAs (miRNAs), transcription factors (TFs) and messenger RNAs (mRNAs).

MiRNAs are small non-coding (□22 nucleotides) RNA molecules present in viruses, plants and animals and are involved in post-transcriptional and post-translational regulation of gene expression. First miRNA molecule (lin-4) was discovered in *C. elegans* by Lee et al. [5]. Later on, second miRNA (let-7) was characterized by Reinhart et al. [6]. Both plants and animals undergo a similar biogenesis mechanism. A two-step procedure catalyzed by RNA pol III like enzyme is required in the miRNA processing of primary precursor. For further processing, these miRNAs are loaded into a protein complex known as RNA induced silencing complex (RISC) [4]. An open access miRNA database is managed by Griffiths-Jones Lab, University of Manchester (http://www.mirbase.org/index.shtml). This public database contains a total of 28,685 miRNAs from viruses, animals and plants [7] and is involved in regulation and modification of several biological pathways by controlling particular genes [8]. Therefore, identifying possible miRNA targets is an effective methodology to thoroughly study miRNA-mediated regulatory function at post-transcriptional level. Earlier studies carried out in Arabidopsis to explore some experimental parameters and procedures inferred for known miRNA-target interaction using bioinformatics tools have been utilized to reveal miRNA target genes in other plants [9]. Wet lab experiments like PAGE, Northern Blot, RAPD and Degradome sequencing were carried out to further validate the computational predictions [10].

Presently, 320,370 TFs have been identified from 58 families of 165 plant species [11]. Various repositories for plant TFs are available, which identify and collect TF from various plant species and are publically available for use (**Table 1**). MiRNAs and TFs are involved in upregulation and downregulation of the target genes, ultimately determine the destiny of specific gene, by turning "on/off" [12]. Mainly, miRNAs are involved in targeting DNA-binding proteins (TFs) [13]. Since a great impact on plant genetic system is exhibited by both the regulators, the interplay of miRNA-TFs will help in understanding the organization of several biological pathways.

Recently, miRNA-based research is focused on biotic and abiotic stress tolerance in plants. These stresses have a significant effect on plant growth and development and cause a great loss to yield. This chapter will provide deeper insights into miRNA-mediated gene regulation and their crosstalk with TFs, which will provide better understanding of plant responses to

http://dx.doi.org/10.5772/intechopen.75942

Database	Acronym	Public URL	Description
Plant transcription factor database	PlantTFDB	http://planttfdb.cbi.pku.edu.cn/	PlantTFDB contains 320,370 TFs from 165 plant species
Arabidopsis thaliana—Plant transcription factor database	PlnTFDB	http://plntfdb.bio.uni-potsdam.de/v3.0/index.php?sp_id=ATH	PlnTFDB contains 2657 protein models, 2451 distinct protein sequences of *A. thaliana*
Database collection: Plant transcription factor database	PlantTFDB	https://www.ebi.ac.uk/miriam/main/datatypes/MIR:00000579	Systematically identifies TFs for plant species
Plant TFDB∣ Transcription factor data: Sequence database	PlantTFDB	http://planttfdb.cbi.pku.edu.cn/	A database of functional and evolutionary study of TFs
Pigeon pea transcription factor database	PpTFDB	http://14.139.229.199/PpTFDB/Home.aspx	Provides a range of information about pigeon pea TFs, encompasses about 1829 TFs and classifies them into 55 TF families
Phaseolus vulgaris transcription factor database	PvTFDB	http://www.multiomics.in/PvTFDB/	Provides comprehensive information about each of the identified TF, encompasses 2370 TFs and classifies them into 49 TF families
Chickpea transcription factor database	CicerTransDB	http://www.cicertransdb.esy.es/documents/about.html	Facilitates uses with a platform for unified and comprehensive study of chickpea TFs
Arabidopsis gene regulatory information server	AGRIS	http://agris-knowledgebase.org/	Provides information about Arabidopsis promoter sequences, TFs and their target gene
Arabidopsis thaliana transcription factor database	AtTFDB	http://agris-knowledgebase.org/	Contains information about 1770 TFs and group them into 50 families on the basis of conserved domains
Database of rice transcription factors	DRTF	http://planttfdb.cbi.pku.edu.cn/	2048 TFs have been identified and are grouped into 56 families from subsp. japonica
Rice stress-responsive transcription factor database	RiceSRTFDB	http://www.nipgr.res.in/RiceSRTFDB.html	Provides most comprehensive information about the expression pattern of rice TFs during drought and salinity stress conditions
Database of populus transcription factors	DPTF	http://planttfdb.cbi.pku.edu.cn/	4287 TFs have been identified and are grouped into 58 families
Database of maize transcription factors	DMTF	http://planttfdb.cbi.pku.edu.cn/	3308 TFs have been identified and are grouped into 56 families
Database of tomato transcription factors	DTTF	http://planttfdb.cbi.pku.edu.cn/	1845 TFs have been identified and are grouped into 58 families
Database of wheat transcription factor	wDBTF	http://wwwappli.nantes.inra.fr:8180/wDBFT/	It contains about 1127 predicted TFs from bread wheat
Stress-responsive transcription factor database	STIFDB	http://caps.ncbs.res.in/stifdb2/	It is a comprehensive collection of biotic and abiotic stress-responsive genes in Arabidopsis and rice

Database	Acronym	Public URL	Description
Transcription factor prediction database	DBD	http://www.transcriptionfactor.org/index.cgi?Home	DBD is a database of predicted TFs in completely sequenced genomes
Interspecies TF function finder for plants	IT3F	http://jicbio.nbi.ac.uk/IT3F/	Provides information about function of TFs

Table 1. Plant transcription factors database.

various biotic and abiotic stresses and will help in developing high yielding and stress tolerant varieties, which is the ultimate aim of the agricultural scientists.

2. Regulatory roles of transcription factors in plants

TFs genes are regulated at both transcriptional and post-transcriptional level in plants [14]. Therefore, to build regulatory networks, understanding the expression of TFs is of great importance. Mainly, TFs act by binding the *cis* element present inside the transcription initiation (promoter) region of their target gene [15]. Recent studies have shown that changes in gene expression are closely related with changes in expression of TFs [16] affecting growth and development in plants [17]. Manipulation of desired traits in plants by engineering TF genes is considered as a major future outlook [18].

Nuclear factor Y (NF-Y) is a class of transcription factor that has three subunits and all are vital for DNA-binding ability (NF-YA, NF-YB and NF-YC) [19]. The function of these TFs varies with the type of subunit. For example, NF-YA and NF-YB are involved in plant responses to drought stress, whereas NF-YC is involved in the regulation of flower development and light-mediated plant growth and development (photomorphogenesis) [20]. NF-Y transcription factors are also involved in plant-microbe interaction, root development and responses to stress [21]. Dark-grown phenotype was exhibited by *NF-Y* mutant plants even in the presence of light; this indicates that NFY TF is a positive regulator of photomorphogenesis [20]. In combination with NF-YB/NF-YC, NF-YA was found to be involved in flowering by triggering *FLOWERING LOCUS T* (FT) gene [22]. Overexpression of NF-YA5 in Arabidopsis resulted in tolerance to drought stress [23]. ABA disruptive phenotype was exhibited by *NF-YC* mutant Arabidopsis plants [24]. Nuclear factor Y complex binds with a unique *cis*-element within the SOC1 promoter region of Arabidopsis and regulates flowering time [25]. In Arabidopsis, leaf development is regulated by NF-YA2 and NF-YA10 via auxin signaling [26]. Arabidopsis nuclear transcription factor genes NF-YA1, 5, 6 and 9 play an important role in the regulation of male gametogenesis, embryogenesis and seed germination [27]. NF-YB confers drought tolerance and leads to improved yield in maize under water-limited conditions [28]. In Arabidopsis, NF-YC3, 4 and 9 are required for regulation of CONSTANTS (CO)-mediated photoperiod-independent flowering [29]. During the early seedling stage in Arabidopsis, under photomorphogenesis, hypocotyl elongation is suppressed by NF-YC1, 3, 4 and 9 [30]. Wheat *TaNF-YB3* gene imparts drought tolerance by regulating ABA-associated signaling pathway [31]. Overexpression of NF-YC9 confers ABA hypersensitivity in Arabidopsis [32].

MYB (myeloblastosis), a huge family protein, is characteristic of all eukaryotes and plays a diverse role in gene networking. Generally, MYB functions as transcription factor and their DNA-binding ability varies with the number of MYB domains [33]. In plants, MYB proteins are classified in four different classes depending upon the number of DNA-binding MYB domains: MYB-related, R2R3-MYBs, R1R2R3-MYBs and atypical MYBs [34]. The first plant MYB gene C1 was identified from maize [35]. Since their identification, they have been found to be extensively dispersed in plants and communicate with additional transcription factors [36]. MYB transcription factors are involved in the regulation of plant growth and development in various species like in soybean, they are involved in regulation of flower color [37] and regulation of signal transduction pathways in Arabidopsis, rice and cassava [38]. Biosynthesis of secondary metabolites is regulated in Arabidopsis and Medicago [36]. In Arabidopsis, sugarcane, potato, cotton, wheat, rice and *Camelina sativa*, they are involved in drought tolerance [39]. Chilling tolerance is imparted in Arabidopsis, wheat and rice [40]. MYB transcription factor genes are also involved in combating salt stress [41].

Arabidopsis transcription factor APETALA2 (AP2) is involved in the regulation of complicated processes of plant growth and development, which includes seed development, maintenance of stem cells and flower development [42]. APETALA2 family, also known as "A" class, acts together with B and C class to determine the final floral organ development, and this interaction of transcription factors forms the well-known ABC model of flower development [43]. Pandey et al. identified an APETALA2 (AP2) domain TF in Arabidopsis that suppresses ABA response during seed germination and ABA and stress-induced gene expression. They also observed that *abr1* mutant plants were hypersensitive to osmotic stress and higher level of ABA was found in mutant plants; this supports that ABA-mediated gene regulation is suppressed by AP2 [44]. Overexpression of *Nicotiana tabacum Tsi1* gene encoding an EREBP/AP2 TF in tobacco enhances resistance against osmotic stress and pathogen attack [45]. Overexpression of *WXP1,* an AP2 domain-containing TF gene of *Medicago truncatula*, enhances wax accumulation and drought tolerance in transgenic alfalfa [46]. Overexpression of *ORA59*, an AP2/ERF transcription factor domain, results in enhanced resistance against fungus *Botrytis cinerea* [47]. WIND1 and AP2/ERF TFs regulate cell differentiation in Arabidopsis [48]. WRINKLED1 (WRI1), an AP2-type transcription factor, was found to be associated with triacylglycerol (TAGs) accumulation in Arabidopsis [49].

TCF transcription factors comprise a domain, called TCP domain, which shares a motif that forms a basic helix-loop-helix (bHLH) structure that has DNA-binding properties [50]. The name TCP came from TEOSINTE BRANCHED1, CYCLOIDEA (CYC) and PROLIFERATING CELL NUCLEAR ANTIGEN FACTOR1 (PCF1) and PCF2, first four members of the TCP family derived from maize, snapdragon and rice, respectively [51]. Earlier studies have shown that TCP has been involved in the regulation of leaf formation by regulating cell cycle [52]. TCP transcription factors are also involved in flower development [53], leaf senescence [54], shoot development [55], jasmonic acid and auxin signaling [56], cell proliferation [57], leaf shape regulation [58], development of macro and micro [50], mitochondrial biogenesis [59] and regulating circadian cycle [60].

One of the largest and diverse families of plant regulators is WRKY transcription factors, with nearly 74 members in Arabidopsis, over 100 in rice, soybean and poplar [61]. There is at least one conserved DNA-binding domain called WRKY domain, which comprises a preserved protein

sequence (WRKYGQK) and a zinc-finger domain. Both of these sequences (hexapeptide and zinc finger domain) are required for binding to *cis* element known as W box (TTGACT/C) [62]. WRKY transcription factors are involved in several molecular and genetic pathways to regulate multiple responses simultaneously, whether it is abiotic or biotic stress [63]. Production of few secondary metabolites like lignin, flavanols and tannins is also regulated by WRKY TFs [64].

NAC transcription factors are one of the major class of plant regulators, engaged in stress responses. The name NAC is derived from three genes initially having the NAC domain; no apical meristem (NAM), Arabidopsis transcription activation factor (ATAF1/2) and cup-shaped cotyledon (CUC2) [65]. The availability of genome sequencing technology has led to the identification of several NAC TFs genes in various species like 117 in Arabidopsis, 151 in rice, 79 in grape, 26 in citrus, 163 in poplar, 152 each in soybean and tobacco, 145 in cotton, 45 in tea plant, 172 in radish, 152 in maize and 110 in potato [66]. In Arabidopsis, of 10 NAC domains 9 domains bind to a conserved DNA target with a GGT[GA] core [67]. NAC TFs are mainly involved in the regulation of plant growth and development under biotic and abiotic stress [68].

Another important class of TFs that belong to plant kingdom is homeodomain-leucine zippers (HD-Zip). In Arabidopsis, there are more than 25 genes that encode these TFs. The HD-Zip protein is characterized by the presence of two important domains: a homeodomain (HD) involved in DNA binding and leucine zipper domain (Zip) responsible for protein-protein interactions [69]. On the basis of earlier sequence similarity findings, HD-Zip class of TFs has been grouped into four different classes (HD-Zip I, II, III and IV). Class I TFs (HD-Zip I) are engaged in ABA (abscisic acid) signaling, embryo development and responses to abiotic stress. Class II (HD-Zip II) TFs are involved hormone signaling (auxin), responses to light and shade. Likewise, class III (HD-Zip III) regulate embryo development, initiation of lateral organs, leaf polarity and meristem functioning, whereas class IV (HD-Zip IV) governs trichome development, root development, epidermal cell differentiation and accumulation of anthocyanin [69].

3. Interplay between transcription factors and miRNA

Plant miRNAs are involved in regulatory networks, which control differential gene expression at tissue and developmental levels. MiRNAs and TFs provide combinatorial gene regulation involving diverse functions which can further be exploited in crop improvement. Combination of microRNA and their targets, which are mainly transcription factors that depict an integrated image for designing regulatory relationship but it could be very difficult at times to develop a clear cut relationship as interaction could take place with each other leading to some novel regulatory pathway. With the advancement in bioinformatic softwares and use of advanced techniques, it is comparatively easy to develop an interaction. MiRNA and TFs are among the primary regulators of gene expression, thus affect plant phenotype in relation to growth and development (**Table 2**).

3.1. Root architecture

MiRNAs and TFs together govern the regulatory network involved in the development of root architecture in various species. In *A. thaliana*, miR160 is known to play key role in root growth

http://dx.doi.org/10.5772/intechopen.75942

miRNA	TF family	Plant	Role
169	NY-FA	*A. thaliana*	Root architecture, nodule formation, drought and salinity stress, abscisic acid response
159	MYB	*A. thaliana*	Seed germination, senescence, ABA hypersensitivity
		O. sativa	
828 and 858	MYB	*G. hirsutum*	Fiber development, response to high temperature
164	NAC1	*A. thaliana*	Lateral root development
		Z. mays	
		T. aestivum	Contribute resistance against *P. striiformis f. sp. Tritici (Pst*)
396	GRF	*A. thaliana*	Leaf and grain development
		Z. mays	
		O. sativa	Response to arsenic treatment
	WRKY	*H. annuus* L.	Response to high temperature
319	TCP	*A. thaliana*	Leaf and floral development, jasmonic acid biosynthesis
164	NAC	*A. thaliana*	Lateral root development
	NAC1	*Z. mays*	Drought tolerance
166	HD-Zip III	*A. thaliana*	Shoot apical meristem, organ polarity and vascular development
156	SPL	*A. thaliana*	Floral development
		Z. mays	
		O. sativa	
		S. lycopersicum	
172	AP2	*Glycine max,*	Nodule formation
		P. vulgaris	
447 and 5255	MYB	*G. hirsutum*	Root and fiber development

Table 2. Differential role of TF-MiRNA interaction in plants.

by negatively regulating AUXIN RESPONSE FACTORs (ARF 10, 16 and 17) and resulted into shorter roots with tumor like puffed-up apex, if overexpression of miRNA160 occurs [70]. Apart from this, another miR164 targets transcription factors of the NAC (NAMATAF-CUC) family and regulates lateral root initiation by limiting NAC1expression [71]. Similarly, in legumes such as *M. truncatula*, miR166 and HD-Zip regulate cell-to-cell communication in root vascular and meristematic tissues [72]. In *A. thaliana*, miR169 isoforms are engaged in targeting NF-YA TF and control primary root growth. The prevention of miR169 expression affects lateral root initiation led to altered dimensions in root meristem [73]. The cross talk between miR166/165 and their target HD-Zip III ensures root development in *Arabidopsis thaliana* as well as in Maize.

3.2. Phosphate content

Phosphorus is essential nutrient for plants and can be acquired by plants only as inorganic phosphate. Certain transcription factors, such as AtPHR1, AtWRKY75, AtZAT6 and

AtBHLH32, regulate phosphate starvation responsive genes in plants. The interplay between miR399 and transcription factor AtMYB2 is known to function in abiotic stress signaling in Arabidopsis, and overexpression of AtMYB2 results into increased phosphorous uptake and changes in root architecture [39].

3.3. Leaf senescence

Leaf senescence is a physiological process, which affects vegetative and productive developmental processes in plants. Increased seed yield and prolonged life span are observed during delayed. The conversion, which occurs from leaf maturation to senescence, is complex and is associated with several genes and transcription factors such as MYB, SQUAMOSA PRMOTER BINDING-LIKE (SPL), WRKY, etc. [74]. Transcription factor MYB was targeted by zms-miR 159d and was downregulated in maize inbred line ELS-1, whereas in Yu87-1inbreed line, zms-miR 159d was found to be upregulated [75].

3.4. Fiber development

Various studies have reported that different transcription factors play an important role in fiber initiation. For example, MYB transcription factors are involved in fiber trichome development in cotton. TFs are predicted to be targeted by certain miRNAs such as MYB3 and MYB88 are targeted by miR447, which is significantly expressed during different fiber initiation, elongation and secondary wall synthesis and play important role in fiber development under salinity and drought stress [76]. In a recent study, MYB genes, including MYB2, MYB3 and MYB12, are targeted by miR828 and are known to play negative role in fiber elongation [77].

3.5. Floral development

Different microRNAs function and play role throughout flower development from early stages to late stages. These microRNAs target various transcription factors by targeting and downregulation and affect floral timing [78]. There are around 11 different miRNA families (miR156, miR159, miR160, miR164, miR165/miR166, miR167, miR169, miR172, miR319, miR390 and miR399) that regulate flower development at several stages. MiR156, miR172 and miR399 mediate plant changes from juvenile to adult, whereas mR159, miR169, miR172 and miR399 mediate transition from vegetative to adult. MiR156 controls flower development in rice, tomato and maize, and its role is found to be conserved [78]. The targets of miR156 are SPL (SQUAMOSA PROMOTER BINDING-LIKE) TFs, which are being downregulated in Arabidopsis, and miR172 targets expression of APETELA2, which resulted in delayed flowering by inhibiting translation [79].

3.6. Nodule formation

Nodule formation and establishment of symbiotic relationship are complex processes. Various miRNA and transcription factors are associated with nodule development. It was suggested that miR169-mediated repression of MtHAP2, a transcription factor, was required for nodule

development in *M. truncatula* [76]. In legumes, such as soybean and common beans, miR172 interacts with AP2 TFs to regulate nodule organogenesis [80].

3.7. Leaf morphogenesis and grain filling

MicroRNA and TFs play a vital role in leaf morphogenesis such as miR319 and TCP are involved in regulation of leaf size. Increase in leaf size was observed with loss of function of miR319 [81]. Similarly, miR319 overexpression resulted in enlarged leaf formation in tomato [82]. Another miRNA family (miR396) targets GRF (GROWTH-REGULATING FACTOR) TF family and regulates leaf morphogenesis [83]. Cell division in leaves is enhanced by suppression of six GRF genes and GIF1 by overexpression of miR396 [83]. MiR396 and GRF TFs are found to be associated with effective grain filling in maize [84]. Similar findings were observed in rice where LOC_Os02g47280 was downregulated by miR396 and was found to be responsible for grain shape [85]. These studies approved the networking between miR396 and GRF transcription factor and suggested the strong role in leaf development and grain filling.

3.8. Shoot apical meristem and vascular patterning

Plants exhibit a long period of organogenesis and give rise to new leaves throughout their life cycle depending upon the activity of shoot meristems. The transcripts of miR165 and miR166 are detected in shoot apical meristem, leaf primordial and vascular tissues in Arabidopsis. The interaction of HD-Zip III with miR165 and miR166 is well known [86]. It regulates diverse functions including plant development, apical and lateral meristem formation, vascular growth and leaf polarity. Downregulation of three HD-Zip genes (ATHB-9/PHV, ATHB-14/PHB and ATHB-15) resulted into recapitulate phenotype upon overexpression of miR166. Similarly, downregulation of five HD-Zip genes by overexpression of miR165 resulted in loss of SAM (shoot apical meristem), changed organ polarity and defected vascular development [87]. MiR165 and miR166 are involved in the regulation of leaf asymmetry patterning in maize and Arabidopsis. The suppression of HD-Zip by miRNA is responsible for vascular patterning in leaves and stem in both monocots and dicots [86].

3.9. Flavonoid biosynthesis pathway

In Arabidopsis, miR858a is supposed to target R2R3-MYB transcription factor. Genomic analysis suggested that miR858a targets various regulatory factors involved in plant growth and development. Overexpression of miR858a led to downregulation of several MYB transcription factors, which in turn regulates and redirects the metabolic flux towards flavonoid biosynthesis [88].

3.10. Jasmonic acid biosynthesis

Jasmonic acid (JA) acts as systemic signaling molecule, which is effective against tomato root knot disease (RKN). This can reduce the number of root knots from nematode invasion resulting into JA-mediated RKN resistance in roots. Several miRNAs are found responsive

to jasmonic acid against pathogen infection. Recent study demonstrated negative correlation between miR319 and its target TCP4 in tomato using reverse genetic approaches. This interaction leads to change in levels of jasmonic acid in leaves. The potential cross talk between miR319 and TCP4 modulates systemic defensive response [89].

3.11. High temperature tolerance

An environmental fluctuation such as high temperature imparts detrimental effect on plants. Some plants show tolerance to these stresses than others and are regulated by a wide network of transcriptional cross talk between transcription factors such as WRKY, ERF, NAC, MADS and miRNA. WRKY TFs found most exclusively in plants and are involved in various developmental and physiological processes. When plants are exposed to high temperature or salicylic acid in case of sunflower, opposite expression of HaWRKY6 and miR396 was observed [90]. In case of cotton, MYB transcription factor is known to be upregulated against high temperature and was targeted by miR828a and miR858 [91].

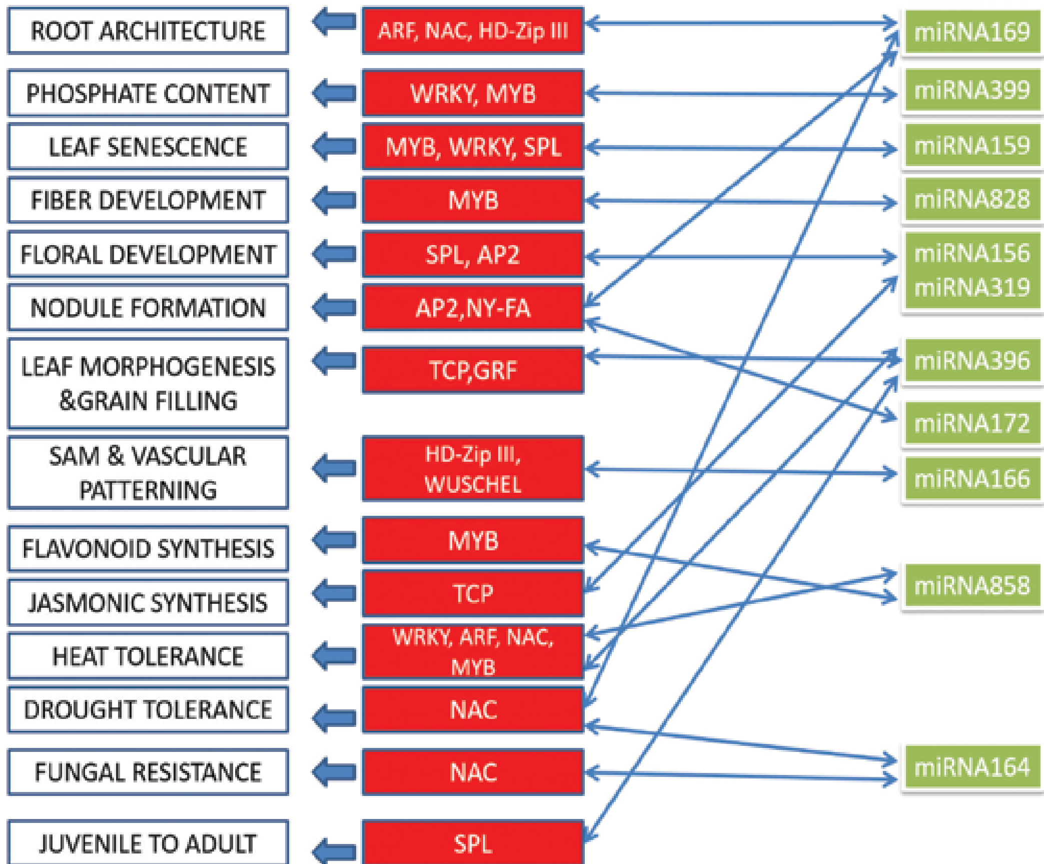

Figure 1. Interaction between miRNAs and TFs for gene regulation in plants.

http://dx.doi.org/10.5772/intechopen.75942

3.12. Drought tolerance

Communication between miR164 and NAC TF genes confer negative regulatory role in drought resistance in rice in addition to developmental roles. In transgenic Arabidopsis plants, overexpression of miR169a in NF-YA5 mutants resulted in increased susceptibility towards water stress in comparison to wild-type plants. Enhanced drought tolerance was observed in plants overexpressing NF-YA5. In addition to drought tolerance, miR169 is also related with salt stress [92]. This phenomenon was also observed with miR393 [93].

3.13. Fungal pathogen resistance

The molecular crosstalk between miRNA and transcription factor is necessary to better understand the disease development. In wheat, stripe rust caused by Puccinia is a serious disease occurring during growing season. Crosstalk between miR164 and NAC21/22 TF resulted into reduced stripe rust resistance. These results conclude that mir164 and novel transcription factor are imperative in the development of stripe rust resistance in wheat [94].

3.14. Juvenile to adult plant development

The conversion from juvenile to adult is accompanied by changes in vegetative morphology and increase in reproductive potential. The regulatory mechanism of this transition involves miR156, miR172 and SPL gene family in case of Arabidopsis. SQUAMOSA PROMOTER BINDING-LIKE (SPL) TF family is a major target of miR156, and 11 SPL genes are repressed through translational inhibition and mRNA cleavage [95]. MiR156 and miR172 are positively regulated by transcription factors they target, and negative feedback loops contribute to stability of juvenile and adult phases (**Figure 1**) [79].

4. Conclusion

Regulatory network involving TFs and miRNA provides deep insight in understanding the complexity of gene regulation in plants. Till date, the computationally and experimentally mapped networks portray considerable information on gene regulation. The complete spectrum of miRNA and their interactions with transcription factors need to be considered in order to study regulatory interactions at particular developmental times or in a tissue specific manner. However, it will be imperative to incorporate all accessible miRNA, TF and target expression blueprint to confine the network to just those communications that can happen and to extend the studies in different set of conditions. For the computational researchers, the particular issues will be to gather and analyze the accessible information, make predictions and to approve the speculations in view of literature or wet lab experiments for set up of regulatory network. In near future, better understanding of regulatory networks is expected, which will enable us for manipulating gene expression for crop improvement and industrial applications. At present, it is, by all accounts, a difficult work to build complete real-time networks

for more experimental information. Still, it is a long way to establish complete miRNA-mediated regulatory network in plants.

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References

[1] Morris KV, Mattick JS. The rise of regulatory RNA. Nature Reviews Genetics [Internet]. Jun 29, 2014;**15**(6):423-437. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24776770 [Accessed: Jan 19, 2018]

[2] Osório J. Landscape and mechanisms of transcription factor cooperativity. Nature Reviews Genetics [Internet]. Jan 23, 2016;**17**(1):5-5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26593418 [Accessed: Jan 19, 2008]

[3] Duval I, Lachance D, Giguère I, Bomal C, Morency M-J, Pelletier G, et al. Large-scale screening of transcription factor-promoter interactions in spruce reveals a transcriptional network involved in vascular development. Journal of Experimental Botany [Internet]. Jun 2014;**65**(9):2319-2333. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24713992 [Accessed: Jan 19, 2018]

[4] Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell. 2004;**116**: 281-297

[5] Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell [Internet]. Dec 1993;**75**(5):843-854. Available from: http://www.cell.com/cell/pdf/0092-8674(93)90529-Y.pdf?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2F009286749390529Y%3Fshowall%3Dtrue [Accessed: Feb 17, 2018]

[6] Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, et al. The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature [Internet]. Feb 24, 2000;**403**(6772):901-906. Available from: http://www.nature.com/articles/35002607 [Accessed: Feb 17, 2008]

[7] Wang Y, Lan Q, Zhao X, Xu W, Li F, Wang Q, et al. Comparative profiling of microRNA expression in soybean seeds from genetically modified plants and their near-isogenic parental lines (Xue Y, editor). PLoS One [Internet]. May 23, 2016;**11**(5):e0155896. Available from: http://dx.plos.org/10.1371/journal.pone.0155896 [Accessed: Jan 19, 2018]

[8] Nazarov P V, Reinsbach SE, Muller A, Nicot N, Philippidou D, Vallar L, et al. Interplay of microRNAs, transcription factors and target genes: Linking dynamic expression changes to function. Nucleic Acids Research [Internet]. Mar 1, 2013;**41**(5):2817-2831. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23335783 [Accessed: Jan 19, 2008]

[9] Cammaerts S, Strazisar M, De Rijk P, Del Favero J. Genetic variants in microRNA genes: Impact on microRNA expression, function, and disease. Frontiers in Genetics [Internet]. 2015;**6**:186. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26052338 [Accessed: Jan 19, 2018]

[10] Akhtar MM, Micolucci L, Islam MS, Olivieri F, Procopio AD. Bioinformatic tools for microRNA dissection. Nucleic Acids Research [Internet]. Jan 8, 2016;**44**(1):24-44. DOI: 10.1093/nar/gkv1221 [Accessed: Jan 19, 2018]

[11] Jin J, Tian F, Yang D-C, Meng Y-Q, Kong L, Luo J, et al. PlantTFDB 4.0: Toward a central hub for transcription factors and regulatory interactions in plants. Nucleic Acids Research [Internet]. Jan 4, 2017;**45**(D1):D1040-D10405. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27924042 [Accessed: Jan 20, 2018]

[12] Chow C-N, Zheng H-Q, Wu N-Y, Chien C-H, Huang H-D, Lee T-Y, et al. PlantPAN 2.0: An update of plant promoter analysis navigator for reconstructing transcriptional regulatory networks in plants. Nucleic Acids Research [Internet]. Jan 4, 2016;**44**(D1):D1154-D1160. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26476450 [Accessed: Jan 20, 2018]

[13] Mitsuda N, Ohme-Takagi M. Functional analysis of transcription factors in arabidopsis. Plant and Cell Physiology [Internet]. Jul 2009;**50**(7):1232-1248. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19478073 [Accessed: Jan 20, 2018]

[14] Payne JL, Wagner A. Mechanisms of mutational robustness in transcriptional regulation. Frontiers in Genetics [Internet]. Oct 27, 2015;**6**(Oct):322. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26579194 [Accessed: Jan 20, 2018]

[15] Biłas R, Szafran K, Hnatuszko-Konka K, Kononowicz AK. Cis-regulatory elements used to control gene expression in plants. Plant Cell, Tissue and Organ Culture [Internet]. Nov 10, 2016;**127**(2):269-287. Available from: http://link.springer.com/10.1007/s11240-016-1057-7 [Accessed: Jan 20, 2018]

[16] Yan X, Dong C, Yu J, Liu W, Jiang C, Liu J, et al. Transcriptome profile analysis of young floral buds of fertile and sterile plants from the self-pollinated offspring of the hybrid between novel restorer line NR1 and Nsa CMS line in *Brassica napus*. BMC Genomics [Internet]. Jan 16, 2013;**14**(1):26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23324545 [Accessed: Jan 21, 2018]

[17] Li J, Han S, Ding X, He T, Dai J, Yang S, et al. Comparative transcriptome analysis between the cytoplasmic male sterile line NJCMS1A and its maintainer NJCMS1B in soybean (*Glycine max* (L.) Merr.). Tian Z, editor. PLoS One [Internet]. May 18, 2015;**10**(5):e0126771. Available from: http://dx.plos.org/10.1371/journal.pone.0126771 [Accessed: Jan 21, 2018]

[18] Weng L, Bai X, Zhao F, Li R, Xiao H. Manipulation of flowering time and branching by overexpression of the tomato transcription factor SlZFP2. Plant Biotechnology Journal

[Internet]. Dec 2016;**14**(12):2310-2321. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27214796 [Accessed: Jan 21, 2018]

[19] Ren C, Zhang Z, Wang Y, Li S, Liang Z. Genome-wide identification and characterization of the NF-Y gene family in grape (*Vitis vinifera* L.). BMC Genomics [Internet]. 2016;**17**(1):605. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27516172 [Accessed: Jan 21, 2018]

[20] Myers ZA, Kumimoto RW, Siriwardana CL, Gayler KK, Risinger JR, Pezzetta D, et al. Nuclear factor Y, subunit C (NF-YC) transcription factors are positive regulators of photomorphogenesis in *Arabidopsis thaliana*. Hake S, editor. PLoS Genet [Internet]. Sep 29, 2016;**12**(9):e1006333. Available from: http://dx.plos.org/10.1371/journal.pgen.1006333 [Accessed: Jan 21, 2018]

[21] Zanetti ME, Rípodas C, Niebel A. Plant NF-Y transcription factors: Key players in plant-microbe interactions, root development and adaptation to stress. Biochimica et Biophysica Acta [Internet]. May 2017;**1860**(5):645-654. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27939756 [Accessed: Jan 22, 2018]

[22] Siriwardana CL, Gnesutta N, Kumimoto RW, Jones DS, Myers ZA, Mantovani R, et al. Nuclear factor Y, subunit A (NF-YA) proteins positively regulate flowering and act through *FLOWERING LOCUS T*. Muday GK, editor. PLOS Genetics [Internet]. Dec 15, 2016;**12**(12):e1006496. Available from: http://dx.plos.org/10.1371/journal.pgen.1006496 [Accessed: Jan 21, 2018]

[23] Petroni K, Kumimoto RW, Gnesutta N, Calvenzani V, Fornari M, Tonelli C, et al. The promiscuous life of plant nuclear factor Y transcription factors. Plant Cell [Internet]. Dec 1, 2012;**24**(12):4777-4792. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23275578 [Accessed: Jan 21, 2018]

[24] Kumimoto RW, Siriwardana CL, Gayler KK, Risinger JR, Siefers N, Holt BF. Nuclear factor Y transcription factors have both opposing and additive roles in ABA-mediated seed germination. Huq E, editor. PLoS One [Internet]. Mar 19, 2013;**8**(3):e59481. Available from: http://dx.plos.org/10.1371/journal.pone.0059481 [Accessed: Jan 22, 2018]

[25] Hou X, Zhou J, Liu C, Liu L, Shen L, Yu H. Nuclear factor Y-mediated H3K27me3 demethylation of the SOC1 locus orchestrates flowering responses of Arabidopsis. Nature Communications [Internet]. Aug 8, 2014;**5**:4601. DOI: 10.1038/ncomms5601 [Accessed: Jan 21, 2018]

[26] Zhang M, Hu X, Zhu M, Xu M, Wang L. Transcription factors NF-YA2 and NF-YA10 regulate leaf growth via auxin signaling in Arabidopsis. Scientific Reports [Internet]. May 3, 2017;**7**(1):1395. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28469131 [Accessed: Jan 22, 2018]

[27] Mu J, Tan H, Hong S, Liang Y, Zuo J. Arabidopsis transcription factor genes NF-YA1, 5, 6, and 9 play redundant roles in male gametogenesis, embryogenesis, and seed development. Molecular Plant [Internet]. 2013;**6**(1):188-201. Available from: http://www.cell.com/molecular-plant/pdf/S1674-2052(14)60890-X.pdf [Accessed: Jan 22, 2018]

[28] Nelson DE, Repetti PP, Adams TR, Creelman RA, Wu J, Warner DC, et al. Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields

on water-limited acres. Proceedings of the National Academy of Sciences of the United States of America [Internet]. Oct 16, 2007;**104**(42):16450-16455. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17923671 [Accessed: Jan 22, 2018]

[29] Kumimoto RW, Zhang Y, Siefers N, Holt BF. NF-YC3, NF-YC4 and NF-YC9 are required for CONSTANS-mediated, photoperiod-dependent flowering in *Arabidopsis thaliana*. Plant Journal [Internet]. 2010 Aug 1, 2010;**63**(3):379-391. DOI: 10.1111/j.1365-313X.2010.04247.x [Accessed: Jan 22, 2018]

[30] Tang Y, Liu X, Liu X, Li Y, Wu K, Hou X. Arabidopsis NF-YCs mediate the light-controlled hypocotyl elongation via modulating histone acetylation. Molecular Plant [Internet]. Feb 13, 2017;**10**(2):260-273. Available from: http://www.sciencedirect.com/science/article/pii/S1674205216302775 [Accessed: Jan 22, 2018]

[31] Yang M, Zhao Y, Shi S, Du X, Gu J, Xiao K. Wheat nuclear factor Y (NF-Y) B subfamily gene TaNF-YB3;l confers critical drought tolerance through modulation of the ABA-associated signaling pathway. Plant Cell, Tissue and Organ Culture [Internet]. Jan 19, 2017;**128**(1):97-111. Available from: http://link.springer.com/10.1007/s11240-016-1088-0 [Accessed: Jan 22, 2018]

[32] Bi C, Ma Y, Wang X-F, Zhang D-P. Overexpression of the transcription factor NF-YC9 confers abscisic acid hypersensitivity in Arabidopsis. Plant Molecular Biology [Internet]. Nov 18, 2017;**95**(4-5):425-439. Available from: http://link.springer.com/10.1007/s11103-017-0661-1 [Accessed: 2018 Jan 22]

[33] Ambawat S, Sharma P, Yadav NR, Yadav RC. MYB transcription factor genes as regulators for plant responses: an overview. Physiology and Molecular Biology of Plants [Internet]. Jul 2013;**19**(3):307-321. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24431500 [Accessed: Jan 22, 2018]

[34] Dubos C, Stracke R, Grotewold E, Weisshaar B, Martin C, Lepiniec L. MYB transcription factors in Arabidopsis. Trends in Plant Sciences [Internet]. Oct 2010;**15**(10):573-581. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20674465 [Accessed: Jan 22, 2018]

[35] Paz-Ares J, Ghosal D, Wienand U, Peterson PA, Saedler H. The regulatory c1 locus of *Zea mays* encodes a protein with homology to myb proto-oncogene products and with structural similarities to transcriptional activators. EMBO Journal [Internet]. Dec 1, 1987;**6**(12):3553-3558. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3428265 [Accessed: Jan 22, 2018]

[36] Nguyen NH, Lee H. MYB-related transcription factors function as regulators of the circadian clock and anthocyanin biosynthesis in Arabidopsis. Plant Signaling & Behavior [Internet]. Mar 3, 2016;**11**(3):e1139278. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26905954 [Accessed: Jan 22, 2018]

[37] Takahashi R, Yamagishi N, Yoshikawa N. A MYB transcription factor controls flower color in soybean. Journal of Heredity [Internet]. Jan 1, 2013;**104**(1):149-153. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23048163 [Accessed: Jan 22, 2018]

[38] Liao W, Yang Y, Li Y, Wang G, Peng M. Genome-wide identification of cassava R2R3 MYB family genes related to abscission zone separation after environmental-stress-induced

abscission. Scientific Reports [Internet]. Oct 30, 2016;**6**(1):32006. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27573926 [Accessed: Jan 22, 2018]

[39] Baldoni E, Genga A, Cominelli E. Plant MYB transcription factors: Their role in drought response mechanisms. International Journal of Molecular Sciences [Internet]. Jul 13, 2015;**16**(7):15811-15851. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26184177 [Accessed: Jan 22, 2018]

[40] Yang A, Dai X, Zhang W-H. A R2R3-type MYB gene, OsMYB2, is involved in salt, cold, and dehydration tolerance in rice. Journal of Experimental Botany [Internet]. Apr 1, 2012;**63**(7):2541-2556. Available from: 10.1093/jxb/err431 [Accessed: Jan 22, 2018]

[41] Kim JH, Nguyen NH, Jeong CY, Nguyen NT, Hong S-W, Lee H. Loss of the R2R3 MYB, AtMyb73, causes hyper-induction of the SOS1 and SOS3 genes in response to high salinity in Arabidopsis. Journal of Plant Physiology [Internet]. Nov 1, 2013;**170**(16):1461-1465. Available from: http://www.sciencedirect.com/science/article/pii/S0176161713002241 [Accessed: Jan 22, 2018]

[42] Liu Z, Gu C, Chen F, Jiang J, Yang Y, Li P, et al. Identification and expression of an APETALA2-like gene from *Nelumbo nucifera*. Applied Biochemistry and Biotechnology [Internet]. Sep 22, 2012;**168**(2):383-391. Available from: http://link.springer.com/10.1007/s12010-012-9782-9 [Accessed: Jan 22, 2018]

[43] Xie W, Huang J, Liu Y, Rao J, Luo D, He M. Exploring potential new floral organ morphogenesis genes of *Arabidopsis thaliana* using systems biology approach. Frontiers in Plant Science [Internet]. Oct 13, 2015;**6**:829. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26528302 [Accessed: Jan 22, 2018]

[44] Pandey GK, Grant JJ, Cheong YH, Kim BG, Li L, Luan S. ABR1, an APETALA2-domain transcription factor that functions as a repressor of ABA response in Arabidopsis. Plant Physiology [Internet]. Nov 1, 2005;**139**(3):1185-1193. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16227468 [Accessed: Jan 22, 2018]

[45] Park JM, Park CJ, Lee SB, Ham BK, Shin R, Paek KH. Overexpression of the tobacco Tsi1 gene encoding an EREBP/AP2-type transcription factor enhances resistance against pathogen attack and osmotic stress in tobacco. Plant Cell [Internet]. May 1, 2001;**13**(5):1035-1046. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11340180 [Accessed: Jan 22, 2018]

[46] Zhang J-Y, Broeckling CD, Blancaflor EB, Sledge MK, Sumner LW, Wang Z-Y. Overexpression of WXP1, a putative *Medicago truncatula* AP2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (*Medicago sativa*). Plant Journal [Internet]. Apr 21, 2005;**42**(5):689-707. DOI: 10.1111/j.1365-313X.2005.02405.x [Accessed: Jan 22, 2018]

[47] Pré M, Atallah M, Champion A, De Vos M, Pieterse CMJ, Memelink J. The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. Plant Physiol [Internet]. Jul 1, 2008;**147**(3):1347-1357. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18467450 [Accessed: Jan 22, 2018]

[48] Iwase A, Mitsuda N, Koyama T, Hiratsu K, Kojima M, Arai T, et al. The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in arabidopsis. Current Biology [Internet]. Mar 22, 2011;**21**(6):508-514. Available from: http://www.sciencedirect.com/science/article/pii/S0960982211002119 [Accessed: Jan 22, 2018]

[49] Maeo K, Tokuda T, Ayame A, Mitsui N, Kawai T, Tsukagoshi H, et al. An AP2-type transcription factor, WRINKLED1, of *Arabidopsis thaliana* binds to the AW-box sequence conserved among proximal upstream regions of genes involved in fatty acid synthesis. Plant Journal [Internet]. Nov 1, 2009;**60**(3):476-487. DOI: 10.1111/j.1365-313X.2009.03967.x [Accessed: Jan 22, 2018]

[50] Li S. The *Arabidopsis thaliana* TCP transcription factors: A broadening horizon beyond development. Plant Signaling & Behavior [Internet]. Jun 3, 2015;**10**(7):1-12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26039357 [Accessed: Jan 22, 2018]

[51] Danisman S. TCP transcription factors at the interface between environmental challenges and the plant's growth responses. Frontiers in Plant Science [Internet]. Dec 21, 2016;**7**:1930. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28066483 [Accessed: Jan 22, 2018]

[52] Bresso EG, Chorostecki U, Rodriguez RE, Palatnik JF, Schommer C. Spatial control of gene expression by miR319-regulated TCP transcription factors in leaf development. Plant Physiology [Internet]. Nov 13, 2017;**176**(2):1694-1708. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29133375 [Accessed: Jan 22, 2018]

[53] Chai W, Jiang P, Huang G, Jiang H, Li X. Identification and expression profiling analysis of TCP family genes involved in growth and development in maize. Physiology and Molecular Biology of Plants [Internet]. Oct 11, 2017;**23**(4):779-791. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29158628 [Accessed: Jan 22, 2018]

[54] Schommer C, Palatnik JF, Aggarwal P, Chételat A, Cubas P, Farmer EE, et al. Control of jasmonate biosynthesis and senescence by miR319 targets. Carrington JC, editor. PLoS Biology [Internet]. Sep 23, 2008;**6**(9):e230. Available from: http://dx.plos.org/10.1371/journal.pbio.0060230 [Accessed: Jan 22, 2018]

[55] Koyama T, Furutani M, Tasaka M, Ohme-Takagi M. TCP transcription factors control the morphology of shoot lateral organs via negative regulation of the expression of boundary-specific genes in Arabidopsis. Plant Cell [Internet]. Feb 9, 2007;**19**(2):473-484. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17307931 [Accessed: Jan 22, 2018]

[56] Danisman S, van der Wal F, Dhondt S, Waites R, de Folter S, Bimbo A, et al. Arabidopsis class I and class II TCP transcription factors regulate jasmonic acid metabolism and leaf development antagonistically. Plant Physiology [Internet]. Aug 1, 2012;**159**(4):1511-1523. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22718775 [Accessed: Jan 22, 2018]

[57] Davière J-M, Wild M, Regnault T, Baumberger N, Eisler H, Genschik P, et al. Class I TCP-DELLA interactions in inflorescence shoot apex determine plant height. Current Biology [Internet]. Aug 18, 2014;**24**(16):1923-1928. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25127215 [Accessed: Jan 22, 2018]

[58] Ma X, Ma J, Fan D, Li C, Jiang Y, Luo K. Genome-wide identification of TCP family transcription factors from populus euphratica and their involvement in leaf shape regulation. Scientific Reports [Internet]. Dec 8, 2016;**6**(1):32795. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27605130 [Accessed: Jan 22, 2018]

[59] Welchen E, García L, Mansilla N, Gonzalez DH. Coordination of plant mitochondrial biogenesis: Keeping pace with cellular requirements. Frontiers in Plant Science [Internet]. Jan 8, 2014;**4**:551. Available from: http://journal.frontiersin.org/article/10.3389/fpls.2013.00551/abstract [Accessed: Jan 22, 2018]

[60] Giraud E, Ng S, Carrie C, Duncan O, Low J, Lee CP, et al. TCP transcription factors link the regulation of genes encoding mitochondrial proteins with the circadian clock in *Arabidopsis thaliana*. Plant Cell [Internet]. Dec 2010;**22**(12):3921-3934. DOI: 10.1105/tpc.110.074518 [Accessed: Jan 22, 2018]

[61] Bakshi M, Oelmüller R. WRKY transcription factors: Jack of many trades in plants. Plant Signaling & Behavior [Internet]. 2014;**9**(2):e27700. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24492469 [Accessed: Jan 23, 2018]

[62] Samad AFA, Sajad M, Nazaruddin N, Fauzi IA, Murad AMA, Zainal Z, et al. MicroRNA and transcription factor: Key players in plant regulatory network. Frontiers in Plant Science [Internet]. Apr 12, 2017;**8**:565. Available from: http://journal.frontiersin.org/article/10.3389/fpls.2017.00565/full [Accessed: Jan 23, 2018]

[63] Hichri I, Muhovski Y, Žižková E, Dobrev PI, Gharbi E, Franco-Zorrilla JM, et al. The *Solanum lycopersicum* WRKY3 transcription factor SlWRKY3 is involved in salt stress tolerance in tomato. Frontiers in Plant Science [Internet]. Jul 31, 2017;**8**:1343. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28824679 [Accessed: Jan 25, 2018]

[64] Amato A, Cavallini E, Zenoni S, Finezzo L, Begheldo M, Ruperti B, et al. A grapevine TTG2-like WRKY transcription factor is involved in regulating vacuolar transport and flavonoid biosynthesis. Frontiers in Plant Science [Internet]. Jan 5, 2017;**7**:1979. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28105033 [Accessed: Jan 25, 2018]

[65] Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M. Genes involved in organ separation in arabidopsis: an analysis of the cup-shaped cotyledon mutant. Plant Cell Online [Internet]. Jun 1, 1997;**9**(6):841-857. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9212461 [Accessed: Jan 25, 2018]

[66] Singh AK, Sharma V, Pal AK, Acharya V, Ahuja PS. Genome-wide organization and expression profiling of the NAC transcription factor family in potato (*Solanum tuberosum* L.). DNA Research [Internet]. Aug 1, 2013;**20**(4):403-423. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23649897 [Accessed: Jan 25, 2018]

[67] Lindemose S, Jensen MK, Van de Velde J, O'Shea C, Heyndrickx KS, Workman CT, et al. A DNA-binding-site landscape and regulatory network analysis for NAC transcription factors in Arabidopsis thaliana. Nucleic Acids Research [Internet]. Jul 2014;**42**(12):7681-7693. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24914054 [Accessed: Jan 25, 2018]

[68] Nuruzzaman M, Sharoni AM, Kikuchi S. Roles of NAC transcription factors in the regulation of biotic and abiotic stress responses in plants. Frontiers in Microbiology [Internet]. Sep 3, 2013;**4**:248. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24058359 [Accessed: Jan 25, 2018]

[69] Mao H, Yu L, Li Z, Liu H, Han R. Molecular evolution and gene expression differences within the HD-Zip transcription factor family of *Zea mays* L. Genetica [Internet]. Apr 15, 2016;**144**(2):243-257. Available from: http://link.springer.com/10.1007/s10709-016-9896-z [Accessed: Jan 25, 2018]

[70] Wang J-W, Wang L-J, Mao Y-B, Cai W-J, Xue H-W, Chen X-Y. control of root cap formation by MicroRNA-targeted auxin response factors in arabidopsis. Plant Cell Online [Internet]. Aug 1, 2005;**17**(8):2204-2216. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16006581 [Accessed: Feb 18, 2018]

[71] Guo H-S, Xie Q, Fei J-F, Chua N-H. MicroRNA directs mRNA cleavage of the transcription factor NAC1 to downregulate auxin signals for arabidopsis lateral root development. Plant Cell Online [Internet]. May 1, 2005;**17**(5):1376-1386. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15829603 [Accessed: Feb 18, 2018]

[72] Boualem A, Laporte P, Jovanovic M, Laffont C, Plet J, Combier J-P, et al. MicroRNA166 controls root and nodule development in Medicago truncatula. Plant Journal [Internet]. Jun 2008;**54**(5):876-887. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18298674 [Accessed: Feb 18, 2018]

[73] Sorin C, Declerck M, Christ A, Blein T, Ma L, Lelandais-Brière C, et al. A miR169 isoform regulates specific NF-YA targets and root architecture in Arabidopsis. New Phytologist [Internet]. Jun 2014;**202**(4):1197-1211. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24533947 [Accessed: Feb 11, 2018]

[74] Balazadeh S, Riaño-Pachón DM, Mueller-Roeber B. Transcription factors regulating leaf senescence in *Arabidopsis thaliana*. Plant Biology [Internet]. Sep 1, 2008;**10**(s1):63-75. DOI: 10.1111/j.1438-8677.2008.00088.x [Accessed: Feb 16, 2018]

[75] Wu X, Ding D, Shi C, Xue Y, Zhang Z, Tang G, et al. microRNA-dependent gene regulatory networks in maize leaf senescence. BMC Plant Biology [Internet]. Dec 22, 2016;**16**(1):73. Available from: http://www.biomedcentral.com/1471-2229/16/73 [Accessed: Feb 11, 2018]

[76] Xie F, Wang Q, Sun R, Zhang B. Deep sequencing reveals important roles of microRNAs in response to drought and salinity stress in cotton. Journal of Experimental Botany [Internet]. Feb 1, 2015;**66**(3):789-804. DOI: 10.1093/jxb/eru437 [Accessed: Feb 18, 2018]

[77] Wang M, Sun R, Li C, Wang Q, Zhang B. MicroRNA expression profiles during cotton (*Gossypium hirsutum* L) fiber early development. Scientific Reports [Internet]. Mar 22, 2017;**7**:44454. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28327647 [Accessed: Feb 16, 2018]

[78] Hong Y, Jackson S. Floral induction and flower formation-the role and potential applications of miRNAs. Plant Biotechnology Journal [Internet]. Apr 1, 2015;**13**(3):282-292. DOI: 10.1111/pbi.12340 [Accessed: Feb 11, 2018]

[79] Wu G, Park MY, Conway SR, Wang J-W, Weigel D, Poethig RS. The sequential action of miR156 and miR172 regulates developmental timing in arabidopsis. Cell [Internet]. Aug 21, 2009;**138**(4):750-759. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19703400 [Accessed: Feb 11, 2018]

[80] Nova-Franco B, Íñiguez LP, Valdés-López O, Alvarado-Affantranger X, Leija A, Fuentes SI, et al. The Micro-RNA172c-APETALA2-1 node as a key regulator of the common bean – *Rhizobium etli* nitrogen fixation symbiosis. Plant Physiol [Internet]. May 2015;**168**(1):273-291. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25739700 [Accessed: Feb 11, 2018]

[81] Schommer C, Palatnik JF, Aggarwal P, Chételat A, Cubas P, Farmer EE, et al. Control of jasmonate biosynthesis and senescence by miR319 targets. Carrington JC, editor. PLoS Biology [Internet]. Sep 23, 2008;**6**(9):e230. Available from: http://dx.plos.org/10.1371/journal.pbio.0060230 [Accessed: Feb 11, 2018]

[82] Parapunova V, Busscher M, Busscher-Lange J, Lammers M, Karlova R, Bovy AG, et al. Identification, cloning and characterization of the tomato TCP transcription factor family. BMC Plant Biology [Internet]. Jun 6, 2014;**14**(1):157. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24903607 [Accessed: Feb 11, 2018]

[83] Baucher M, Moussawi J, Vandeputte OM, Monteyne D, Mol A, Pérez-Morga D, et al. A role for the miR396/GRF network in specification of organ type during flower development, as supported by ectopic expression of *Populus trichocarpa* miR396c in transgenic tobacco. Piechulla B, editor. Plant Biology [Internet]. Sep 2013;**15**(5):892-898. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23173976 [Accessed: Feb 11, 2018]

[84] Zhang K, Shi X, Zhao X, Ding D, Tang J, Niu J. Investigation of miR396 and growth-regulating factor regulatory network in maize grain filling. Acta Physiologiae Plantarum [Internet]. Feb 21, 2015;**37**(2):28. Available from: http://link.springer.com/10.1007/s11738-014-1767-6 [Accessed: Feb 11, 2018]

[85] Zhang W, Sun P, He Q, Shu F, Wang J, Deng H. Fine mapping of GS2, a dominant gene for big grain rice. Crop Journal [Internet]. Dec 1, 2013;**1**(2):160-165. Available from: https://www.sciencedirect.com/science/article/pii/S2214514113000238 [Accessed: Feb 11, 2018]

[86] Ramachandran P, Carlsbecker A, Etchells JP, Turner S. Class III HD-ZIPs govern vascular cell fate: An HD view on patterning and differentiation. Journal of Experimental Botany [Internet]. 2016;**68**:55-69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27794018 [Accessed: Feb 11, 2018]

[87] Zhou G-K, Kubo M, Zhong R, Demura T, Ye Z-H. Overexpression of miR165 affects apical meristem formation, organ polarity establishment and vascular development in arabidopsis. Plant and Cell Physiology [Internet]. Mar 2007;**48**(3):391-404. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17237362 [Accessed: Feb 11, 2018]

[88] Sharma D, Tiwari M, Pandey A, Bhatia C, Sharma A, Trivedi PK. MicroRNA858 is a potential regulator of phenylpropanoid pathway and plant development in Arabidopsis.

Plant Physiology [Internet]. Apr 27, 2016;**171**(2):01831.2015. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27208307 [Accessed: Feb 11, 2018]

[89] Zhao W, Li Z, Fan J, Hu C, Yang R, Qi X, et al. Identification of jasmonic acid-associated microRNAs and characterization of the regulatory roles of the miR319/TCP4 module under root-knot nematode stress in tomato. Journal of Experimental Botany [Internet]. Aug 2015;**66**(15):4653-4667. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26002970 [Accessed: Feb 11, 2018]

[90] Giacomelli JI, Weigel D, Chan RL, Manavella PA. Role of recently evolved miRNA regulation of sunflower *HaWRKY6* in response to temperature damage. New Phytologist [Internet]. Sep 1, 2012;**195**(4):766-773. DOI: 10.1111/j.1469-8137.2012.04259.x [Accessed: Feb 11, 2018]

[91] Wang H, Wang H, Shao H, Tang X. Recent advances in utilizing transcription factors to improve plant abiotic stress tolerance by transgenic technology. Frontiers in Plant Science [Internet]. Feb 9, 2016;**7**:67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26904044 [Accessed: Jan 20, 2018]

[92] Zhao B, Ge L, Liang R, Li W, Ruan K, Lin H, et al. Members of miR-169 family are induced by high salinity and transiently inhibit the NF-YA transcription factor. BMC Molecular Biology [Internet]. Apr 8, 2009;**10**(1):29. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19351418 [Accessed: Jan 26, 2018]

[93] Xia K, Wang R, Ou X, Fang Z, Tian C, Duan J, et al. OsTIR1 and OsAFB2 downregulation via OsmiR393 overexpression leads to more tillers, early flowering and less tolerance to salt and drought in rice. Zhang B, editor. PLoS One [Internet]. Jan 10, 2012;**7**(1):e30039. Available from: http://dx.plos.org/10.1371/journal.pone.0030039 [Accessed: Feb 18, 2018]

[94] Feng H, Duan X, Zhang Q, Li X, Wang B, Huang L, et al. The target gene of tae-miR164, a novel NAC transcription factor from the NAM subfamily, negatively regulates resistance of wheat to stripe rust. Molecular Plant Pathology [Internet]. Apr 2014;**15**(3):284-296. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24128392 [Accessed: Jan 25, 2018]

[95] Teotia S, Tang G. To bloom or not to bloom: Role of MicroRNAs in plant flowering. Molecular Plant [Internet]. Mar 2015;**8**(3):359-377. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25737467 [Accessed: Feb 11, 2018]

Edited by Kais Ghedira

This book focuses on the transcriptional and post-transcriptional gene regulations and presents a detailed portrait of many novel aspects related to highlighting the importance of key TFs in some vital biological processes, the role of certain TFs to control some infectious diseases, the role of non-coding RNAs in controlling mRNA expression, the involvement of these non-coding RNAs in diseases, and the interplay between TFs and microRNAs as key players for gene expression regulation giving a complete picture of how genes are regulated at the cellular level.

The editor embarked upon this writing project entitled "Transcriptional and Post-transcriptional Regulation" to make pertinent contributions accessible to the scientific community. Hopefully, a large audience will enjoy reading and benefit from the chapters of this book.

Published in London, UK

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ISBN 978-1-83881-641-4