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Gaetano Santulli *Editor*

microRNA: Medical Evidence

From Molecular Biology to Clinical Practice

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Gaetano Santulli
Editor

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Editor
Gaetano Santulli
Columbia University Medical Center
New York Presbyterian Hospital—Manhattan
New York, NY, USA

"Federico II" University Hospital
Naples, Italy

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Foreword

It gives me immense pleasure to introduce *microRNA: Medical Evidence—From Molecular Biology to Clinical Practice* to the medical and scientific community. The book you are holding was developed to provide medical students, researchers, and physicians with the knowledge on an emerging fundamental section of biomedicine: microRNA.

This book represents one volume—focused on clinical practice—of a trilogy exploring the functional role of microRNAs from basic science to the clinical scenario. The other two volumes explore the importance of microRNA in molecular biology and in cancer, respectively. The books have been edited by Dr. Gaetano Santulli, MD, PhD, who reunited the major experts in the microRNA field in order to have a comprehensive, up-to-date, and systematic overview of the mechanistic roles of these tiny molecules in physiology and disease.

MicroRNAs are small endogenous noncoding RNAs (~22 nucleotides) that fine-tune gene expression at the posttranscriptional level through mainly binding 3′-UTR of mRNAs. They are involved in numerous pathophysiological processes within cells and represent major regulators of gene expression by virtue of their preponderance to target transcription factors.

Following an introduction to precision medicine and personalized therapies, the book propones diverse chapters discussing the role of microRNAs in neurologic disorders, including epilepsy, autism, chronic pain, fragile X syndrome, and neurodegenerative disease. Then, a series of chapters extensively describes the clinical aspects of microRNAs in both diagnosis and therapy of metabolic and cardiovascular disorders, focusing on mitochondrial fitness, arterial hypertension, cardiovascular remodeling, cerebrovascular disease, pulmonary hypertension, diabetic kidney disease, and kidney transplantation. In the following chapters the experts discuss the importance of microRNAs in the wound healing process and in skin disease, in the pathogenesis of allergy, in human ovulation, and in infection. An interesting outline on the emerging role of microRNAs in the field of doping and a chapter explaining in detail microRNA profiling conclude the book.

This book highlights the functional roles of microRNAs in various human disorders, discussed in a detailed manner by expert contributors. Worldwide renowned experts also emphasize the current challenges and outstanding questions for the application of microRNA in clinical practice. The book includes many color pictures, schemes, and diagrams that will be very helpful to students and physicians and eloquent tables that support the text.

The clinical profile is evident in each chapter. The authors have done a terrific job in presenting such complex topics in an easy and comprehensible manner.

Milan, Italy Gianluigi Condorelli

The original version of the editor affiliation has been revised. An erratum can be found at DOI 10.1007/978-3-319-22671-2_22

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Contributors

Ayyappan Anitha Department of Neurogenetics, Institute for Communicative and Cognitive Neurosciences (ICCONS), Palakkad, Kerala, India

Jaideep Banerjee Extremity Trauma and Regenerative Medicine Division, US Army Institute of Surgical Research, San Antonio, TX, USA

Sébastien Bonnet Pulmonary Hypertension Research Group of the Quebec Heart and Lung Institute Research Center, Laval University, Quebec City, QC, Canada

Olivier Boucherat Pulmonary Hypertension Research Group of the University Institute of Cardiology and Pneumology Research Center, Laval University, Quebec City, QC, Canada

Fadi J. Charchar School of Applied and Biomedical Sciences, Faculty of Science and Technology, Federation University Australia, Mount Helen, VIC, Australia

Arthur C. K. Chung Partner State Key Laboratory of Environmental and Biological Analysis, and Department of Chemistry, The Hong Kong Baptist University, Hong Kong, China

HKBU Institute for Research and Continuing Education, Shenzhen, China

Petra Cimflová Department of Radiology, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno, Czech Republic

Dominika Coufalová Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic

International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic

Filipe V. Duarte Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

Iris Eisenberg Department of Obstetrics and Gynecology, The Magda and Richard Hoffman Center for Human Placenta Research, Hadassah Mt. Scopus—Hebrew University Medical Center, Jerusalem, Israel

Damjan Glavač Department of Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Debra Goldman-Wohl Department of Obstetrics and Gynecology, The Magda and Richard Hoffman Center for Human Placenta Research, Hadassah Mt. Scopus—Hebrew University Medical Center, Jerusalem, Israel

David C. Henshall Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland

Tal Imbar Department of Obstetrics and Gynecology, The Magda and Richard Hoffman Center for Human Placenta Research, Hadassah Mt. Scopus—Hebrew University Medical Center, Jerusalem, Israel

Kíra Jelencsics Medical University of Vienna, Vienna, Austria

Linda Kašičková Department of Neurology, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno, Czech Republic

Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Noora Kotaja Department of Physiology, Institute of Biomedicine, University of Turku, Turku, Finland

Charles-Henri Lecellier University of Montpellier, Montpellier, France

Institute of Molecular Genetics of Montpellier, Montpellier, France

Computational Biology Institute, Montpellier, France

Nicolas Leuenberger Swiss Laboratory for Doping Analyses, University Center of Legal Medicine, Lausanne–Geneva, Switzerland

Lausanne University Hospital, University of Lausanne, Epalinges, Switzerland

Shi-Lung Lin Division of Regenerative Medicine, WJWU & LYNN Institute for Stem Cell Research, Santa Fe Springs, CA, USA

Jun Lu Department of Genetics, Yale Stem Cell Center and Yale Cancer Center, Yale University School of Medicine, New Haven, CT, USA

Yale Center for RNA Science and Medicine, New Haven, CT, USA

Francine Z. Marques School of Applied and Biomedical Sciences, Faculty of Science and Technology, Federation University Australia, Mount Helen, VIC, Australia

Heart Failure Research Group, Baker IDI Heart and Diabetes Institute, Melbourne, VIC, Australia

Stephen Mastriano Department of Genetics, Yale Stem Cell Center and Yale Cancer Center, Yale University School of Medicine, New Haven, CT, USA

Yutaka Naito Division of Molecular and Cellular Medicine, National Cancer Center Research Institute, Tokyo, Japan

Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

Jan Novák 2nd Department of Internal Medicine, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno, Czech Republic

Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Rainer Oberbauer Medical University of Vienna, Vienna, Austria

Takahiro Ochiya Division of Molecular and Cellular Medicine, National Cancer Center Research Institute, Tokyo, Japan

Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

Koh Ono Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan

Carlos M. Palmeira Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

Department of Life Sciences, University of Coimbra, Coimbra, Portugal

François Potus Pulmonary Hypertension Research Group of the Quebec Heart and Lung Institute Research Center, Laval University, Quebec City, QC, Canada

Lifeng Qiu Neural Stem Cell Research Lab, Department of Research, National Neuroscience Institute, Singapore, Singapore

Metka Ravnik-Glavač Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Ana Rebane Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia

Cristina R. Reschke Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin 2, Ireland

Christine Roden Department of Genetics, Yale Stem Cell Center and Yale Cancer Center, Yale University School of Medicine, New Haven, CT, USA

Anabela P. Rolo Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

Department of Life Sciences, University of Coimbra, Coimbra, Portugal

Atsushi Sakai Department of Pharmacology, Graduate School of Medicine, Nippon Medical School, Tokyo, Japan

Gaetano Santulli Columbia University Medical Center, New York Presbyterian Hospital—Manhattan, New York, NY, USA

"Federico II" University Hospital, Naples, Italy

Martial Saugy Swiss Laboratory for Doping Analyses, University Center of Legal Medicine, Lausanne–Geneva, Switzerland

Lausanne University Hospital and University of Lausanne, Epalinges, Switzerland

Anne Saumet University of Montpelier, Montpellier, France

Faculty of Medicine, Montpellier, France

Chandan K. Sen The Ohio State University Wexner Medical Center, Davis Heart & Lung Research Institute, Comprehensive Wound Center, Center for Regenerative Medicine & Cell-Based Therapies, Columbus, OH, USA

Hidenori Suzuki Department of Pharmacology, Graduate School of Medicine, Nippon Medical School, Tokyo, Japan

Eng King Tan Department of Neurology, National Neuroscience Institute, Singapore, Singapore

Department of Research, National Neuroscience Institute, Singapore, Singapore

Neuroscience and Behavioral Disorders program, Duke-National University of Singapore Graduate Medical School, Singapore, Singapore

Yasuhito Tanaka Division of Molecular and Cellular Medicine, National Cancer Center Research Institute, Tokyo, Japan

Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

Ismail Thanseem Department of Neurogenetics, Institute for Communicative and Cognitive Neurosciences (ICCONS), Palakkad, Kerala, India

Ondřej Volný Department of Neurology, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno, Czech Republic

Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic

International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic

Nayi Wang The Biomedical Engineering Graduate Program, New Haven, CT, USA

Li Zeng Neural Stem Cell Research Lab, Department of Research, National Neuroscience Institute, Singapore, Singapore

Department of Research, National Neuroscience Institute, Singapore, Singapore

Neuroscience and Behavioral Disorders program, Duke-National University of Singapore Graduate Medical School, Singapore, Singapore

Chapter 1
Exploiting microRNA Specificity and Selectivity: Paving a Sustainable Path Towards Precision Medicine

Gaetano Santulli

Abstract In his State of the Union address before both chambers of the US Congress, President Barack Obama called for increased investment in US infrastructure and research and announced the launch of a new Precision Medicine Initiative, aiming to accelerate biomedical discovery. Due to their well-established selectivity and specificity, microRNAs can represent a useful tool, both in diagnosis and therapy, in forging the path towards the achievement of precision medicine. This introductory chapter represents a guide for the Reader in examining the functional roles of microRNAs in the most diverse aspects of clinical practice, which will be explored in this third volume of the microRNA trilogy.

Keywords miRNA • Pharmacogenomics • Precision medicine • Initiative • Selectivity • Specificity • Pharmacogenetics

In his last State of the Union address before both chambers of the US Congress, President Barack Obama called for increased investment in US infrastructure and research and announced the launch of an innovative *Precision Medicine Initiative*. "I want the country that eliminated polio and mapped the human genome to lead a new era of medicine—one that delivers the right treatment at the right time," he said. "Tonight, I'm launching a new Precision Medicine Initiative to bring us closer to curing diseases like cancer and diabetes—and to give all of us access to the personalized information we need to keep ourselves and our families healthier," he continued [1].

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G. Santulli, M.D., Ph.D. (✉)
Columbia University Medical Center, New York Presbyterian Hospital—Manhattan, New York, NY, USA

"Federico II" University Hospital, Naples, Italy
e-mail: gsantulli001@gmail.com

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Such an announcement offers an illustration of the considerable interest that exists in achieving greater progress in treating disease [2].

President Obama has long expressed a strong conviction that research offers great potential for improving health [3]. One million or more US citizens will be powering President Barack Obama's Precision Medicine Initiative. This bold volunteer-driven move to collect and link genotypic, phenotypic, and lifestyle data, including crowdsourcing and social media tools, aims to accelerate biomedical discovery with an initial focus on cancer [4].

The patient-participant cohort at the core of the initiative will enable new approaches to prevention, diagnosis, and treatments tailored to individual patients. "It's a new model for doing medical research," says National Institutes of Health's (NIH) director Francis Collins, while discussing the precision medicine approach [3, 4].

Do microRNAs (miRs) have a role in precision medicine? The answer is yes, and apparently not only at an interindividual level but also at an intercellular level. Indeed, miRs are exquisite regulators of gene expression that inhibit translation and/or promote mRNA degradation by base pairing to precise complementary sequences within the 3′-untranslated region.

They are expressed in a cell-specific manner and give us the possibility to generate selective treatments that target the bad cells and preserve the good cells, with major implication in cancer (see the second volume of the trilogy, where these aspects are discussed in detail) but also in other disorders [5–14].

This introductory chapter opens the third volume, where miRs will be analyzed in the clinical scenario. In the next years, clinicians will have to deal with miRs, not just as diagnostic biomarkers but also as potential tools to design selective treatments, alongside with their emerging important role in prognostic signatures and prediction models.

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References

1. McCarthy M. Obama promises to defend health law and promote "precision medicine". BMJ. 2015;350:h385.
2. Elwood TW. The usefulness of discerning patterns. J Allied Health. 2015;44:1.
3. Collins FS, Varmus H. A new initiative on precision medicine. N Engl J Med. 2015;372:793–5.
4. Fox JL. Obama catapults patient-empowered Precision Medicine. Nat Biotechnol. 2015;33:325.
5. Lin AH, Timberlake N, Logg CR, Liu Y, Kamijima S, Diago O, et al. MicroRNA 142-3p attenuates spread of replicating retroviral vector in hematopoietic lineage-derived cells while maintaining an antiviral immune response. Hum Gene Ther. 2014;25:759–71.
6. Santulli G, Wronska A, Uryu K, Diacovo TG, Gao M, Marx SO, et al. A selective microRNA-based strategy inhibits restenosis while preserving endothelial function. J Clin Invest. 2014;124:4102–14.

7. Edwards JK, Pasqualini R, Arap W, Calin GA. MicroRNAs and ultraconserved genes as diagnostic markers and therapeutic targets in cancer and cardiovascular diseases. J Cardiovasc Transl Res. 2010;3:271–9.
8. Ristori E, Lopez-Ramirez MA, Narayanan A, Hill-Teran G, Moro A, Calvo CF, et al. A dicer-miR-107 interaction regulates biogenesis of specific miRNAs crucial for neurogenesis. Dev Cell. 2015;32:546–60.
9. Santulli G, Iaccarino G, De Luca N, Trimarco B, Condorelli G. Atrial fibrillation and microRNAs. Front Physiol. 2014;5:15.
10. Della Peruta M, Badar A, Rosales C, Chokshi S, Kia A, Nathwani D, et al. Preferential targeting of disseminated liver tumors using a recombinant adeno-associated viral vector. Hum Gene Ther. 2015;26:94–103.
11. Sardu C, Marfella R, Santulli G, Paolisso G. Functional role of miRNA in cardiac resynchronization therapy. Pharmacogenomics. 2014;15:1159–68.
12. Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, et al. A brain-specific microRNA regulates dendritic spine development. Nature. 2006;439:283–9.
13. Taulli R, Pandolfi PP. "Snorkeling" for missing players in cancer. J Clin Invest. 2012;122:2765–8.
14. Wronska A, Kurkowska-Jastrzebska I, Santulli G. Application of microRNAs in diagnosis and treatment of cardiovascular disease. Acta Physiol (Oxf). 2015;213:60–83.

Chapter 2
microRNAs and Personalized Medicine: Evaluating Their Potential as Cancer Biomarkers

Anne Saumet and Charles-Henri Lecellier

Abstract microRNA deregulations are often, if not invariably, associated with human malignancies, including cancers. Though most of these deregulations may not be functionally implicated in tumorigenesis, the fact that microRNA expression can be monitored in a variety of human specimens, including biological fluids, supports studies aimed at characterizing microRNA signatures able to detect various cancers (diagnosis), predict their outcome (prognosis), monitor their treatment (theranosis), and adapt therapy to a patient (precision medicine). Here, we review and discuss pros and cons of microRNA-based approaches that can support their exploitation as cancer biomarkers.

Keywords Cancer • Theranosis • Diagnosis • Prognosis • Precision medicine • Tumorigenesis

microRNA Biogenesis

The microRNAs (miRNAs) are a class of 18–25 nucleotides long RNAs involved in the repression of translation and in the adjustment of protein production in response to various stimuli [1–3]. Their expression must be accurately controlled to ensure

A. Saumet, M.Sc., Ph.D.
University of Montpellier, 163 rue Auguste Broussonnet, Montpellier 34090, France

Faculty of Medicine, 2 rue de l'Ecole de Médecine, Montpellier 34060, France
e-mail: annesaumet@yahoo.fr

C.-H. Lecellier, M.Sc., Ph.D. (✉)
University of Montpellier, 163 rue Auguste Broussonnet, Montpellier 34090, France

Institute of Molecular Genetics of Montpellier, CNRS UMR 5535,
1919 Route de Mende, Montpellier 34293, France

Computational Biology Institute, 860 rue de Saint Priest, Montpellier 34095, France
e-mail: charles.lecellier@igmm.cnrs.fr

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plethora of cellular processes [4–6]. The miRNA biogenesis involves several steps, each step being subject to specific controls (for review [7]). Briefly, a long (thousand nucleotides long) RNA called the primary-miRNA (pri-miRNA) is transcribed from the genome mostly by the RNA polymerase II. This pri-miRNA contains one or several local stem-loop structures (called precursor(pre)-miRNA) in which the mature miRNA sequence is embedded. Next, a specific complex, called the Microprocessor and containing the RNAse III Drosha, crops the pre-miRNA from the pri-miRNA. The pre-miRNA is exported to the cytoplasm where another RNAse III, Dicer, processes the pre-miRNA into duplex of miRNAs. Only one strand of this duplex will guide a protein complex onto mRNAs harboring partial sequence homology and eventually trigger translation repression mostly by mRNA exonucleolytic cleavage [8]. The first two steps are believed to be the main control points for miRNA regulation [7, 9]. Similarly to protein coding genes (PCGs), control of pri-miRNA transcription involves DNA-binding proteins (i.e., transcription factors, TFs) that recognize specific *cis*-regulatory DNA motifs in the promoter region of the pri-miRNA. The definition of miRNA promoters remains elusive. The pri-miRNAs are unstable molecules making hard the precise identification of their 5′ end, i.e., miRNA Transcription Start Sites (TSSs). Numerous studies have tackled that problem and proposed different approaches to characterize miRNA TSSs, mostly based on features of PCG promoters such as CpG content, epigenetic marks, nucleosome positioning [10–19] but the results are quite mixed. A precise and complete map of miRNA TSSs/promoters is thus still missing precluding a genome-wide view of miRNA transcriptional regulations and the identification of potential miRNA-specific regulations. This lack of knowledge does not impede the study of specific miRNA loci though. We and others have shown that miRNA genes and PCGs are regulated by the same TFs. For instance, we have demonstrated that the PML-RARA oncogenic protein, which is associated with the Acute Promyelocytic Leukemia, represses the transcription of retinoic acid-responsive miRNA genes similarly to its action on PCGs [20]. Likewise, we showed that the antagonism between retinoic acid and estrogen signaling initially reported for PCGs [21] is also observed on miRNA genes [22].

At the posttranscriptional level, control of the miRNA biogenesis can be subjected to RNA-binding proteins (RBPs), which recognize specific RNA motifs on or at the vicinity of the pre-miRNAs. For instance, the LIN28 protein, a developmentally regulated RBP, can recognize a specific motif in the loop of the pre-miRNAs belonging to the let-7 family and selectively blocks their processing [23]. Also the p72 DEAD Box RNA Helicase binds a motif located in the 3′ flanking region of the pre-miRNAs and this binding can be controlled—in a cell-density-dependent manner—by the sequestration of p72 by YAP, a downstream target of the tumor-suppressive Hippo-signaling pathway [24].

These transcriptional and posttranscriptional regulations make miRNA extremely sensitive to various intra- and extracellular stimuli (e.g., hormones, vitamins, nutrients, pharmacological molecules, or hypoxia). They notably ensure that the miRNA repertoire is controlled in a temporal and cell-specific manner. These features were first reported by Chen et al., who observed that the miR-181 was preferentially expressed in the B-lymphoid cells and that its ectopic expression in hematopoietic progenitor

cells redirects lymphopoiesis towards the B-cell lineage [25]. On the other hand, these tight regulations can have severe consequences in human diseases in particular cancer [22, 26–30].

microRNA Deregulation in Cancers

The miRNAs are key players in cancer initiation and progression, including metastasis formation [31–33]. This field of research is probably one of the most productive in terms of publications (16,022 publications related to "miRNA and Cancer" listed in PubMed in March 2015 with an increase throughout the years). The miRNAs can act as oncogenes ("oncomirs") or tumor suppressors [34]. He et al. first reported the potential of one miRNA cluster, the miR-17/92, to act as an oncogene [35]. In 2007, Chang et al. showed that the miR-34a, which is transcriptionally regulated by p53, has a tumor suppressor activity [36]. Several databases have now been created to list the miRNA activity in specific cancer type [37, 38]. As observed for PCGs [39–43], the oncogene/tumor suppressor activity of miRNAs depends on the cellular context and/or the type of cancer considered. For example, the miR-221 can act as an oncogene in liver cancer [44] while playing a tumor suppressor role in erythroblastic leukemia [45].

The miRNA deregulations observed in cancer (i.e., forced expression for oncomiRs and downregulation for tumor suppressor miRNAs) can occur at the gene (deletions, amplifications, or mutations of miRNA genes), the transcriptional (epigenetic silencing, deregulation of transcription factors), and/or the posttranscriptional (deregulation of the miRNA biogenesis pathway) levels (for review [29]). The action of miRNAs can also be impaired without affecting miRNA expression levels by, for example, genomic mutations that can modify either the sequence of the miRNAs and/or the sequence of their targets [46]. We provided earlier some examples of specific transcriptional regulations responsible for miRNA deregulations [20, 36]. Likewise, the miR-15a and miR-16 are downregulated in the majority of chronic lymphocytic leukemia cases because the corresponding gene is frequently deleted [47]. The transcription of miRNA genes can also be silenced by DNA methylation [48]. At the posttranscriptional level, the reactivation of LIN28 is many human tumors can lead to the exclusive downregulation of let-7 miRNAs [49]. The expression of several key proteins involved in the processing or the action of miRNAs (e.g., Dicer, Drosha, Argonaute 2) is perturbed in certain cancers [50, 51] with presumably broad impact on cell biology.

These deregulations ultimately generate miRNA profiles that can be associated with cancer types/subtypes and/or response to chemotherapies [52–57]. Most of these profiles have been made available in several databases. PhenomiR provides data from several studies that investigate deregulation of microRNA expression in various diseases (not only cancer) and biological processes as a systematic, manually curated resource [58]. OncomiRDB is specifically dedicated to cancers [37]. Wang et al. manually curated 2259 entries of cancer-related miRNA regulations with direct experimental evidence from approximately 9000 abstracts, covering

more than 300 miRNAs and 829 target genes across 25 cancer tissues [37]. PROGmiR is aimed at providing potential prognostic properties of miRNAs in several cancer types derived from publicly available data from Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) [59]. The next question remains to determine whether these profiles contain clinically relevant biomarkers that could serve in diagnostic, prognostic, and/or theranostic tests.

Specific Advantages of microRNAs in Cancer Diagnosis

In addition to specific mutations associated to specific cancers [46], miRNA levels can also be indicative of cancer initiation, progression, and metastasis formation. Measuring miRNA levels is relatively straightforward. Several technologies are now available to profile either a specific set of miRNAs (RT-qPCR, Nanostring, microarrays) or the whole miRNA repertoire (small RNA sequencing). Advantageously, RT-qPCR does not necessitate large amount of RNA and is highly sensitive and specific. Moreover, several assays are commercially available rendering miRNA profiling easy even in clinical practice. It is important to note that each platform has, however, its advantages and drawbacks. For instance, the use of specific RT primers [60] could be a heavy procedure compared to the universal method, which uses linkers and one common RT primer. Problems with cross-priming can also lead to specificity issues and make it difficult to distinguish miRNAs belonging to the same family and differing by 1 or 2 nucleotides only. The Nanostring technology utilizes color-coded barcodes, which hybridize with the targeted miRNAs without the need of amplification thereby providing very sensitive digital data. However, similar to microarrays, RT-qPCR and Nanostring technologies are targeted approaches that do not allow the detection of novel miRNAs that can be species- and tissue-specific [61, 62]. In that context, RNA sequencing is definitely the best way to discover novel miRNAs. It can also detect sequence variation and posttranscriptional modifications thereby providing a more complete picture of the miRNA repertoire. However, its cost is still high to be envisaged in clinics. Besides, analysis of sequencing data is still a complex process, which requires rigorous bioinformatics approaches and refined sequence algorithms.

The miRNAs can be detected in a variety of human tissue specimens, fresh or Formalin-Fixed Paraffin Embedded (FFPE), and in almost all human biological fluids (e.g., serum, plasma, saliva, urine) [63–67]. In contrast to most RNAs, circulating miRNAs are remarkably stable [68]. In fact, circulating miRNAs represent a potent mode of intercellular communication [69, 70]. The secretion of miR-105 through exosome destroys tight junctions between endothelial cells thereby facilitating metastasis propagation [70]. The molecular mechanisms responsible for the secretion of miRNAs remain largely unknown. Circulating miRNAs can be free, packed into exosomes or other microvesicles present in body fluids [71] or can be associated with (lipo)proteins (HDL [72] and Argonaute 2 protein [73]). Plethora of studies showed association between the presence of one or several extracellular circulating miRNAs in a given biological fluid and cancer initiation/progression or response to

chemotherapy. These profiles have been listed and classified in the miRandola database [74]. miRandola contains 2132 entries, with 581 unique mature miRNAs and 21 types of samples. miRNAs are classified into four categories, based on their extracellular form: miRNA-Ago2 (173 entries), miRNA-exosome (856 entries), miRNA-HDL (20 entries), and miRNA-circulating (1083 entries) [74]. miRandola is also connected to miRò, a compendium, which integrates various online resources (ontologies, diseases, and targets) to provide users with miRNA-phenotype associations in humans [75].

All these features make miRNAs appealing candidates for non-invasive diagnostic tests and several companies have indeed decided to meet the challenge (e.g., Santaris Pharma, Rosetta Genomics, Cepheid, Prestizia-Theradiag, and IntegraGen; [76]). However, at this stage, miRNA signatures per cancer type are still inconsistent [77, 78] impeding their usage in clinics and calling for further development and research.

Challenges in microRNA-Based Diagnosis

One important challenge in the field of microRNA-based diagnosis is to find the sources of inconsistencies in order to propose standardized protocols. Inconsistencies in miRNA signatures could come from sample procurement and could be the results of, for instance, platelet contamination of the plasma [79, 80] or hemolysis occurring during blood collection [81–84]. The protocols used to extract miRNAs also differ and can introduce significant variability. One important point to compare miRNA extraction protocols is to evaluate the quantity and the quality of the extracted miRNAs. Though the size and abundance of ribosomal RNAs is traditionally used as a quality marker for large RNAs, these RNAs cannot be informative on the quality of the miRNA extraction and specific methods are required (e.g., Agilent Small RNA Kit, synthetic miRNA standards). Moreover the quantification of miRNAs is only accurate in samples where larger RNAs are not degraded. The low concentration of RNAs in body fluids also makes the estimation of miRNAs abundance particularly difficult [85]. Besides, protein and lipid content of plasma and serum samples could affect efficiency of RNA extraction and introduce potential inhibitors of PCR [86]. This can be estimated using a spiked non-human synthetic miRNAs (typically from Arabidopsis thaliana or *Caenorhabditis elegans*) that will go through the entire RNA isolation procedure and will eventually be measured by RT-qPCR. Another aspect that should be considered is that the extraction methods could affect the nature (i.e., nucleotide composition) of the miRNAs extracted. Notably, depending on the protocol used, the quantity of the biological samples can impact the GC content of the miRNAs detected [87, 88]. Since these observations were also made in serum [88] (where large RNAs are barely detected), it is likely that the selective lost of miRNAs is linked to the presence of additional compounds (proteins and/or lipids), which are associated with miRNAs. Together these studies [87, 88] argue for standardization in quantities/volumes of starting materials to allow strict comparison of miRNA profiles. In fact, all these considerations point to the urgent need of consistency in all the steps of miRNA extraction procedures.

Analytical aspects also impact the definition of miRNA signatures. Among them, normalization of the data, which is required to remove unwanted technical variation present in the samples, is critical. On common approach is to use other abundant noncoding RNAs, such as U6 small nuclear RNAs, as normalizers of miRNA expression. However, the biology of such RNAs is quite distinct from miRNA biology in terms of transcription, processing, and tissue-specific expression [89]. An alternative is to use miRNAs whose expression is supposed to be stable in various conditions. However, this strategy can be limited by the fact that the chosen reference miRNAs are sensitive to other biological processes and/or other diseases commonly encountered in clinics. In that case, the expression of the normalizer miRNAs could fluctuate in patients and introduce serious bias. In fact miRNA levels are extremely sensitive to various stimuli and conditions, even nonpathogenic, from gender [90, 91] and age [92, 93] to nutrients such as amino acids, carbohydrates, fatty acids, vitamins, and phytochemicals (curcumin, resveratrol) [94, 95]. If clinically relevant, these aspects should be invariably taken into account in the cohort used to define a miRNA signature. The ideal strategy would be to restrict potential miRNA signature to miRNAs whose transcriptional/posttranscriptional regulations are relevant for the cancer or the chemotherapy considered. This is where translational research meets fundamental research as this strategy clearly depends on a better understanding of miRNA regulations.

Conclusion

The discovery of miRNAs [96] has opened up new avenues of research in biomedicine, in particular in cancer, and contributed to a large extent to the "Noncoding RNA revolution" [97]. It is remarkable to note not only the fast rate of fundamental discoveries made in two decades (illustrated by the exponentially growing number of publications) but also the velocity with which "miRNA gets to business" [76]. These molecules indeed harbor specific features (stability, easy manipulation, reasonably simple detection, tissue specificity) that make them appealing candidates as diagnostic, prognostic, or theranostic biomarkers and even therapeutic targets [64, 98]. However some uncertainties remain [77, 78] that may prevent their immediate large-scale exploitation. Collective efforts made by clinicians, academic and industrial researchers are needed to circumvent these limitations and promote the transfer of miRNAs from bench to bedside.

References

1. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol. 2009;10:126–39.
2. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet. 2010;11:597–610.

3. Tran N, Hutvagner G. Biogenesis and the regulation of the maturation of miRNAs. Essays Biochem. 2013;54:17–28.
4. Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the microprocessor complex. Nature. 2004;432:231–5.
5. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S, Kim VN. The nuclear RNase III Drosha initiates microRNA processing. Nature. 2003;425:415–9.
6. Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. The Drosha-DGCR8 complex in primary microRNA processing. Genes Dev. 2004;18:3016–27.
7. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol. 2014;15:509–24.
8. Huntzinger E, Izaurralde E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. Nat Rev Genet. 2011;12:99–110.
9. Conrad T, Marsico A, Gehre M, Orom UA. Microprocessor activity controls differential miRNA biogenesis In Vivo. Cell Rep. 2014;9:542–54.
10. Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, Lin C, Socci ND, Hermida L, Fulci V, Chiaretti S, Foa R, Schliwka J, Fuchs U, Novosel A, Muller RU, Schermer B, Bissels U, Inman J, Phan Q, Chien M, Weir DB, Choksi R, De Vita G, Frezzetti D, Trompeter HI, Hornung V, Teng G, Hartmann G, Palkovits M, Di Lauro R, Wernet P, Macino G, Rogler CE, Nagle JW, Ju J, Papavasiliou FN, Benzing T, Lichter P, Tam W, Brownstein MJ, Bosio A, Borkhardt A, Russo JJ, Sander C, Zavolan M, Tuschl T. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell. 2007;129:1401–14.
11. Saini HK, Griffiths-Jones S, Enright AJ. Genomic analysis of human microRNA transcripts. Proc Natl Acad Sci U S A. 2007;104:17719–24.
12. Fujita S, Iba H. Putative promoter regions of miRNA genes involved in evolutionarily conserved regulatory systems among vertebrates. Bioinformatics. 2008;24:303–8.
13. Marsico A, Huska MR, Lasserre J, Hu H, Vucicevic D, Musahl A, Orom U, Vingron M. PROmiRNA: a new miRNA promoter recognition method uncovers the complex regulation of intronic miRNAs. Genome Biol. 2013;14:R84.
14. Ozsolak F, Poling LL, Wang Z, Liu H, Liu XS, Roeder RG, Zhang X, Song JS, Fisher DE. Chromatin structure analyses identify miRNA promoters. Genes Dev. 2008;22:3172–83.
15. Marson A, Levine SS, Cole MF, Frampton GM, Brambrink T, Johnstone S, Guenther MG, Johnston WK, Wernig M, Newman J, Calabrese JM, Dennis LM, Volkert TL, Gupta S, Love J, Hannett N, Sharp PA, Bartel DP, Jaenisch R, Young RA. Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells. Cell. 2008;134:521–33.
16. Corcoran DL, Pandit KV, Gordon B, Bhattacharjee A, Kaminski N, Benos PV. Features of mammalian microRNA promoters emerge from polymerase II chromatin immunoprecipitation data. PLoS One. 2009;4, e5279.
17. Chien CH, Sun YM, Chang WC, Chiang-Hsieh PY, Lee TY, Tsai WC, Horng JT, Tsou AP, Huang HD. Identifying transcriptional start sites of human microRNAs based on high-throughput sequencing data. Nucleic Acids Res. 2011;39:9345–56.
18. Chen D, Fu LY, Zhang Z, Li G, Zhang H, Jiang L, Harrison AP, Shanahan HP, Klukas C, Zhang HY, Ruan Y, Chen LL, Chen M. Dissecting the chromatin interactome of microRNA genes. Nucleic Acids Res. 2014;42:3028–43.
19. Georgakilas G, Vlachos IS, Paraskevopoulou MD, Yang P, Zhang Y, Economides AN, Hatzigeorgiou AG. microTSS: accurate microRNA transcription start site identification reveals a significant number of divergent pri-miRNAs. Nat Commun. 2014;5:5700.
20. Saumet A, Vetter G, Bouttier M, Portales-Casamar E, Wasserman WW, Maurin T, Mari B, Barbry P, Vallar L, Friederich E, Arar K, Cassinat B, Chomienne C, Lecellier CH. Transcriptional repression of microRNA genes by PML-RARA increases expression of key cancer proteins in acute promyelocytic leukemia. Blood. 2009;113:412–21.
21. Hua S, Kittler R, White KP. Genomic antagonism between retinoic acid and estrogen signaling in breast cancer. Cell. 2009;137:1259–71.
22. Saumet A, Vetter G, Bouttier M, Antoine E, Roubert C, Orsetti B, Theillet C, Lecellier CH. Estrogen and retinoic acid antagonistically regulate several microRNA genes to control aerobic glycolysis in breast cancer cells. Mol Biosyst. 2012;8:3242–53.

23. Viswanathan SR, Daley GQ, Gregory RI. Selective blockade of microRNA processing by Lin28. Science. 2008;320:97–100.
24. Mori M, Triboulet R, Mohseni M, Schlegelmilch K, Shrestha K, Camargo FD, Gregory RI. Hippo signaling regulates microprocessor and links cell-density-dependent miRNA biogenesis to cancer. Cell. 2014;156:893–906.
25. Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. Science. 2004;303:83–6.
26. Waltering KK, Porkka KP, Jalava SE, Urbanucci A, Kohonen PJ, Latonen LM, Kallioniemi OP, Jenster G, Visakorpi T. Androgen regulation of micro-RNAs in prostate cancer. Prostate. 2011;71:604–14.
27. Wang WL, Chatterjee N, Chittur SV, Welsh J, Tenniswood MP. Effects of 1alpha,25 dihydroxyvitamin D3 and testosterone on miRNA and mRNA expression in LNCaP cells. Mol Cancer. 2011;10:58.
28. Kulshreshtha R, Ferracin M, Wojcik SE, Garzon R, Alder H, Agosto-Perez FJ, Davuluri R, Liu CG, Croce CM, Negrini M, Calin GA, Ivan M. A microRNA signature of hypoxia. Mol Cell Biol. 2007;27:1859–67.
29. Iorio MV, Croce CM. Causes and consequences of microRNA dysregulation. Cancer J. 2012;18:215–22.
30. Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet. 2009;10:704–14.
31. Pencheva N, Tavazoie SF. Control of metastatic progression by microRNA regulatory networks. Nat Cell Biol. 2013;15:546–54.
32. Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H, Patrawala L, Yan H, Jeter C, Honorio S, Wiggins JF, Bader AG, Fagin R, Brown D, Tang DG. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. Nat Med. 2011;17:211–5.
33. Huang Q, Gumireddy K, Schrier M, le Sage C, Nagel R, Nair S, Egan DA, Li A, Huang G, Klein-Szanto AJ, Gimotty PA, Katsaros D, Coukos G, Zhang L, Pure E, Agami R. The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nat Cell Biol. 2008;10:202–10.
34. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer. 2006;6:857–66.
35. He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, Hammond SM. A microRNA polycistron as a potential human oncogene. Nature. 2005;435:828–33.
36. Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, Feldmann G, Yamakuchi M, Ferlito M, Lowenstein CJ, Arking DE, Beer MA, Maitra A, Mendell JT. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell. 2007;26:745–52.
37. Wang D, Gu J, Wang T, Ding Z. OncomiRDB: a database for the experimentally verified oncogenic and tumor-suppressive microRNAs. Bioinformatics. 2014;30:2237–8.
38. Li Y, Qiu C, Tu J, Geng B, Yang J, Jiang T, Cui Q. HMDD v2.0: a database for experimentally supported human microRNA and disease associations. Nucleic Acids Res. 2014;42:D1070–4.
39. Johnson DG. The paradox of E2F1: oncogene and tumor suppressor gene. Mol Carcinog. 2000;27:151–7.
40. Rowland BD, Bernards R, Peeper DS. The KLF4 tumour suppressor is a transcriptional repressor of p53 that acts as a context-dependent oncogene. Nat Cell Biol. 2005;7:1074–82.
41. Liang J, Mills GB. AMPK: a contextual oncogene or tumor suppressor? Cancer Res. 2013;73:2929–35.
42. Toker A, Chin YR. Akt-ing up on SRPK1: oncogene or tumor suppressor? Mol Cell. 2014;54:329–30.
43. Lobry C, Oh P, Mansour MR, Look AT, Aifantis I. Notch signaling: switching an oncogene to a tumor suppressor. Blood. 2014;123:2451–9.
44. Pineau P, Volinia S, McJunkin K, Marchio A, Battiston C, Terris B, Mazzaferro V, Lowe SW, Croce CM, Dejean A. miR-221 overexpression contributes to liver tumorigenesis. Proc Natl Acad Sci U S A. 2010;107:264–9.

45. Felli N, Fontana L, Pelosi E, Botta R, Bonci D, Facchiano F, Liuzzi F, Lulli V, Morsilli O, Santoro S, Valtieri M, Calin GA, Liu CG, Sorrentino A, Croce CM, Peschle C. MicroRNAs 221 and 222 inhibit normal erythropoiesis and erythroleukemic cell growth via kit receptor down-modulation. Proc Natl Acad Sci U S A. 2005;102:18081–6.
46. Mishra PJ, Banerjee D, Bertino JR. MiRSNPs or MiR-polymorphisms, new players in microRNA mediated regulation of the cell: Introducing microRNA pharmacogenomics. Cell Cycle. 2008;7:853–8.
47. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A. 2002;99:15524–9.
48. Lopez-Serra P, Esteller M. DNA methylation-associated silencing of tumor-suppressor microRNAs in cancer. Oncogene. 2012;31:1609–22.
49. Thornton JE, Gregory RI. How does Lin28 let-7 control development and disease? Trends Cell Biol. 2012;22:474–82.
50. Blenkiron C, Goldstein LD, Thorne NP, Spiteri I, Chin SF, Dunning MJ, Barbosa-Morais NL, Teschendorff AE, Green AR, Ellis IO, Tavare S, Caldas C, Miska EA. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype. Genome Biol. 2007;8:R214.
51. Avery-Kiejda KA, Braye SG, Forbes JF, Scott RJ. The expression of Dicer and Drosha in matched normal tissues, tumours and lymph node metastases in triple negative breast cancer. BMC Cancer. 2014;14:253.
52. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A. 2006;103:2257–61.
53. Di Leva G, Croce CM. miRNA profiling of cancer. Curr Opin Genet Dev. 2013;23:3–11.
54. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Menard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, Croce CM. MicroRNA gene expression deregulation in human breast cancer. Cancer Res. 2005;65:7065–70.
55. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. Nature. 2005;435:834–8.
56. Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, Iuliano R, Palumbo T, Pichiorri F, Roldo C, Garzon R, Sevignani C, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med. 2005;353:1793–801.
57. Lowery AJ, Miller N, Devaney A, McNeill RE, Davoren PA, Lemetre C, Benes V, Schmidt S, Blake J, Ball G, Kerin MJ. MicroRNA signatures predict oestrogen receptor, progesterone receptor and HER2/neu receptor status in breast cancer. Breast Cancer Res. 2009;11:R27.
58. Ruepp A, Kowarsch A, Schmidl D, Bruggenthin F, Brauner B, Dunger I, Fobo G, Frishman G, Montrone C, Theis FJ. PhenomiR: a knowledgebase for microRNA expression in diseases and biological processes. Genome Biol. 2010;11:R6.
59. Goswami CP, Nakshatri H. PROGmiR: a tool for identifying prognostic miRNA biomarkers in multiple cancers using publicly available data. J Clin Bioinforma. 2012;2:23.
60. Chen C, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, Nguyen JT, Barbisin M, Xu NL, Mahuvakar VR, Andersen MR, Lao KQ, Livak KJ, Guegler KJ. Real-time quantification of microRNAs by stem-loop RT-PCR. Nucleic Acids Res. 2005;33, e179.
61. Friedlander MR, Lizano E, Houben AJ, Bezdan D, Banez-Coronel M, Kudla G, Mateu-Huertas E, Kagerbauer B, Gonzalez J, Chen KC, LeProust EM, Marti E, Estivill X. Evidence for the biogenesis of more than 1,000 novel human microRNAs. Genome Biol. 2014;15:R57.
62. Londin E, Loher P, Telonis AG, Quann K, Clark P, Jing Y, Hatzimichael E, Kirino Y, Honda S, Lally M, Ramratnam B, Comstock CE, Knudsen KE, Gomella L, Spaeth GL, Hark L, Katz LJ,

Witkiewicz A, Rostami A, Jimenez SA, Hollingsworth MA, Yeh JJ, Shaw CA, McKenzie SE, Bray P, Nelson PT, Zupo S, Van Roosbroeck K, Keating MJ, Calin GA, Yeo C, Jimbo M, Cozzitorto J, Brody JR, Delgrosso K, Mattick JS, Fortina P, Rigoutsos I. Analysis of 13 cell types reveals evidence for the expression of numerous novel primate- and tissue-specific microRNAs. Proc Natl Acad Sci U S A. 2015;112:E1106–15.
63. Etheridge A, Lee I, Hood L, Galas D, Wang K. Extracellular microRNA: a new source of biomarkers. Mutat Res. 2011;717:85–90.
64. Heneghan HM, Miller N, Kerin MJ. MiRNAs as biomarkers and therapeutic targets in cancer. Curr Opin Pharmacol. 2010;10:543–50.
65. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008;105:10513–8.
66. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Zen K, Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 2008;18:997–1006.
67. Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids--the mix of hormones and biomarkers. Nat Rev Clin Oncol. 2011;8:467–77.
68. Chevillet JR, Lee I, Briggs HA, He Y, Wang K. Issues and prospects of microRNA-based biomarkers in blood and other body fluids. Molecules. 2014;19:6080–105.
69. Fabbri M, Paone A, Calore F, Galli R, Gaudio E, Santhanam R, Lovat F, Fadda P, Mao C, Nuovo GJ, Zanesi N, Crawford M, Ozer GH, Wernicke D, Alder H, Caligiuri MA, Nana-Sinkam P, Perrotti D, Croce CM. MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. Proc Natl Acad Sci U S A. 2012;109:E2110–6.
70. Zhou W, Fong MY, Min Y, Somlo G, Liu L, Palomares MR, Yu Y, Chow A, O'Connor ST, Chin AR, Yen Y, Wang Y, Marcusson EG, Chu P, Wu J, Wu X, Li AX, Li Z, Gao H, Ren X, Boldin MP, Lin PC, Wang SE. Cancer-secreted miR-105 destroys vascular endothelial barriers to promote metastasis. Cancer Cell. 2014;25:501–15.
71. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9:654–9.
72. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol. 2011;13:423–33.
73. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci U S A. 2011;108:5003–8.
74. Russo F, Di Bella S, Nigita G, Macca V, Lagana A, Giugno R, Pulvirenti A, Ferro A. miRandola: extracellular circulating microRNAs database. PLoS One. 2012;7, e47786.
75. Lagana A, Forte S, Giudice A, Arena MR, Puglisi PL, Giugno R, Pulvirenti A, Shasha D, Ferro A. miRo: a miRNA knowledge base. Database (Oxford). 2009;2009:bap008.
76. Mack GS. MicroRNA gets down to business. Nat Biotechnol. 2007;25:631–8.
77. Leidner RS, Li L, Thompson CL. Dampening enthusiasm for circulating microRNA in breast cancer. PLoS One. 2013;8, e57841.
78. Jarry J, Schadendorf D, Greenwood C, Spatz A, van Kempen LC. The validity of circulating microRNAs in oncology: five years of challenges and contradictions. Mol Oncol. 2014;8:819–29.
79. Cheng HH, Yi HS, Kim Y, Kroh EM, Chien JW, Eaton KD, Goodman MT, Tait JF, Tewari M, Pritchard CC. Plasma processing conditions substantially influence circulating microRNA biomarker levels. PLoS One. 2013;8, e64795.
80. Wang K, Yuan Y, Cho JH, McClarty S, Baxter D, Galas DJ. Comparing the MicroRNA spectrum between serum and plasma. PLoS One. 2012;7, e41561.

81. Kirschner MB, Edelman JJ, Kao SC, Vallely MP, van Zandwijk N, Reid G. The impact of hemolysis on cell-free microRNA biomarkers. Front Genet. 2013;4:94.
82. Kirschner MB, Kao SC, Edelman JJ, Armstrong NJ, Vallely MP, van Zandwijk N, Reid G. Haemolysis during sample preparation alters microRNA content of plasma. PLoS One. 2011;6, e24145.
83. Blondal T, Jensby Nielsen S, Baker A, Andreasen D, Mouritzen P, Wrang Teilum M, Dahlsveen IK. Assessing sample and miRNA profile quality in serum and plasma or other biofluids. Methods. 2013;59:S1–6.
84. Pritchard CC, Kroh E, Wood B, Arroyo JD, Dougherty KJ, Miyaji MM, Tait JF, Tewari M. Blood cell origin of circulating microRNAs: a cautionary note for cancer biomarker studies. Cancer Prev Res (Phila). 2012;5:492–7.
85. Tzimagiorgis G, Michailidou EZ, Kritis A, Markopoulos AK, Kouidou S. Recovering circulating extracellular or cell-free RNA from bodily fluids. Cancer Epidemiol. 2011;35:580–9.
86. Kroh EM, Parkin RK, Mitchell PS, Tewari M. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods. 2010;50:298–301.
87. Kim YK, Yeo J, Kim B, Ha M, Kim VN. Short structured RNAs with low GC content are selectively lost during extraction from a small number of cells. Mol Cell. 2012;46:893–5.
88. Monleau M, Bonnel S, Gostan T, Blanchard D, Courgnaud V, Lecellier CH. Comparison of different extraction techniques to profile microRNAs from human sera and peripheral blood mononuclear cells. BMC Genomics. 2014;15:395.
89. Chugh P, Dittmer DP. Potential pitfalls in microRNA profiling. Wiley Interdiscip Rev RNA. 2012;3:601–16.
90. Morgan CP, Bale TL. Sex differences in microRNA regulation of gene expression: no smoke, just miRs. Biol Sex Differ. 2012;3:22.
91. Sharma S, Eghbali M. Influence of sex differences on microRNA gene regulation in disease. Biol Sex Differ. 2014;5:3.
92. Lai CY, Wu YT, Yu SL, Yu YH, Lee SY, Liu CM, Hsieh WS, Hwu HG, Chen PC, Jeng SF, Chen WJ. Modulated expression of human peripheral blood microRNAs from infancy to adulthood and its role in aging. Aging Cell. 2014;13:679–89.
93. Noren Hooten N, Fitzpatrick M, Wood 3rd WH, De S, Ejiogu N, Zhang Y, Mattison JA, Becker KG, Zonderman AB, Evans MK. Age-related changes in microRNA levels in serum. Aging (Albany NY). 2013;5:725–40.
94. Palmer JD, Soule BP, Simone BA, Zaorsky NG, Jin L, Simone NL. MicroRNA expression altered by diet: can food be medicinal? Ageing Res Rev. 2014;17:16–24.
95. Garcia-Segura L, Perez-Andrade M, Miranda-Rios J. The emerging role of MicroRNAs in the regulation of gene expression by nutrients. J Nutrigenet Nutrigenomics. 2013;6:16–31.
96. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75:843–54.
97. Cech TR, Steitz JA. The noncoding RNA revolution-trashing old rules to forge new ones. Cell. 2014;157:77–94.
98. Hauptman N, Glavac D. MicroRNAs and long non-coding RNAs: prospects in diagnostics and therapy of cancer. Radiol Oncol. 2013;47:311–8.

Chapter 3
microRNA and Pain

Atsushi Sakai and Hidenori Suzuki

Abstract Pain is an important protective system that alerts organisms to actual or possible tissue damage. However, a variety of pathologies can lead to chronic pain that is no longer beneficial. Lesions or diseases of the somatosensory nervous system cause intractable neuropathic pain that occasionally lasts even after the original pathology subsides. Chronic inflammatory diseases like arthritis are also associated with severe pain. Because conventional analgesics such as non-steroidal anti-inflammatory drugs and opioids have limited efficacy and/or severe adverse events associated with long-term use, chronic pain remains a major problem in clinical practice. Recently, causal roles of microRNAs in chronic pain and their therapeutic potential have been emerging. microRNA expressions are altered not only at the primary origin of pain, but also along the somatosensory pathways. Notably, microRNA expressions are differentially affected depending on the causes of chronic pain. This chapter summarizes current insights into the roles of microRNAs in pain based on the underlying pathologies.

Keywords Arthritis • Cancer pain • Inflammatory pain • Neuropathic pain • Somatosensory pathways

Introduction

In general, pain is elicited by nociceptive stimuli applied to the body or through pathology in an internal organ, and is perceived in the brain [1]. A subset of primary afferents, or nociceptors, detect various forms of nociceptive stimuli, including mechanical, thermal (hot or cold), and chemical stimuli, in the body surface (skin), deep tissue, viscera, and others. Primary afferents are the axons of primary sensory neurons referred to as dorsal root ganglion (DRG) or trigeminal ganglion (TG) neurons after the locations of their cell bodies. Thus, a subset of DRG and TG neurons are the first-line nociceptive neurons that detect noxious stimuli and tissue lesions,

A. Sakai • H. Suzuki (✉)
Department of Pharmacology, Graduate School of Medicine, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan
e-mail: sa19@nms.ac.jp; hsuzuki@nms.ac.jp

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while another subset of DRG neurons detect non-noxious stimuli such as tactile stimuli. Once stimulated, DRG and TG neurons transduce sensory stimuli into electrical signals and transmit these signals to the spinal and medullary dorsal horns, respectively. The sensory information is then synaptically transmitted to spinal neurons and considerably modulated by a complex spinal network interconnected with excitatory and inhibitory interneurons, descending axons from the brainstem and glial cells (microglia and astrocytes) [2, 3]. The processed sensory information is further transmitted through direct or indirect connections to multiple brain areas, including not only somatosensory cortices, but also limbic systems and other cortices. The somatosensory cortices are mainly involved in the sensory aspects of pain such as intensity and location, while the limbic systems are involved in the affective and emotional aspects of pain as well as pain perception and attention [4].

Although pain is an essential protective system that alerts organisms to actual or possible tissue damage, unnecessary or long-lasting pain is debilitating and requires medical intervention. In particular, chronic pain is a major clinical problem because conventional analgesics such as nonsteroidal anti-inflammatory drugs and opioids have problems associated with long-term treatment. In addition, neuropathic pain, a form of chronic pain caused by lesions or diseases of the somatosensory system, is no longer beneficial, and is poorly controlled by the currently available analgesics [5]. However, there are many obstacles for the development of ideal analgesics. Processing of nociceptive information is readily disrupted in injured or pathological conditions through neural plasticity that develops at multiple points along the sensory circuits [1, 6]. These changes in nociceptive processing can occur over a wide range of time scales (acute to chronic) and at multiple levels in molecules, synapses, cells, and networks [2]. For example, hyperexcitability of primary sensory neurons is commonly observed in pathological pain and is caused by changes in the expression level, intracellular distribution, and posttranslational modulation of ion channels, such as voltage-gated sodium channels [7]. In the spinal dorsal horn, aberrant processing of sensory inputs occurs through neuronal or synaptic changes such as long-term potentiation and disinhibition and contributes to pathological pain with both spinal and peripheral origins [8]. Consistent with these long-term changes in the nociceptive pathway, there is increasing evidence that epigenetic mechanisms such as DNA methylation, histone modification, and miRNA expressions are implicated in chronic pain syndromes [9]. On the other hand, spinal glial cells, especially microglia and astrocytes, are also recognized as major players in pain modulation by regulating neurotransmission and neuroinflammation [10]. In the brain, nociceptive inputs negatively affect emotion, cognition, and motivation, most notably in the chronic pain state, and chronic pain is correlated with comorbid cognitive, mood and anxiety disorders [11]. Reciprocally, the cortical activity underlying these higher brain functions can affect pain perception [12, 13].

Critical roles of microRNAs (miRNAs) have been emerging in the development and pathophysiology of the nervous system [14–16], including chronic pain [17]. miRNA dysregulation leads to abnormal neuronal excitability through regulation of ion channel expressions [18]. Specific deletion of Dicer in nociceptive DRG neurons resulted in expression changes of three nociceptor-enriched voltage-gated