Preface

microRNAs (miRNAs) are small noncoding RNAs that regulate various biological phenomena, such as development and homeostasis. The dysregulation of miRNA leads to disease progression, particularly of cancer. Recently, circulating miRNAs have been observed in human body fluids. Furthermore, miRNAs have been shown to be located inside exosomes, which are small membrane vesicles, and these exosomal miRNAs are proposed to be novel mediators of cell–cell communication. Although the clinical and industrial importance of this research is promising, this research field is still just beginning. The purpose of this book is to inform the readers of recent advances in the isolation, purification, and analysis of circulating miRNAs from a variety of sources for research. This book is divided into three main topics. The first involves the study of secretory miRNAs in cell–cell communication, and the second, the study of circulating miRNAs in body fluids. The last describes the novel techniques used to study circulating miRNAs.

Cell–cell communication, which is essential for certain physiological phenomena, including the development and homeostasis of an organism, is also crucial for the development of a variety of diseases, such as immune disease, cardiac disease, neuronal disease, and cancer. The study of cell–cell interactions has revealed many aspects of the physiological and pathological conditions associated with various cell types, such as immune cells, stem cells, and cancer cells. A variety of cytokines and chemokines are also involved in these cell–cell interactions; however, these molecules cannot fully explain the biology of cell–cell communication. For this reason, secretory miRNAs, including exosomal miRNAs and other types of extracellular miRNAs, have been the focus of recent studies. How do these secretory miRNAs contribute to the multiple stages of disease development? Analyzing the involvement of secretory miRNAs in cell–cell communication may shed light on these unknown biological phenomena, thereby leading to the identification of novel therapeutic approaches for a variety of diseases. Chapters 1–6 describe a variety of research fields including cancer cells (Chapters 1 and 2), immune cells (Chapters 3–5), and stem cells (Chapter 6) with respect to secretory miRNAs. Another type of miRNA transfer was also reported. Chapter 7 describes the non-exosomal secretory type of circulating miRNAs associated with RNA-binding proteins. Recent findings clearly demonstrated that various types of secretory miRNAs from cells and circulating miRNAs in human body fluids can be found. In particular, the RNA-binding protein-associated miRNAs are found at high levels in the circulating blood. Not only exosomes but also RNA-binding proteins can protect miRNAs from environmental attacks, particularly from RNases. This chapter describes the analysis methods used on these newly identified circulating miRNAs. Chapter 8 presents the analysis methods to examine miRNA transfer mediated by gap junction. A gap junction is an intercellular connection between various cell types that directly connects the cytoplasm of two cells and allows various molecules to pass freely between the cells. The transfer of miRNAs through gap junctions has led to the idea that different types of cell contact affect the gene expression of each cell because of the regulatory roles played by miRNAs in gene expression.

Biomarkers are essential not only to evaluate a patient's disease state but also to monitor the efficiency of the patient's treatment. The currently known protein-based biomarkers

are not fully applicable for these functions. Additionally, the acquisition of biomarkers that provide detailed information about patient status is desirable. For example, in the case of cancer patients, biomarkers that provide information regarding the recurrence of cancer cells and the specific features of those cancer cells, such as drug resistance or increased metastatic potential, provide crucial data that allow for appropriate treatment design. Moreover, this detailed information prevents the waste of medical goods and finances. Therefore, the establishment of novel biomarkers is essential. Recently, many researchers confirmed that circulating miRNAs can be found in a variety of human body fluids, and these circulating miRNAs may represent novel biomarkers that can provide insight into the detailed disease status of patients. In this section, the methods used for the isolation and analysis of circulating miRNAs from various types of human body fluids are given. Chapters 9–13 provide the variety of methods for the analysis of circulating miRNAs in serum and/or plasma. Serum and plasma are the most popular sources of noninvasive diagnosis, and there are large amounts of serum and plasma stock in the refrigerator located in laboratories worldwide. Thus, readers might be able to start to identify novel biomarkers using the protocols described in this book. Chapters 14–18 offer information on the more specialized body fluids, including saliva, breast milk, cerebrospinal fluid, urine, and forensically relevant body fluids. Those body fluids containing miRNAs can be used as specific biomarkers, such as saliva for oral disease, breast milk for allergies, cerebrospinal fluid for neuronal disease, urine for urinary disease, and forensically relevant body fluids for the identification of the body fluid origin of forensic biological stains. Chapter 19 describes the methods to identify biomarkers using animal models. Mouse models serve as experimental models and are also used to study disease progression in detail. For the further development of circulating miRNAs as disease biomarkers, the use of mouse models might provide useful data.

The development of novel instruments to study the roles of secretory miRNAs and to develop circulating miRNAs as biomarkers is essential. Chapter 20 provides novel extraction and detection methods that have been optimized for circulating miRNAs. Although the extraction and detection methods for cellular miRNAs are now widely applicable to circulating miRNAs, optimized methods for circulating miRNAs are required. This chapter provides an example of such an optimized method. Chapter 21 describes a novel detection method using nanopore technology, which is a molecular-sized pore that can electrically detect single target molecules that interact with the pore. This technique can detect target miRNAs without the amplification of the target miRNA.

These three sections are not independent protocols but are instead interrelated. For example, clarifying the function of secretory miRNAs in cell–cell communication will lead to the understanding of the development of biomarkers for assessing disease status. In addition, comparing the protocols described in this book will be useful for the readers.

The topics covered in this volume will be of interest to researchers, teachers, students, and biotech companies interested in circulating miRNAs. I hope that the readers will benefit from this collection of excellent chapters dealing with the recent advances of RNAi technology from the bench to the bedside.

Finally, I would like to thank the authors for their contributions, Nami Kosaka for her excellent editorial assistance, the series editors, John Walkers, and all those involved in the production of the book.

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