PREFACE

MicroRNAs are small non-coding RNAs involved in posttranscriptional regulation of gene expression. Thousands of miRNAs have been identified in different organisms including viruses, insects, plants and animals. MiRNAs has emerged as key regulators of important biological processes. The differential expression of miRNAs in various human diseases has made them potential candidates for developing novel therapies and personalized medicines. This book is focused on microRNA *Let-7*, the second miRNA discovered in the year 2000 and one of the most studied miRNA. This book discusses various aspects of miRNA *Let-7* starting from its discovery, biogenesis, transcriptional and posttranscriptional regulation to its crucial role in various fundamental cellular processes such as development, stem cell maintenance and differentiation, regulation of signaling pathways in cancer, drug resistance and therapeutic potential in different human diseases.

Chapter 1 - MiRNAs are small non coding RNAs capable of regulating gene expression at post-transcriptional level. The discovery of miRNAs has revolutionized the field of biomedical research. A large portion of human genome is regulated by miRNAs. They target a number of genes such as cell cycle regulators, transcription factors, and are involved in many aspects of tumor development, progression and metastasis. Tumor suppressive microRNA *Let-7* is an important miRNA, originally discovered in the *Caenorhabditis elegans*. Several *Let-7* targets have been already identified among which RAS and HMGA2 has oncogenic roles. The tumor suppressive function of *Let-7* has made it an important therapeutic target. Thus, *Let-7* may be a useful tumor marker and a potential candidate gene for miRNA-based therapies for the treatment of cancer. This chapter will discuss about *Let-7* discovery, biogenesis and its common functions.

Chapter 2 - MicroRNAs have emerged as novel molecules involved in post-transcriptional regulation of genes. They play important roles in development, differentiation, cellular metabolism and are implicated in a number of human diseases including cancer. A number of studies have shown miRNA mediated regulation of gene expression. To understand the complex network of miRNA:mRNA function, it is important to understand the molecular players of miRNA biogenesis and post-transcriptional processing. MicroRNA *Let-7* is the first known human miRNAs and is highly conserved in different organisms. Most of the human cancers show down-regulation of *Let-7* family members suggesting a tumor suppressive function of these miRNAs. *Let-7* has been shown to be an important candidate for predicting survival of cancer patients. For developing novel *Let-7* based therapeutic approaches, it is very important to understand the biogenesis and post-transcriptional

regulation of this miRNA. This chapter summarizes the recent advances on regulatory molecules controlling transcriptional and post-transcriptional processing of *Let-7*.

Chapter 3 - The *Let-7* family is one of the first identified miRNA families, and is highly conserved across animal species in sequence. The *Let-7* family members have identical seed sequences and may overlap sets of targets. *Let-7* interwines with cellular networks to exert its function. *Let-7* has been identified as cancer repressor or oncogene. In this chapter, the authors focus on discussion of *Let-7* and cancer signaling. Particular *Let-7* family members have been shown to be specifically deregulated in certain cancers. Epigenetic regulation, and processing at transcription and post-transcriptional level are probably involved in *Let-7* deregulation in cancers. Deregulation of *Let-7* may contribute to pathological process by changing the threshold of apoptosis and cell cycle checkpoints in a tissue or stage dependent manner. The *Let-7* family regulates not only a single important pathway protein, but rather coordinate gene expression levels on a pathway-wide scale. *Let-7*-regulated genes were found enriched in focal adhesion, apoptosis, MAPK and cell cycle signaling pathways. Manipulation of *Let-7* expression may provide a potential strategy for designing novel anti-cancer therapies.

Chapter 4 - *Let-7* was identified in a genetic screen in *C. elegans*. *Let-7* regulates a number of fundamental activities in the cells such as cell division, proliferation and differentiation. *Let-7* is involved in a regulatory feed back loop with LIN28, which is one of the factors required for pluripotency of cells suggesting role of *Let-7* in stem cell differentiation and maintenance. In addition to the *Let-7*/LIN28 feedback loop which is important in cancer stem cell, other *Let-7* targets such as RAS and HMGA2 are essential for self renewal of cancer stem cells and maintenance of the undifferentiated state. *Let-7* promotes differentiation by suppressing self renewal pathways in the cells. The ability of *Let-7* members to regulate multiple pathways plays an important in fine tuning of cellular processes of self-renewal and differentiation.

Chapter 5 - One of the first discovered human microRNAs, *Let-7* and its family members, are highly conserved across species in the regulation of embryonic development and stemness. Misregulation of *Let-7* results in a dedifferentiated cellular state and the development of cell-based disorders such as cancer. Although, many preclinical studies have been devoted to the *Let-7*-based cancer therapy, research into what is the physiological function of *Let-7* in the regeneration of adult tissues has only just begun. The exact role of *Let-7* in cancer is not yet fully understood. There is a need to understand the biological meanings of *Let-7* alterations in normal stem cells and cancers before proceeding to clinical applications. This chapter highlights an outlook on certain pivotal aspects of *Let-7* in stemness and tumorigenesis for future therapeutic potential.

Chapter 6 - MiRNAs (miRNAs) represent a class of naturally occurring, small (19 to 25-nucleotides), non-coding RNA molecules. Among 16000 miRNA genes which have been identified in various species, more than 1,000 miRNAs have been reported in the humans. The lethal-7 (*Let-7*) gene family, which was initially found as an essential developmental gene family in *Caenorhabditis elegans,* is one of the most extensively studied miRNA families, and there is high evidence that these miRNAs play key roles in the initiation, growth and progression of a variety of (malignant) tumors. Based on these data, there is now a growing interest in using these molecules for clinical purposes. The present chapter therefore discusses and highlights potential diagnostic applications of members of the *Let-7* family as predictors of clinical outcome and as markers for response monitoring during chemo- and/ or

radiotherapy. In addition, the authors present first promising data on the *Let-7* family concerning their use as primary anticancer agents and, perhaps more importantly, as modifiers and enhancers of well-established anticancer therapy strategies. In this context, modulation of *Let-7* expression seems to provide a new and very promising first-line treatment option in cancer, as well as an additive therapeutic modality to chemo- and radiotherapy, augmenting their effects and possibly reversing drug resistance – a major obstacle in modern anticancer treatment.

Chapter 7 - MicroRNAs (MiRNAs) are emerging as important therapeutic molecules. Their role in regulating fundamental processes of life and altered expression in a number of human diseases has made it necessary to study their diagnostic and therapeutic potential. A number of human cancers shows unique miRNA signature useful in distinguishing different subtypes of tumors. MiRNAs has been reported to function both as oncogenes and tumor suppressors. For example members of *Let-7* family are well known tumor suppressors whereas miR-17-92 family members function as oncogenes. Most of the *Let-7* family members are downregulated in cancers and two well know oncogenic targets regulated by *Let-7* are RAS and HMGA2. *Let-7* is also involved in development of drug resistance. In addition to cancer altered expression of *Let-7* has been reported in other diseases such as lung fibrosis, inflammation etc. Because of its involvement in development and progression of the diseases, *Let-7* is being studied for its potential as a therapeutic target. This chapter summarizes the current knowledge of *Let-7* functions in cancer and other disease conditions and challenges associated with development of miRNA based therapeutic approaches.

Chapter 8 - In cancer, microRNAs dysregulation affects the post-transcriptional control of oncogenes and tumor suppressors. *Let-7* importance emerged in cancer when its implication in regulating the expression of RAS oncogene family was uncovered. Frequently reduced in several types of cancer, *Let-7* exerts a tumor suppressor role in tumorigenesis, and loss of its expression is associated with poor prognosis. The molecular mechanisms of *Let-7* down-regulation involve genetic alterations such as deletions and loss of heterozygosity, and more recently, post-transcriptional regulation by LIN28, which impairs *Let-7* miRNA maturation. The biological role of *Let-7* is being elucidated gradually by the validation of its targets in relevant pathways such as cyclins, CDKs, E2F2 and MYC in cell cycle; RAS in MAPK pathway; IGF2BP1 in drug resistance; FAS and CASP3 in apoptotic process. As the functional understanding of *Let-7* targets improves, new insights into *Let-7* regulatory network and its potential implication in therapeutics arise. Restoring *Let-7* levels *in vitro* leads to growth restrain and apoptosis of cancer cells, suggesting that in the future therapeutical replacement of *Let-7* could improve or potentiate cancer patient response to common treatment. Thus, unraveling molecular *Let-7* network in cancer is essential to address novel perspectives to translational medicine.

Chapter 9 - MiRNAs (miRNAs) are a class of 18 to 25-nucleotide, short, non-coding RNAs that regulate gene expression post-transcriptionally. The lethal-7 (*Let-7*) gene is one of the founding members of the first two miRNA families identified in *C. elegans*, and the first miRNA known to be found in humans. *Let-7* family miRNAs are expressed from early development through maturity in both invertebrates and vertebrates. The majority of miRNAs are not essential for viability or development in *C. elegans*. However, *Let-7* family miRNAs are critical regulators of development that control both early and late developmental timing decisions, and were elucidated by extensive genetic and molecular analysis using *C. elegans* mutants as well as *Drosophila* mutants. In contrast to invertebrates, the authors'

understanding of the functional roles of *Let-7* family miRNAs in vertebrate development is still limited because there are many redundant *Let-7* family miRNAs, and thus experiments to clarify *in vivo* functions of *Let-7* family miRNAs using *Let-7* family miRNA mutants in vertebrates are practically difficult. However, indirect but significant *in vivo* evidence has suggested that *Let-7* is essential for gene regulation in mouse development. In addition, ectopic expression of *Let-7* in zebrafish embryos leads to early down-regulation of endogenous *Let-7* targets, resulting in severe embryonic developmental abnormalities, and suggesting that *Let-7* plays a significant role in zebrafish development. Although studies on vertebrate *Let-7* family miRNAs are still in the early stages, these studies have provided us with evidence of important biological roles for *Let-7*. Based on past and recent findings, the authors review the expression patterns, biological roles, and functional mechanisms of *Let-7* family miRNAs in normal development of both invertebrates and vertebrates. In addition, the authors describe *in vitro* analysis on the molecular roles of *Let-7* family miRNAs underlying cellular differentiation. Future studies on *Let-7* family miRNAs will provide us with not only important clues to understanding cell differentiation in development, but also useful tools for diagnosis and/or therapies in the medical field.