Advances in Experimental Medicine and Biology 889

Gaetano Santulli *Editor*

microRNA: Cancer

From Molecular Biology to Clinical Practice

Advances in Experimental Medicine and Biology

Volume 889

Editorial Board:

IRUN R. COHEN, *The Weizmann Institute of Science, Rehovot, Israel*
ABEL LAJTHA, *N.S. Kline Institute for Psychiatric Research, Orangeburg, NY, USA*
JOHN D. LAMBRIS, *University of Pennsylvania, Philadelphia, PA, USA*
RODOLFO PAOLETTI, *University of Milan, Milan, Italy*

More information about this series at http://www.springer.com/series/5584

Gaetano Santulli
Editor

microRNA: Cancer

From Molecular Biology to Clinical Practice

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

"Federico II" University Hospital
Naples, Italy

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-319-23729-9 ISBN 978-3-319-23730-5 (eBook)
DOI 10.1007/978-3-319-23730-5

Library of Congress Control Number: 2015955611

Springer Cham Heidelberg New York Dordrecht London
© Springer International Publishing Switzerland 2015
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Foreword

I am delighted to introduce the book *microRNA Cancer: From Molecular Biology to Clinical Practice*, edited by Dr. Gaetano Santulli. This book makes an ideal companion to *microRNA: Basic Science* and *microRNA: Medical Evidence*. In these three volumes, Gaetano has been able to reunite several renowned experts in the microRNA field in order to provide an up-to-date overview of the functional roles of microRNAs in human pathophysiology.

microRNAs are small endogenous noncoding RNAs that regulate the gene expression at the posttranscriptional level. These molecules are involved in a plethora of cellular processes, both in physiology and disease. The present book elegantly highlights the functional roles of microRNAs in human cancer discussed in detail by prominent experts in the field, who present intricate and complicated topics in a very clear and understandable way while also highlighting intriguing questions and challenges.

A simple and innovative examination of the malignant transformation process, which addresses the main pathway modulated by microRNAs, introduces the book. The following chapters address established evidence and recent advances concerning the role of microRNAs in specific forms of cancer, from lung cancer to leukemia/lymphomas and prostate cancer. Of note, the book includes valuable color pictures, tables, diagrams, and schemes that support the text and in my opinion are very useful to the reader.

In summary, you will find in this book a well-organized and informative assessment of the state-of-the-art of a rapidly growing field of investigation: microRNAs and oncology. Every single chapter is a valuable tool for scholars and will certainly bring anyone rapidly up to speed in current progress in this exciting field.

Columbus, OH, USA Carlo M. Croce

The original version of the editor affiliation has been revised. An erratum can be found at DOI 10.1007/978-3-319-23730-5_12

Contents

1 **microRNAs in the Malignant Transformation Process** 1
Anne E. Sarver, Lihua Li, Reena V. Kartha, and Subbaya Subramanian

2 **microRNA and Chronic Lymphocytic Leukemia** 23
Dana Elena Giza and George A. Calin

3 **microRNA in Malignant Lymphoma** 41
Hiroyuki Tagawa

4 **MicroRNAs and Gastrointestinal Stromal Tumor** 51
Pinar Akçakaya and Weng-Onn Lui

5 **Insights into the Role of microRNAs in Pancreatic Cancer Pathogenesis: Potential for Diagnosis, Prognosis, and Therapy** 71
Mohammad Aslam Khan, Haseeb Zubair, Sanjeev Kumar Srivastava, Seema Singh, and Ajay Pratap Singh

6 **microRNAs and Colorectal Cancer** 89
Anna Lena Ress, Samantha Perakis, and Martin Pichler

7 **microRNAs and Prostate Cancer** 105
Sajni Josson, Leland W.K. Chung, and Murali Gururajan

8 **microRNA and Ovarian Cancer** 119
Ream Langhe

9 **microRNA and Lung Cancer** 153
Valerio Del Vescovo and Michela A. Denti

10 microRNAs and Soft Tissue Sarcomas 179
Tomohiro Fujiwara, Toshiyuki Kunisada, Koji Uotani,
Aki Yoshida, Ken Takeda, and Toshifumi Ozaki

11 microRNA and Bone Cancer 201
Mary Nugent

Erratum E1

Index 231

Contributors

Pinar Akçakaya Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden

Cancer Center Karolinska, Karolinska University Hospital, Stockholm, Sweden

George A. Calin Experimental Therapeutics Department, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

The RNA Interference and Non-coding RNA Center, MD Anderson Cancer Center, Texas State University, Houston, TX, USA

Leland W. K. Chung Uro-Oncology Research Program, Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Michela A. Denti Laboratory of RNA Biology and Biotechnology, Centre for Integrative Biology, University of Trento, Trento, Italy

Tomohiro Fujiwara Center for Innovative Clinical Medicine, Okayama University Hospital, Okayama, Japan

Department of Orthopedic Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

Dana Elena Giza Experimental Therapeutics Department, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Department of Hematology, Fundeni Clinical Institute, Bucharest, Romania

Murali Gururajan Uro-Oncology Research Program, Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Bristol-Myers Squibb Inc., Princeton, NJ, USA

Sajni Josson Uro-Oncology Research Program, Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Neostrata Inc., Princeton, NJ, USA

Reena V. Kartha Department of Experimental and Clinical Pharmacology, Center for Orphan Drug Research, University of Minnesota, Minneapolis, MN, USA

Mohammad Aslam Khan Department of Oncologic Sciences, Mitchell Cancer Institute, University of South Alabama, Mobile, AL, USA

Toshiyuki Kunisada Department of Orthopedic Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

Department of Medical Materials for Musculoskeletal Reconstruction, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Ream Langhe Department of Obstetrics and Gynaecology, Trinity Centre for Health Sciences, St. James's Hospital, Dublin, Ireland

Lihua Li Department of Surgery, University of Minnesota, Minneapolis, MN, USA

Weng-Onn Lui Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden

Cancer Center Karolinska, Karolinska University Hospital, Stockholm, Sweden

Mary Nugent Department of Trauma and Orthopaedics, St. James Hospital, Dublin 8, Ireland

Toshifumi Ozaki Department of Orthopedic Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

Samantha Perakis Division of Oncology, Medical University of Graz, Graz, Austria

Martin Pichler Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Anna Lena Ress Division of Oncology, Medical University of Graz, Graz, Austria

Anne E. Sarver Department of Surgery, University of Minnesota, Minneapolis, MN, USA

Seema Singh Department of Oncologic Sciences, Mitchell Cancer Institute, University of South Alabama, Mobile, AL, USA

Ajay Pratap Singh Department of Oncologic Sciences, Mitchell Cancer Institute, University of South Alabama, Mobile, AL, USA

Department of Biochemistry and Molecular Biology, College of Medicine, University of South Alabama, Mobile, AL, USA

Sanjeev Kumar Srivastava Department of Oncologic Sciences, Mitchell Cancer Institute, University of South Alabama, Mobile, AL, USA

Subbaya Subramanian Department of Surgery, University of Minnesota, Minneapolis, MN, USA

Hiroyuki Tagawa Department of Hematology, Nephrology, and Rheumatology, Akita University Graduate School of Medicine, Akita, Japan

Ken Takeda Department of Intelligent Orthopaedic System, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Department of Orthopedic Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

Koji Uotani Department of Orthopaedic Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

Valerio Del Vescovo Laboratory of RNA Biology and Biotechnology, Centre for Integrative Biology, University of Trento, Trento, Italy

Aki Yoshida Department of Orthopedic Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

Haseeb Zubair Department of Oncologic Sciences, Mitchell Cancer Institute, University of South Alabama, Mobile, AL, USA

Chapter 1
microRNAs in the Malignant Transformation Process

Anne E. Sarver, Lihua Li, Reena V. Kartha, and Subbaya Subramanian

Abstract Many cancers originate as benign neoplasms that transform into malignant cancerous tumors in a multistep progression that is regulated, in part, by microRNAs. Benign neoplasms, by definition, lack the ability to invade adjacent tissues or spread to distant sites through metastasis. The benign to malignant transition is a critical intervention stage as tumors diagnosed in subsequent nonlocalized and malignant stages are exponentially more difficult to treat successfully. This chapter explores the critical roles that microRNAs play in the transformation from benign to malignant in four representative cancers: colorectal cancer, pancreatic cancer, malignant peripheral nerve sheath tumor, and prostate cancer. Understanding how these microRNAs control this progression and transformation will lead to new therapeutic targets and diagnostic biomarkers, resulting in improved treatments and patient outcomes.

Keywords microRNA • Benign to malignant transformation • Biomarkers • Colorectal cancer • Pancreatic cancer • Malignant peripheral nerve sheath tumor • Prostate cancer

Introduction

Benign neoplasms, by definition, lack the ability to invade adjacent tissues or spread to distant sites through metastasis. Many cancers originate as benign neoplasms (sometimes referred to as a benign tumor, polyp, or cyst) that transform into malignant cancerous tumors in a multistep progression [1–4]. A cancer patient's prognosis is highly dependent on whether their cancer was detected at an early (more benign/

A.E. Sarver • L. Li • S. Subramanian, Ph.D. (✉)
Department of Surgery, University of Minnesota, MMC 195, 11-211 Moos Tower,
515 Delaware Street, S.E., Minneapolis, MN 55455, USA
e-mail: subree@umn.edu

R.V. Kartha
Department of Experimental and Clinical Pharmacology, Center for Orphan Drug Research,
University of Minnesota, Minneapolis, MN, USA

© Springer International Publishing Switzerland 2015

G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental
Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5_1

localized) or late (more aggressive/metastatic) stage. More intensive early screening efforts have significantly decreased cancer mortality because cancers in early, localized stages are more successfully treated, through surgical resection, chemotherapy, and/or radiation treatment. Once a cancer progresses to a metastatic stage there is the possibility of cancer cells escaping treatment and remaining dormant in the body, only to reawaken months or years later resulting in new malignancies and a poorer prognosis (Surveillance, Epidemiology, and End Results (SEER) Program and the National Center for Health Statistics) [5]. The benign to malignant transition is a critical intervention stage as there are fewer dysregulated signaling pathways, making it significantly more likely that therapeutic intervention will be successful in either eliminating the cancer from the patient or turning it into more of a benign, chronic disease that can be successfully managed over time.

microRNAs (miRNAs) play critical roles in the majority of canonical cellular signaling networks and their dysregulation is implicated in many cancers including breast cancer, colon cancer, gastric cancer, lung cancer, and sarcomas [6, 7]. As elucidated in the other two volumes of the present trilogy ("microRNA: Basic Science" and "MicroRNA: Medical Evidence"), a single miRNA has the potential to regulate hundreds of target genes, and their associated functional pathways. Small changes in the expression level of a few miRNAs can therefore have a dramatic biological impact, particularly when dysregulated. They are also, therefore, attractive therapeutic targets (see Chap. 1 of the volume "microRNA: Medical Evidence"). If we can identify and understand the master regulatory roles miRNAs play in cancer processes then we can devise therapeutic interventions that target these miRNAs, stopping the cascade of events that results in a malignant cancer (see Chap. 2 of the volume "microRNA: Basic Science" for an introduction to miRNA biological machinery).

This chapter focuses on the critical roles these miRNAs play in the transformation from a benign neoplasm to a malignant cancer in four representative cancer types that include some of the most common and some of the most deadly malignancies: colorectal cancer, pancreatic cancer, malignant peripheral nerve sheath tumor, and prostate cancer. miRNAs function as both tumor suppressors and tumor promoters (oncomirs) and their function may be tissue-specific. Recent studies, such as those summarized in this chapter, have established that there are many common miRNAs that appear to play consistent roles across many different types of cancer. Understanding how these miRNAs control this progression and transformation will lead to new therapeutic targets and diagnostic biomarkers, resulting in improved treatments and patient outcomes.

Colorectal Cancer

Colorectal cancer is the third most common cancer in both men and women, and the second leading cause of cancer death in the United States (*Cancer Facts & Figures 2015*. Atlanta: American Cancer Society) [8]. Increased screening with sigmoidoscopy, colonoscopy, and fecal occult blood tests, is credited with significantly

decreasing the mortality rate, primarily through early detection of precancerous polyps or early-stage colon cancer. The 5-year survival rate for early-stage detection (90 %) is about seven times that of late stage colon cancer (13 %). Despite the increased preventive screening, colorectal cancer remains a deadly disease, with an estimated 136,830 new cases and 50,310 deaths in the United States in 2014 (American Cancer Society. Colorectal Cancer Facts & Figures) [8].

Colorectal cancer arises typically from a benign polyp, also termed a colon adenoma, and proceeds through several well-defined clinical stages that are associated with characteristic genomic and molecular events [9, 10]. The transition of a colon adenoma to a cancerous adenocarcinoma (i.e., colon cancer) has been actively investigated by many researchers and key-conserved driver mutations have been identified [11, 12]. The key initiating step in the transformation from adenoma to adenocarcinoma is the dysregulation of the Wnt/β-catenin signaling pathway (found in ~93–97 % of genetically characterized tumors), specifically mutations that result in the inactivation of the adenomatous polyposis coli (APC) protein or the activation of β-catenin (CTNNB1), both resulting in the accumulation and increased translocation to the nucleus of β-catenin [13]. Additional mutations in genes such as *KRAS*, *TP53*, and *SMAD4* may result in the progression from benign polyp to colorectal cancer [14]. Mutations in *RAS* drive the progression from adenoma to adenocarcinoma in part by stimulating an increase in polyp size. Adenomas lacking a *RAS* mutation are typically small (<1 cm) in size. Over half of adenomas >1 cm in size (the size at which malignant potential dramatically increases [15]) carry a *RAS* mutation, a frequency similar to adenocarcinomas [16]. The TGF-β/Smad signaling pathway is also dysregulated in a majority of colorectal cancers, mutations may be present in TGF-β itself, one or more SMAD proteins, or in regulators of the pathway, such as miRNAs [17, 18]. Dysregulation of either the Wnt/β-catenin or TGF-β/Smad signaling pathways can result in activation of the *MYC* oncogene, a central player in many cancers, including colorectal cancer [11]. Interestingly, mutations in *TP53* tend to occur relatively late in the progression from adenoma to adenocarcinoma and may play a critical transformative role [19].

Dysregulation of microRNAs is an established feature of colon cancer pathogenesis and progression [20–22]. Our work has highlighted several critical pathways mediated by miRNAs with powerful implications in cellular adaptations for tumorigenesis, survival, and growth [23–25]. Just as there is an established progression of protein-coding gene mutations in the transformation from benign polyp to colon cancer, there is also a stepwise progression of dysregulated miRNAs. Aberrant expression of the majority of these differentially regulated miRNAs is detectable prior to histopathological changes, indicating that they may be helping to drive the progression [25].

Perhaps the most consistently upregulated miRNA across all tumor types is the antiapoptotic miR-21 [26–29]. In many cancers, including colorectal cancer, expression levels of miR-21 are correlated with the progression from benign neoplasm to malignant cancer [29, 30]. Protein levels of PDCD4, a tumor suppressor gene targeted by miR-21, are inversely correlated with miR-21 expression during colorectal cancer development [31].

Our analysis of miRNA expression data from 225 colon cancer patient samples revealed that a key feature of colon cancer is the overexpression of miR-182 and miR-503. Twelve-year survival data from these patients found a strong correlation between poor overall survival and a high combined level of these miRNAs. We found that miR-182 and miR-503 act cooperatively to downregulate *FBXW7*, a tumor suppressor driver gene in colon cancer, and are critical to the malignant transformation from adenoma to adenocarcinoma [32]. These oncomirs are sequentially upregulated, first miR-182 is upregulated in the benign polyp compared with normal colon tissue, and subsequently miR-503 is upregulated, potentially triggering the transformation of benign polyp to colon cancer (Fig. 1.1) [32].

miR-182's cluster members, miR-183 and miR-96, are also highly expressed in benign colon polyps compared to normal colon tissue, and likely contribute to initial transformation and progression from dysplasia towards colon cancer [20, 21]. This cluster is highly expressed and has been studied in a variety of cancer types including colon cancer, breast cancer, ovarian cancer, bladder cancer, lung cancer, and hepatocellular carcinoma [20, 33–36]. Identified and validated targets of miR-183 in these different cancers include EGR1 [25, 37], PDCD4 [38–40], RAB21 [33], and SMAD4 [41]. Identified and validated targets of miR-182 include BRCA1 [42], CADM1 [43], FOXF2, and MTSS1 [44]. miR-182 and miR-96 and numerous

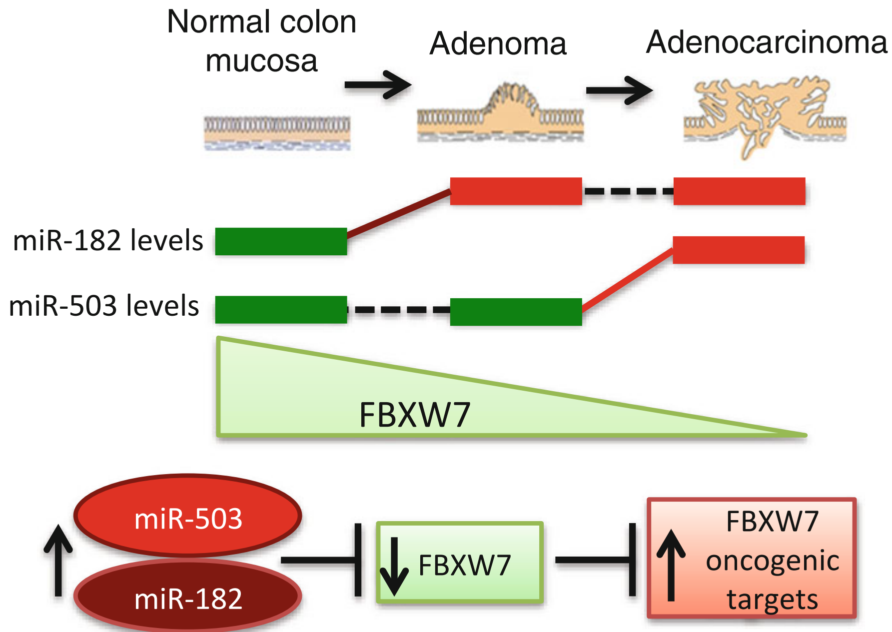

Fig. 1.1 Schematic representation of the stepwise expression of miR-182 and miR-503 levels during colon adenoma-to-adenocarcinoma progression and corresponding levels of FBWX7. Reprinted with permission from Li et al. [32]

common targets including FOXO1 [45–47], FOXO3 [48–50], and RECK [44, 51]. These three cluster members appear to cooperatively and complementarily target multiple critical oncogenic and tumor suppressor pathways whose dysregulation is known to contribute to tumor formation and progression.

Colorectal cancer could be classified into two major biological categories based on the type of genetic aberration underlying the disease: chromosomal instability and microsatellite instability [11, 52, 53]. Chromosomal instability is responsible for the majority (~75–85 %) of colorectal cancers and is characterized by chromosomal aberrations [52, 54]. Microsatellite instability is due to failure of the DNA mismatch repair (MMR) system and these tumors are highly associated with inactivation or loss of the MMR gene products (MLH1, MSH2, MSH3, MSH6, and PMS2) [55–57]. In an analysis of 80 colon tumors compared with 28 normal colon tissue samples, significant expression differences were seen in six miRNAs (miR-31, miR-181c, miR-196b, miR-552, miR-592, and miR-625) in MLH1-deficient tumors [20]. In contrast, one of the hallmarks of chromosomal instability tumors is the overexpression of the miR-17-92 cluster, located on chromosome 13q31.3 and comprising six miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a-1) over an 800-nucleotide genomic region [22]. The miR-17-92 cluster is frequently upregulated in many different cancers including lung cancer, lymphoma, osteosarcoma, and colorectal cancer [22, 58–60]. Not all of these cancers overexpress the miR-17-92 cluster due to chromosomal instability. The oncogenic transcription factor cMYC (frequently upregulated in colon cancer due to instability at chromosomal location 8q34) induces miR-17-92 expression [61]. Whether due to chromosomal instability or upregulation by another oncogene/oncomir, cMYC and miR-17-92 overexpression is a significant step in the progression of colorectal adenoma to adenocarcinoma [22].

Many miRNAs serve as tumor suppressors, providing critical regulators of cell growth. Early in the development of most adenomas, miR-137 is downregulated via epigenetic silencing [62]. This silencing persists throughout the progression to adenocarcinoma and has not been observed in healthy individuals nor in the normal mucosa from colorectal cancer patients [63, 64]. miR-137 targets CDC42 and acts as an inhibitor of cell cycle G1 arrest and ectopic expression of miR-137 inhibited invasion of colorectal cancer cells [65]. Other miRNAs involved in regulation of cell growth that are also downregulated in the early phase of adenoma formation include miR-143 and miR-145. Downregulation of these two tumor suppressor miRNAs is likely a critical step in the initial formation of an adenoma [66].

The mechanism by which many of these miRNAs become dysregulated is unclear. It is highly likely that expression of these miRNAs is being regulated, at least in part, by other miRNAs and by well-known tumor suppressor and oncogenic signaling pathways. Inactivation of TP53 typically occurs late in adenoma development [19]. TP53 not only functions as a tumor suppressor by regulating expression of numerous proteins and miRNAs through regulatory sites in their promoter regions [67] but also regulates the posttranscriptional maturation of miRNAs by interacting with the Drosha processing complex, promoting the processing of primary miRNAs to precursor miRNAs [68]. Loss of TP53 activity therefore represents a major dis-

ruptive event that, coupled with the other genetic changes, may solidly commit the adenoma cells progression to malignant adenocarcinoma.

The signals that promote progression from a benign polyp to malignant cancer do not necessarily follow a single path or stepwise progression. Disruptions at any one of multiple points along the major tumor suppressor and/or oncogenic pathways can result in the formation of an adenoma (or other benign neoplasm) and subsequent progression to cancer. For example, miR-34a and miR-34b/c are tumor suppressors that help regulate apoptosis, cell cycle arrest, and senescence. They can be inactivated either through a loss in TP53 activity or by epigenetic silencing. In samples from 114 colorectal cancer patients, there was a significant correlation of miR-34a methylation (i.e. epigenetic silencing) and the absence of TP53 mutation, indicating that either path could be sufficient to drive cancer progression [69, 70].

While dysregulation of miRNAs such as miR-182 and miR-137 appear critical to formation of adenomas, dysregulation of additional miRNAs such as miR-503 and the miR-34 family appear necessary to continue the progression to adenocarcinoma. Further elucidation of the miRNAs involved both in adenoma formation and in progression to adenocarcinoma may result in both better diagnostics and in improved therapeutics for colorectal cancer (see Chap. 6 for a detailed discussion on miRNAs and colorectal cancer).

Pancreatic Cancer

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States despite being only the twelfth most common cancer. In 2014, it is estimated that out of 46,000 patients diagnosed with pancreatic cancer in the United States, 40,000 will die from the disease (Surveillance, Epidemiology, and End Results (SEER) Program and the National Center for Health Statistics) [5]. Pancreatic cancer's extremely high mortality rate (less than 7 % survival at 5 years) is due to its early metastatic nature, lack of routine screening, and the fact that early-stage pancreatic cancer is usually asymptomatic. At diagnosis, more than 80 % of pancreatic cancer patients already have an invasive form of the disease that is largely unresponsive to surgical and chemotherapeutic interventions, resulting in a very poor prognosis [71].

Pancreatic cancer is comprised of four distinct tumor subtypes, defined by their histological features, which have distinct clinical behaviors and genetic mutation profiles: adenocarcinoma, acinar-cell carcinoma, pancreatic endocrine tumors, and serous cystadenoma. The most common subtype is pancreatic adenocarcinoma (ductal-cell histology), accounting for more than 85 % of pancreatic neoplasms [72]. Pancreatic intraepithelial neoplasias (PanINs) are precursors to pancreatic adenocarcinomas that represent progressive stages of neoplastic growth with accompanying genetic alterations [73]. Early mutations typically appearing in the PanIN-1A/B stages include activating KRAS, ERBB2, and EGFR mutations, followed by loss of function mutations in INK4A and TP53 as the lesion progresses to PanIN-2. In the PanIN-3 stage, which is marked by budding into the lumen and severe nuclear atypia, loss of function mutations arise in SMAD4/DPC4 and BRCA2, and mutations

in all are typically present in the invasive adenocarcinoma [72]. In addition to mutations in these key tumor suppressor genes, the dysregulation of miRNAs is an established feature of pancreatic cancer progression and pathogenesis [74].

Many studies have compared the global miRNA expression profiles in pancreatic cancer to normal or benign pancreatic tissue and/or chronic pancreatitis [75, 76]. Kent et al. [77] profiled global miRNA expression in 21 human pancreatic ductal adenocarcinoma cell lines and compared expression levels to control nontransformed pancreatic ductal-cell lines. To better understand the transformation from benign to malignant it is necessary to compare miRNA profiles across multiple PanIN stages.

Many miRNAs are dysregulated in PanIN lesions and likely play critical roles in the progression to pancreatic cancer (see Chap. 5 for a detailed discussion on miRNAs and pancreatic cancer). Yu et al. [78] performed one of the most extensive studies of miRNA expression in PanINs, screening 735 miRNAs for aberrant expression in laser capture-microdissected tissue samples taken from different stage PanINs. They identified 107 dysregulated miRNAs, only some of which had previously been reported to be dysregulated in pancreatic ductal adenocarcinoma [78]. Other studies have focused on miRNAs previously identified as significantly dysregulated in pancreatic cancer such as miR-21, miR-145, miR-155, miR-200a/b/c, miR-205, miR-221, miR-375, and let-7a [79–81]. Interestingly, the progression of miRNA upregulation parallels the changes seen in protein levels as the neoplasm progresses through the PanIN stages into adenocarcinoma and metastasis. Some miRNAs, such as miR-21, begin to be upregulated early in PanIN development while others, such as miR-196b are not upregulated until the PanIN-3 lesion stage [78]. The majority of these miRNAs are also overexpressed in a wide range of cancers including colon, lung, and breast, indicating they may play common critical roles in cancer development and progression [75]. Figure 1.2 illustrates some of the key dysregulated miRNA regulators of signaling pathways involved in the molecular pathogenesis of pancreatic ductal adenocarcinoma [82].

One example of a well-characterized oncomir is miR-21, which is responsible for increased cell proliferation and decreased apoptosis [83]. miR-21 regulates key signaling molecules including BCL-2 [83], FASL [84], PDCD4 [85], and the PTEN/AKT pathway [86]. In pancreatic cancer patients, miR-21 overexpression is correlated with chemoresistance and poor prognosis [84, 87]. In a mouse model of pancreatic cancer (the KRAS(G12D) model) miR-21 expression not only paralleled PanIN progression but its overexpression preceded phenotypic changes [79], suggesting that an increase in miR-21 is not only correlated but also causative in pancreatic, and other, cancers.

Another common oncomir, miR-221, is one of the most consistently overexpressed miRNAs in pancreatic cancer tissues. miR-221 and its cluster member miR-222 promote cell proliferation in numerous tumor types via their targeting of *CDKN1B/p27* and the cyclin-dependent kinase inhibitor *CDKN1C/p27* [75, 88–90]. Circulating levels of miR-221 was a significant prognostic factor for overall survival in colorectal cancer patients [91]. Circulating levels of miR-221 were significantly higher in pancreatic cancer patients versus those with a benign tumor, suggesting that miR-221 could be a useful noninvasive marker for monitoring transformation and progression along the PanIN stages to adenocarcinoma and metastasis [92].

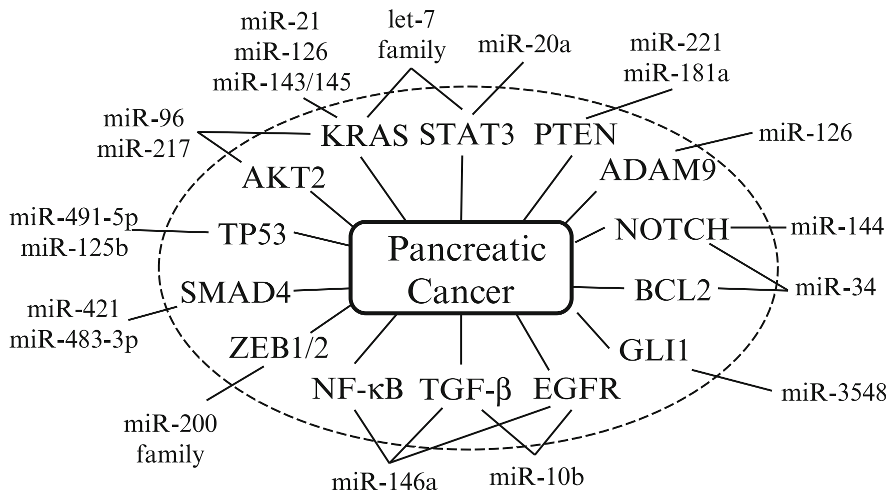

Fig. 1.2 Key dysregulated miRNA regulators of signaling pathways involved in the molecular pathogenesis of pancreatic ductal adenocarcinoma. Adapted from Chitkara et al. [82]

miR-155 is highly overexpressed in pancreatic cancer compared to normal pancreas and chronic pancreatitis tissues [75]. miR-155 is expressed as part of BIC, a noncoding transcript also highly upregulated in activated lymphocytes, and upregulation of miR-155 in mice results in cancer [93]. miR-155 targets key genes in the inactivation of mismatch repair pathway, a pathway whose dysregulation has been identified as a significant contributing factor to development of colorectal, endometrial, ovarian, gastric, and urothelial cancer [94]. In pancreatic cancer, miR-155 downregulates TP53INP1, a proapoptotic stress-induced p53 target gene whose expression is significantly decreased early in pancreatic cancer development, correlating with the induction of miR-155 in PanINs [95].

Early diagnosis of pancreatic cancer is difficult, with no approved blood biomarkers to identify patients with early-stage pancreatic cancer [96]. Research efforts are underway to discover specific biomolecules that can be used for early diagnosis, prognosis, and therapy of pancreatic cancer, as the currently used ones lack adequate sensitivity and specificity to detect early-stage pancreatic cancer or to therapeutically target cancer cells [97]. Serum cancer antigen 19-9 (CA19-9) is elevated in approximately 80 % of patients with pancreatic cancer and is approved for use as a treatment guide and prognosis indicator, it may prove to also have useful diagnostic attributes [98, 99]. Whole blood-derived miRNA profiles may be developed into a new tool for early detection of pancreatic cancer and other adenocarcinomas [100–102]. Advantages of whole blood versus serum or plasma include: higher miRNA content, elimination of many technical handling problems, the possibility of measuring both tumor- and host-secreted miRNAs, and following changes over time, especially during and after treatment [102, 103]. Cho [104]

reviewed the most common potential miRNA biomarkers and their key known targets in specific cancer tissues, in subsequent years these same miRNAs (miR-21, miR-155, miR-196a/b, etc.) have been found to be significantly dysregulated in many other cancer types, including pancreatic cancer.

Malignant Peripheral Nerve Sheath Tumor

Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive soft tissue tumors that occur either sporadically or in patients with Neurofibromatosis Type 1 (NF1). Approximately half of MPNSTs occur as sporadic cases; the remainder arises in patients with the autosomal dominant genetic disorder NF1. NF1 is caused by inactivating mutations in the *NF1* gene and affects 1:3000 live births. It is associated with a significant risk of developing malignancies, especially MPNSTs that occur in NF1 patients with an incidence of ~10 % [105–107]. MPNSTs, particularly in NF1, are highly aggressive tumors that appear largely unresponsive to conventional chemotherapy [108]. Overall, these tumors have a 5-year survival rate of about 40 % [109]. In NF1 patients, MPNSTs most often develop from pre-existing neurofibromas. Screening for malignant transformation in NF1 patients is difficult due to the large number and diverse anatomical sites of neurofibromas that occur in these patients. As a result, most MPNSTs are identified at a late clinical stage [105, 110].

The development of MPNSTs from neurofibromas is a complex process and a number of studies have described different molecular findings in these lesions. Both NF1-associated and sporadic MPNSTs are characterized by loss of *NF1* expression [111] that leads to increased RAS signaling and increased cell proliferation [112]. Molecular events such as DNA amplification with gain of expression of *TOP2A* and *EGFR* [113, 114], and inactivation of *CDKN2A* and *p53* [115–117] have been implicated in malignant transformation towards MPNSTs. Yang et al. [118] using a mouse model of NF1, demonstrated that for neurofibroma formation, *Nf1* haploinsufficiency is required in the nonneoplastic cells of the tumor microenvironment and also implicated mast cells as critical mediators of neurofibroma initiation. Earlier studies have shown differences in gene expression patterns between neurofibromas and MPNSTs and between dermal and plexiform neurofibromas [119, 120]. However, NF1-associated and sporadic MPNSTs could not be distinguished by gene expression profiling [121]. Miller et al. [122] demonstrated downregulation of Schwann cell differentiation markers in MPNST and showed that reduction of TWIST1 expression inhibited chemotaxis.

Subsets of MPNSTs are characterized by the presence of a 1.4-Mb microdeletion. Pasmant et al. identified the presence of 2 miRNAs, miR-193a, and miR-365-2 in this microdeletion [123]. However, expression analysis of these miRNAs did not show any significant difference between human dermal and plexiform neurofibromas and MPNSTs.

Gene expression analysis performed by our group has identified an expression signature indicating p53 inactivation in the majority of MPNSTs [124]. Subsequently, we performed miRNA profiling in benign and malignant PNSTs. This analysis indicated a relative downregulation of miR-34a in most MPNSTs compared to neurofibromas. Using functional studies we demonstrated that exogenous expression of TP53 or miR-34a promotes apoptotic cell death in MPNSTs. In addition, miR-214 was highly upregulated in MPNSTs compared to neurofibromas. miR-214 is a direct transcriptional target of TWIST1 [125], a regulator of metastasis [126]. It is to be noted that TWIST1 is highly expressed in MPNSTs. miR-214 targets PTEN, hence the *TWIST1-miR-214-PTEN* pathway could be further explored to potentially decipher MPNST pathogenesis. Collectively, our findings suggest that deregulation of miRNAs has a potential role in the malignant transformation process in PNSTs [124]. This was further confirmed by a series of studies examining the role of several candidate miRNAs by other groups. Figure 1.3 shows the miRNAs dysregulated in the malignant transformation of neurofibroma.

Chai et al. reported upregulation of miR-10b in primary Schwann cells isolated from neurofibromas and in MPNST tumors and cell lines [127]. Using functional studies in multiple cell lines, the authors demonstrated that the inhibition of miR-10b reduced cell proliferation, migration, and invasion by affecting NF1 expression and RAS signaling. miR-21 also appears to play an important role in MPNST tumorigenesis and progression through its target, PDCD4, similar to other cancers being studied [128]. Masliah-Planchon et al. analyzed the expression of 377 miRNAs in NF1 benign neurofibromas and MPNSTs. They found aberrant expression in the neurofibromas of additional four miRNAs involved in the RAS-MAPK pathway (miR-370, miR-143, miR-181a, and miR-145). The most significantly upregulated miRNA in the neurofibroma samples was miR-486-3p, which targets the major tumor suppressor gene PTEN, and they confirmed downregulation of PTEN in these samples [129]. The downregulation of miR-210 has been observed in many types of cancer and is also downregulated in MPNST cells compared to their benign neurofibroma precursors, indicating that dysregulation of this miRNA may be a common key step in the progression from benign to cancer [130].

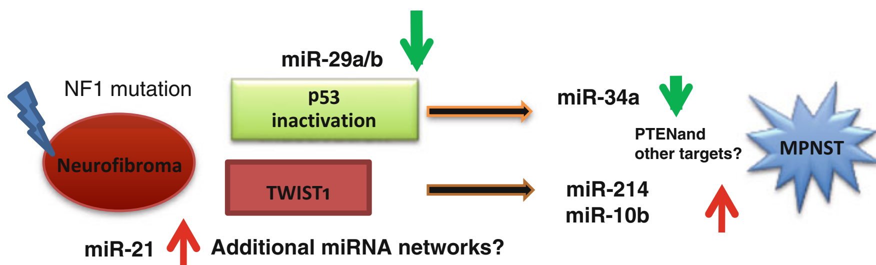

Fig. 1.3 miRNA gene networks dysregulated in the malignant transformation of neurofibroma to MPNSTs. Adapted from Subramanian and Kartha [23]

Prostate Cancer

Prostate cancer is the most common nonskin cancer, with approximately one in seven men being diagnosed with this malignancy during their lifetime, and the second leading cause of death among men in the United States. The American Cancer Society estimates that in 2015 there will be approximately 220,800 new cases and 27,540 deaths from prostate cancer in the United States [8]. With advances in diagnosis and treatment techniques, the relative 5-year survival rate when including all stages of prostate cancer is almost 100 %. However, the relative 5-year survival for late stage cancers (that have spread to distant lymph nodes, bones, and other organs) is only 28 %.

Only a few studies that have addressed the role of miRNAs in the progression of a benign prostate tumor to malignant prostate cancer (see Chap. 7 for a detailed discussion on miRNAs and prostate cancer). Initiation and progression of prostate cancer depends on androgen-receptor signaling and this pathway regulates many miRNAs that have been demonstrated to be dysregulated in malignant prostate cancer [131, 132].

In the prostate, neoplastic transformation is first recognized as a noninvasive lesion that precedes invasive cancer and is called a high-grade prostatic intraepithelial neoplasia (PIN) [133]. A PIN lesion then progresses to prostate cancer. Prostate cancer tissue is graded using a Gleason score that ranges from 2 to 10 and is based on the histology and morphology of the tissue. Prostate cancers with a Gleason score in the 2–4 range are small benign tissues that are usually found incidentally. Most treatable tumors that are found in response to an abnormal screening result are in the 5–7 Gleason score range. Prostate tumors that have advanced and may be aggressively malignant (Gleason score 8–10) have a very poor prognosis [134].

Comparison of large gene expression data sets generated from normal tissues with those from more invasive lesions demonstrated enrichment of developmental genes in invasive transitions. The enrichment was particularly significant for categories indicating malignant transformation (normal vs. PIN), invasion (PIN vs. cancer), and aggressiveness [135]. Interestingly the authors observed that more than 50 % of the suppressed genes contained one or more predicted binding sites for miRNAs, thus providing a mechanism for rapid suppression of gene expression in response to androgen. Specifically there was activation of predicted targets of miR-21 and miR-17-5p in PIN samples compared to prostate cancer tissues [135].

Chronic inorganic arsenic exposure in vitro of human prostate cancer stem cells can induce their malignant transformation [136]. Investigation aimed at understanding the molecular mechanisms underlying this transformation ruled out DNA damage during this process [137]. However, aberrant miRNA expression was observed to have a significant role in the transformation of prostate epithelium by arsenic, impacting activation of RAS oncogenes [138]. Several miRNAs that target KRAS or other RAS superfamily members, including miR-134, miR-373, miR-34c-5p, miR-155, miR-138, miR-181d, miR-96, miR-181c, miR-143, miR-148a, and let-7 were observed to be dysregulated with corresponding activation of KRAS in the prostate epithelium and stem cells malignantly transformed by arsenic [138].

For instance, miR-143 is a well-known tumor suppressor targeting KRAS and the RAS/MAPK signaling pathway and is downregulated in prostate cancer [139, 140]. However, KRAS activation is not mandatory for malignant transformation of prostate epithelial cells since treatment of these cells with N-methyl-N-nitrosourea, a genotoxin, does not cause KRAS activation [138].

A number of miRNAs also interact with apoptotic pathway genes such as p53 and E2F1-3 transcription factors and dysregulation of their expression in prostate cancer may promote apoptosis avoidance and thus contribute to malignant transformation [141]. For instance, miR-125b modulates p53-dependent and p53-independent apoptosis in prostate cancer cells by modulating p14 (ARF) levels [142]. Likewise, miR-17-92 cluster expression is upregulated in prostate cancer cells resulting in suppression of E2F1-3 expression and thus apoptosis avoidance [143]. miR-20a expression levels have been shown to increase with prostate cancer progression, with more dedifferentiated cells (Gleason score 7–10) having higher miR-20a levels suggesting a possible role of miR-20a in prostate cancer progression [144]. Further study is needed to understand the roles that miRNAs play in transformation of normal tissue to PIN lesions and from PIN lesions to early, relatively benign, prostate cancer.

Conclusion

Progressive dysregulation of miRNAs is a hallmark of cancer's benign to malignant transformation. Just as many oncogenic proteins are dysregulated across many different tumor types, so too are there commonly dysregulated oncomirs such as miR-21. Tissue- and tumor-specific proteins and miRNAs further contribute to cancer formation and progression. Once any cancer has progressed to a nonlocalized and/or metastatic stage, the disease becomes exponentially more difficult to treat and the patient's prognosis becomes increasingly poor. The key stage for both detection and intervention is thus at the benign stage or in an early, localized stage. Therefore, development of reliable benign/early-stage biomarkers is critical, and circulating miRNAs provide attractive potential biomarkers. Recent studies have shown miRNAs are very stable in blood serum and plasma, and extensive efforts are underway to develop circulating miRNA-based diagnostic and prognostic markers [145]. Major technical challenges in developing circulating miRNA-based markers still need to be addressed, including standardization of pre-analytical, analytical, and post-analytical methods for effective reproducibility. Wang et al. recently completed a meta-analysis of 107 studies from 42 articles and concluded that diagnostic panels consisting of multiple miRNAs were highly accurate in diagnosis of gastric cancer and colorectal cancer from plasma and serum samples, respectively [146]. As we have shown in this chapter, dysregulation of miRNAs occurs early in the transformation from benign to malignant, highlighting their potential importance as biomarkers for the noninvasive detection of early-stage cancers.

Therapeutics targeting miRNAs represent a largely untapped pool of potential therapies for many different diseases, including cancer. The first miRNA targeted drug, miravirsen, to show efficacy in human clinical trials is an inhibitor of miR-122, a liver-specific miRNA required by the Hepatitis C virus (HCV) for replication. Initial Phase 2a results demonstrated that miravirsen, being developed by Santaris Pharma A/S, was associated with dose-dependent reductions in HCV RNA and that 4 out of 9 patients treated with the highest dose no longer had detectable HCV RNA after 5 weekly doses [147]. Additional Phase 2 trials of miravirsen were still ongoing in early 2015 (NCT01872936, NCT01727934, and NCT02031133).

In addition to numerous completed and ongoing miRNA-based biomarker clinical trials, the first miRNA-mimic based drug targeting cancer entered Phase 1 clinical trials in April 2013. MRX34, a liposome-formulated double-stranded mimic of tumor suppressor miR-34 being developed by Mirna Therapeutics Inc., is initially being tested as a therapeutic in patients with unresectable primary liver cancer or those with liver metastasis from other cancers (NCT01829971). Another miRNA tumor suppressor, miR-16, is the basis of a second Phase 1 clinical trial in patients with malignant pleural mesothelioma or nonsmall cell lung cancer. Double-stranded mimics of miR-16 are being formulated in targeted nonliving bacterial minicell delivery vehicles that are targeted to EGFR-expressing cancer cells via an anti-EGFR-bispecific antibody (NCT02369198). Results from both of these studies are anticipated to be reported in 2016.

It is critical that we continue to increase our understanding of how the benign to cancer transformation is regulated and controlled in order to develop more effective early-stage treatments and/or preventive drugs. If well-tolerated treatments could halt progression of benign polyps/neoplasms or return early-stage cancers to a more quiescent, benign stage, then patient survival and quality of life outcomes could be significantly increased, perhaps even turning cancer into a chronic, benign disease under tight control. miRNAs are critical regulators of the benign to malignant transition and should continue to be the target of active research.

Acknowledgements SS is supported by research grants funded by the American Cancer Society (RSG-13-381-01), the Children's Cancer Research Fund, the Zach Sobiech Osteosarcoma Fund, and the Karen Wyckoff Rein in Sarcoma Foundation. Due to space restrictions, we could not cite many other significant contributions made by numerous researchers and laboratories in this important and rapidly progressing field.

References

1. Cowan RW, Maitra A. Genetic progression of pancreatic cancer. Cancer J. 2014;20:80–4.
2. Grady WM, Markowitz SD. The molecular pathogenesis of colorectal cancer and its potential application to colorectal cancer screening. Dig Dis Sci. 2015;60(3):762–72.
3. Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, van der Kwast T, Mason M, Matveev V, Wiegel T, Zattoni F, Mottet N. European Association of U. EAU guidelines on prostate cancer. part 1: screening, diagnosis, and local treatment with curative intent-update 2013. Eur Urol. 2014;65:124–37.

4. Farid M, Demicco EG, Garcia R, Ahn L, Merola PR, Cioffi A, Maki RG. Malignant peripheral nerve sheath tumors. Oncologist. 2014;19:193–201.
5. http://seer.cancer.gov/.
6. Thayanithy V, Sarver AL, Kartha RV, Li L, Angstadt AY, Breen M, Steer CJ, Modiano JF, Subramanian S. Perturbation of 14q32 miRNAs-cMYC gene network in osteosarcoma. Bone. 2012;50:171–81.
7. Drury R, Verghese ET, Hughes TA. The roles of microRNAs in sarcomas. J Pathol. 2012;227: 385–91.
8. American Cancer Society. Colorectal cancer facts & figures 2014–2016. Atlanta: American Cancer Society; 2014.
9. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990;61: 759–67.
10. Jones S, Chen WD, Parmigiani G, Diehl F, Beerenwinkel N, Antal T, Traulsen A, Nowak MA, Siegel C, Velculescu VE, Kinzler KW, Vogelstein B, Willis J, Markowitz SD. Comparative lesion sequencing provides insights into tumor evolution. Proc Natl Acad Sci U S A. 2008;105:4283–8.
11. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012;487:330–7.
12. Markowitz SD, Bertagnolli MM. Molecular origins of cancer: Molecular basis of colorectal cancer. N Engl J Med. 2009;361:2449–60.
13. Goss KH, Groden J. Biology of the adenomatous polyposis coli tumor suppressor. J Clin Oncol. 2000;18:1967–79.
14. Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. Cell. 2012;149:1192–205.
15. Zhu H, Zhang G, Yi X, Zhu X, Wu Y, Liang J, Zhang S, Zeng Y, Fan D, Yu X, He J, He G, Chen Z, Duan S, Zhang L. Histology subtypes and polyp size are associated with synchronous colorectal carcinoma of colorectal serrated polyps: a study of 499 serrated polyps. Am J Cancer Res. 2015;5:363–74.
16. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. N Engl J Med. 1988;319:525–32.
17. Derynck R, Akhurst RJ, Balmain A. TGF-beta signaling in tumor suppression and cancer progression. Nat Genet. 2001;29:117–29.
18. Lampropoulos P, Zizi-Sermpetzoglou A, Rizos S, Kostakis A, Nikiteas N, Papavassiliou AG. TGF-beta signalling in colon carcinogenesis. Cancer Lett. 2012;314:1–7.
19. Baker SJ, Preisinger AC, Jessup JM, Paraskeva C, Markowitz S, Willson JK, Hamilton S, Vogelstein B. p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. Cancer Res. 1990;50:7717–22.
20. Sarver AL, French AJ, Borralho PM, Thayanithy V, Oberg AL, Silverstein KA, Morlan BW, Riska SM, Boardman LA, Cunningham JM, Subramanian S, Wang L, Smyrk TC, Rodrigues CM, Thibodeau SN, Steer CJ. Human colon cancer profiles show differential microRNA expression depending on mismatch repair status and are characteristic of undifferentiated proliferative states. BMC Cancer. 2009;9:401.
21. Oberg AL, French AJ, Sarver AL, Subramanian S, Morlan BW, Riska SM, Borralho PM, Cunningham JM, Boardman LA, Wang L, Smyrk TC, Asmann Y, Steer CJ. Thibodeau SN miRNA expression in colon polyps provides evidence for a multihit model of colon cancer. PloS One. 2011;6, e20465.
22. Diosdado B, van de Wiel MA, Terhaar Sive Droste JS, Mongera S, Postma C, Meijerink WJ, Carvalho B, Meijer GA. MiR-17-92 cluster is associated with 13q gain and c-myc expression during colorectal adenoma to adenocarcinoma progression. Br J Cancer. 2009;101:707–14.
23. Subramanian S, Kartha RV. MicroRNA-mediated gene regulations in human sarcomas. Cell Mol Life Sci. 2012;69:3571–85.
24. Subramanian S, Steer CJ. MicroRNAs as gatekeepers of apoptosis. J Cell Physiol. 2010; 223:289–98.

25. Sarver AL, Li L, Subramanian S. MicroRNA miR-183 functions as an oncogene by targeting the transcription factor EGR1 and promoting tumor cell migration. Cancer Res. 2010;70: 9570–80.
26. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res. 2005;65:6029–33.
27. 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.
28. 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.
29. Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, Yuen ST, Chan TL, Kwong DL, Au GK, Liu CG, Calin GA, Croce CM, Harris CC. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA. 2008;299:425–36.
30. Schmitz KJ, Hey S, Schinwald A, Wohlschlaeger J, Baba HA, Worm K, Schmid KW. Differential expression of microRNA 181b and microRNA 21 in hyperplastic polyps and sessile serrated adenomas of the colon. Virchows Arch. 2009;455:49–54.
31. Yamamichi N, Shimomura R, Inada K, Sakurai K, Haraguchi T, Ozaki Y, Fujita S, Mizutani T, Furukawa C, Fujishiro M, Ichinose M, Shiogama K, Tsutsumi Y, Omata M, Iba H. Locked nucleic acid in situ hybridization analysis of miR-21 expression during colorectal cancer development. Clin Cancer Res. 2009;15:4009–16.
32. Li L, Sarver AL, Khatri R, Hajeri PB, Kamenev I, French AJ, Thibodeau SN, Steer CJ, Subramanian S. Sequential expression of miR-182 and miR-503 cooperatively targets FBXW7, contributing to the malignant transformation of colon adenoma to adenocarcinoma. J Pathol. 2014;234:488–501.
33. Li P, Sheng C, Huang L, Zhang H, Huang L, Cheng Z, Zhu Q. MiR-183/-96/-182 cluster is up-regulated in most breast cancers and increases cell proliferation and migration. Breast Cancer Res. 2014;16:473.
34. McMillen BD, Aponte MM, Liu Z, Helenowski IB, Scholtens DM, Buttin BM, Wei JJ. Expression analysis of MIR182 and its associated target genes in advanced ovarian carcinoma. Mod Pathol. 2012;25:1644–53.
35. Wang L, Zhu MJ, Ren AM, Wu HF, Han WM, Tan RY, Tu RQ. A ten-microRNA signature identified from a genome-wide microRNA expression profiling in human epithelial ovarian cancer. PLoS One. 2014;9, e96472.
36. Zhang QH, Sun HM, Zheng RZ, Li YC, Zhang Q, Cheng P, Tang ZH, Huang F. Meta-analysis of microRNA-183 family expression in human cancer studies comparing cancer tissues with noncancerous tissues. Gene. 2013;527:26–32.
37. Hannafon BN, Sebastiani P, de las Morenas A, Lu J, Rosenberg CL. Expression of microRNA and their gene targets are dysregulated in preinvasive breast cancer. Breast Cancer Res. 2011;13:R24.
38. Ren LH, Chen WX, Li S, He XY, Zhang ZM, Li M, Cao RS, Hao B, Zhang HJ, Qiu HQ, Shi RH. MicroRNA-183 promotes proliferation and invasion in esophageal squamous cell carcinoma by targeting programmed cell death 4. Br J Cancer. 2014;111:2003–13.
39. Yang M, Liu R, Li X, Liao J, Pu Y, Pan E, Yin L, Wang Y. miRNA-183 suppresses apoptosis and promotes proliferation in esophageal cancer by targeting PDCD4. Mol Cells. 2014;37: 873–80.
40. Li J, Fu H, Xu C, Tie Y, Xing R, Zhu J, Qin Y, Sun Z, Zheng X. miR-183 inhibits TGF-beta1-induced apoptosis by downregulation of PDCD4 expression in human hepatocellular carcinoma cells. BMC Cancer. 2010;10:354.
41. Ueno K, Hirata H, Shahryari V, Deng G, Tanaka Y, Tabatabai ZL, Hinoda Y, Dahiya R. microRNA-183 is an oncogene targeting Dkk-3 and SMAD4 in prostate cancer. Br J Cancer. 2013;108:1659–67.

42. Moskwa P, Buffa FM, Pan Y, Panchakshari R, Gottipati P, Muschel RJ, Beech J, Kulshrestha R, Abdelmohsen K, Weinstock DM, Gorospe M, Harris AL, Helleday T, Chowdhury D. miR-182-mediated downregulation of BRCA1 impacts DNA repair and sensitivity to PARP inhibitors. Mol Cell. 2011;41:210–20.
43. Qiu Y, Luo X, Kan T, Zhang Y, Yu W, Wei Y, Shen N, Yi B, Jiang X. TGF-beta upregulates miR-182 expression to promote gallbladder cancer metastasis by targeting CADM1. Mol Biosyst. 2014;10:679–85.
44. Hirata H, Ueno K, Shahryari V, Deng G, Tanaka Y, Tabatabai ZL, Hinoda Y, Dahiya R. MicroRNA-182-5p promotes cell invasion and proliferation by down regulating FOXF2, RECK and MTSS1 genes in human prostate cancer. PLoS One. 2013;8, e55502.
45. Guttilla IK, White BA. Coordinate regulation of FOXO1 by miR-27a, miR-96, and miR-182 in breast cancer cells. J Biol Chem. 2009;284:23204–16.
46. Myatt SS, Wang J, Monteiro LJ, Christian M, Ho KK, Fusi L, Dina RE, Brosens JJ, Ghaem-Maghami S, Lam EW. Definition of microRNAs that repress expression of the tumor suppressor gene FOXO1 in endometrial cancer. Cancer Res. 2010;70:367–77.
47. Fendler A, Jung M, Stephan C, Erbersdobler A, Jung K, Yousef GM. The antiapoptotic function of miR-96 in prostate cancer by inhibition of FOXO1. PLoS One. 2013;8, e80807.
48. Segura MF, Hanniford D, Menendez S, Reavie L, Zou X, Alvarez-Diaz S, Zakrzewski J, Blochin E, Rose A, Bogunovic D, Polsky D, Wei J, Lee P, Belitskaya-Levy I, Bhardwaj N, Osman I, Hernando E. Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor. Proc Natl Acad Sci U S A. 2009;106:1814–9.
49. Li J, Li P, Chen T, Gao G, Chen X, Du Y, Zhang R, Yang R, Zhao W, Dun S, Gao F, Zhang G. Expression of microRNA-96 and its potential functions by targeting FOXO3 in non-small cell lung cancer. Tumour Biol. 2015;36:685–92.
50. Zhang J, Kong X, Li J, Luo Q, Li X, Shen L, Chen L, Fang L. miR-96 promotes tumor proliferation and invasion by targeting RECK in breast cancer. Oncol Rep. 2014;31:1357–63.
51. Xia H, Chen S, Chen K, Huang H, Ma H. MiR-96 promotes proliferation and chemo- or radioresistance by down-regulating RECK in esophageal cancer. Biomed Pharmacother. 2014;68:951–8.
52. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell. 1996;87:159–70.
53. Worthley DL, Leggett BA. Colorectal cancer: molecular features and clinical opportunities. Clin Biochem Rev. 2010;31:31–8.
54. Hermsen M, Postma C, Baak J, Weiss M, Rapallo A, Sciutto A, Roemen G, Arends JW, Williams R, Giaretti W, De Goeij A, Meijer G. Colorectal adenoma to carcinoma progression follows multiple pathways of chromosomal instability. Gastroenterology. 2002;123: 1109–19.
55. Ahuja N, Li Q, Mohan AL, Baylin SB, Issa JP. Aging and DNA methylation in colorectal mucosa and cancer. Cancer Res. 1998;58:5489–94.
56. Curtin K, Slattery ML, Samowitz WS. CpG island methylation in colorectal cancer: past, present and future. Patholog Res Int. 2011;2011:902674.
57. Kuismanen SA, Holmberg MT, Salovaara R, de la Chapelle A, Peltomaki P. Genetic and epigenetic modification of MLH1 accounts for a major share of microsatellite-unstable colorectal cancers. Am J Pathol. 2000;156:1773–9.
58. Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, Yatabe Y, Kawahara K, Sekido Y, Takahashi T. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res. 2005;65: 9628–32.
59. Campora J, Palma P, del Rio D, Lopez JA, Valerga P. Reactivity of an anionic Pd(II) metallacycle with CH2X2 (X=Cl, Br, I): formal insertion of methylene into a Pd-C(aryl) bond. Chem Commun. 2004:1490–1.
60. Petrocca F, Vecchione A, Croce CM. Emerging role of miR-106b-25/miR-17-92 clusters in the control of transforming growth factor beta signaling. Cancer Res. 2008;68:8191–4.

61. O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. c-Myc-regulated microRNAs modulate E2F1 expression. Nature. 2005;435:839–43.
62. Steponaitiene R, Kupcinskas J, Langner C, Balaguer F, Venclauskas L, Pauzas H, Tamelis A, Skieceviciene J, Kupcinskas L, Malfertheiner P, Link A. Epigenetic silencing of miR-137 is a frequent event in gastric carcinogenesis. Mol Carcinog. 2015.
63. Lasjaunias P, Magufis G, Goulao A, Piske R, Suthipongchai S, Rodesch R, Alvarez H. Anatomoclinical aspects of dural arteriovenous shunts in children. Review of 29 cases. Interv Neuroradiol. 1996;2:179–91.
64. Balaguer F, Link A, Lozano JJ, Cuatrecasas M, Nagasaka T, Boland CR, Goel A. Epigenetic silencing of miR-137 is an early event in colorectal carcinogenesis. Cancer Res. 2010;70: 6609–18.
65. Liu M, Lang N, Qiu M, Xu F, Li Q, Tang Q, Chen J, Chen X, Zhang S, Liu Z, Zhou J, Zhu Y, Deng Y, Zheng Y, Bi F. miR-137 targets Cdc42 expression, induces cell cycle G1 arrest and inhibits invasion in colorectal cancer cells. Int J Cancer. 2011;128:1269–79.
66. Akao Y, Nakagawa Y, Hirata I, Iio A, Itoh T, Kojima K, Nakashima R, Kitade Y, Naoe T. Role of anti-oncomirs miR-143 and -145 in human colorectal tumors. Cancer Gene Ther. 2010;17:398–408.
67. Tarasov V, Jung P, Verdoodt B, Lodygin D, Epanchintsev A, Menssen A, Meister G, Hermeking H. Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest. Cell Cycle. 2007;6:1586–93.
68. Suzuki HI, Yamagata K, Sugimoto K, Iwamoto T, Kato S, Miyazono K. Modulation of microRNA processing by p53. Nature. 2009;460:529–33.
69. Toyota M, Suzuki H, Sasaki Y, Maruyama R, Imai K, Shinomura Y, Tokino T. Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in colorectal cancer. Cancer Res. 2008;68:4123–32.
70. Vogt M, Munding J, Gruner M, Liffers ST, Verdoodt B, Hauk J, Steinstraesser L, Tannapfel A, Hermeking H. Frequent concomitant inactivation of miR-34a and miR-34b/c by CpG methylation in colorectal, pancreatic, mammary, ovarian, urothelial, and renal cell carcinomas and soft tissue sarcomas. Virchows Arch. 2011;458:313–22.
71. Sultana A, Tudur Smith C, Cunningham D, Starling N, Neoptolemos JP, Ghaneh P. Meta-analyses of chemotherapy for locally advanced and metastatic pancreatic cancer: results of secondary end points analyses. Br J Cancer. 2008;99:6–13.
72. Bardeesy N, DePinho RA. Pancreatic cancer biology and genetics. Nat Rev Cancer. 2002;2:897–909.
73. Hruban RH, Adsay NV, Albores-Saavedra J, Compton C, Garrett ES, Goodman SN, Kern SE, Klimstra DS, Kloppel G, Longnecker DS, Luttges J, Offerhaus GJ. Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. Am J Surg Pathol. 2001;25:579–86.
74. Jiao LR, Frampton AE, Jacob J, Pellegrino L, Krell J, Giamas G, Tsim N, Vlavianos P, Cohen P, Ahmad R, Keller A, Habib NA, Stebbing J, Castellano L. MicroRNAs targeting oncogenes are down-regulated in pancreatic malignant transformation from benign tumors. PLoS One. 2012;7, e32068.
75. Bloomston M, Frankel WL, Petrocca F, Volinia S, Alder H, Hagan JP, Liu CG, Bhatt D, Taccioli C, Croce CM. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. JAMA. 2007;297:1901–8.
76. Lee EJ, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, Morgan DL, Postier RG, Brackett DJ, Schmittgen TD. Expression profiling identifies microRNA signature in pancreatic cancer. Int J Cancer. 2007;120:1046–54.
77. Kent OA, Mullendore M, Wentzel EA, Lopez-Romero P, Tan AC, Alvarez H, West K, Ochs MF, Hidalgo M, Arking DE, Maitra A, Mendell JT. A resource for analysis of microRNA expression and function in pancreatic ductal adenocarcinoma cells. Cancer Biol Ther. 2009;8:2013–24.

78. Yu J, Li A, Hong SM, Hruban RH, Goggins M. MicroRNA alterations of pancreatic intraepithelial neoplasias. Clin Cancer Res. 2012;18:981–92.
79. du Rieu MC, Torrisani J, Selves J, Al Saati T, Souque A, Dufresne M, Tsongalis GJ, Suriawinata AA, Carrere N, Buscail L, Cordelier P. MicroRNA-21 is induced early in pancreatic ductal adenocarcinoma precursor lesions. Clin Chem. 2010;56:603–12.
80. Ryu JK, Hong SM, Karikari CA, Hruban RH, Goggins MG, Maitra A. Aberrant MicroRNA-155 expression is an early event in the multistep progression of pancreatic adenocarcinoma. Pancreatology. 2010;10:66–73.
81. Xue Y, Abou Tayoun AN, Abo KM, Pipas JM, Gordon SR, Gardner TB, Barth Jr RJ, Suriawinata AA, Tsongalis GJ. MicroRNAs as diagnostic markers for pancreatic ductal adenocarcinoma and its precursor, pancreatic intraepithelial neoplasm. Cancer Genet. 2013;206:217–21.
82. Chitkara D, Mittal A, Mahato RI. miRNAs in pancreatic cancer: therapeutic potential, delivery challenges and strategies. Adv Drug Deliv Rev. 2015;81:34–52.
83. Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. Oncogene. 2007;26:2799–803.
84. Wang P, Zhuang L, Zhang J, Fan J, Luo J, Chen H, Wang K, Liu L, Chen Z, Meng Z. The serum miR-21 level serves as a predictor for the chemosensitivity of advanced pancreatic cancer, and miR-21 expression confers chemoresistance by targeting FasL. Mol Oncol. 2013;7:334–45.
85. Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. J Biol Chem. 2008;283:1026–33.
86. Hu Y, Wang C, Li Y, Zhao J, Chen C, Zhou Y, Tao Y, Guo M, Qin N, Ren T, Wen Z, Xu L. MiR-21 controls in situ expansion of CCR6 regulatory T cells through PTEN/AKT pathway in breast cancer. Immunol Cell Biol. 2015.
87. Giovannetti E, Funel N, Peters GJ, Del Chiaro M, Erozenci LA, Vasile E, Leon LG, Pollina LE, Groen A, Falcone A, Danesi R, Campani D, Verheul HM, Boggi U. MicroRNA-21 in pancreatic cancer: correlation with clinical outcome and pharmacologic aspects underlying its role in the modulation of gemcitabine activity. Cancer Res. 2010;70:4528–38.
88. Fornari F, Gramantieri L, Ferracin M, Veronese A, Sabbioni S, Calin GA, Grazi GL, Giovannini C, Croce CM, Bolondi L, Negrini M. MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. Oncogene. 2008;27:5651–61.
89. 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.
90. Galardi S, Mercatelli N, Giorda E, Massalini S, Frajese GV, Ciafre SA, Farace MG. miR-221 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines by targeting p27Kip1. J Biol Chem. 2007;282:23716–24.
91. Pu XX, Huang GL, Guo HQ, Guo CC, Li H, Ye S, Ling S, Jiang L, Tian Y, Lin TY. Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression. J Gastroenterol Hepatol. 2010; 25:1674–80.
92. Kawaguchi T, Komatsu S, Ichikawa D, Morimura R, Tsujiura M, Konishi H, Takeshita H, Nagata H, Arita T, Hirajima S, Shiozaki A, Ikoma H, Okamoto K, Ochiai T, Taniguchi H, Otsuji E. Clinical impact of circulating miR-221 in plasma of patients with pancreatic cancer. Br J Cancer. 2013;108:361–9.
93. Tili E, Croce CM, Michaille JJ. miR-155: on the crosstalk between inflammation and cancer. Int Rev Immunol. 2009;28:264–84.
94. Valeri N, Gasparini P, Fabbri M, Braconi C, Veronese A, Lovat F, Adair B, Vannini I, Fanini F, Bottoni A, Costinean S, Sandhu SK, Nuovo GJ, Alder H, Gafa R, Calore F, Ferracin M, Lanza G, Volinia S, Negrini M, McIlhatton MA, Amadori D, Fishel R, Croce CM. Modulation of mismatch repair and genomic stability by miR-155. Proc Natl Acad Sci U S A. 2010;107: 6982–7.

95. Gironella M, Seux M, Xie MJ, Cano C, Tomasini R, Gommeaux J, Garcia S, Nowak J, Yeung ML, Jeang KT, Chaix A, Fazli L, Motoo Y, Wang Q, Rocchi P, Russo A, Gleave M, Dagorn JC, Iovanna JL, Carrier A, Pebusque MJ, Dusetti NJ. Tumor protein 53-induced nuclear protein 1 expression is repressed by miR-155, and its restoration inhibits pancreatic tumor development. Proc Natl Acad Sci U S A. 2007;104:16170–5.
96. Locker GY, Hamilton S, Harris J, Jessup JM, Kemeny N, Macdonald JS, Somerfield MR, Hayes DF, Bast Jr RC, ASCO. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. J Clin Oncol. 2006;24:5313–27.
97. Rachagani S, Macha MA, Heimann N, Seshacharyulu P, Haridas D, Chugh S, Batra SK. Clinical implications of miRNAs in the pathogenesis, diagnosis and therapy of pancreatic cancer. Adv Drug Deliv Rev. 2015;81:16–33.
98. Hidalgo M. Pancreatic cancer. N Engl J Med. 2010;362:1605–17.
99. Ballehaninna UK, Chamberlain RS. The clinical utility of serum CA 19-9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: an evidence based appraisal. J Gastrointest Oncol. 2012;3:105–19.
100. Bauer AS, Keller A, Costello E, Greenhalf W, Bier M, Borries A, Beier M, Neoptolemos J, Buchler M, Werner J, Giese N, Hoheisel JD. Diagnosis of pancreatic ductal adenocarcinoma and chronic pancreatitis by measurement of microRNA abundance in blood and tissue. PLoS One. 2012;7, e34151.
101. Hausler SF, Keller A, Chandran PA, Ziegler K, Zipp K, Heuer S, Krockenberger M, Engel JB, Honig A, Scheffler M, Dietl J, Wischhusen J. Whole blood-derived miRNA profiles as potential new tools for ovarian cancer screening. Br J Cancer. 2010;103:693–700.
102. Patnaik SK, Yendamuri S, Kannisto E, Kucharczuk JC, Singhal S, Vachani A. MicroRNA expression profiles of whole blood in lung adenocarcinoma. PLoS One. 2012;7, e46045.
103. Chen X, Liang H, Zhang J, Zen K, Zhang CY. Secreted microRNAs: a new form of intercellular communication. Trends Cell Biol. 2012;22:125–32.
104. Cho WC. MicroRNAs: potential biomarkers for cancer diagnosis, prognosis and targets for therapy. Int J Biochem Cell Biol. 2010;42:1273–81.
105. Kleihues P, Cavanee W. World Health Organization classification of tumours: pathology and genetics: tumours of the nervous system. Lyon: IARC Press; 2000.
106. King AA, Debaun MR, Riccardi VM, Gutmann DH. Malignant peripheral nerve sheath tumors in neurofibromatosis 1. Am J Med Genet. 2000;93:388–92.
107. Evans DG, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. J Med Genet. 2002;39:311–4.
108. Zehou O, Fabre E, Zelek L, Sbidian E, Ortonne N, Banu E, Wolkenstein P, Valeyrie-Allanore L. Chemotherapy for the treatment of malignant peripheral nerve sheath tumors in neurofibromatosis 1: a 10-year institutional review. Orphanet J Rare Dis. 2013;8:127.
109. Weiss S, Goldblum J. Soft tissue tumors. St Louis: Elsevier, Mosby; 2008.
110. Weiss SW, Goldbum JR. Soft tissue tumors. St Louis: Mosby; 2001.
111. Cichowski K, Jacks T. NF1 tumor suppressor gene function: narrowing the GAP. Cell. 2001;104:593–604.
112. DeClue JE, Papageorge AG, Fletcher JA, Diehl SR, Ratner N, Vass WC, Lowy DR. Abnormal regulation of mammalian p21ras contributes to malignant tumor growth in von Recklinghausen (type 1) neurofibromatosis. Cell. 1992;69:265–73.
113. Skotheim RI, Kallioniemi A, Bjerkhagen B, Mertens F, Brekke HR, Monni O, Mousses S, Mandahl N, Soeter G, Nesland JM, Smeland S, Kallioniemi OP, Lothe RA. Topoisomerase-II alpha is upregulated in malignant peripheral nerve sheath tumors and associated with clinical outcome. J Clin Oncol. 2003;21:4586–91.
114. Ling BC, Wu J, Miller SJ, Monk KR, Shamekh R, Rizvi TA, Decourten-Myers G, Vogel KS, DeClue JE, Ratner N. Role for the epidermal growth factor receptor in neurofibromatosis-related peripheral nerve tumorigenesis. Cancer Cell. 2005;7:65–75.
115. Nielsen GP, Stemmer-Rachamimov AO, Ino Y, Moller MB, Rosenberg AE, Louis DN. Malignant transformation of neurofibromas in neurofibromatosis 1 is associated with CDKN2A/p16 inactivation. Am J Pathol. 1999;155:1879–84.

116. Menon AG, Anderson KM, Riccardi VM, Chung RY, Whaley JM, Yandell DW, Farmer GE, Freiman RN, Lee JK, Li FP, et al. Chromosome 17p deletions and p53 gene mutations associated with the formation of malignant neurofibrosarcomas in von Recklinghausen neurofibromatosis. Proc Natl Acad Sci U S A. 1990;87:5435–9.
117. Legius E, Dierick H, Wu R, Hall BK, Marynen P, Cassiman JJ, Glover TW. TP53 mutations are frequent in malignant NF1 tumors. Genes Chromosomes Cancer. 1994;10:250–5.
118. Yang FC, Ingram DA, Chen S, Zhu Y, Yuan J, Li X, Yang X, Knowles S, Horn W, Li Y, Zhang S, Yang Y, Vakili ST, Yu M, Burns D, Robertson K, Hutchins G, Parada LF, Clapp DW. Nf1-dependent tumors require a microenvironment containing Nf1+/− and c-kit-dependent bone marrow. Cell. 2008;135:437–48.
119. Watson MA, Perry A, Tihan T, Prayson RA, Guha A, Bridge J, Ferner R, Gutmann DH. Gene expression profiling reveals unique molecular subtypes of Neurofibromatosis Type I-associated and sporadic malignant peripheral nerve sheath tumors. Brain Pathol. 2004;14: 297–303.
120. Levy P, Vidaud D, Leroy K, Laurendeau I, Wechsler J, Bolasco G, Parfait B, Wolkenstein P, Vidaud M, Bieche I. Molecular profiling of malignant peripheral nerve sheath tumors associated with neurofibromatosis type 1, based on large-scale real-time RT-PCR. Mol Cancer. 2004;3:20.
121. Holtkamp N, Reuss DE, Atallah I, Kuban RJ, Hartmann C, Mautner VF, Frahm S, Friedrich RE, Algermissen B, Pham VA, Prietz S, Rosenbaum T, Estevez-Schwarz L, von Deimling A. Subclassification of nerve sheath tumors by gene expression profiling. Brain Pathol. 2004;14:258–64.
122. Miller SJ, Rangwala F, Williams J, Ackerman P, Kong S, Jegga AG, Kaiser S, Aronow BJ, Frahm S, Kluwe L, Mautner V, Upadhyaya M, Muir D, Wallace M, Hagen J, Quelle DE, Watson MA, Perry A, Gutmann DH, Ratner N. Large-scale molecular comparison of human schwann cells to malignant peripheral nerve sheath tumor cell lines and tissues. Cancer Res. 2006;66:2584–91.
123. Pasmant E, Masliah-Planchon J, Levy P, Laurendeau I, Ortonne N, Parfait B, Valeyrie-Allanore L, Leroy K, Wolkenstein P, Vidaud M, Vidaud D, Bieche I. Identification of genes potentially involved in the increased risk of malignancy in NF1-microdeleted patients. Mol Med. 2011;17:79–87.
124. Subramanian S, Thayanithy V, West RB, Lee CH, Beck AH, Zhu S, Downs-Kelly E, Montgomery K, Goldblum JR, Hogendoorn PC, Corless CL, Oliveira AM, Dry SM, Nielsen TO, Rubin BP, Fletcher JA, Fletcher CD, van de Rijn M. Genome-wide transcriptome analyses reveal p53 inactivation mediated loss of miR-34a expression in malignant peripheral nerve sheath tumours. J Pathol. 2010;220:58–70.
125. Lee YB, Bantounas I, Lee DY, Phylactou L, Caldwell MA, Uney JB. Twist-1 regulates the miR-199a/214 cluster during development. Nucleic Acids Res. 2009;37:123–8.
126. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell. 2004;117:927–39.
127. Chai G, Liu N, Ma J, Li H, Oblinger JL, Prahalad AK, Gong M, Chang LS, Wallace M, Muir D, Guha A, Phipps RJ, Hock JM, Yu X. MicroRNA-10b regulates tumorigenesis in neurofibromatosis type 1. Cancer Sci. 2010;101:1997–2004.
128. Itani S, Kunisada T, Morimoto Y, Yoshida A, Sasaki T, Ito S, Ouchida M, Sugihara S, Shimizu K, Ozaki T. MicroRNA-21 correlates with tumorigenesis in malignant peripheral nerve sheath tumor (MPNST) via programmed cell death protein 4 (PDCD4). J Cancer Res Clin Oncol. 2012;138(9):1501–9.
129. Masliah-Planchon J, Pasmant E, Luscan A, Laurendeau I, Ortonne N, Hivelin M, Varin J, Valeyrie-Allanore L, Dumaine V, Lantieri L, Leroy K, Parfait B, Wolkenstein P, Vidaud M, Vidaud D, Bieche I. MicroRNAome profiling in benign and malignant neurofibromatosis type 1-associated nerve sheath tumors: evidences of PTEN pathway alterations in early NF1 tumorigenesis. BMC Genomics. 2013;14:473.

130. Wang Z, Yin B, Wang B, Ma Z, Liu W, Lv G. MicroRNA-210 promotes proliferation and invasion of peripheral nerve sheath tumor cells targeting EFNA3. Oncol Res. 2013;21: 145–54.
131. Scher HI, Sawyers CL. Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis. J Clin Oncol. 2005;23:8253–61.
132. Catto JW, Alcaraz A, Bjartell AS, De Vere WR, Evans CP, Fussel S, Hamdy FC, Kallioniemi O, Mengual L, Schlomm T, Visakorpi T. MicroRNA in prostate, bladder, and kidney cancer: a systematic review. Eur Urol. 2011;59:671–81.
133. Bostwick DG, Qian J. High-grade prostatic intraepithelial neoplasia. Mod Pathol. 2004;17:360–79.
134. Epstein JI, Allsbrook Jr WC, Amin MB, Egevad LL, Committee IG. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Surg Pathol. 2005;29:1228–42.
135. Schaeffer EM, Marchionni L, Huang Z, Simons B, Blackman A, Yu W, Parmigiani G, Berman DM. Androgen-induced programs for prostate epithelial growth and invasion arise in embryogenesis and are reactivated in cancer. Oncogene. 2008;27:7180–91.
136. Tokar EJ, Diwan BA, Waalkes MP. Arsenic exposure transforms human epithelial stem/progenitor cells into a cancer stem-like phenotype. Environ Health Perspect. 2010;118:108–15.
137. Kojima C, Ramirez DC, Tokar EJ, Himeno S, Drobna Z, Styblo M, Mason RP, Waalkes MP. Requirement of arsenic biomethylation for oxidative DNA damage. J Natl Cancer Inst. 2009;101:1670–81.
138. Ngalame NN, Tokar EJ, Person RJ, Xu Y, Waalkes MP. Aberrant microRNA expression likely controls RAS oncogene activation during malignant transformation of human prostate epithelial and stem cells by arsenic. Toxicol Sci. 2014;138:268–77.
139. Clape C, Fritz V, Henriquet C, Apparailly F, Fernandez PL, Iborra F, Avances C, Villalba M, Culine S, Fajas L. miR-143 interferes with ERK5 signaling, and abrogates prostate cancer progression in mice. PLoS One. 2009;4:e7542.
140. Xu B, Niu X, Zhang X, Tao J, Wu D, Wang Z, Li P, Zhang W, Wu H, Feng N, Wang Z, Hua L, Wang X. miR-143 decreases prostate cancer cells proliferation and migration and enhances their sensitivity to docetaxel through suppression of KRAS. Mol Cell Biochem. 2011;350: 207–13.
141. Jackson BL, Grabowska A, Ratan HL. MicroRNA in prostate cancer: functional importance and potential as circulating biomarkers. BMC Cancer. 2014;14:930.
142. Amir S, Ma AH, Shi XB, Xue L, Kung HJ, Devere White RW. Oncomir miR-125b suppresses p14(ARF) to modulate p53-dependent and p53-independent apoptosis in prostate cancer. PLoS One. 2013;8, e61064.
143. Sylvestre Y, De Guire V, Querido E, Mukhopadhyay UK, Bourdeau V, Major F, Ferbeyre G, Chartrand P. An E2F/miR-20a autoregulatory feedback loop. J Biol Chem. 2007;282: 2135–43.
144. Pesta M, Klecka J, Kulda V, Topolcan O, Hora M, Eret V, Ludvikova M, Babjuk M, Novak K, Stolz J, Holubec L. Importance of miR-20a expression in prostate cancer tissue. Anticancer Res. 2010;30:3579–83.
145. 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.
146. Wang R, Wen H, Xu Y, Chen Q, Luo Y, Lin Y, Luo Y, Xu A. Circulating microRNAs as a novel class of diagnostic biomarkers in gastrointestinal tumors detection: a meta-analysis based on 42 articles. PLoS One. 2014;9, e113401.
147. Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, van der Meer AJ, Patick AK, Chen A, Zhou Y, Persson R, King BD, Kauppinen S, Levin AA, Hodges MR. Treatment of HCV infection by targeting microRNA. N Engl J Med. 2013;368:1685–94.

Chapter 2
microRNA and Chronic Lymphocytic Leukemia

Dana Elena Giza and George A. Calin

Abstract Expression profiling of microRNAs identified important differences in microRNA expression between CLL samples and normal CD5+ B-cells. Researchers have first discussed the dual role of miRNAs working as tumor suppressors (inhibiting malignant potential) or as oncogenes (activating malignant potential) in CLL pathogenesis. Understanding the roles of miRNAs in leukemic cells brings information on a new layer of gene regulation and also provides new markers for improved diagnosis and prognosis, as well as novel therapeutic options for CLL patients. Herein we will focus on the roles of miRNAs in CLL, highlighting what is already known about their function, proposing a novel model of CLL predisposition and progression, and describing the challenges for the near future.

Keywords CLL • miRNAs • ZAP-70 • IGHV • ATM mutation

Chronic Lymphocytic Leukemia Before miRNAs Revolution

Chronic lymphocytic leukemia (CLL) is a disease of mature B-cells and represents the most common leukemia in Western countries, with an age-adjusted incidence of 4.3/100,000 inhabitants in United States [1]. More than 15,000 newly diagnosed cases and almost 4,500 deaths occur every year worldwide [2]. The median age at diagnostic ranges from 67 to 72 years, with a predominant number of men being

D.E. Giza
Experimental Therapeutics Department, The University of Texas MD Anderson Cancer Center, Unit 1950, 1515 Holcombe Blvd, Houston, TX 77030-4009, USA

Department of Hematology, Fundeni Clinical Institute, Bucharest, Romania
e-mail: DEGiza@mdanderson.org

G.A. Calin (✉)
Experimental Therapeutics Department, The University of Texas MD Anderson Cancer Center, Unit 1950, 1515 Holcombe Blvd, Houston, TX 77030-4009, USA

The RNA Interference and Non-coding RNA Center, MD Anderson Cancer Center, Texas State University, Houston, TX 77030-4009, USA
e-mail: gecalin2003@yahoo.com

© Springer International Publishing Switzerland 2015

G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5_2

affected. Some of the patients progress very slowly towards more aggressive forms of the disease, while others require early therapeutic intervention. The pathological pattern of CLL is characterized by the clonal proliferation and accumulation of mature, CD5+ B-cells within blood, bone marrow, lymph nodes, and spleen [3].

Historically, it has been generally accepted that CLL results from the continuous accumulation in lymphatic tissues of mature lymphocytes that never undergo apoptosis [4]. Recently published studies have shown that although the majority of circulating lymphocytes in peripheral blood in CLL are resting, there are several proliferation centers in lymph nodes and in blood marrow that continuously replenish the clonal cell population [5]. It has been supposed that the CLL cells enter the proliferation centers and proliferate due to the stimulation of unknown antigen and microenvironment stimuli, thus nourishing the peripheral compartment and before becoming quiescent [6].

The survival of the CLL cells is attributed to genomic abnormalities that lead to deregulation of the cell cycle and on a permissive microenvironment composed of macrophages, T-cells or stromal follicular cells using chemokines, angiogenic factors and cytokines to promote activation of proliferative signaling pathways of transformed cells [7]. The 90 % of the leukemic cells are not dividing and are at the G0/G1 phase of the cell cycle [8]. Dysregulation of several signaling pathways, such as B-cell receptor, toll-like receptor, PI3K, nuclear factor κB, notch signaling pathway, Wnt/Fzd signaling pathway, and Hedgehog and Janus kinases/signal transducers and activators of transcription signaling pathway, were found to be responsible for the aberrant gene expression and the pro-survival effects of microenvironment. Therefore, CLL is the result of a combination of genetic (chromosomal abnormalities, gene mutations) as well as epigenetic (altered microRNA expression, DNA methylation) modifications.

CLL shows significant clinical heterogeneity among the incidence of individuals related to the time of progression, treatment response, time to next treatment, and overall survival. Several prognostic markers have been identified so far to facilitate the clinical management of CLL patients, such as the mutational status of the immunoglobulin heavy-chain variable-region gene (IGHV), the expression levels of the 70 kDa zeta-associated protein (ZAP-70) and CD38, and the presence of different chromosomal alterations [9]. The unmutated IGHV gene status and high expression (>20 %) of ZAP-70 or CD38 are usually associated with an aggressive course, whereas patients with mutated IGVH clones and low ZAP-70 or CD38 expression have an indolent course [10].

Genomic alterations in CLL are also important independent predictors of disease progression and survival; however, the molecular basis of these associations was largely unknown until recently [11]. Genomic aberrations are detected by fluorescence in situ hybridization (FISH) in over 80 % of CLL cases and include 13q, 11q, 17p, and 6q deletions, and trisomy 12. The incidence of these genetic abnormalities are ~50 % for deletion of 13q14, ~10 % for deletion of 11q23, ~15 % for trisomy 12, 7–10 % for deletion of 17p, and 2–5 % for deletion of 6q [12]. These cytogenetic abnormalities can be used to identify subsets of patients

with different clinical course, time to progression, and survival rates. Based on the genomic studies, three risk groups can be differentiated: (1) low-risk: patients with a normal karyotype or isolated 13q deletion; (2) intermediate-risk: subjects with del 11q deletion, trisomy 12 or 6q deletion; and (3) high-risk: patients with 17p deletion or a complex karyotype [13]. Approximately one-third of patients never require treatment; in another third the initial indolent phase is followed by progression of the disease, and the remaining third has aggressive disease at the onset and needs immediate treatment [14]. In addition, BCR stimulation activates different tyrosine kinases (BTK—Bruton tyrosine kinase, SyK—spleen tyrosine kinase, ZAP70, PI3K—phosphatidyl inositol 3 kinase) that stimulate malignant B-cell survival.

In 2002, a link between small noncoding RNAs, known as miRNAs, and cancer was made by the observation that in CLL, the most common genetic aberration 13q14 deletion, was associated with downregulation of miR-15a and miR-16-1, which reside in the minimally deleted region within 13q14.2 [8]. This region was found to be the most frequently deleted in CLL and with several other hematological malignancies like multiple myeloma, mantle cell lymphoma or solid cancers like prostate cancer. In a search for a tumor-suppressor gene located in or close to the deletion, somatic cell hybrids between mouse and CLL cells carrying 13q14 deletions and translocations showed that 13q14 tumor-suppressor gene lies within a 30 kb region between exons 2 and 5 of the DLEU2 gene. Within this 30 kb region a cluster of two miRNAs genes, miR-15a and miR-16-1 was found to be located very close to a translocation breakdown point. The analysis of the expression of miR-15/16 in CLL patients revealed that miR-15/16 cluster was deleted or the expression of both miR-15 and miR-16 were decreased in two-thirds of the total cases analyzed [15]. This observation led to many studies focused on the role of miRs in the pathogenesis of CLL. The pioneering work of Calin et al. on miR-15a and 16-1 brought new information about the complexity of aberrant miR expression and its possible relation with alterations in cancer-specific biological pathways. Although early studies suggested that in CLL, miR-15a/16-1-mediated control of BCL2 expression and survival, it took until 2010 to learn that in fact the function of these miRs in B-cell malignancies is exerted mainly by downregulation of genes controlling cell-cycle entry [16].

Moreover, the deletion of the long arm of chromosome 11, which is found in 10–20 % of the total cases of CLL, is associated with ATM mutation interfering with the process of DNA repair during p53-induced apoptosis [17]. Often located in this deleted regions are two members of miR-34 family, miR-34b, and miR-34c that proved to be transcriptionally regulated by TP53 [18]. Finally, the incidence of 17p deletion or/and TP53 mutations may vary according to stage disease, ranging from 2 to 3 % at the time of diagnosis and 20–30 % among patients who have relapsed/refractory disease [19].

The results of the studies published in the last decade suggest that CLL might be a genetic disease where the main alterations occur at the level of transcriptional/posttranscriptional regulations in malignant cells genome because of deregulations of this new class of gene, miRNAs.

What Are miRNAs?

The miRNAs are a large family of highly conserved noncoding genes thought to be involved in temporal and tissue-specific gene regulation [20]. miRNAs are an evolving class of gene products with generally unknown function and are usually excised from 70- to 80-nt stem-loop RNA precursor structures. Derived from transcripts transcribed by RNA polymerase II, microRNAs are made via a two-step processing mechanism from a primary transcript (pri-miRNA) through an intermediate 60–90 nucleotide stem-loop structure (pre-mRNA) to the final mature microRNA [21]. Dicer and Argonaute family members are required for the miRNA precursor processing reaction. Single-stranded miRNA binds specific messangerRNA (mRNA) through sequences that are significantly, although not by completely complementarity to the target mRNA, mainly on the 3′ UTR. The bounded mRNA will remain untranslated due to mechanisms not fully understood at the moment, resulting in reduced levels of corresponding protein, therefore microRNAs have the ability to regulate gene expression [22].

It was estimated that the human genome contains more than 1000 miRNAs and that miRNAs negatively regulate the expression of the majority of protein-coding genes (PCGs). miRNAs activate mRNA expression via AU-rich elements inside mRNA 3′ UTRs and initiate the translation of proteins whose expression they normally repress during cell proliferation. Although the relative contribution of each of the miRNAs mechanism of gene regulation is still unknown, it is believed that mRNA destabilization is the predominant mechanism of action to decrease the target levels. Moreover, it was shown that the miRNAs mechanisms of action suffer plasticity, in the sense that miRNAs can also activate translation of targeted mRNAs, switching between translation repression and activation in coordination with the cell cycle. Most of the studies investigating miRNAs-target interaction focus on the 3′ UTR of mRNAs. Lytle et al. brought a new perspective in the study of this interaction suggesting that miRNAs could actually associate with any position of target mRNAs, efficiently repressing them by binding to miRNA-binding site in 5′ UTR [23]. Other mechanisms of action for miRNAs include targeting gene promoters and also decoy activity that interferes with the function of regulatory proteins [24, 25]. Furthermore, miRNAs can also regulate gene expression at the transcriptional level by directly binding to DNA regulatory elements [26].

microRNAs are involved in a variety of cellular processes and expressed in a tissue-specific manner. In physiological conditions, microRNAs act as gene expression controllers by targeting the mRNA or inhibiting its translation. Recent reports revealed the following functions for miRNAs: regulating the hematopoietic B-cell lineage fate (miR-181), B-cell survival (miR-15a and miR-16), cell proliferation control (miR-125b and let7), brain patterning (miR-430), pancreatic cell insulin secretion (miR-375), and adipocyte development (miR-375) [27].

Deregulation of miRNA regulation in these processes can lead to an alteration of the expression level of many genes which can induce the development or promote the progression of tumors. First association between cancer and miRNAs has been demonstrated by Calin et al. in 2002, which triggered many further reports confirming the involvement of miRNAs in the pathogenesis of many diseases [15].

Most experimental approaches focus on identifying miRNA targets and investigating how the expression of each miRNA can control the expression of tens or hundreds of mRNA due to imperfect complementarity, while several miRNAs can control a single mRNA. As these small miRNA molecules regulate a significant part of the mammalian transcriptome, recent published studies suggest that they are organized in gene regulatory networks that are larger than previously believed [28]. An increasing effort has been made in investigating these complex gene regulatory networks and their role in normal and pathological conditions. Changes in miRNA's expressions occur in response to environmental stimuli and play an important role in regulating the immune response, inflammation primarily, by regulating the pathways associated with the nuclear factor kappa beta (NF-kB), the central mediator of inflammatory response [29]. These findings not only provided insights about miRNA-mediated inflammatory responses, but also inspired researchers to look at miRNAs as potential drug targets for fine-tuning the immune system. Aberrant miRNA expression should proportionally affect these critical processes leading to various pathological outcomes. A more comprehensive profiling of the miRNA expression will be able to identify the miRNA patterns that hold great prognostic values for a specific disease.

microRNA Signature in Chronic Lymphocytic Leukemia

The development of microarray containing oligonucleotides probes for precursors and mature miRNAs led to the identification of more than 200 miRNAs that revealed a distinct signature between CLL cells and normal CD 5+ B-cells, underlying the fact miRNAs play an important role in the pathogenesis of CLL (Table 2.1) [1].

In CLL, miRNA's expression levels are used mostly to discriminate the clinical forms of CLL and to follow disease's progression, with only recent interest in interrogating the molecular impact of miRNA's deregulation in CLL. A signature profile was reported, describing 13 microRNAs that can differentiate aggressive and indolent CLL [1]. Another report showed that a profile of 32 microRNAs is able to discriminate between cytogenetic subgroups [30]. Patients with high levels of miR-21 had a higher risk of death compared to patients with low expression levels [30]. Likewise, high expression of miR-155 was reported in the aggressive form of CLL [31]. miR-181b was found not only to distinguish between indolent and aggressive cohorts of patients but also to predict time to treatment, acting as a biomarker of the disease progression.

microRNAs can function in CLL as oncogenes, tumor-suppressor genes or can be used as markers for disease onset/progression. For example, in indolent CLL, 13q14 deletions targeting miR-15/16 initiate the disease, while in aggressive CLL miR-181 targets the critical TCL1 oncogene and can also be used as a progression marker. Here we discuss the foremost findings about the role of microRNAs in CLL pathogenesis and how this knowledge can be used to identify new approaches to treat CLL [32].

Table 2.1 Expression of miRNAs in CLL and their implication as diagnostic and prognostic markers

miRNA (genomic location)	Expression in CLL	Functional targets	Diagnostic and prognostic markers
Let-7 family (various location)	Downregulated	CCDN1, CDC25a, CDK6, HOXA9, IMP-1, MYC, RAS	Affects response to chemotherapy
miR-16-1/miR-15a cluster(13q14.3 intron)	Downregulated	BCL2, CARD10, CCND1, CDK6, CDC27, DMTF1, MCL1, NGN2, VEGF	Involved in de novo aggressive CLL
miR-21(17q23.1, 3′ UTR TMEM49)	Overexpressed	BLC2, MASPIN, PDCD4, PTEN, TPM1, MCL1	Involved in de novo DLBCL, affects chemotherapy response
miR-29 family (various locations)	Downregulated	DNMT3A, DNMT3B	Correlates with short intervals from diagnosis to therapy
miR-34 family (1p36.23 and 11q23.1)	Downregulated	BLC2, CCDN1, CCNE2, CDK4/6, DLL1, E23, Notch 1, MYCN, MET	Associated with impaired DNA damage response and fludarabine-refractory CLL
miR-143/145 cluster (intergenic, 5q32)	Downregulated	ERK5, HOXA9, PARP8	
miR-155 (21q21.3)		Uc.364, uc 160	Associated with poor prognosis
miR-181 family	Overexpressed	HOXA11, TLC1	Correlates with short interval from diagnosis to therapy

Table 2.1 is a reproduction after Calin et al. [35] focused only on the miRNA clinical correlation with CLL

miR-29 and miR-181 families were found to play a role in CLL pathogenesis by influencing TLC1 expression. TLC1 (T-cell leukemia lymphoma 1) functions as a promoter for PI3K-Akt(PKB) oncogenic pathway, activating Akt, driving its nuclear translocation, which leads to an increased proliferation, inhibition of apoptosis, and transformation [33]. Activation of TLC1 oncogene correlates with an aggressive course of the disease, miR-29a and miR-29b are located in the 7q23 region, a high expression of miR-29a in indolent B-CLL is inversely correlated with the expression of TLC1. In both indolent and aggressive CLL, the expression of miR-29 is high. From the miRNA signature of 13 miRNAs found to differentiate aggressive and indolent CLL [1], out of four miRNAs downregulated in aggressive CLL, three are the isoforms of miR-29 family (miR-29a-2, miR-29b-2, miR-29c). The upregulation of miR-29 in indolent CLL compared to normal B-cells suggests that miR-29 has an oncogenic function, contributing to the pathogenesis of CLL. Moreover, the difference of expression of miR-29 family can discriminate between CLL with good and bad prognosis [34].

miR-181 is differentially expressed in B-cells and TLC1 is mostly a B-cell-specific gene, suggesting that TLC1 might be a target of miR-181 not only in CLL

cells but also in normal B lymphocytes [35]. Inversely correlated expression of miR-181b and miR-29 and TLC1 was found in CLL cells, supporting the idea that the TLC1 expression in CLL is regulated by these two miRNAs.

As all miRNAs are transcribed by the RNA polymerase II, an important role can be attributed to transcription factors in regulating their expression. p53 tumor protein is a transcription factor with a crucial role in cell-cycle regulation, stress response, DNA damage and repair and antiproliferative process [36]. It is involved in antiapoptotic processes, regulating the expression of the genes as BCL-2, Bax, BID, PUMA, and Noxa. Recently, it was discovered that members of miR-34 family, miR-34b and miR-34c, are direct targets of p53 and components of the p53 network [37]. Binding sites for p53 were identified on miR-34b and miR-34c using computational analysis, 3 kb upstream from the coding sequence. Another member of miR-34 family, miR-34a, is induced by p53 by binding a p53 responsive element situated within miR-34a gene. The lack of miR-34a expression prevents p53-dependent apoptosis, when miR-34a is expressed ectopically (Fig. 2.1). miR-34a regulates the pro- to pre-B-cell transition and also appears to be expressed at the highest levels in pro-B-cells. miR-

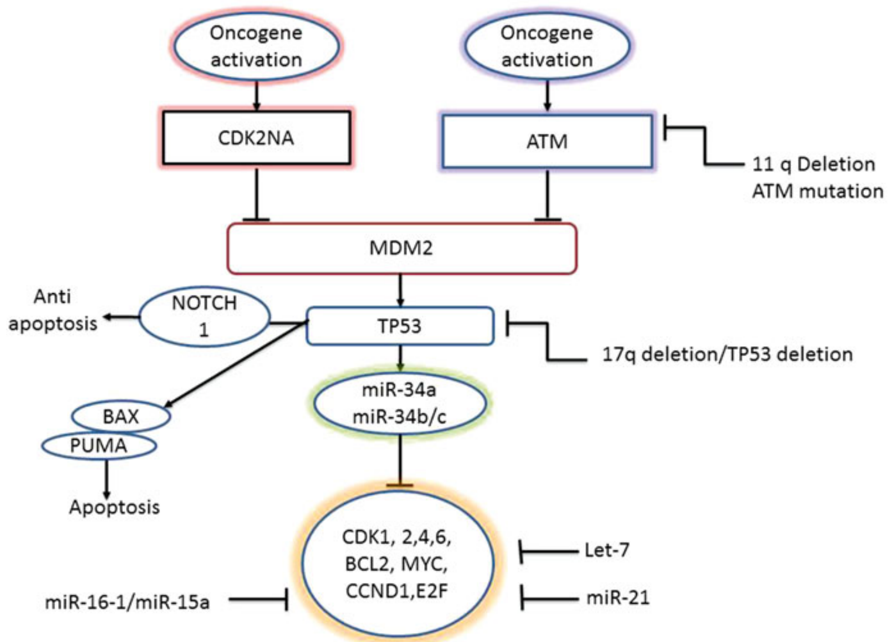

Fig. 2.1 The TP53-dependent miR-34 family and other aberrantly expressed miRNAs in chronic lymphocytic leukemia. DNA damage results in the activation of ATM which inactivates MDM2, and hence upregulating functional TP53, which results in transcription of TP53-dependent miRNAs genes including the miR-34 family. miR-34a and miR-34b/c may target and repress translation of genes including *cyclin-dependent kinases 1, 2, 4* or *6; BCL2; MYC; SIRT* along with other miRNAs found deregulated in CLL, inducing cycle arrest or apoptosis

34a knockdown results in increased amounts of mature B-cells. The key mediator of the miR-34a effect is the transcription factor FOXP1, which was found to be its direct target required for B-cell development [38].

A new pathogenic model in CLL that involves miRNAs (miR-15a/miR-16-1 and miR-34b/mi-R-34c) and protein-coding genes like TP53 and ZAP70, was recently proposed [39]. In this model TP53 gene is the link between miR-15a/miR-16-1 and miR-34b/miR-34c clusters. The loss of miR-15a/miR-16-1 expression together with 13q deletions leads to higher levels of antiapoptotic proteins like BCL-2 and myeloid cell leukemia sequence 1 (BCL-2 related)- MCL1, and also toward higher levels of the tumor-suppressor protein p53. Although the number of apoptotic cells may decrease because of the increased levels of antiapoptotic proteins, the p53 remains intact keeping the increased tumor growth relatively low. Increased levels of p53 in patients with 13q deletions is associated with miR-34b/miR-34c activation which leads to an increased level of ZAP70, a tyrosine kinase which plays a key role in T-cell receptor-mediated signal transduction.

Negrini et al. performed a comparison between CLL cells and the most normal "comparator" cells led to the identification of 106 deregulated miRNAs [40]. The authors found that miR-155 and miR-150 were upregulated while miR-181b, miR-222, and miR-92 were downregulated, in concordance with other published studies. miR-155 is implicated in lymphocyte development and is aberrantly upregulated in B-cell malignancies including Diffuse large B-cell Lymphoma (DLBCL), Follicular Lymphoma (FL), CLL, and mantle cell lymphoma (MCL). The expression of miR-155 in B-cells is directly activated by NFkB and MyB through its promoter and also promotes cell migration and lymphomagenesis by targeting HGAL, a lymphocyte motility inhibitor. miR-150 levels were found also to be low in different hematological malignancies like DLBCL, MCL, Burkitt lymphoma, and aggressive CLL, restoration of miR-150 levels leading to reduced cell proliferation. Similar to miR-155, miR-150 targets MYB gene, which was the only target validated in progenitor B-cells.

miR-155 is also upregulated in response to BCR ligation, and its upregulation may result in the enhanced expression of a large number or target genes, most notably the transcription factor PU.1, activation-induced cytidine deaminase (CID) and BCR-phosphatase SHIP-1 [41]. As is likely targeted by miR-155, which is expressed depending upon the relative expression of numerous target mRNA in any given cell type, CLL cells with low levels of the miR-155 tend to have higher expression of miR-155 and higher capacity for BCR signaling. In CLL, the miR-155-mediated regulation of SHIP-1 phosphatase is involved in counterbalancing (inhibiting) the BCR signaling capacity.

Several investigators looked at CLL cells and plasma from patients with CLL in order to establish if miR-155 can be used as biomarker in monitoring the response to therapy [42]. Plasma miR-155 was as effective a biomarker as miR-155 isolated from cells. Plasma is easier to work with for routine clinical biomarker screening than cellular material. Plasma has also been shown in a number of studies [43] to contain tiny microvesicles that package miRNA and other biomolecules. Although only a small number of samples was analyzed, the data published demonstrated that microvesicles isolated from the plasma of patients with CLL also contained miR-155. Having a

plasma-based biomarker to predict which patients will respond to therapy is the next important step in cancer therapeutics.

Other miRNAs have been reported to play a role in CLL: miR-193b, miR-33b*, and miR-196 were found downregulated whereas miR-23b, miR-26a, and miR-532 (5p and 3p) were upregulated [40]. The large number of deregulated miRNAs made the authors to doubt their involvement in CLL pathogenesis and speculate that the deregulation of these miRNAs might be related to Dicer dysfunction or to the fact that CLL cells are constitutively activated in vivo as demonstrated by the expression of surface activation markers.

miRNA's Mechanism of Action in CLL

The majority of CLL cells are nondividing and arrest at G0 to G1 phase of cell cycle, which made researchers to conclude that this is a result of decreased apoptosis and deregulation of cell-cycle control, rather than an increased proliferation rate. Hallmarks of malignant cells seem to be influenced by the altered expression of miRNAs in CLL-like evasion of apoptosis, self-sufficiency in growth and stimulation of angiogenesis, and dissemination as described below.

1. Evasion of apoptosis
   The malignant, nonproliferating B-cell in CLL has an overexpression of the anti-apoptotic protein BCL-2, which is responsible for maintaining an equilibrium between proliferation and apoptosis [44]. One of the mechanisms of BCL-2 gene activation is the translocation t(14,18)(q32;q21) which places the BCL-2 gene under the control of immunoglobulin heavy-chain enhancers, resulting in deregulated expression of the gene [45]. Investigators have shown that miR-15a and miR-16-1 are major direct negative regulators of the BCL2 antiapoptotic program leading to apoptotic peptidase activating factor1/caspase-9/poly(adenosine diphosphate-ribose) polymerase pathway activation [46]. MCL1 protein, which belongs to the Bcl2 family of antiapoptotic factors, is targeted by miR-29b, another miRNA whose expression is known to be downregulated in patients with CLL with a poor prognostic [47]. A novel mechanism by which miRNAs regulate the process of apoptosis is by repressing the itchy E3 ubiquitin protein ligase homolog (ITCH) and inducing the expression of the proapoptotic regulator p73 in CLL [48]. miR-106b activation by E2F1 and MYC after treatment of primary CLL cells with deacetylase inhibitors, led to downregulation of E3-ubiquitin ligase ITCH expression and reciprocal accumulation of the proapoptotic substrate, p73, which triggers mitochondrial dysfunction and processing of caspase-9 and apoptosis of CLL cells. In CLL, as in many other malignancies, miR-21 acts as an antiapoptotic protein by blocking the expression of critical apoptosis-related genes. Among the apoptotic targets of miR-21 are the tumor-suppressor PDCD4 and the tumor-suppressor PTEN, downregulation of which further promote the antiapoptotic survival signals through phosphoinositide 3-kinase/AKT pathway.

2. Self-sufficiency in growth
   One of the main differences between normal and cancer cells is the ability of the latter to proliferate without relying on the mitogens in the surrounding environment. RAS activation is the most commonly used way by tumor cells to escape the growth factor dependency and become oncogene dependent. Let-7i is involved in regulating posttranscriptionally RAS and its expression is downregulated in carcinomas, lymphomas, and leukemias [49]. In leukemias, RAS signaling is increased and in CLL it was shown to be linked to let7i loss of expression.

   Other important miRNAs found to be downregulated in many different solid tumors such as breast, colon, ovarian tumors and in also B-cell malignancies, including CLL, Burkitt lymphoma and Epstein-Barr virus transformed B-cell lymphomas, is miR-143 and miR 145 cluster located on chromosome 5 [50]. Downregulation of these miRNAs leads to an accelerated cell proliferation by deregulating the extracellular signal-related kinase 5 (ERK 5) because they target ERK5 mRNA. Moreover, miR-145 was found to have an antiproliferative effect on tumor cells by suppressing insulin receptor substrate expression 1 which is a docking protein for the insulin-like growth factor type-1 receptor and the insulin receptor, which sends mitogenic and antiapoptotic signals and is differentially expressed in B-cells and leukemia cells. miR-145 is involved in p53-dependent regulation of the oncogene c-Myc as its expression is directly induced at transcriptional level by p53 and directly repressed at the posttranscriptional level at c-Myc.
3. Hypoxia and angiogenesis
   The hypoxic environment created with the rapid growth of tumor cells stimulates hypoxia-inducible factor-dependent pathway and angiogenesis. Due to increased endogenous levels of VEGF mRNA found in CLL B the lymph nodes of the patients and bone marrow have a high degree of vascularization and high levels of VEGF were associated with advanced disease even in early stage cases. miRNAs modulated by HIF-1 transcriptional activation were found to be engaged in antiapoptotic programs and participating in the angiogenetic program [51]. Aberrantly expressed miRNAs in CLL target the von Hippel–Lindau gene product which is responsible for HIF-1α degradation and expressed at lower levels in CLL B than in normal cells. miR-19-2-1 is targets pVHL transcript and repress expression of it, participating in stabilization of HIF-1α, which, in turn can form a transcriptionally active complex at the VEGF promoter.

miRNAs in CLL Mouse Models

The discovery of miR-15a/16-1 deletion in CLL was followed closely by similar genetic manipulations in mice in order to confirm the findings. Klein and Dalla-Favera designed the first mouse model with conditional alleles that resemble the loss of the minimal deleted region (Mdr), which spans entirely the DLEU2 gene or

the miR-15a/16-1 cluster [52]. Mdr and miR-15a/16-1 cluster knockout strains at 1 year of age presented 50 % CD5+ B220+ B-cells among mononuclear cells in the peritoneum versus 15 % in control animals. Moreover, it was observed that Mdr Knockout (KO) animals which have shown a more aggressive disease lived less than WT siblings and further developed leukemias. One of the mechanisms leading to B-cell proliferation investigated has shown that miR-15a/16-1 KO B-cells begin DNA synthesis earlier than WT B-cells. The authors analyzed the levels of phosphorylated retinoblastoma (pRb) protein, an indicator of entry into the cell cycle in mitogen-stimulated B-cells isolated from miR-15a/16-1 KO or Mdr KO and WT animals and noticed that PRb was produced in both KO cells at earlier points than in WT B-cells. An impaired proliferation occurred in miR-15a/16-1 expressing cells, with a higher fraction of cells in G0/G1 phase, but not in those expressing DLEU2, therefore suggesting a possible control of the inhibition of G0/G1 phase transition by miR-15a/16-1.

The importance of miR-15a/16-1 cluster in CLL was confirmed by similar findings in New Zealand black (NZB) mice, which naturally develops CLL later in life with an autoimmune phenotype [53]. Linkage analysis has shown that the genomic region homologous to 13q14 is one of the loci associated with CLL development. After performing DNA sequencing, a point mutation in miR-15a/16-1 precursor which causes a decrease of miR-16-1 showed reduced expression of this miRNA. Exogenous delivery of miR-16-1 to a NZB malignant cell line led to the cell-cycle alterations such as a decrease in S phase cells and G1 arrest.

miR-15a and miR-16-1 expression is negatively regulating the expression BCL-2 in CLL; downregulation of these two miRNAs leads to an increase of Bcl-2 expression with consequent inhibition of apoptosis [46]. Since 13q14 deletion is associated with the indolent form of the disease, it is likely an upregulation of BCL-2 that plays a major role in this subset of CLLs. TRAF2 binds to TNF receptor family and mediates the activation of NF-kB by TNF proteins, which increases lymphocyte proliferation and survival [54]. Although TRAF2 transgenic mice showed an increased number of B-cells accompanied by lymphadenopathy and splenomegaly, transgenic TRAF2 mice failed to develop leukemia. BCL2 transgenic animals, designed with a construct mimicking t(14,18) translocation juxtaposing Bcl2 gene with the immunoglobulin heavy-chain locus at 14q32 as reported in human follicular lymphomas, failed to develop malignancies presenting only prolonged in vitro B-cell survival and in vivo polyclonal B-cell expansion [55]. TRAF2DN-BCL2 double transgenic mice displaced severe splenomegaly and most of the animals developed CLL-like disease with high B-cell blood count [56]. All these findings suggest that 13q14 deletions induce CLL development by a molecular mechanism similar to the one is TRAF2DN/BCL2 transgenic mice.

Santanam et al. designed a transgenic mouse characterized by overexpression of miR-29 in B-cells to study the role of miR 29 in B-cell leukemias. CD5+ CD19+ IgM+ B-cell population was increased in splenocytes from these transgenic mice [57]. 85 % of miR-29 animals showed a marked increase in the growth of B-cells between 12 and 14 months of age, representing 50 % of the total B-cells, which lead to the conclusion that miR-29 mice mimicked the indolent form of CLL. A gradual

increase of the percentage of leukemic cells was observed with aging, from 20 % of all B-cells in mice below 15 months of age to more than 65 % in mice above 20 months old. Moreover, immune response to SRBC antigen and serum levels of immunoglobulins were analyzed in miR-29 mice and wild-type littermates, and both of the parameters were decreased in transgenic mice, confirming miR-29 involvement in the indolent form of CLL disease [57].

miRNAs as Diagnostic and Prognostic Factors for CLL

The clinical course of CLL is variable, therefore several prognostic markers have been identified to guide the clinical management of CLL patients including the IGHV status, the expression of ZAP-70 or/and CD38 and the presence of different chromosomal alterations. The clinical outcome of CLL can be predicted based on the cellular and molecular markers, including the presence or the absence of somatic mutations at the IGHV locus or ZAP70 and CD38 expression on neoplastic cell surface [10]. A unique signature of 13 miRNAs with most frequently deregulated miRNAs in hematological malignancies and with prognostic implications was found to be correlated with ZAP-70 and IGHV mutational status [34]. Different studies confirmed that from this signature, members of the family miR-29a and miR-223 were downregulated in CLL and associated with prognostic factors of aggressive disease [58]. A quantitative method for the simultaneous determination of the expression levels of miR-223, miR-29c, ZAP-70, and lipoprotein lipase in sample by devising a polymerase chain reaction was developed.

Although Fabbri et al. showed that members of miR-29 family target DNA methyltransferase (DNMTs) and can reactivate silenced tumor-suppressor genes in CLL as well as in myelogenous leukemia, a new role for miR-29 in causing epigenetic changes in CLL cells needs to be further investigated [59].

CLL cases harboring mutated IGHV (M-CLL) generally present with an indolent disease; on the contrary, cases with unmutated IGHV (UM-CLL) more often have a progressive disease [60]. The presence of somatic IGHV hypermutation in M-CLL and the utilization of a stereotyped IGHV/IGHL gene repertoire supports the notion that CLL cells are antigen-experienced B-cells. However, the similarity of CLL cells with antigen-experienced B-cells reflects maybe only the maturation stage reached by CLL cells in the context of a leukemic stem cell hierarchical model [61].

The correlation between the IGHV status and clinical behavior reflects the differential responsiveness of CLL cells to the B-cell receptor (BCR) stimulation. In UM-CLL cases, IgM crosslinking induces the upregulation of the cell-cycle-related genes and an increased proliferation, while most M-CLL appear unresponsive to BCR stimulation. Several published studies have linked the most abundantly expressed miRNAs in CLL to the BCR signaling pathway. Mraz et al. showed that miR-150 levels were significantly lower in cases that used unmutated IGHV or that expressed ZAP-70 [62]. Using transcriptome analysis data, two genes with evolutionary conserved binding sides for miR-150 were identified, GAB1 and FOXP1.

Patient survival studies further indicated that there is an association between expression levels of miR-150, GAB1, FOXP1, and clinical outcome. GAB1 and FOXP1 are involved in essential signaling cascades in both normal and malignant B-cells. GAB1 is an adaptor molecule of phosphoinositide 3 kinase (PI3K), which can be activated by a variety of receptor tyrosine kinases and cytokine receptors. Although miR-150 expression correlates with ZAP-70 or IGHV status, low-level expressions of miR-150 have an independent prognostic value for both overall survival and treatment-free survival. The importance of BCR signaling in CLL is suggested by the significant clinical activity of inhibitors blocking BCR-associated kinase, specifically Bruton tyrosine kinase and PI3K. Therefore, the data published by Mraz underscores the fact that miR-150 expression and its target genes might influence the sensitivity of malignant B-cells to these inhibitors.

In Negrini's study, the high expression of miR-29c*, miR 532-3p, miR 146b-5p, miR139-3p, miR-222, and miR-29c was associated with a reduced risk of disease progression, whereas the opposite was true for miR-338-3p, miR-575-3p, and miR-16. High levels of miR 146b-5p appeared to have a protective effect from disease progression, while miR-155, miR- 338-3p, and miR-16 showed the opposite [40].

Moreover, microRNA signature can also be used to predict refractoriness to chemotherapeutic treatment in CLL [63]. A link between cytogenetically well-defined CLL samples and expression of miR-34a, a member of a miRNA family positively regulated by p53, was observed. Low expression of miR-34a was significantly associated with impaired DNA damage response, p53 mutations, and fludarabine-refractory CLL either with or without p53 mutations [64]. Moreover, an 829C>T polymorphism in the dihydrofolate reductase binding site for miR-24 led to loss of function and resulted in the dihydrofolate reductase overexpression and methotrexate resistance in cancer cells. To clarify whether microRNAs are directly involved in the development of fludarabine resistance, Ferrajoli et al. analyzed the expression of microRNAs before and after Fludarabine therapy in patients classified as responder or refractory and identified a microRNA signature able to distinguish between these two classes. Expression levels of several microRNAs were also able to predict fludarabine resistance in an independent test cohort. Among these microRNAs, miR-148a, miR-222, and miR-21 exhibited a significantly higher expression in nonresponders either before or after treatment. Recently, Zenz and colleagues found that fludarabine-refractory CLLs are frequently characterized by lower levels of miR-34a, and low expression of miR-34a was associated with fludarabine resistance even in the absence of p53 aberrations [64].

miRNAs and RNA-Inhibition Therapy in CLL

Different progression rates and need for therapy are characteristic for CLL, therefore patient-specific therapeutic drugs will be designed for CLL patients harboring abnormalities in miRNA expression. Knocking down the overexpression of miRNAs and inducing expression of silenced miRNAs in cancer may contribute to

selective tumor inhibition. Loss of expression in patients with CLL may selectively suppress proapoptotic pathways providing suck malignancies with a survival advantage. Chemotherapeutic drugs activating miR-106b may initiate a p53-independent mechanism that targets CLL cells, whereas restoration of miR-34a expression can overcome P53-dependent resistance to chemotherapy [65]. Differently from previously tested RNA-inhibition therapies such as the use of antisense oligonucleotides, ribozymes, or siRNAs that are working on a one-to-one basis with their targets, the microRNAs are targeting several members of pathways important for a disease. For example, the cluster miR-15a/miR-16-1 was shown to target not only BCL-2 but also MCL1, both important antiapoptotic oncogenes, as well as other significant cancer-related genes, such as Jun, MSH2, or WT1 (Wilms tumor 1).

Two strategies for inhibition of RNA expression to treat CLL are already in plan. “The sandwich RNA-inhibition strategy” is one of them and focuses on a major molecular alteration clearly linked with CLL pathogenesis via the use of multiple agents. Given recently published studies showing the relative efficacy of oblimersen sodium in treating relapsed or refractory CLL, designing regimens using a cocktail of anti-BCL-2 ASOs and miRNAs targeting BCL2, such as miR-15 and miR-16, for indolent CLL would be feasible. “The multiplex RNA-inhibition strategy” targets various molecular defects in the same pathway, for example, multiple synthetic miRNAs targeting the overexpressed apoptosis regulators BCL2 (miR-15 and miR-16) and MCL1 (the miR-29 family) have a better chance of consistently and robustly reducing these proteins’ expression levels than single-agent therapy. The use of miRNAs and/or their antisense inhibitors in cancer treatment has only recently been approached, and clinical trials of their use in this manner certainly will be further developed [35].

Conclusions

It is clear by now that miRNAs alterations are involved in the initiation and progression of CLL. A possible explanation for the more widespread differential expression of miRNA genes in malignant B-cells versus their normal counterpart can be explained by the locations of these genes in cancer-associated genomic regions, epigenetic mechanisms, and alterations in the miRNA processing machinery. miRNA expression profiling of human CLLs signatures can be associated with diagnosis, staging, progression, prognosis, and response to treatment. In addition, this profiling has been used to identify miRNA genes that may be downstream targets of activated oncogenic pathways or to target PCGs involved in cancer. Although they may lead to a new understanding of CLL in particular and cancer pathogenesis in general, explaining the involvement of miRNAs and ncRNAs in CLL and using this knowledge for the patient’s benefit are still challenging.

Acknowledgments Dr. Dana Elena Giza was supported in part by CNCS-UEFISCDI project number 22 from 28/08/2013 (PN-II-ID-PCE-2012-4-0018), and by the Romanian National Research Council (CNCS) Complex Exploratory Research Projects (Grant CEEX 187/2006). Dr. Calin is The Alan M. Gewirtz Leukemia & Lymphoma Society Scholar. Work in Dr. Calin’s

laboratory is supported in part by the NIH/NCI grants 1UH2TR00943-01 and 1 R01 CA182905-01, The UT MD Anderson Cancer Center SPORE in Melanoma grant from NCI (P50 CA093459), Aim at Melanoma Foundation and the Miriam and Jim Mulva research funds, the Brain SPORE (2P50CA127001), the Center for radiation Oncology Research Project, the Center for Cancer Epigenetics Pilot project, a 2014 Knowledge GAP MDACC grant, a CLL Moonshot pilot project, The UT MD Anderson Cancer Center Duncan Family Institute for Cancer Prevention and Risk Assessment, a SINF grant in colon cancer, the Laura and John Arnold Foundation, the RGK Foundation, and the Estate of C. G. Johnson, Jr. We would like to give special thanks to Dr. Mircea Ionescu for helping with text for editorial assistance.

References

1. Calin GA, Liu CG, Sevignani C, Ferracin M, Felli N, Dumitru CD, Shimizu M, Cimmino A, Zupo S, Dono M, Dell'Aquila ML, Alder H, Rassenti L, Kipps TJ, Bullrich F, Negrini M, Croce CM. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci U S A. 2004;101:11755–60.
2. Hallek M. Chronic lymphocytic leukemia: 2013 update on diagnosis, risk stratification and treatment. Am J Hematol. 2013;88:803–16.
3. Montserrat E, Rozman C. Chronic lymphocytic leukemia: present status. Ann Oncol. 1995; 6:219–35.
4. Bottoni A, Calin GA. MicroRNAs as main players in the pathogenesis of chronic lymphocytic leukemia. Microrna. 2014;2:158–64.
5. Ponzoni M, Doglioni C, Caligaris-Cappio F. Chronic lymphocytic leukemia: the pathologist's view of lymph node microenvironment. Semin Diagn Pathol. 2011;28:161–6.
6. Gaidano G, Foa R, Dalla-Favera R. Molecular pathogenesis of chronic lymphocytic leukemia. J Clin Invest. 2012;122:3432–8.
7. Hallek M. Signaling the end of chronic lymphocytic leukemia: new frontline treatment strategies. Hematology Am Soc Hematol Educ Program. 2013;2013:138–50.
8. Bullrich F, Fujii H, Calin G, Mabuchi H, Negrini M, Pekarsky Y, Rassenti L, Alder H, Reed JC, Keating MJ, Kipps TJ, Croce CM. Characterization of the 13q14 tumor suppressor locus in CLL: identification of ALT1, an alternative splice variant of the LEU2 gene. Cancer Res. 2001;61:6640–8.
9. Orchard JA, Ibbotson RE, Davis Z, Wiestner A, Rosenwald A, Thomas PW, Hamblin TJ, Staudt LM, Oscier DG. ZAP-70 expression and prognosis in chronic lymphocytic leukaemia. Lancet. 2004;363:105–11.
10. Chiorazzi N, Allen SL, Ferrarini M. Clinical and laboratory parameters that define clinically relevant B-CLL subgroups. Curr Top Microbiol Immunol. 2005;294:109–33.
11. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, Dohner K, Bentz M, Lichter P. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000;343:1910–6.
12. Stilgenbauer S, Bullinger L, Benner A, Wildenberger K, Bentz M, Dohner K, Ho AD, Lichter P, Dohner H. Incidence and clinical significance of 6q deletions in B cell chronic lymphocytic leukemia. Leukemia. 1999;13:1331–4.
13. Moreno C, Montserrat E. Genetic lesions in chronic lymphocytic leukemia: what's ready for prime time use? Haematologica. 2010;95:12–5.
14. Dighiero G, Binet JL. When and how to treat chronic lymphocytic leukemia. N Engl J Med. 2000;343:1799–801.
15. 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.

16. Palamarchuk A, Efanov A, Nazaryan N, Santanam U, Alder H, Rassenti L, Kipps T, Croce CM, Pekarsky Y. 13q14 deletions in CLL involve cooperating tumor suppressors. Blood. 2010; 115:3916–22.
17. Roos WP, Kaina B. DNA damage-induced cell death by apoptosis. Trends Mol Med. 2006; 12:440–50.
18. Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. Cell. 2012; 148:1172–87.
19. Austen B, Powell JE, Alvi A, Edwards I, Hooper L, Starczynski J, Taylor AM, Fegan C, Moss P, Stankovic T. Mutations in the ATM gene lead to impaired overall and treatment-free survival that is independent of IGVH mutation status in patients with B-CLL. Blood. 2005;106:3175–82.
20. Ambros V. MicroRNAs and developmental timing. Curr Opin Genet Dev. 2011;21:511–7.
21. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116: 281–97.
22. Ambros V, Lee RC, Lavanway A, Williams PT, Jewell D. MicroRNAs and other tiny endogenous RNAs in C. elegans. Curr Biol. 2003;13:807–18.
23. Lytle JR, Yario TA, Steitz JA. Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5′ UTR as in the 3′ UTR. Proc Natl Acad Sci U S A. 2007;104:9667–72.
24. Eiring AM, Harb JG, Neviani P, Garton C, Oaks JJ, Spizzo R, Liu S, Schwind S, Santhanam R, Hickey CJ, Becker H, Chandler JC, Andino R, Cortes J, Hokland P, Huettner CS, Bhatia R, Roy DC, Liebhaber SA, Caligiuri MA, Marcucci G, Garzon R, Croce CM, Calin GA, Perrotti D. miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts. Cell. 2010;140:652–65.
25. Place RF, Li LC, Pookot D, Noonan EJ, Dahiya R. MicroRNA-373 induces expression of genes with complementary promoter sequences. Proc Natl Acad Sci U S A. 2008;105:1608–13.
26. Kim DH, Saetrom P, Snove Jr O, Rossi JJ. MicroRNA-directed transcriptional gene silencing in mammalian cells. Proc Natl Acad Sci U S A. 2008;105:16230–5.
27. Harfe BD. MicroRNAs in vertebrate development. Curr Opin Genet Dev. 2005;15:410–5.
28. Tudor S, Giza DE, Lin HY, Fabris L, Yoshiaki K, D'Abundo L, Toale KM, Shimizu M, Ferracin M, Challagundla KB, Cortez MA, Fuentes-Mattei E, Tulbure D, Gonzalez C, Henderson J, Row M, Rice TW, Ivan C, Negrini M, Fabbri M, Morris JS, Yeung SC, Vasilescu C, Calin GA. Cellular and Kaposi's sarcoma-associated herpes virus microRNAs in sepsis and surgical trauma. Cell Death Dis. 2014;5, e1559.
29. Schulte LN, Westermann AJ, Vogel J. Differential activation and functional specialization of miR-146 and miR-155 in innate immune sensing. Nucleic Acids Res. 2013;41:542–53.
30. Rossi S, Shimizu M, Barbarotto E, Nicoloso MS, Dimitri F, Sampath D, Fabbri M, Lerner S, Barron LL, Rassenti LZ, Jiang L, Xiao L, Hu J, Secchiero P, Zauli G, Volinia S, Negrini M, Wierda W, Kipps TJ, Plunkett W, Coombes KR, Abruzzo LV, Keating MJ, Calin GA. microRNA fingerprinting of CLL patients with chromosome 17p deletion identify a miR-21 score that stratifies early survival. Blood. 2010;116:945–52.
31. Calin GA, Pekarsky Y, Croce CM. The role of microRNA and other non-coding RNA in the pathogenesis of chronic lymphocytic leukemia. Best Pract Res Clin Haematol. 2007;20:425–37.
32. Zhu DX, Zhu W, Fang C, Fan L, Zou ZJ, Wang YH, Liu P, Hong M, Miao KR, Liu P, Xu W, Li JY. miR-181a/b significantly enhances drug sensitivity in chronic lymphocytic leukemia cells via targeting multiple anti-apoptosis genes. Carcinogenesis. 2012;33:1294–301.
33. Pekarsky Y, Santanam U, Cimmino A, Palamarchuk A, Efanov A, Maximov V, Volinia S, Alder H, Liu CG, Rassenti L, Calin GA, Hagan JP, Kipps T, Croce CM. Tcl1 expression in chronic lymphocytic leukemia is regulated by miR-29 and miR-181. Cancer Res. 2006;66:11590–3.
34. 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 associ-

ated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med. 2005;353:1793–801.
35. Calin GA, Croce CM. Chronic lymphocytic leukemia: interplay between noncoding RNAs and protein-coding genes. Blood. 2009;114:4761–70.
36. Dohner H, Fischer K, Bentz M, Hansen K, Benner A, Cabot G, Diehl D, Schlenk R, Coy J, Stilgenbauer S, et al. p53 gene deletion predicts for poor survival and non-response to therapy with purine analogs in chronic B-cell leukemias. Blood. 1995;85:1580–9.
37. Rokavec M, Li H, Jiang L, Hermeking H. The p53/miR-34 axis in development and disease. J Mol Cell Biol. 2014;6:214–30.
38. Rao DS, O'Connell RM, Chaudhuri AA, Garcia-Flores Y, Geiger TL, Baltimore D. MicroRNA-34a perturbs B lymphocyte development by repressing the forkhead box transcription factor Foxp1. Immunity. 2010;33:48–59.
39. Fabbri M, Bottoni A, Shimizu M, Spizzo R, Nicoloso MS, Rossi S, Barbarotto E, Cimmino A, Adair B, Wojcik SE, Valeri N, Calore F, Sampath D, Fanini F, Vannini I, Musuraca G, Dell'Aquila M, Alder H, Davuluri RV, Rassenti LZ, Negrini M, Nakamura T, Amadori D, Kay NE, Rai KR, Keating MJ, Kipps TJ, Calin GA, Croce CM. Association of a microRNA/TP53 feedback circuitry with pathogenesis and outcome of B-cell chronic lymphocytic leukemia. JAMA. 2011;305:59–67.
40. Negrini M, Cutrona G, Bassi C, Fabris S, Zagatti B, Colombo M, Ferracin M, D'Abundo L, Saccenti E, Matis S, Lionetti M, Agnelli L, Gentile M, Recchia AG, Bossio S, Reverberi D, Rigolin G, Calin GA, Sabbioni S, Russo G, Tassone P, Morabito F, Ferrarini M, Neri A. microRNAome expression in chronic lymphocytic leukemia: comparison with normal B-cell subsets and correlations with prognostic and clinical parameters. Clin Cancer Res. 2014;20:4141–53.
41. Cui B, Chen L, Zhang S, Mraz M, Fecteau JF, Yu J, Ghia EM, Zhang L, Bao L, Rassenti LZ, Messer K, Calin GA, Croce CM, Kipps TJ. MicroRNA-155 influences B-cell receptor signaling and associates with aggressive disease in chronic lymphocytic leukemia. Blood. 2014;124:546–54.
42. Ferrajoli A, Shanafelt TD, Ivan C, Shimizu M, Rabe KG, Nouraee N, Ikuo M, Ghosh AK, Lerner S, Rassenti LZ, Xiao L, Hu J, Reuben JM, Calin S, You MJ, Manning JT, Wierda WG, Estrov Z, O'Brien S, Kipps TJ, Keating MJ, Kay NE, Calin GA. Prognostic value of miR-155 in individuals with monoclonal B-cell lymphocytosis and patients with B chronic lymphocytic leukemia. Blood. 2013;122:1891–9.
43. 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.
44. Kitada S, Andersen J, Akar S, Zapata JM, Takayama S, Krajewski S, Wang HG, Zhang X, Bullrich F, Croce CM, Rai K, Hines J, Reed JC. Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: correlations with In vitro and In vivo chemoresponses. Blood. 1998;91:3379–89.
45. Tsujimoto Y, Louie E, Bashir MM, Croce CM. The reciprocal partners of both the t(14; 18) and the t(11; 14) translocations involved in B-cell neoplasms are rearranged by the same mechanism. Oncogene. 1988;2:347–51.
46. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A. 2005;102:13944–9.
47. Calin GA, Cimmino A, Fabbri M, Ferracin M, Wojcik SE, Shimizu M, Taccioli C, Zanesi N, Garzon R, Aqeilan RI, Alder H, Volinia S, Rassenti L, Liu X, Liu CG, Kipps TJ, Negrini M, Croce CM. MiR-15a and miR-16-1 cluster functions in human leukemia. Proc Natl Acad Sci U S A. 2008;105:5166–71.
48. Sampath D, Calin GA, Puduvalli VK, Gopisetty G, Taccioli C, Liu CG, Ewald B, Liu C, Keating MJ, Plunkett W. Specific activation of microRNA106b enables the p73 apoptotic response in chronic lymphocytic leukemia by targeting the ubiquitin ligase Itch for degradation. Blood. 2009;113:3744–53.
49. Roush S, Slack FJ. The let-7 family of microRNAs. Trends Cell Biol. 2008;18:505–16.

50. Akao Y, Nakagawa Y, Hirata I, Iio A, Itoh T, Kojima K, Nakashima R, Kitade Y, Naoe T. Role of anti-oncomirs miR-143 and -145 in human colorectal tumors. Cancer Gene Ther. 2010;17:398–408.
51. Ghosh AK, Shanafelt TD, Cimmino A, Taccioli C, Volinia S, Liu CG, Calin GA, Croce CM, Chan DA, Giaccia AJ, Secreto C, Wellik LE, Lee YK, Mukhopadhyay D, Kay NE. Aberrant regulation of pVHL levels by microRNA promotes the HIF/VEGF axis in CLL B cells. Blood. 2009;113:5568–74.
52. Klein U, Lia M, Crespo M, Siegel R, Shen Q, Mo T, Ambesi-Impiombato A, Califano A, Migliazza A, Bhagat G, Dalla-Favera R. The DLEU2/miR-15a/16-1 cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. Cancer Cell. 2010;17:28–40.
53. Raveche ES, Salerno E, Scaglione BJ, Manohar V, Abbasi F, Lin YC, Fredrickson T, Landgraf P, Ramachandra S, Huppi K, Toro JR, Zenger VE, Metcalf RA, Marti GE. Abnormal microRNA-16 locus with synteny to human 13q14 linked to CLL in NZB mice. Blood. 2007;109:5079–86.
54. Haiat S, Billard C, Quiney C, Ajchenbaum-Cymbalista F, Kolb JP. Role of BAFF and APRIL in human B-cell chronic lymphocytic leukaemia. Immunology. 2006;118:281–92.
55. Balatti V, Pekarky Y, Rizzotto L, Croce CM. miR deregulation in CLL. Adv Exp Med Biol. 2013;792:309–25.
56. Zapata JM, Krajewska M, Morse 3rd HC, Choi Y, Reed JC. TNF receptor-associated factor (TRAF) domain and Bcl-2 cooperate to induce small B cell lymphoma/chronic lymphocytic leukemia in transgenic mice. Proc Natl Acad Sci U S A. 2004;101:16600–5.
57. Santanam U, Zanesi N, Efanov A, Costinean S, Palamarchuk A, Hagan JP, Volinia S, Alder H, Rassenti L, Kipps T, Croce CM, Pekarsky Y. Chronic lymphocytic leukemia modeled in mouse by targeted miR-29 expression. Proc Natl Acad Sci U S A. 2010;107:12210–5.
58. Stamatopoulos B, Meuleman N, Haibe-Kains B, Saussoy P, Van Den Neste E, Michaux L, Heimann P, Martiat P, Bron D, Lagneaux L. microRNA-29c and microRNA-223 down-regulation has in vivo significance in chronic lymphocytic leukemia and improves disease risk stratification. Blood. 2009;113:5237–45.
59. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, Liu S, Alder H, Costinean S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chan KK, Marcucci G, Calin GA, Huebner K, Croce CM. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci U S A. 2007;104:15805–10.
60. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, Buchbinder A, Budman D, Dittmar K, Kolitz J, Lichtman SM, Schulman P, Vinciguerra VP, Rai KR, Ferrarini M, Chiorazzi N. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999;94:1840–7.
61. Kikushige Y, Ishikawa F, Miyamoto T, Shima T, Urata S, Yoshimoto G, Mori Y, Iino T, Yamauchi T, Eto T, Niiro H, Iwasaki H, Takenaka K, Akashi K. Self-renewing hematopoietic stem cell is the primary target in pathogenesis of human chronic lymphocytic leukemia. Cancer Cell. 2011;20:246–59.
62. Mraz M, Chen L, Rassenti LZ, Ghia EM, Li H, Jepsen K, Smith EN, Messer K, Frazer KA, Kipps TJ. miR-150 influences B-cell receptor signaling in chronic lymphocytic leukemia by regulating expression of GAB1 and FOXP1. Blood. 2014;124:84–95.
63. Ferracin M, Zagatti B, Rizzotto L, Cavazzini F, Veronese A, Ciccone M, Saccenti E, Lupini L, Grilli A, De Angeli C, Negrini M, Cuneo A. MicroRNAs involvement in fludarabine refractory chronic lymphocytic leukemia. Mol Cancer. 2010;9:123.
64. Zenz T, Mohr J, Eldering E, Kater AP, Buhler A, Kienle D, Winkler D, Durig J, van Oers MH, Mertens D, Dohner H, Stilgenbauer S. miR-34a as part of the resistance network in chronic lymphocytic leukemia. Blood. 2009;113:3801–8.
65. Calin GA, Croce CM. Genomics of chronic lymphocytic leukemia microRNAs as new players with clinical significance. Semin Oncol. 2006;33:167–73.

Chapter 3
microRNA in Malignant Lymphoma

Hiroyuki Tagawa

Abstract microRNAs (miRNAs) are noncoding regulatory RNAs usually consisting of 20–24 nucleotides. During the past decade, increases and decreases in miRNA expression have been shown to associate with various types of diseases, including cancer. Over 4500 miRNAs have been identified in humans, and it is known that nearly all human protein-encoding genes can be controlled by miRNAs in both healthy and malignant cells. Detailed genome-wide miRNA expression analysis has been performed in various malignant lymphoma subtypes, and these analyses have led to the discovery of subtype-specific miRNA alterations. In this chapter, I describe several key miRNAs and their targets in distinct malignant lymphoma subsets and their roles in their pathogenesis, studies of which will lead new therapeutic strategies against aggressive lymphomas.

Keywords miRNAs • Noncoding RNAs • Malignant lymphoma • Burkitt's lymphoma • Mantle cell lymphoma • NK/T-cell lymphoma

Introduction

Malignant lymphoma is classified into Hodgkin's or non-Hodgkin's lymphoma. On the basis of normal correspondence lymphoid cells, non-Hodgkin's lymphoma is further classified into B- and T/NK-cell lymphomas. These can also be divided into a number of subtypes; for instance, B-cell lymphoma can be classified into diffuse large B-cell lymphoma (DLBCL), Burkitt's lymphoma (BL), mantle cell lymphoma (MCL), follicular lymphoma, MALT (mucosa-associated lymphoid tissue) lymphoma, and other subtypes [1]. These subtypes are further classified into a few or several subtypes. For instance, DLBCL can be classified into two subtypes such as activated B-cell (ABC) type and germinal B-cell (GCB) types; MALT lymphoma is

H. Tagawa, M.D. (✉)
Department of Hematology, Nephrology, and Rheumatology, Akita University Graduate School of Medicine, Akita 0108543, Japan
e-mail: htagawa0279jp@yahoo.co.jp

© Springer International Publishing Switzerland 2015

G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5_3

also classified into nodal and extranodal type [1]. These classifications are usually made on the basis of subtype-specific translocation, origin of their normal parts or difference of genetic signature and clinical course. In addition to these classifications, subtype-based miRNA classification may be useful in malignant lymphomas because miRNA alteration is frequently occurred in malignant lymphoma.

Abnormal miRNA expression is known to occur in many cancers [2, 3], and the first two miRNA aberrations known resulted from altered expression of *DLEU2* and *C13orf25* in hematological malignancies. *DLEU2*, which encodes miR-15a and miR-16-1, was discovered from 13q14 deletion in chronic lymphocytic leukemia (CLL) [4, 5]. *C13orf25*, which encodes six mature miRNAs (miR-17, miR-18, miR-19a, miR-19b, miR-20a, and miR-92a), was identified from 13q31 amplification in aggressive B-cell lymphomas [6–8]. These miRNAs were downregulated or upregulated in accordance with genomic deletion or amplification, which suggests they contribute to tumorigenesis through altered regulation of target oncogenes or tumor suppressors.

miRNA dysregulation and the role in the pathogenesis malignant lymphomas and other cancers is being actively studied, worldwide. However, the available information remains incomplete and complex, sometimes making it difficult to understand the precise function of miRNA in carcinogenesis. In this chapter, I have summarized the actions of miRNAs in distinct subtypes of malignant lymphomas, focusing in particular on their involvement in disease progression and transformation.

Discovery of miRNA Dysregulations in Malignant Lymphomas

13q31-32 genomic amplification is a well-known genomic alteration in DLBCL that was recognized well before its response gene was identified. This is because there was no protein-coding gene in that region. In 2004, however, Seto's group carefully examined expression of about 60 expressed sequence tags (ESTs) and found that EST, BCO40320, was strongly expressed in accordance with 13q31 amplification in DLBCL. They then identified a novel gene, *C13orf25*, from the EST [6]. *C13orf25* contains the miR-17-92 polycistron, encoding six miRNAs (miR-17-5p, miR-18, miR-19a, miR-19b, miR-20a, and miR-92-1), which can be divided into the miR-19 and miR-17 families. Subsequent investigation revealed that the miR-17-92 is overexpressed in aggressive B-cell lymphomas with genomic amplification of 13q31 [6, 7]. Based on that report, Hannon's group investigated the polycistron's potential to act as an oncogene. They first transduced miR-17-19b into stem cells from the fetal liver of Eu-Myc mice, and then inoculated the transduced cells into irradiated Eu-Myc mice. They found that the death rate among mice inoculated with miR-17-19b was higher than among control mice due to the induction of B-cell leukemia. They also showed that miR-17-92 could induce tumor formation by acting in concert with Myc [8]. In 2008, two groups respectively demonstrated miR-17-92 target gene(s) involved in normal B-cell development.

Using miR-17-92 knockout mice, Jacks' lab showed that deletion of miR-17-92 could induce upregulation of the pro-apoptotic protein Bim with inhibition of differentiation from pro- to pre-B-cell transition [9, 10]. In addition, Rajewsky and coworkers established miR-17-92 transgenic mice [10], which developed lymphproliferative disease because the miR-17-92 reduced expression of Bim protein; however, they did not develop lymphomas. Nonetheless, these reports suggest the pro-apoptotic protein Bim is a likely target of miR-17-92 during B-cell lymphomagenesis.

The findings summarized above strongly suggest that by enhancing anti-apoptotic capability in B-cell lymphomas, downregulation of Bim by miR-17-92 contributes to lymphomagenesis.

Tagawa's group found that not only Bim but also p21 is a possible target of miR-17-92 in B-cell lymphoma cells [11]. Then in 2009, two groups (He's and Ventura's group) demonstrated that miR-19 could regulate the tumor suppressor Pten, thereby enhancing anti-apoptotic potential via upregulation of the AKT/mTOR pathway [12, 13]. Collectively, these reports suggest that miR-17-92 regulates several targets in different B-cell lymphoma subtypes (miR-17 family: Bim and p21, miR-19 family: Pten), and that the upregulation of miR-17-92 is an additional genetic event that enhances the tumorigenicity of the original cancer.

Abnormal downregulation of miRNA is now known to occur in many cancers, but it was first reported in CLL. In 2001, Dalla-Favera's group found reduced expression of the gene *DLEU2* through a detailed search of the minimal deleted region of 13q14 [2]. In 2002, Croce's group reported reduced expression of miR-15a and miR-16-1 from the common loss region of 13q14 in CLL [2]. They also showed that miR-15a and 16-1 were present in the noncoding region of *DLEU2*. miR-16 family has been known to possess tumor-suppressive function in various cancer by inhibiting apoptosis but also var cell-cycle related genes and their products [14–26].

Differences in miRNA Expression Can Define Lymphoma Subtype

miRNA expression is different in lymphoma subtypes. As following we discuss about (1) DLBCL, (2) anaplastic large cell lymphoma (ALCL) and (3) NK/T-cell lymphomas. Since the discovery of the upregulated expression of miR-17-92 in malignant lymphomas, the genomes of various subtypes of malignant lymphomas have been screened for miRNA expression. miRNA may even be differentially expressed within a single tumor entity, such as DLBCL and ALCL, and NK/T-cell lymphomas.

1. DLBCL can be divided into two distinct subtypes: the ABC and the GCB types [27]. Lenz and coworkers reported that 13q31 amplification (*C13orf25*) frequently occurs in GCB but not in ABC type [28]. They further showed that

DLBCLs overexpressing miR-17-92 also express *MYC* and their target genes at significantly higher levels than those without this abnormality. Interestingly, 10q23 (*PTEN*) deletion is frequently detected in GCB DLBCL without 13q amplification. Both 13q31 amplification and 10q23 deletion could downregulate Pten, suggesting altered AKT-mTOR signaling may be important in the pathogenesis of GCB DLBCL. Malumbres and coworkers (2009) recently provided evidence that germinal center-enriched miR-125b downregulates expression of *IRF4* and *PRDM1*, and memory B-cell-enriched miR-223 downregulates expression of LMO2 [29]. These reports also suggest miRNAs play crucial roles in different DLBCL subtypes. Furthermore, miR-181a and miR-222 have been shown to predict overall survival and progression-free survival in rituximab cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP)-treated DLBCL patients [30, 31].

2. In ALK^+ALCL, miRNA dysregulation is well documented. Merkel and coworkers [32] compared miRNA expression differences between ALK^+ and ALK^- subgroups and found several candidate miRNAs likely to be associated with tumorigenesis [32]. They found that an oncogenic miRNA, miR-155, was frequently observed in ALK^- ALCL. Moreover, they showed that the tumor-suppressive miRNA, miR-101, was commonly downregulated in both ALK^+ and ALK^- ALCL when compared with normal $CD3^+$ cells. However, they reported that enforced expression of miR-101 led to inhibition of cell proliferation only in ALK^+ type. ALK^+ and ALK^- subtypes can be distinguished based on their distinct miR-17-92 polycistron profiles; miR-17-92 polycistron was more strongly expressed in the ALK^+ ALCL [33, 34]. Activation of the transcription factor STAT3 in ALK^+ ALCL is crucial and essential in the pathogenesis, as STAT3 activates or deactivates transcription and expression of various miRNA genes [35]. STAT3, which is activated by NPM-ALK, epigenetically dysregulates miRNAs, which then contribute to cancer development via alteration of downstream target genes. Recently Miyazono's group (2011) reported the role of miR-135b, which is aberrantly overexpressed in ALK^+ ALCL [36]. NPM-ALK activates miR-135b through STAT3 signaling; miR-135b then directly downregulates forkhead box protein O1 (FOXO1), leading to enhancement of antitumor agent resistance. They found that miR-135b contributes to the T_H17 phenotype of ALK^+ ALCL, as miR-135b inhibits T_H2 differentiation-related transcription factors, such as STAT6 and GATA3, and upregulates IL-17A and IL-17 F expression in ALK^+ ALCL. They further showed that cytokines produced by T_H17 cells contribute to cancer progression via induction of angiogenesis.
3. NK/T-cell lymphoma/leukemia can be classified into leukemic and extra nodal (ENKL) types [1, 37]. Tagawa's group showed that upregulation of miR-21 or miR-155 and downregulation of miR-150 occur frequently in NK/T-cell leukemia/lymphoma [38, 39]. We found that miR-21 is upregulated in NK-cell leukemia, and miR-155 in ENKL type. These miRNAs respectively regulate protein tyrosine phosphatases, Pten or Ship1, whose downregulation commonly activates AKT signaling. Downregulation of miR-150 also contributes to inhibit cellular senescence [38].

miRNA Is Additional Genetic Alteration and the Dysregulation Contributes to Their Aggressive Clinical Behavior

Functional analyses of miRNA have been conducted with aggressive lymphomas, including BL, MCL, and NK/T-cell lymphoma. BL is characterized by the dysregulated expression of *MYC* as a consequence of translocations involving the *MYC* (8q24) and immunoglobulin genes. It has also been shown that AID is required for the *MYC* translocation and development of BL [40]. miR-155 expression is reduced in BL, and recent work by Dorsett and coworkers demonstrated that miR-155 suppresses AID-mediated MYC-IGH translocation [41]. This suggests that downregulation of miR-155 in germinal center lymphoid tissue is a deeply associated first hit event in BL. Further, several epidemiologic subtypes of BL (endemic, sporadic, and HIV-associated) share a homogeneous microRNA profile, distinct from that of DLBCL [42], which confirms the potential relevance of this signature in the diagnosis of BL.

MCL is characterized by t(11;14)(q13;q32), which results in overexpression of *CCND1*/CyclinD1, and is presumed to derive from naive pre-germinal center CD5+ B-cells [1]. Underlying MCL is a larger number of genetic alterations than is seen in other lymphoma subtypes, and a lot of miRNA aberrations and miRNA dysregulation have also been identified in MCL [43–45]. Essential is dysregulation of miR-29, miR-15a/16-1, miR-26, and miR-17-92. miR-17-92 is frequently upregulated in MCL, and since miR-17-92 appears to negatively regulate *CDKN1A*/p21 [11], increases in its expression could enhance cell-cycle progression. miR-16-1 is expressed normally in MCL, as compared to its expression in normal CD5+ B-cells, [46] but Chen and coworkers demonstrated that the 3′UTR of *CCND1* is frequently truncated or mutated in MCL, which inhibits the interaction of miR-16-1 with the "seed" sequence of the 3′UTR of *CCND1*, thereby contributing to continuous upregulation of CyclinD1 [47]. In addition, some miRNAs have been shown to act as regulators of polycomb-group repressive complex (PRC) proteins. miR-16-1 can regulate BMI1 translation, which would enhance anti-apoptotic potential by negatively regulating pro-apoptotic genes such as *PMAIP1*/Nox*a* and *BCL2L11*/Bim [46].

In aggressive B-cell lymphomas such as BL, DLBCL, and MCL, overexpression of *MYC* is strongly associated with their aggressiveness. Recently Zhang and coworkers showed that MYC, HDAC3, and EZH2 form a PRC tethered to miR-29 promoter elements to epigenetically repress miR-29 transcription in MYC-expressing lymphoma cells. Downregulation of miR-29 induces upregulation of CDK6 (cell-cycle progression) and IGF-1R (anti-apoptosis). Furthermore, MYC can regulate transcription of miR-26a, whose downregulation leads to upregulation of EZH2. This in turn reduces miR-494 expression, leading to upregulation of MYC. This MYC-miR-26a-EZH2-miR-494 positive feedback loop is observed in aggressive MCL, especially in the cases with MYC upregulation [48].

Multistep genetic alterations are occurred during disease progression of cancer. Genetic alterations that inhibit cellular senescence can be the initial step for developing cancer, while metastasis or invasion is an additional tumorigenic capability during disease progression and likely occurred in advanced cases. Cutaneous T-cell lymphoma (CTCL) is the best model for understanding the multiple steps of genetic alterations in cancer development. It has been suggested that IL-22 and/or chemokine (C-C motif) ligand 20 (CCL20)/CCR6 interaction may play an important role in the relationship between CTCL and keratinocytes or dendritic cells. Recently, Tarawa's group showed that (1) CTCL cells can produce IL-22 (but not IL-17), and express the IL-22 receptor, whose expression is not found in normal lymphocytes, and that (2) IL-22 stimulation against IL-22RA1 may trigger CCL20 production via activation of JAK/STAT pathway, leading to the enhancement of CCL20/CCR6 interaction in advanced CTCL. This interaction may enhance the migration potential of advanced CTCL, resulting in multiple invasion and metastasis of CTCL cells into various visceral organs, following a nutrition-dependent concentration gradient. As CCR6 is strictly controlled by miR-150, downregulation of miR-150 in CTCL may contribute to the constitutive activation of the IL-22/CCL20/CCR6 autocrine pathway [49]. These findings suggest that inhibition of the autocrine pathway may be therapeutically useful in advanced CTCL. Antibody for CCL20 could represent novel molecular targeting therapies against advanced CTCL.

miRNA Dysregulation Contributes to the Transformation from Early to Advanced Lymphomas

miRNAs do not appear to be as important for the pathogenesis of low-grade B-cell lymphomas (e.g., follicular lymphoma (FL), marginal zone lymphoma (MZL) as for high-grade and transformed lymphomas (e.g., FL/MZL to DLBCL).

FL is characterized as a indolent B-cell lymphoma, with approximately 80 % of cases possessing t(14;18)(q32;q21). In FLs with the translocation, the miRNA profile showed upregulation of miR-20a/b and miR-194, which target *CDKN1A* and *SOCS2*, respectively, potentially contributing to tumor-cell proliferation and survival [50]. CD10-negative FL cases are usually t(14;18)-negative and/or morphologically high-grade (Grade 3a or b), and therefore high dose intensive chemotherapy (R-CHOP) is required [51]. t(14;18)-negative cases, have a distinct miRNA profile frequently characterized by downregulation of miR-16-1, miR-26a, miR-101, miR-29, and miR-138 [52]. Because these miRNAs are known to function as tumor-suppressive miRNA, their downregulation may associate with the pathogenesis of some FL subtypes and high-grade FL.

Expression of miRNA in marginal zone lymphomas (MZLs) has also been analyzed. The results suggest they are likely important in advanced stage or transformed cases of MZL and DLBCL. miRNA expression has been analyzed in MALT type, nodal type, and splenic type MZL. A study of gastric MALT type MZL revealed that high levels of miR-223 expression are a marker of MALT stratification

and correlate with increased E2A+ expression, higher clinical stage, and diminished response to Helicobacter pylori eradication therapy [53]. Large B-cell lymphomas that originate in the stomach, and which are presumably derived from the MALT, exhibit a MYC-miRNA signature, and transformation of MALT to DLBCL is associated with MYC, which negatively regulates miR-34a, leading to downregulation of FOXP1 [54]. Studies of nodal MZL confirm that these tumors have distinctive features that distinguish them from FL cases. As compared to FL, nodal MZL shows greater expression of miR-221, miR-223, and let-7f, which is a signature very similar to that exhibited by memory B-cells and cells isolated from the normal marginal zone. Expression of these miRNAs is enhanced in nodal MZL, whereas FL strongly expresses miR-494. Upregulation of miR-223 and miR-221, which targets the germinal center-related genes LMO2 and CD10, could be partially responsible for expression of a marginal zone signature. In splenic MZL, the miR-29 cluster is commonly lost and its expression silenced [55].

Conclusion

miRNAs have now been shown to play both oncogenic and tumor-suppressive roles in aggressive lymphoma subtypes. Some miRNAs have also been shown to contribute to phenotypic transformation of malignant lymphoma. More often, however, miRNA dysregulation likely adds to original genetic events, and the resultant aberrations enhance tumorigenicity through activation of additional signaling pathways. Consequently, analysis of miRNA expression may be more useful for evaluation of disease progression and transformation than for classification of various lymphoma entities. For novel treatments against lymphoma, miRNA itself or the appropriate antisense could be useful therapeutic agents, but future functional studies with distinct lymphoma subtypes will be required to determine whether that is the case.

References

1. Jaffe ES, Harris NL, Stein H, Campo E, Pileri SA, Swerdlow SH. Introduction and over review of the classification of the lymphoid neoplasms. In: Swerdloe AH, Campo E, Harris NL, Jaffe ES, Stein H, Thiele J, Vardiman JW, editors. World health classification of tumors. Pathology & Genetics of tumors of haematopoietic and lymphoid tissues. Washington/Lyon: IARC press; 2008. p. 158–78.
2. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer. 2006;6: 857–66.
3. Baer C, Claus R, Plass C. Genome-wide epigenetic regulation of miRNAs in cancer. Cancer Res. 2013;73:473–7.
4. Migliazza A, Bosch F, Komatsu H, et al. Nucleotide sequence, transcription map, and mutation analysis of the 13q14 chromosomal region deleted in B-cell chronic lymphocytic leukemia. Blood. 2001;97:2098–104.

5. Calin GA, Dumitru CD, Shimizu M, et al. 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.
6. Ota A, Tagawa H, Karnan S, et al. Identification and characterization of a novel gene, C13orf25, as a target for 13q31-q32 amplification in malignant lymphoma. Cancer Res. 2004;64:3087–95.
7. Tagawa H, Seto M. A microRNA cluster as a target of genomic amplification in malignant lymphoma. Leukemia. 2005;19:2013–6.
8. He L, Thomson JM, Hemann MT, et al. A microRNA polycistron as a potential human oncogene. Nature. 2005;435:828–33.
9. Ventura A, Young AG, Winslow MM, et al. Targeted deletion reveals essential and overlapping functions of the miR-17-92 family of miRNA clusters. Cell. 2008;132:875–86.
10. Xiao C, Srinivasan L, Calado DP, et al. Lymphoproliferative disease and autoimmunity in mice with elevated miR-17-92 expression in lymphocytes. Nat Immunol. 2008;9:405–14.
11. Inomata M, Tagawa H, Guo Y-M, et al. MicroRNA-17-92 downregulates expression of distinct targets in different B-cell lymphoma subtypes. Blood. 2009;113:396–402.
12. Olive V, Bennett MJ, Walker JC, et al. miR-19 is a key oncogenic component of mir-17-92. Genes Dev. 2009;23:2839–49.
13. Mu P, Han YC, Betel D, Yao E, et al. Genetic dissection of the miR-17-92 cluster of microRNAs in Myc-induced B-cell lymphomas. Genes Dev. 2009;23:2806–11.
14. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A. 2005;102(39):13944–9.
15. Linsley PS, Schelter J, Burchard J, Kibukawa M, Martin MM, Bartz SR, Johnson JM, Cummins JM, Raymond CK, Dai H, Chau N, Cleary M, Jackson AL, Carleton M, Lim L. Transcripts targeted by the microRNA-16 family cooperatively regulate cell cycle progression. Mol Cell Biol. 2007;27(6):2240–52.
16. Bonci D, Coppola V, Musumeci M, Addario A, Giuffrida R, Memeo L, D'Urso L, Pagliuca A, Biffoni M, Labbaye C, Bartucci M, Muto G, Peschle C, De Maria R. The miR-15a and miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. Nat Med. 2008;14(11):1271–7.
17. Liu Q, Fu H, Sun F, Zhang H, Tie Y, Zhu J, Xing R, Sun Z, Zheng X. miR-16 family induces cell cycle arrest by regulating multiple cell cycle genes. Nucleic Acids Res. 2008;36(16): 5391–404.
18. Pothof J, Verkaik NS, van IJcken W, Wiemer EA, Ta VT, van der Horst GT, Jaspers NG, van Gent DC, Hoeijmakers JH, Persengiev SP. MicroRNA-mediated gene silencing modulates the UV-induced DNA-damage response. EMBO J. 2009;28(14):2090–9.
19. Zhang X, Wan G, Mlotshwa S, Vance V, Berger FG, Chen H, Lu X. Oncogenic Wip1 phosphatase is inhibited by miR-16 in the DNA damage signaling pathway. Cancer Res. 2010;70(18): 7176–86.
20. Ofir M, Hacohen D, Ginsberg D. miR-15 and miR-16 are direct transcriptional targets of E2F1 that limit E2F-induced proliferation by targeting cyclin E. Mol Cancer Res. 2011;9(4):440–7.
21. Rivas MA, Venturutti L, Huang YW, Schillaci R, Huang THM, Elizalde PV. Downregulation of the tumor-suppressor miR-16 via progestin-mediated oncogenic signaling contributes to breast cancer development. Breast Cancer Res. 2012;14(3):R77.
22. Pouliot LM, Chen YC, Bai J, Guha R, Martin SE, Gottesman MM, Hall MD. Cisplatin sensitivity mediated by WEE1 and CHK1 is mediated by miR-155 and the miR-15 family. Cancer Res. 2012;72(22):5945–55.
23. Kim KT, Carroll AP, Mashkani B, Cairns MJ, Small D, Scott RJ. MicroRNA-16 is down-regulated in mutated FLT3 expressing murine myeloid FDC-P1 cells and Interacts with Pim-1. PLoS One. 2012;7(9), e44546.
24. Zhou R, Li X, Hu G, Gong AY, Drescher KM, Chen XM. MiR-16 targets transcriptional corepressor SMRT and modulates NF-kappaB-regulated transactivation of interleukin-8 gene. PLoS One. 2012;7(1), e30772.

25. Young LE, Moore AE, Sokol L, Meisner-Kober N, Dixon DA. The mRNA stability factor HuR inhibits MicroRNA-16 targeting of COX-2. Mol Cancer Res. 2012;10(1):167–80.
26. Rissland OS, Hong S-L, Bartel DP. MicroRNA destabilization enables dynamic regulation of the miR-16 family in response to cell-cycle changes. Mol Cell. 2011;43(6):993–1004.
27. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;403:503–11.
28. Lenz G, Wright GW, Emre NC, et al. Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. Proc Natl Acad Sci U S A. 2008;105:13520–5.29.
29. Malumbres R, Sarosiek KA, Cubedo E, Ruiz JW, Jiang X, Gascoyne RD, et al. Differentiation stage-specific expression of microRNAs in B lymphocytes and diffuse large B-cell lymphomas. Blood. 2009;113:3754–3764.
30. Montes-Moreno S, Martinez N, et al. miRNA expression in diffuse large B-cell lymphoma treated with chemoimmunotherapy. Blood. 2011;118:1034–40.
31. Alencar AJ, Malumbres R, Kozloski GA, et al. MicroRNAs are independent predictors of outcome in diffuse large B-cell lymphoma patients treated with R-CHOP. Clin Cancer Res. 2011;17:4125–35.
32. Merkel O, Hamacher F, Laimer D, et al. Identification of differential and functionally active miRNAs in both anaplastic lymphoma kinase (ALK) + and ALK- anaplastic large-cell lymphoma. Proc Natl Acad Sci U S A. 2010;107:16228–33.
33. Liu C, Iqbal J, Teruya-Feldstein J, Shen Y, Dabrowska MJ, Dybkaer K, et al. MicroRNA expression profiling identifies molecular signatures associated with anaplastic large cell lymphoma. Blood. 2013;122(12):2083–92.
34. Spaccarotella E, Pellegrino E, Ferracin M, Ferreri C, Cuccuru G, Liu C, et al. STAT3-mediated activation of microRNA cluster 17 ~ 92 promotes proliferation and survival of ALK positive anaplastic large cell lymphoma. Haematologica. 2013;99(1):116–24.
35. Zhu H, Vishwamitra D, Curry CV, Manshouri R, Diao L, Khan A, et al. NPM-ALK up-regulates iNOS expression through a STAT3/microRNA-26a-dependent mechanism. J Pathol. 2013;230(1):82–94.
36. Matsuyama H, Suzuki HI, Nishimori H, Noguchi M, Yao T, Komatsu N, et al. miR-135b mediates NPM-ALK-driven oncogenicity and renders IL-17-producing immunophenotype to anaplastic large cell lymphoma. Blood. 2011;118(26):6881–92.
37. Nakashima Y, Tagawa H, Suzuki R, et al. Genome-wide array-based comparative genomic hybridization of natural killer cell lymphoma/leukemia: different genomic alteration patterns of aggressive NK-cell leukemia and extranodal NK/T lymphoma, nasal type. Genes Chromosomes Cancer. 2005;19:247–55.
38. Yamanaka Y, Tagawa H, Takahashi N, et al. Aberrant overexpression of microRNAs activate AKT signaling via down-regulation of tumor suppressors in natural killer-cell lymphoma/leukemia. Blood. 2009;114:3265–75.
39. Watanabe A, Tagawa H, Yamashita J, Teshima K, Nara M, Iwamoto K, et al. The role of microRNA-150 as a tumor suppressor in malignant lymphoma. Leukemia. 2011;25(8): 1324–34.
40. Robbiani DF, Bothmer A, Callen E, et al. AID is required for the chromosomal breaks in c-myc that lead to c-myc/IgH translocations. Cell. 2008;135:1028–38.
41. Dorsett Y, McBride KM, Jankovic M, et al. MicroRNA-155 suppresses activation-induced cytidine deaminase-mediated Myc-Igh translocation. Immunity. 2008;8:630–8.
42. Lenze D, Leoncini L, Hummel M, et al. The different epidemiologic subtypes of Burkitt lymphoma share a homogenous micro RNA profile distinct from diffuse large B-cell lymphoma. Leukemia. 2011;25:1869–76.
43. Zhao JJ, Lin J, Lwin T, et al. microRNA expression profile and identification of miR-29 as a prognostic marker and pathogenetic factor by targeting CDK6 in mantle cell lymphoma. Blood. 2010;115:2630–9.
44. Navarro A, Beà S, Fernández V, et al. MicroRNA expression, chromosomal alterations, and immunoglobulin variable heavy chain hypermutations in mantle cell lymphomas. Cancer Res. 2009;69:7071–8.

45. Di Lisio L, Gómez-López G, Sánchez-Beato M, et al. Mantle cell lymphoma: transcriptional regulation by microRNAs. Leukemia. 2010;24:1335–42.
46. Teshima K, Nara M, Watanabe A, et al. Dysregulation of BMI1 and microRNA-16 collaborate to enhance an anti-apoptotic potential in the side population of refractory mantle cell lymphoma. Oncogene. 2014;33(17):2191–203.
47. Chen RW, Bemis LT, Amato CM, et al. Truncation in CCND1 mRNA alters miR-16-1 regulation in mantle cell lymphoma. Blood. 2008;112:822–9.
48. Zhang X, Zhao X, Fiskus W, et al. Coordinated silencing of MYC-mediated miR-29 by HDAC3 and EZH2 as a therapeutic target of histone modification in aggressive B-cell lymphomas. Cancer Cell. 2012;22:506–23.
49. Ito M, Teshima K, Ikeda S, Watanabe A, Nara M, Kitadate A, et al. MicroRNA-150 inhibits tumor invasion and metastasis by targeting the chemokine receptor CCR6 in advanced cutaneous T-cell lymphoma. Blood. 2014;123(10):1499–511
50. Wang W, Corrigan-Cummins M, Hudson J, et al. MicroRNA profiling of follicular lymphoma identifies microRNAs related to cell proliferation and tumor response. Haematologica. 2012;97:586–94.
51. Karube K, Guo Y, Suzumiya J, et al. CD10-MUM1+ follicular lymphoma lacks BCL2 gene translocation and shows characteristic biologic and clinical features. Blood. 2007;109: 3076–9.
52. Leich E, Zamo A, Horn H, et al. MicroRNA profiles of t(14;18)-negative follicular lymphoma support a late germinal center B-cell phenotype. Blood. 2011;118:5550–8.
53. Liu TY, Chen SU, Kuo SH, Cheng AL, Lin CW. E2A-positive gastric MALT lymphoma has weaker plasmacytoid infiltrates and stronger expression of the memory B-cell-associated miR-223: possible correlation with stage and treatment response. Mod Pathol. 2010;23:1507–17.
54. Craig VJ, Cogliatti SB, Imig J, et al. Myc-mediated repression of microRNA-34a promotes high-grade transformation of B-cell lymphoma by dysregulation of FoxP1. Blood. 2011;117:6227–36.
55. Arribas AJ, Campos-Martín Y, Gómez-Abad C, et al. Nodal marginal zone lymphoma: gene expression and miRNA profiling identify diagnostic markers and potential therapeutic targets. Blood. 2012;119:e9–21.

Chapter 4
MicroRNAs and Gastrointestinal Stromal Tumor

Pinar Akçakaya and Weng-Onn Lui

Abstract Gastrointestinal stromal tumor (GIST) is the most commonly diagnosed mesenchymal tumor in the gastrointestinal tract. This tumor type is driven by gain-of-function mutations in receptor tyrosine kinases (such as *KIT*, *PDGFRA*, and *BRAF*) or loss-of-function mutations in succinate dehydrogenase complex subunit genes (*SDHx*). Molecular studies on GIST have improved our understanding of the biology of the disease and have led to the use of targeted therapy approach, such as imatinib for *KIT/PDGFRA*-mutated GIST. Recently, microRNAs have emerged as important regulators of *KIT* expression, cancer cell behavior, and imatinib response in GIST. This chapter aims to provide an overview on current understanding of the biological roles of microRNAs in GIST and possible implications in prognosis and therapeutic response.

Keywords microRNA • GIST • Prognosis • Diagnosis • Therapy • Biomarker

Introduction

Gastrointestinal stromal tumors (GISTs) comprise one-fifth of soft tissue sarcomas, making them the most common sarcoma of the gastrointestinal tract [1]. The annual incidence of GIST is between 11 and 19.5 per million [2–5], and it has a prevalence of about 130 cases per million population [2–4]. For many years, GISTs were considered as smooth muscle sarcomas based on their morphology, and had been misdiagnosed as leiomyomas, leiomyosarcomas, or leiomyoblastomas. The prognosis of advanced GIST was very poor due to resistance to conventional chemotherapy and radiotherapy prior to the discovery of targeted therapies [6].

P. Akçakaya (✉) • W.-O. Lui (✉)
Department of Oncology-Pathology, Karolinska Institutet, Stockholm SE-17176, Sweden

Cancer Center Karolinska, Karolinska University Hospital, Stockholm SE-17176, Sweden
e-mail: pinar.akcakaya@ki.se; weng-onn.lui@ki.se

© Springer International Publishing Switzerland 2015

G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5_4

In the late 1990s, two groundbreaking discoveries had revolutionized the approach to diagnosis and treatment of GIST: (1) majority of GISTs (>95 %) were found immunohistochemically positive for the tyrosine kinase receptor KIT (also known as CD117) [7], and (2) *KIT* gene mutations were identified in 70–80 % of GISTs [8]. To date, KIT immunostaining and mutation screening are used as key diagnostic markers in clinical practice for GISTs, and mutant KIT is a clinically important therapeutic target in GISTs. The evolution of understanding the biology of GIST transformed it from a challenging chemotherapy-resistant disease to a model for molecular targeted therapy.

Although the initial events in GIST development are well characterized, the prognosis is clearly influenced by other genetic or epigenetic events that are still poorly understood. Aberrant microRNA expression is common in a wide range of human cancers. Accumulated evidence has shown that microRNAs are associated with clinical and pathological features in GIST, suggesting their important roles in GIST development.

This chapter gives a brief background on clinical features and biology of GIST, and provides an overview of the current knowledge on involvement of microRNAs in GIST tumorigenesis and therapeutic response.

Gastrointestinal Stromal Tumor

GISTs are thought to originate from the interstitial cells of Cajal (ICC) or their stem-like precursors [7, 8]. ICC function as pacemaker in the gastrointestinal tract that controls peristaltic contractions [7]. GISTs can be found anywhere along the gastrointestinal tract, but predominantly occur in the stomach (50–60 %) and the small intestine (30–35 %), less frequently in the colon/rectum (5 %) and esophagus (<1 %) [9]. These tumors can arise at any age, with a median age of diagnosis at 63 years [1, 9]. The tumor size varies between 2 and 30 cm at the time of diagnosis [10].

Oncogenic Mutations

The main initial event in GIST tumorigenesis is gain-of-function mutations in *KIT* (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) or *PDGFRA* (platelet-derived growth factor-α) genes. These genes are located on the long arm of chromosome 4 (4q12), and encode transmembrane proteins that belong to the type III tyrosine kinase receptor family.

Under normal physiological conditions, activation of KIT and PDGFRA receptors is controlled by spatial and temporal expression of their respective ligands, SCF and PDGF. Binding of these ligands to the receptors results in homodimerization, transphosphorylation of the tyrosine residues, and kinase activation that initiates signal

transduction cascades promoting cell proliferation, growth, and survival [11–13]. About 75 % of GISTs harbor *KIT* mutations [14], whereas 10 % of GISTs harbor *PDGFRA* mutations [15, 16]. These mutations disrupt the autoregulatory mechanisms and cause ligand-independent constitutive activation of the encoded tyrosine kinase receptors [17], which results in aberrant cell growth and tumor formation [18]. Activation of KIT or PDGFRA stimulates several downstream signaling pathways such as mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3K)/AKT/mTOR and signal transducer, and activator of transcription 3 (STAT3) [19–21].

About 10–15 % of GISTs do not harbor *KIT* or *PDGFRA* mutations. These tumors display mutations in multiple cancer genes, including succinate dehydrogenase complex subunit genes (*SDHA, SDHB*, *SDHC*, and *SDHD*) (50 %) [22, 23], *BRAF* V600E substitution (13 %) [24], neurofibromin 1 (*NF1*) (7 %) [25, 26], and RAS family members [27]. Different signaling pathways in GIST are illustrated in Fig. 4.1.

Unlike adult GISTs, pediatric GISTs (1–2 % of all GISTs) are rarely positive for *KIT* or *PDGFRA* mutations, despite expressing KIT at similar levels as adult GISTs [28]. Gene expression pattern of these tumors is also different from adult GISTs [29, 30], suggesting alternative mechanisms of KIT activation or distinct pathways in pediatric GISTs.

Chromosomal Changes in GIST

Cytogenetic studies demonstrated that about 65 % of GISTs have either monosomy of chromosome 14 or partial loss of 14q [31–33]. Loss of heterozygosity and comparative genomic hybridization studies identified two hotspot regions (14q11.2 and 14q32), pointing tumor suppressor genes at these loci might be important for GIST development [32, 34]. Several candidate genes are suggested within these regions, such as *PARP2, APEX1*, and *NDRG2* genes at 14q11.2, *SIVA* [35] and microRNA clusters at 14q32 [36, 37].

Several chromosomal abnormalities have been associated with malignant behavior in GIST. Loss of the long arm of chromosome 22 is observed in approximately 50 % of GISTs and associated with malignancy [31, 33, 38]. Chromosome 9p21 deletion causes inactivation of the tumor suppressor gene *CDKN2A* and associated with metastatic behavior [39–42]. Gains on chromosomes 8q (including *MYC*), 3q (including *SMARCA3*) and 17q are associated with metastasis [32, 43–45].

Treatment of GIST

Surgical resection is the main therapy for localized GIST, with the goal of complete resection and avoidance of tumor rupture [46]. However, surgery is sometimes not applicable for metastatic GISTs or clinically unresectable GISTs. A small molecule

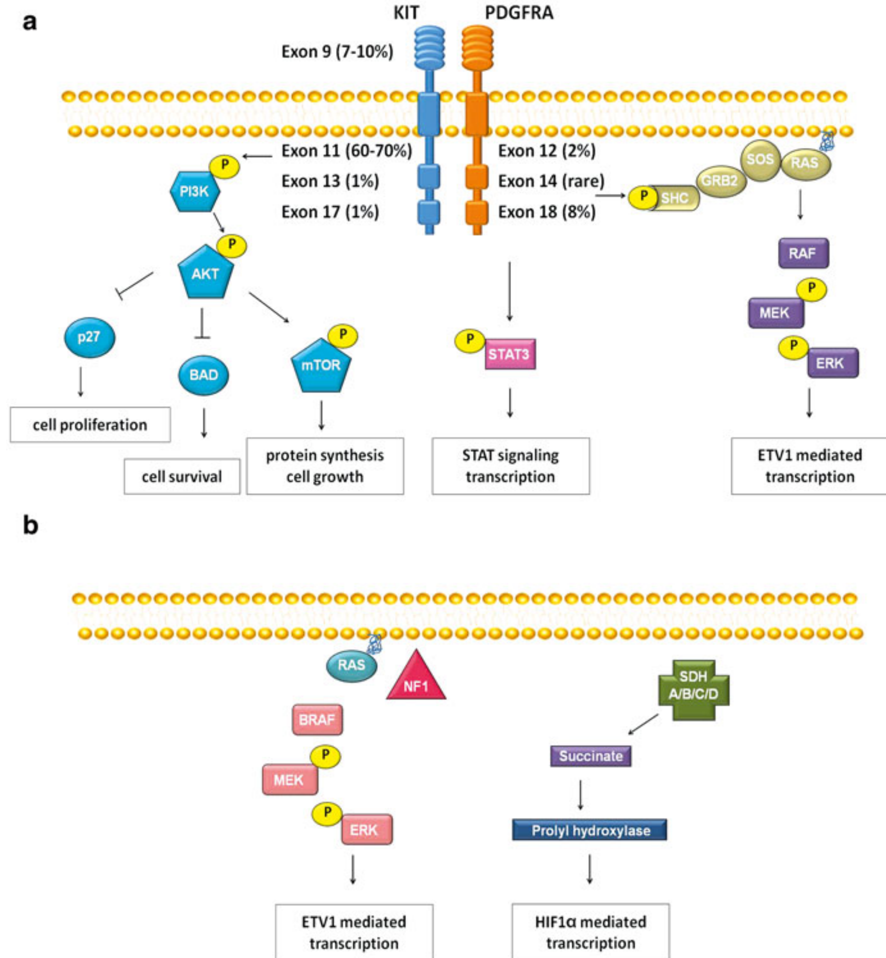

Fig. 4.1 Signaling pathways in GIST. (**a**) KIT and PDGFRA signaling pathways. Mutations in *KIT* or *PDGFRA* activate MAPK, PI3K/AKT/mTOR, and STAT3 pathways. The overall percentage of specific mutation sites is given in parentheses. (**b**) Signaling pathways in "wild-type" GISTs. Mutations in *NF1*, *BRAF*, or *RAS* lead to increased MAPK signaling. Mutations in one of the SDH genes (*SDHA, SDHB, SDHC, or SDHD*) lead to succinate accumulation, which inhibits prolyl hydroxylase-mediated HIF1α degradation and thereby increased HIF1α-mediated transcription of *VEGF* and *IGF. P* Phosphate group (Modified from Akcakaya P, thesis for doctoral degree 2015, ISBN 978-91-7549-730-3)

tyrosine kinase receptor inhibitor, such as imatinib mesylate, is used for the treatment of advanced GISTs.

Imatinib can selectively block the enzymatic activity of both transmembrane receptor tyrosine kinases KIT and PDGFRA [47, 48]. It competes with ATP for the ATP-binding pocket located in the kinase domain, and blocks the phosphorylation of the tyrosine kinase receptors. Binding of imatinib inhibits the activation of

downstream survival pathways such as PI3K-mTOR and MAPK [19], and induces cell apoptosis through BIM [49] and soluble histone H2AX [50]. In addition, imatinib reduces the expression of indoleamine 2,3-dioxygenase (IDO) [51], which is an enzyme that produces immunosuppressive metabolites. Reduction of IDO causes depletion of regulatory T cells and increase of tumor-infiltrating $CD8^+$ T cells. Thus, imatinib stimulates an anticancer immune response by diminishing IDO-mediated immunosuppression.

The majority of GIST patients with advanced disease get a clinical benefit from imatinib treatment. Imatinib achieved disease control in 70–85 % of patients with advanced GIST, median progression-free survival increased from 8–10 months to 20–24 months, and median overall survival increased from 18–20 months to 50 months [52–54]. However, resistance to imatinib is one of the biggest obstacles in current GIST clinical practice.

Approximately 10 % of patients progress within 6 months of initial therapy, which is defined as primary resistance to imatinib [53–56]. Primary resistance shows stronger correlation with certain tumor genotypes, such as wild-type *KIT* or *PDGFRA*, *KIT* exon 9 mutations and *PDGFRA* D842V mutation [57–60]. In addition, 50–60 % of the initially responding patients develop disease progression within 2 years, regarded as secondary or acquired resistance [53–56]. The main mechanism of acquired resistance is the acquisition of secondary mutations in the kinase or loop domain of KIT or PDGFRA [61]. Several alternative mechanisms of resistance have been described. Kinase switching is one of them and several kinases have been involved in such mechanism. AXL is an oncogenic tyrosine kinase receptor that regulates the same downstream signaling pathways as KIT. Kinase switching from KIT to AXL was observed in imatinib-resistant GIST cell lines and clinical samples [62]. Besides AXL, a switch from KIT to FAK and FYN activation has also been reported in GIST cells upon acquisition of imatinib resistance, and phosphorylated FAK inhibition can re-sensitize the resistant cells to imatinib-induced cell death [63]. FAK has also been implicated in growth and survival of imatinib-resistant GIST cells [64]. In addition, gene amplification of *KIT* or *PDGFRA* was shown as a potential mechanism leading to either primary or secondary resistance [65]. Moreover, microRNAs have also been shown to play a role in imatinib resistance in GIST, as described in the following section.

MicroRNA Deregulation in GIST

MicroRNA signature of GISTs was first described by Subramanian and colleagues in 2008 [66]. The study compared microRNA profiles of 27 sarcomas with different histological types, and demonstrated that GISTs were clearly distinguished from other sarcomas based on their microRNA expressions (Table 4.1). This distinction implicates the role of microRNAs in GIST tumorigenesis and their potential applications as diagnostic markers or therapeutic targets in GIST. Compared to other sarcoma types, *miR-221–222* and *miR-17–92* clusters were expressed at lower level

Table 4.1 MicroRNA profiling studies in GIST

	MicroRNAs		
Comparison	Up	Down	Ref
GISTs vs. sarcomas	*miR-140*, miR-29c , miR-29b, miR-22, miR-30a-5p, miR-30d, miR-99b, miR-30e-5p, miR-143, miR-29a, miR-30c, miR-145, miR-125a, let-7b, miR-10a*	*miR-368, miR-133b, miR-1, miR-376a, miR-133a, miR-200b, miR-221, miR-222, miR-92*	[66]
GIST vs. GI-LMS	*miR-29c, miR-497, miR-30a, miR-603, miR-330-3p, miR-96, miR-527*	*miR-222, miR-221, miR-382, miR-938, miR-21, miR-21*, miR-155, miR-645, miR-297, miR-190b, miR-20a, miR-18a, miR-17, miR-19a*	[67]
14q − vs. 14q +		14q.32.31 microRNA cluster, e.g.: *miR-134, miR-370*	[37]
		14q.32.33 microRNA cluster, e.g.: *miR-495, miR-376, miR-134, miR-377, miR-539*	[36]
Gastric vs. intestinal	*miR-504, miR-7-1*, miR-598, miR-24-1-5p*	*miR-220c, miR-329, miR-370, miR-210, miR-409-3p, miR-376a, miR-376c*	[37]
	miR-124a, miR-199b, miR-451, miR-663, miR-10a, miR-218, miR-638, miR-24-3p, miR-27b, miR-128b, miR-588, miR-518c, miR-199a, miR-346, miR-200a, miR-526a, miR-625, miR-489, miR-140, miR-23b*	*miR-383, miR-136, miR-146a, miR-409-3p*	[36]
High risk vs. low risk		*miR-377, miR-409-3p, miR-376a*, miR-376b, miR-127, miR-136, miR-214, miR-150-5p, miR-495, miR-154*, miR-497, miR-381, miR-132, miR-195, miR-487b, miR-335, miR-146b, miR-342, miR-363*, miR-100, miR-21, miR-424, miR-487a, miR-16, miR-133b, miR-140, miR-125b, miR-23b, miR-365, miR-30e-5p, miR-152, miR-26b*	[36]
	miR-196a		[69]
		miR-483-5p, miR-1268, miR-508-5p, miR-1915, miR-762, miR-452, miR-371-5p, miR-638, miR-744, miR-1225-5p, miR-1272, miR-137, miR-885-3p, miR-133b, miR-206, miR-1261, miR-939, miR-572, miR-767-3p, miR-1228, miR-892b, miR-589, miR-149*, miR-526b*	[70]

(continued)

Table 4.1 (continued)

	MicroRNAs		
Comparison	Up	Down	Ref
PDGFRA- vs. *KIT*-mutated	*miR-1229, miR-1234, miR-766, miR-652, miR-629, miR-200c, miR-181a-2*, miR-342-3p, miR-151-5p, miR-185, miR-146b-5p, miR-132*, miR-132, miR-150, miR-509-3p*	*miR-330-5p*	[37]
Pediatric vs. adult mutant	*miR-7-2*, miR-15a, miR-16, miR-34c-5p, miR-125a-3p, miR-125b-1*, miR-126, miR-129-3p, miR-186, miR-190, miR-192, miR-210, miR-214, miR-345, miR-361-5p, miR-383, miR-422a, miR-423-5p, miR-450a, miR-450b-5p, miR-488*, miR-488, miR-491-5p, miR-523, miR-542-5p, miR-551b*, miR-576-3p, miR-590-5p, miR-744, miR-873*	*let-7f-2*, miR-139-5p, miR-152, miR-181a-2*, miR-193b*, miR-340, miR-365, miR-455-3p, miR-886-3p, miR-886-5p*	[71]
Imatinib resistant vs. sensitive	*miR-301a, miR-30c, miR-146b-5p, miR-125a-5p, miR-193b, miR-25, let-7d, miR-365, miR-30a-3p, miR-483-3p, let-7e, miR-103, miR-362-5p, miR-15b, miR-151-3p, let-7f, miR-324-5p, miR-30b, let-7a, let-7i, miR-151-5p, miR-130b, miR-660, miR-107, miR-532-5p, miR-214, miR-143*	*miR-574-5p, miR-134, miR-638, miR-762, miR-1224-5p, miR-1225-5p, miR-150-3p, miR-1207-5p, miR-1915, miR-1268, miR-139-3p, miR-663, miR-1249, miR-296-5p, miR-188-5p, miR-939, miR-1228*	[72]
Metastatic vs. nonmetastatic	*miR-301a-3p, miR-30c, miR-25, miR-455-3p, miR-130b, miR-148a, miR-362-5p, miR-660, miR-532-5p, miR-151-3p, miR-214, miR-193b, miR-146b-5p, miR-99b, miR-181a, miR-107, miR-193a-3p, miR-483-3p, miR-24-1-5p*	*miR-134, miR-939, miR-762, miR-1914-3p, miR-1290, miR-1305, miR-1202, miR-1207-5p, miR-1225-5p, miR-663, miR-575, miR-150-3p, miR-125a-3p, miR-1246, miR-1308, miR-139-3p, miR-188-5p, miR-1268, miR-1224-5p, miR-638, miR-150-5p, miR-1915, miR-501-5p, miR-101, miR-574-5p*	[72]

(continued)

Table 4.1 (continued)

	MicroRNAs		
Comparison	Up	Down	Ref
Double vs. single *KIT* mutant	*let-7d, miR-455-3p, miR-93, miR-106b, miR-130b, miR-103, miR-660, miR-99b, miR-107, miR-210, miR-720, miR-1260, miR-24, miR-151-3p, miR-1280, miR-342-3p, miR-125a-5p, miR-130a, miR-199a-5p, let-7e, miR-1274b, miR-362-5p, miR-25, miR-27b, miR-140-5p, let-7f, miR-1274a, miR-21, miR-886-3p, miR-146a, miR-30d, miR-331-3p, miR-143, miR-324-5p, miR-199a-3p, let-7a, miR-151-5p, miR-181a, miR-22, miR-30b, miR-140-3p, miR-23b, miR-17, miR-214, miR-181b, miR-361-5p, miR-132, let-7i*	*miR-940, miR-939, miR-150-3p, miR-638, miR-134, miR-1225-5p, miR-762, miR-572, miR-1275, miR-1207-5p, miR-1224-5p, miR-1268, miR-1915, miR-663, miR-1202, miR-296-5p, miR-1249, miR-1228, miR-188-5p, miR-1238, miR-101, miR-139-3p, miR-574-5p, miR-150-5p*	[72]

GIST gastrointestinal stromal tumor, *GI-LMS* gastrointestinal leiomyosarcomas, *e.g.* for example, *vs.* versus, *Ref* Reference

Note: Only top ranked differentially expressed miRNAs are listed. Up- and downregulated microRNAs refer to the first group in each comparison

in GIST [66, 67]. These microRNAs have been shown to target the two key factors *KIT* and *ETV1* in GIST tumorigenesis [67] (Table 4.2), suggesting that lower expression of these microRNAs in GIST could be important for the pathogenesis of this tumor type. The current known microRNAs involved in regulating key genes in GIST development and progression are shown in Fig. 4.2.

MicroRNAs Associated with Clinical and Pathological Features in GIST

Morphology, clinical behavior, and molecular biology of GISTs differ according to their anatomical localization [68]. Likewise, microRNA expression profiles of GISTs located in stomach are distinct from the GISTs found in small intestine [36, 37]. Notably, different sets of microRNAs associated with anatomical location were observed in different studies. For example, Haller et al. showed that gastric GISTs presented higher expressions of *miR-504*, *miR-7-1**, *miR-598*, and *miR-24-1**, while the intestinal GISTs had higher levels of *miR-220c*, *miR-229*, *miR-370*,

Table 4.2 Examples of aberrantly expressed microRNAs with functional role in GIST

microRNA	Target	Cellular function	Ref
miR-196a	*ANXA1*	Invasion	[69]
miR-494	*KIT*	Proliferation, apoptosis	[74]
miR-221/222	*KIT*	Proliferation, apoptosis	[67]
miR-17	*ETV1*	Proliferation, apoptosis	[67]
miR-20a	*ETV1*	Proliferation, apoptosis	[67]
miR-137	*TWIST1*	EMT, migration, cell cycle arrest, apoptosis	[73]
miR-125a-5p	*PTPN18*	Imatinib resistance	[72]
miR-218	*KIT*	Imatinib resistance, proliferation, invasion, apoptosis	[75, 101]

EMT Epithelial-mesenchymal transition, *Ref* Reference

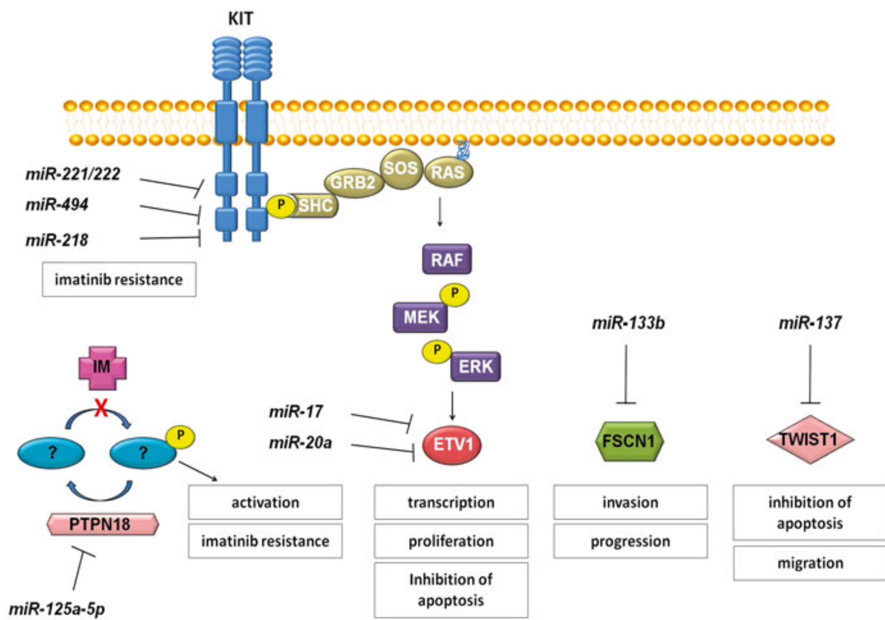

Fig. 4.2 MicroRNAs involved in the regulation of GIST development, progression, and imatinib response. In brief, *miR-221*, *miR-222*, *miR-494*, and *miR-218* directly target the *KIT* expression, while *miR-17* and *miR-20a* regulates the survival factor *ETV1*. *miR-133b* and *miR-137* regulate GIST progression by targeting *FSCN1* and *TWIST1*, respectively. *miR-125a-5p* regulates imatinib response through the regulation of *PTPN18*. *miR-218* also regulates imatinib response. *IM* Imatinib, *P* Phosphate group

miR-210, *miR-409-3p*, *miR-376a*, and *miR-376c* [37]. Choi et al. demonstrated higher expressions of *miR-383*, *miR-136*, *miR-146a*, and *miR-409a-3p*, and lower expressions of *miR-124a, miR-199b, miR-451, miR-663, miR-10a*, and *miR-218* in the intestinal compared to gastric GISTs [36]. The discrepancy is likely due to additional factors (e.g., risk grade and mutation status) that may contribute to differences besides anatomical locations in the tumors analyzed in both studies.

Several microRNA signatures have been described in GIST progression. In terms of tumor-risk group, a number of studies revealed distinct microRNA expression patterns between the high-risk and the low-risk GISTs, and identified a number of tumor-risk associated microRNAs (Table 4.1) [36, 69–71]. In the study of Choi et al., they compared microRNA profiles of 10 high-risk and 4 low-risk GISTs, and identified 28 microRNAs to be expressed at lower level in the high-risk group [36]. Yamamoto et al. reported 24 microRNAs with lower expression in the high-risk GISTs compared to low-to-intermediate risk tumors [70]. Kelly et al. found only *miR-150* to be expressed at higher level in the low-risk tumors [71], and Niinuma et al. reported higher *miR-196a* expression in the high-risk group [69].

Besides tumor risk, several microRNAs are associated with tumor metastasis in GISTs. For example, low expression of *miR-150-3p* and high expressions of *miR-301a-3p* and *miR-196a* are associated with metastasis in GIST [69, 72]. In experimental cell culture systems, two microRNAs have been evaluated for their effect on tumor progression. Overexpression of *miR-137* can inhibit cell migration and regulates epithelial-to-mesenchymal transition (EMT) by targeting *TWIST1* [73], and inhibition of *miR-196a* can suppress cell invasion in GIST cells [69].

In terms of survival, low expression of *miR-1915* is associated with disease-free and overall survival [72], while higher *miR-196a* expression is associated with poorer overall survival of GIST patients [69].

MicroRNAs Associated with Chromosomal and Genetic Alterations in GIST

As previously described, loss of 14q is common in GIST [31–33]. Downregulation of multiple microRNA clusters located at chromosome 14q (i.e., 14q32.31 and 14q32.33) has been reported in GISTs with 14q loss (Table 4.1) [36, 37]. One of the microRNAs located in this region, i.e., *miR-494*, was shown to directly target *KIT* and suppress its expression, and activates downstream signaling components such as AKT and STAT3 [74]. Functionally, inhibition of *miR-494* suppresses proliferation and induces apoptosis in GIST cells [74].

Given that *KIT* and *PDGFRA* are key factors involved in GIST tumorigenesis, microRNA-mediated regulation of these factors is important for GIST development. As aforementioned, *miR-494*, *miR-221*, and *miR-222* have been shown to directly regulate KIT expression in GIST cells [67, 74]. Recently, *miR-218* was also found directly targeting *KIT*, and its overexpression suppresses proliferation and invasion, and induces apoptosis in GIST-T1 cells [75]. On the other hand, *PDGFRA* is known to be regulated by several microRNAs in different cell types, such as *miR-126* in osteoblasts [76], *miR-34a* in gastric cancer [77], lung cancer [78], and glioma [79], and *miR-146a/146b-5p* in endothelial [80] and hematopoietic cells [81]; however, no microRNA has been experimentally validated to target *PDGFRA* in GIST.

GISTs show differential microRNA expression patterns according to their mutation status [37]. Several microRNAs are associated with *KIT* or *PDGFRA*-mutated

GISTs. For example, *miR-132*, *miR-766*, *miR-652*, *miR-629*, *miR-200c*, *miR-342-3p*, *miR-185*, *miR-146b-5p*, and *miR-150* levels are higher, whereas *miR-330-3p* is lower in *PDGFRA*-mutated GISTs as compared to *KIT*-mutated GISTs [37]. Higher expressions of *miR-221* and *miR-222* were found in the wild-type tumors compared to the tumors with *KIT* or *PDGFRA* mutation [37]. Concordantly, several studies have also revealed distinct mRNA expression profiles between GISTs with *KIT* and *PDGFRA* mutations [66, 82]. These findings suggest that, despite the common pathways activated by both mutations (e.g., PI3K/AKT and MAPK) [15], differences exist in the signal transduction networks between GISTs with *KIT* and *PDGFRA* mutations. In addition, several microRNAs are differentially expressed between GISTs with a single and double *KIT* mutations [72], suggesting that these microRNAs may be involved in partly distinct pathways [72].

Besides *KIT* and *PDGFRA*, several microRNAs are also associated with *SDHB* mutation. The *SDHB*-mutated GISTs show several microRNAs with higher (*miR-132*, *miR-146a*, *miR-193b*, *miR-193b**, *miR-455-3p*, *miR-455-5p*, *miR-484*, and *miR-886-5p*) and lower (*miR-125b*, *miR-450b*, *miR-488**, *miR-542-3p*, *miR-551b*, *miR-576-3p* and *miR-769-5p*) expressions compared to non-*SDHB*-mutated tumors [71].

MicroRNAs in Imatinib Resistance in GIST

MicroRNAs are known to play a role in tyrosine kinase inhibitor resistance [83–88]. The best example is the EGFR-inhibitor resistance in lung cancer. Numerous microRNAs (e.g., *miR-205*, *miR-374a*, *miR-548b*, *miR-30b*, *miR-30c*, *miR-221*, *miR-222*, and *miR-200* family members) have been shown to regulate EGFR-inhibitor response in lung cancer [84, 85, 89, 90]. In chronic myelogenous leukemia (CML), *miR-17–19b*, *miR-30e*, *miR-203*, and *miR-138* have been demonstrated to modulate imatinib sensitivity, while *miR-30a* promotes autophagy that enhances imatinib resistance [91–99].

In GIST, only two microRNAs have been functionally determined to modulate imatinib response [72, 100], despite a number of microRNAs are associated with imatinib resistance [72]. The expression of *miR-218* is lower in imatinib-resistant compared to -sensitive GIST cell lines. Overexpression of *miR-218* increases imatinib-induced cell death in the imatinib-resistant GIST430 cells. On the other hand, inhibition of *miR-218* expression increases cell viability and decreases apoptosis in the imatinib-sensitive GIST882 cells upon imatinib treatment. Although no target gene(s) of *miR-218* was identified, the authors propose that the effect might be mediated through PI3K/AKT signaling pathway.

The second microRNA is *miR-125a-5p*, which was found at higher expression levels in the imatinib-resistant than the -sensitive GISTs [72]. Overexpression of *miR-125a-5p* increases cell viability in the single *KIT*-mutated GIST882 cells upon imatinib treatment. However overexpression or suppression of *miR-125a-5p* in the double *KIT*-mutated GIST48 cells has no effect on imatinib response, suggesting

that microRNA-mediated regulation is an alternative resistance mechanism to secondary *KIT* mutations in GIST. Ectopic expression of *miR-125a-5p* suppresses its target gene *PTPN18* expression and silencing of PTPN18 increases cell viability in GIST882 cells upon imatinib treatment. The authors also observed an increased expression of *miR-125a-5p* and a decreased expression of PTPN18 in the imatinib-resistant subclone of GIST882 cells as compared to its sensitive counterpart, providing the functional evidence of *miR-125a-5p*-mediated regulation in imatinib resistance. PTPN18 is a member of the PEST domain containing protein-tyrosine phosphatase superfamily, which has been shown to dephosphorylate the phosphotyrosine residues of several tyrosine kinases, such as HER2 and SRC [101, 102]. Takahashi et al. recently demonstrated that altered phosphorylation of tyrosine kinases is an alternative mechanism of imatinib resistance in GIST [63]. Further studies have yet to determine whether the tyrosine kinases described by Takahashi et al. could be the substrate(s) of PTPN18.

Clinical Implications of microRNAs in GIST

MicroRNA expression profiles can distinguish GISTs from other sarcomas, and distinct microRNA expression signatures are associated with clinical, molecular, and histopathological features of GIST. These findings suggest a promising role for microRNAs as diagnostic and prognostic indicators in GIST.

Given their relatively higher stability in clinical samples and robust expression patterns, microRNAs have been suggested to have a greater utility as biomarkers in comparison to mRNAs [103]. Importantly, microRNAs can be released into the body fluids through microvesicles, which gives them a potential value as noninvasive biomarkers [104, 105]. Future studies evaluating the potential of circulating microRNAs as response markers for treatment or as reflective markers of GIST biological outcome would have a clinical benefit. However, there are some obstacles for circulating microRNAs, e.g., identification of an appropriate endogenous control and fluctuations in microRNA expression caused by diet, infection, treatment, trauma, or other factors [106].

Inhibition of KIT and PDGFRA by imatinib is the key therapeutic approach for advanced GISTs beside surgery. However, imatinib resistance is one of the biggest challenges in current GIST clinical practice. Post-transcriptional inhibition of oncogenes by microRNA mimics and activation of tumor suppressor genes by microRNA inhibitors are currently under investigation for their potential as therapeutic agents in cancer. KIT-targeting microRNA mimics (e.g., *miR-221, miR-222, miR-494*) [67, 74] may be used directly to target GIST cells to enhance the effect of imatinib for the purpose of overcoming resistance. Likewise, microRNA mimics/inhibitors for microRNAs specific to imatinib resistance, metastasis, risk grade or survival may be used for therapeutic purposes. Off-target effects and delivery of these molecules to specific GIST tissues/cells remain as the biggest challenges. Several strategies have

been developed for delivery of microRNA-based therapeutics, including the use of nanoparticles, liposomes, antibodies and nucleic acid structure modifications [107].

Conclusion

In the last 20 years, growing knowledge of GIST molecular biology has revolutionized the clinical management of this disease, from a treatment-resistant uncontrolled disease to the development of targeted therapies. Despite tyrosine kinase inhibitors improve the outcome of the majority of patients, they fail to provide a permanent cure and resistant clones are observed in most of the initially responding tumors.

Development of alternative treatment strategies is needed in order to overcome resistance to ATP-competitive kinase inhibitors. Complete understanding of molecular biology in GIST development, progression, and treatment response is necessary to establish a ground for developing effective combinational therapies with a goal of not only to temporarily control the disease, but also to permanently eradicate all tumor cells.

MicroRNAs have been shown to play a role not only in the GIST tumorigenesis, but also in the stratification of patients at risk of developing the disease or therapy response. Although this research area is still relatively understudied, the work reported in the last 3 years is indicative of the excitement in this area. Ongoing and future studies will illuminate effectiveness and safeness of microRNAs as novel agents for GIST treatment and their predictive value as novel biomarkers. This will hopefully turn GIST from a model of targeted therapies that control the disease progression to a model of complete cancer cure.

References

1. Ducimetiere F, Lurkin A, Ranchere-Vince D, Decouvelaere AV, Peoc'h M, Istier L, Chalabreysse P, Muller C, Alberti L, Bringuier PP, Scoazec JY, Schott AM, Bergeron C, Cellier D, Blay JY, Ray-Coquard I. Incidence of sarcoma histotypes and molecular subtypes in a prospective epidemiological study with central pathology review and molecular testing. PLoS One. 2011;6, e20294.
2. Chan KH, Chan CW, Chow WH, Kwan WK, Kong CK, Mak KF, Leung MY, Lau LK. Gastrointestinal stromal tumors in a cohort of Chinese patients in Hong Kong. World J Gastroenterol. 2006;12:2223–8.
3. Goettsch WG, Bos SD, Breekveldt-Postma N, Casparie M, Herings RM, Hogendoorn PC. Incidence of gastrointestinal stromal tumours is underestimated: results of a nation-wide study. Eur J Cancer. 2005;41:2868–72.
4. Nilsson B, Bumming P, Meis-Kindblom JM, Oden A, Dortok A, Gustavsson B, Sablinska K, Kindblom LG. Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era—a population-based study in western Sweden. Cancer. 2005;103:821–9.

5. Tryggvason G, Gislason HG, Magnusson MK, Jonasson JG. Gastrointestinal stromal tumors in Iceland, 1990–2003: the icelandic GIST study, a population-based incidence and pathologic risk stratification study. Int J Cancer. 2005;117:289–93.
6. Dematteo RP, Heinrich MC, El-Rifai WM, Demetri G. Clinical management of gastrointestinal stromal tumors: before and after STI-571. Hum Pathol. 2002;33:466–77.
7. Kindblom LG, Remotti HE, Aldenborg F, Meis-Kindblom JM. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol. 1998;152:1259–69.
8. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad Tunio G, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science. 1998;279:577–80.
9. Joensuu H, Vehtari A, Riihimaki J, Nishida T, Steigen SE, Brabec P, Plank L, Nilsson B, Cirilli C, Braconi C, Bordoni A, Magnusson MK, Linke Z, Sufliarsky J, Federico M, Jonasson JG, Dei Tos AP, Rutkowski P. Risk of recurrence of gastrointestinal stromal tumour after surgery: an analysis of pooled population-based cohorts. Lancet Oncol. 2012;13:265–74.
10. Corless CL, McGreevey L, Haley A, Town A, Heinrich MC. KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. Am J Pathol. 2002;160:1567–72.
11. Heldin CH. Dimerization of cell surface receptors in signal transduction. Cell. 1995;80:213–23.
12. Hubbard SR, Mohammadi M, Schlessinger J. Autoregulatory mechanisms in protein-tyrosine kinases. J Biol Chem. 1998;273:11987–90.
13. Roskoski Jr R. Signaling by Kit protein-tyrosine kinase—the stem cell factor receptor. Biochem Biophys Res Commun. 2005;337:1–13.
14. Corless CL, Barnett CM, Heinrich MC. Gastrointestinal stromal tumours: origin and molecular oncology. Nat Rev Cancer. 2011;11:865–78.
15. Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, Demetri GD, Fletcher CD, Fletcher JA. PDGFRA activating mutations in gastrointestinal stromal tumors. Science. 2003;299:708–10.
16. Hirota S, Ohashi A, Nishida T, Isozaki K, Kinoshita K, Shinomura Y, Kitamura Y. Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. Gastroenterology. 2003;125:660–7.
17. Gajiwala KS, Wu JC, Christensen J, Deshmukh GD, Diehl W, DiNitto JP, English JM, Greig MJ, He YA, Jacques SL, Lunney EA, McTigue M, Molina D, Quenzer T, Wells PA, Yu X, Zhang Y, Zou A, Emmett MR, Marshall AG, Zhang HM, Demetri GD. KIT kinase mutants show unique mechanisms of drug resistance to imatinib and sunitinib in gastrointestinal stromal tumor patients. Proc Natl Acad Sci U S A. 2009;106:1542–7.
18. Corless CL, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. J Clin Oncol. 2004;22:3813–25.
19. Bauer S, Duensing A, Demetri GD, Fletcher JA. KIT oncogenic signaling mechanisms in imatinib-resistant gastrointestinal stromal tumor: PI3-kinase/AKT is a crucial survival pathway. Oncogene. 2007;26:7560–8.
20. Duensing A, Medeiros F, McConarty B, Joseph NE, Panigrahy D, Singer S, Fletcher CD, Demetri GD, Fletcher JA. Mechanisms of oncogenic KIT signal transduction in primary gastrointestinal stromal tumors (GISTs). Oncogene. 2004;23:3999–4006.
21. Rossi F, Ehlers I, Agosti V, Socci ND, Viale A, Sommer G, Yozgat Y, Manova K, Antonescu CR, Besmer P. Oncogenic Kit signaling and therapeutic intervention in a mouse model of gastrointestinal stromal tumor. Proc Natl Acad Sci U S A. 2006;103:12843–8.
22. Janeway KA, Kim SY, Lodish M, Nose V, Rustin P, Gaal J, Dahia PL, Liegl B, Ball ER, Raygada M, Lai AH, Kelly L, Hornick JL, O'Sullivan M, de Krijger RR, Dinjens WN, Demetri GD, Antonescu CR, Fletcher JA, Helman L, Stratakis CA. Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations. Proc Natl Acad Sci U S A. 2011;108:314–8.

23. Pantaleo MA, Astolfi A, Indio V, Moore R, Thiessen N, Heinrich MC, Gnocchi C, Santini D, Catena F, Formica S, Martelli PL, Casadio R, Pession A, Biasco G. SDHA loss-of-function mutations in KIT-PDGFRA wild-type gastrointestinal stromal tumors identified by massively parallel sequencing. J Natl Cancer Inst. 2011;103:983–7.
24. Hostein I, Faur N, Primois C, Boury F, Denard J, Emile JF, Bringuier PP, Scoazec JY, Coindre JM. BRAF mutation status in gastrointestinal stromal tumors. Am J Clin Pathol. 2010;133:141–8.
25. Kinoshita K, Hirota S, Isozaki K, Ohashi A, Nishida T, Kitamura Y, Shinomura Y, Matsuzawa Y. Absence of c-kit gene mutations in gastrointestinal stromal tumours from neurofibromatosis type 1 patients. J Pathol. 2004;202:80–5.
26. Andersson J, Sihto H, Meis-Kindblom JM, Joensuu H, Nupponen N, Kindblom LG. NF1-associated gastrointestinal stromal tumors have unique clinical, phenotypic, and genotypic characteristics. Am J Surg Pathol. 2005;29:1170–6.
27. Miranda C, Nucifora M, Molinari F, Conca E, Anania MC, Bordoni A, Saletti P, Mazzucchelli L, Pilotti S, Pierotti MA, Tamborini E, Greco A, Frattini M. KRAS and BRAF mutations predict primary resistance to imatinib in gastrointestinal stromal tumors. Clin Cancer Res. 2012;18:1769–76.
28. Janeway KA, Liegl B, Harlow A, Le C, Perez-Atayde A, Kozakewich H, Corless CL, Heinrich MC, Fletcher JA. Pediatric KIT wild-type and platelet-derived growth factor receptor alpha-wild-type gastrointestinal stromal tumors share KIT activation but not mechanisms of genetic progression with adult gastrointestinal stromal tumors. Cancer Res. 2007;67:9084–8.
29. Agaram NP, Laquaglia MP, Ustun B, Guo T, Wong GC, Socci ND, Maki RG, DeMatteo RP, Besmer P, Antonescu CR. Molecular characterization of pediatric gastrointestinal stromal tumors. Clin Cancer Res. 2008;14:3204–15.
30. Prakash S, Sarran L, Socci N, DeMatteo RP, Eisenstat J, Greco AM, Maki RG, Wexler LH, LaQuaglia MP, Besmer P, Antonescu CR. Gastrointestinal stromal tumors in children and young adults: a clinicopathologic, molecular, and genomic study of 15 cases and review of the literature. J Pediatr Hematol Oncol. 2005;27:179–87.
31. Bergmann F, Gunawan B, Hermanns B, Hoer J, Schumpelick V, Fuzesi L. Cytogenetic and morphologic characteristics of gastrointestinal stromal tumors. Recurrent rearrangement of chromosome 1 and losses of chromosomes 14 and 22 as common anomalies. Verh Dtsch Ges Pathol. 1998;82:275–8.
32. Debiec-Rychter M, Lasota J, Sarlomo-Rikala M, Kordek R, Miettinen M. Chromosomal aberrations in malignant gastrointestinal stromal tumors: correlation with c-KIT gene mutation. Cancer Genet Cytogenet. 2001;128:24–30.
33. Fukasawa T, Chong JM, Sakurai S, Koshiishi N, Ikeno R, Tanaka A, Matsumoto Y, Hayashi Y, Koike M, Fukayama M. Allelic loss of 14q and 22q, NF2 mutation, and genetic instability occur independently of c-kit mutation in gastrointestinal stromal tumor. Jpn J Cancer Res. 2000;91:1241–9.
34. El-Rifai W, Sarlomo-Rikala M, Andersson LC, Miettinen M, Knuutila S. High-resolution deletion mapping of chromosome 14 in stromal tumors of the gastrointestinal tract suggests two distinct tumor suppressor loci. Genes Chromosomes Cancer. 2000;27:387–91.
35. Assamaki R, Sarlomo-Rikala M, Lopez-Guerrero JA, Lasota J, Andersson LC, Llombart-Bosch A, Miettinen M, Knuutila S. Array comparative genomic hybridization analysis of chromosomal imbalances and their target genes in gastrointestinal stromal tumors. Genes Chromosomes Cancer. 2007;46:564–76.
36. Choi HJ, Lee H, Kim H, Kwon JE, Kang HJ, You KT, Rhee H, Noh SH, Paik YK, Hyung WJ. MicroRNA expression profile of gastrointestinal stromal tumors is distinguished by 14q loss and anatomic site. Int J Cancer. 2010;126:1640–50.
37. Haller F, von Heydebreck A, Zhang JD, Gunawan B, Langer C, Ramadori G, Wiemann S, Sahin O. Localization- and mutation-dependent microRNA (miRNA) expression signatures in gastrointestinal stromal tumours (GISTs), with a cluster of co-expressed miRNAs located at 14q32.31. J Pathol. 2010;220:71–86.

38. Kim NG, Kim JJ, Ahn JY, Seong CM, Noh SH, Kim CB, Min JS, Kim H. Putative chromosomal deletions on 9P, 9Q and 22Q occur preferentially in malignant gastrointestinal stromal tumors. Int J Cancer. 2000;85:633–8.
39. Perrone F, Tamborini E, Dagrada GP, Colombo F, Bonadiman L, Albertini V, Lagonigro MS, Gabanti E, Caramuta S, Greco A, Torre GD, Gronchi A, Pierotti MA, Pilotti S. 9p21 locus analysis in high-risk gastrointestinal stromal tumors characterized for c-kit and platelet-derived growth factor receptor alpha gene alterations. Cancer. 2005;104:159–69.
40. Ricci R, Arena V, Castri F, Martini M, Maggiano N, Murazio M, Pacelli F, Potenza AE, Vecchio FM, Larocca LM. Role of p16/INK4a in gastrointestinal stromal tumor progression. Am J Clin Pathol. 2004;122:35–43.
41. Sabah M, Cummins R, Leader M, Kay E. Loss of heterozygosity of chromosome 9p and loss of p16INK4A expression are associated with malignant gastrointestinal stromal tumors. Mod Pathol. 2004;17:1364–71.
42. Schneider-Stock R, Boltze C, Lasota J, Miettinen M, Peters B, Pross M, Roessner A, Gunther T. High prognostic value of p16INK4 alterations in gastrointestinal stromal tumors. J Clin Oncol. 2003;21:1688–97.
43. El-Rifai W, Sarlomo-Rikala M, Andersson LC, Knuutila S, Miettinen M. DNA sequence copy number changes in gastrointestinal stromal tumors: tumor progression and prognostic significance. Cancer Res. 2000;60:3899–903.
44. O'Leary T, Ernst S, Przygodzki R, Emory T, Sobin L. Loss of heterozygosity at 1p36 predicts poor prognosis in gastrointestinal stromal/smooth muscle tumors. Lab Invest. 1999;79:1461–7.
45. Ylipaa A, Hunt KK, Yang J, Lazar AJ, Torres KE, Lev DC, Nykter M, Pollock RE, Trent J, Zhang W. Integrative genomic characterization and a genomic staging system for gastrointestinal stromal tumors. Cancer. 2011;117:380–9.
46. Hohenberger P, Ronellenfitsch U, Oladeji O, Pink D, Strobel P, Wardelmann E, Reichardt P. Pattern of recurrence in patients with ruptured primary gastrointestinal stromal tumour. Br J Surg. 2010;97:1854–9.
47. Buchdunger E, Cioffi CL, Law N, Stover D, Ohno-Jones S, Druker BJ, Lydon NB. Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. J Pharmacol Exp Ther. 2000;295:139–45.
48. Heinrich MC, Griffith DJ, Druker BJ, Wait CL, Ott KA, Zigler AJ. Inhibition of c-kit receptor tyrosine kinase activity by STI 571, a selective tyrosine kinase inhibitor. Blood. 2000;96:925–32.
49. Gordon PM, Fisher DE. Role for the proapoptotic factor BIM in mediating imatinib-induced apoptosis in a c-KIT-dependent gastrointestinal stromal tumor cell line. J Biol Chem. 2010;285:14109–14.
50. Liu Y, Tseng M, Perdreau SA, Rossi F, Antonescu C, Besmer P, Fletcher JA, Duensing S, Duensing A. Histone H2AX is a mediator of gastrointestinal stromal tumor cell apoptosis following treatment with imatinib mesylate. Cancer Res. 2007;67:2685–92.
51. Balachandran VP, Cavnar MJ, Zeng S, Bamboat ZM, Ocuin LM, Obaid H, Sorenson EC, Popow R, Ariyan C, Rossi F, Besmer P, Guo T, Antonescu CR, Taguchi T, Yuan J, Wolchok JD, Allison JP, DeMatteo RP. Imatinib potentiates antitumor T cell responses in gastrointestinal stromal tumor through the inhibition of Ido. Nat Med. 2011;17:1094–100.
52. MetaGIST. Comparison of two doses of imatinib for the treatment of unresectable or metastatic gastrointestinal stromal tumors: a meta-analysis of 1,640 patients. J Clin Oncol. 2010;28:1247–53.
53. Blanke CD, Rankin C, Demetri GD, Ryan CW, von Mehren M, Benjamin RS, Raymond AK, Bramwell VH, Baker LH, Maki RG, Tanaka M, Hecht JR, Heinrich MC, Fletcher CD, Crowley JJ, Borden EC. Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. J Clin Oncol. 2008;26:626–32.
54. Verweij J, Casali PG, Zalcberg J, LeCesne A, Reichardt P, Blay JY, Issels R, van Oosterom A, Hogendoorn PC, Van Glabbeke M, Bertulli R, Judson I. Progression-free survival in gas-

trointestinal stromal tumours with high-dose imatinib: randomised trial. Lancet. 2004;364:1127–34.
55. Blanke CD, Demetri GD, von Mehren M, Heinrich MC, Eisenberg B, Fletcher JA, Corless CL, Fletcher CD, Roberts PJ, Heinz D, Wehre E, Nikolova Z, Joensuu H. Long-term results from a randomized phase II trial of standard- versus higher-dose imatinib mesylate for patients with unresectable or metastatic gastrointestinal stromal tumors expressing KIT. J Clin Oncol. 2008;26:620–5.
56. van Oosterom AT, Judson IR, Verweij J, Stroobants S, Dumez H, Donato di Paola E, Sciot R, Van Glabbeke M, Dimitrijevic S, Nielsen OS. Update of phase I study of imatinib (STI571) in advanced soft tissue sarcomas and gastrointestinal stromal tumors: a report of the EORTC Soft Tissue and Bone Sarcoma Group. Eur J Cancer. 2002;38 Suppl 5:S83–7.
57. Debiec-Rychter M, Sciot R, Le Cesne A, Schlemmer M, Hohenberger P, van Oosterom AT, Blay JY, Leyvraz S, Stul M, Casali PG, Zalcberg J, Verweij J, Van Glabbeke M, Hagemeijer A, Judson I. KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. Eur J Cancer. 2006;42:1093–103.
58. Debiec-Rychter M, Wasag B, Stul M, De Wever I, Van Oosterom A, Hagemeijer A, Sciot R. Gastrointestinal stromal tumours (GISTs) negative for KIT (CD117 antigen) immunoreactivity. J Pathol. 2004;202:430–8.
59. Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, McGreevey LS, Chen CJ, Van den Abbeele AD, Druker BJ, Kiese B, Eisenberg B, Roberts PJ, Singer S, Fletcher CD, Silberman S, Dimitrijevic S, Fletcher JA. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol. 2003;21:4342–9.
60. Heinrich MC, Owzar K, Corless CL, Hollis D, Borden EC, Fletcher CD, Ryan CW, von Mehren M, Blanke CD, Rankin C, Benjamin RS, Bramwell VH, Demetri GD, Bertagnolli MM, Fletcher JA. Correlation of kinase genotype and clinical outcome in the North American Intergroup Phase III Trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 Study by Cancer and Leukemia Group B and Southwest Oncology Group. J Clin Oncol. 2008;26:5360–7.
61. Heinrich MC, Corless CL, Blanke CD, Demetri GD, Joensuu H, Roberts PJ, Eisenberg BL, von Mehren M, Fletcher CD, Sandau K, McDougall K, Ou WB, Chen CJ, Fletcher JA. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol. 2006;24:4764–74.
62. Mahadevan D, Cooke L, Riley C, Swart R, Simons B, Della Croce K, Wisner L, Iorio M, Shakalya K, Garewal H, Nagle R, Bearss D. A novel tyrosine kinase switch is a mechanism of imatinib resistance in gastrointestinal stromal tumors. Oncogene. 2007;26:3909–19.
63. Takahashi T, Serada S, Ako M, Fujimoto M, Miyazaki Y, Nakatsuka R, Ikezoe T, Yokoyama A, Taguchi T, Shimada K, Kurokawa Y, Yamasaki M, Miyata H, Nakajima K, Takiguchi S, Mori M, Doki Y, Naka T, Nishida T. New findings of kinase switching in gastrointestinal stromal tumor under imatinib using phosphoproteomic analysis. Int J Cancer. 2013;133:2737–43.
64. Sakurama K, Noma K, Takaoka M, Tomono Y, Watanabe N, Hatakeyama S, Ohmori O, Hirota S, Motoki T, Shirakawa Y, Yamatsuji T, Haisa M, Matsuoka J, Tanaka N, Naomoto Y. Inhibition of focal adhesion kinase as a potential therapeutic strategy for imatinib-resistant gastrointestinal stromal tumor. Mol Cancer Ther. 2009;8:127–34.
65. Debiec-Rychter M, Cools J, Dumez H, Sciot R, Stul M, Mentens N, Vranckx H, Wasag B, Prenen H, Roesel J, Hagemeijer A, Van Oosterom A, Marynen P. Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. Gastroenterology. 2005;128:270–9.
66. Subramanian S, Lui WO, Lee CH, Espinosa I, Nielsen TO, Heinrich MC, Corless CL, Fire AZ, van de Rijn M. MicroRNA expression signature of human sarcomas. Oncogene. 2008;27:2015–26.
67. Gits CM, van Kuijk PF, Jonkers MB, Boersma AW, van Ijcken WF, Wozniak A, Sciot R, Rutkowski P, Schoffski P, Taguchi T, Mathijssen RH, Verweij J, Sleijfer S, Debiec-Rychter

M, Wiemer EA. MiR-17-92 and miR-221/222 cluster members target KIT and ETV1 in human gastrointestinal stromal tumours. Br J Cancer. 2013;109:1625–35.

68. Miettinen M, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. Semin Diagn Pathol. 2006;23:70–83.
69. Niinuma T, Suzuki H, Nojima M, Nosho K, Yamamoto H, Takamaru H, Yamamoto E, Maruyama R, Nobuoka T, Miyazaki Y, Nishida T, Bamba T, Kanda T, Ajioka Y, Taguchi T, Okahara S, Takahashi H, Nishida Y, Hosokawa M, Hasegawa T, Tokino T, Hirata K, Imai K, Toyota M, Shinomura Y. Upregulation of miR-196a and HOTAIR drive malignant character in gastrointestinal stromal tumors. Cancer Res. 2012;72:1126–36.
70. Yamamoto H, Kohashi K, Fujita A, Oda Y. Fascin-1 overexpression and miR-133b downregulation in the progression of gastrointestinal stromal tumor. Mod Pathol. 2013;26:563–71.
71. Kelly L, Bryan K, Kim SY, Janeway KA, Killian JK, Schildhaus HU, Miettinen M, Helman L, Meltzer PS, van de Rijn M, Debiec-Rychter M, O'Sullivan M. Post-transcriptional dysregulation by miRNAs is implicated in the pathogenesis of gastrointestinal stromal tumor [GIST]. PLoS One. 2013;8, e64102.
72. Akcakaya P, Caramuta S, Ahlen J, Ghaderi M, Berglund E, Ostman A, Branstrom R, Larsson C, Lui WO. microRNA expression signatures of gastrointestinal stromal tumours: associations with imatinib resistance and patient outcome. Br J Cancer. 2014;111:2091–102.
73. Liu S, Cui J, Liao G, Zhang Y, Ye K, Lu T, Qi J, Wan G. miR-137 regulates epithelial-mesenchymal transition in gastrointestinal stromal tumor. Tumour Biol. 2014;35:9131–8.
74. Kim WK, Park M, Kim YK, Tae YK, Yang HK, Lee JM, Kim H. MicroRNA-494 downregulates KIT and inhibits gastrointestinal stromal tumor cell proliferation. Clin Cancer Res. 2011;17:7584–94.
75. Fan R, Zhong J, Zheng S, Wang Z, Xu Y, Li S, Zhou J, Yuan F. MicroRNA-218 inhibits gastrointestinal stromal tumor cell and invasion by targeting KIT. Tumour Biol. 2014;35:4209–17.
76. Schmidt Y, Simunovic F, Strassburg S, Pfeifer D, Stark GB, Finkenzeller G. miR-126 regulates platelet-derived growth factor receptor-alpha expression and migration of primary human osteoblasts. Biol Chem. 2015;396:61–70.
77. Peng Y, Guo JJ, Liu YM, Wu XL. MicroRNA-34A inhibits the growth, invasion and metastasis of gastric cancer by targeting PDGFR and MET expression. Biosci Rep. 2014;34.
78. Garofalo M, Jeon YJ, Nuovo GJ, Middleton J, Secchiero P, Joshi P, Alder H, Nazaryan N, Di Leva G, Romano G, Crawford M, Nana-Sinkam P, Croce CM. MiR-34a/c-Dependent PDGFR-alpha/beta Downregulation Inhibits Tumorigenesis and Enhances TRAIL-Induced Apoptosis in Lung Cancer. PLoS One. 2013;8, e67581.
79. Silber J, Jacobsen A, Ozawa T, Harinath G, Pedraza A, Sander C, Holland EC, Huse JT. miR-34a repression in proneural malignant gliomas upregulates expression of its target PDGFRA and promotes tumorigenesis. PLoS One. 2012;7:e33844.
80. Zhu K, Pan Q, Zhang X, Kong LQ, Fan J, Dai Z, Wang L, Yang XR, Hu J, Wan JL, Zhao YM, Tao ZH, Chai ZT, Zeng HY, Tang ZY, Sun HC, Zhou J. MiR-146a enhances angiogenic activity of endothelial cells in hepatocellular carcinoma by promoting PDGFRA expression. Carcinogenesis. 2013;34:2071–9.
81. Zhai PF, Wang F, Su R, Lin HS, Jiang CL, Yang GH, Yu J, Zhang JW. The regulatory roles of microRNA-146b-5p and its target platelet-derived growth factor receptor alpha (PDGFRA) in erythropoiesis and megakaryocytopoiesis. J Biol Chem. 2014;289:22600–13.
82. Kang HJ, Nam SW, Kim H, Rhee H, Kim NG, Hyung WJ, Noh SH, Kim JH, Yun CO, Liu ET. Correlation of KIT and platelet-derived growth factor receptor alpha mutations with gene activation and expression profiles in gastrointestinal stromal tumors. Oncogene. 2005;24:1066–74.
83. Bryant JL, Britson J, Balko JM, Willian M, Timmons R, Frolov A, Black EP. A microRNA gene expression signature predicts response to erlotinib in epithelial cancer cell lines and targets EMT. Br J Cancer. 2012;106:148–56.
84. Garofalo M, Romano G, Di Leva G, Nuovo G, Jeon YJ, Ngankeu A, Sun J, Lovat F, Alder H, Condorelli G, Engelman JA, Ono M, Rho JK, Cascione L, Volinia S, Nephew KP, Croce CM.

EGFR and MET receptor tyrosine kinase-altered microRNA expression induces tumorigenesis and gefitinib resistance in lung cancers. Nat Med. 2012;18:74–82.
85. Wang Y, Xia H, Zhuang Z, Miao L, Chen X, Cai H. Axl-altered microRNAs regulate tumorigenicity and gefitinib resistance in lung cancer. Cell Death Dis. 2014;5:e1227.
86. Wang YS, Wang YH, Xia HP, Zhou SW, Schmid-Bindert G, Zhou CC. MicroRNA-214 regulates the acquired resistance to gefitinib via the PTEN/AKT pathway in EGFR-mutant cell lines. Asian Pac J Cancer Prev. 2012;13:255–60.
87. Weiss GJ, Bemis LT, Nakajima E, Sugita M, Birks DK, Robinson WA, Varella-Garcia M, Bunn Jr PA, Haney J, Helfrich BA, Kato H, Hirsch FR, Franklin WA. EGFR regulation by microRNA in lung cancer: correlation with clinical response and survival to gefitinib and EGFR expression in cell lines. Ann Oncol. 2008;19:1053–9.
88. Zhong M, Ma X, Sun C, Chen L. MicroRNAs reduce tumor growth and contribute to enhance cytotoxicity induced by gefitinib in non-small cell lung cancer. Chem Biol Interact. 2010;184:431–8.
89. Park KS, Raffeld M, Moon YW, Xi L, Bianco C, Pham T, Lee LC, Mitsudomi T, Yatabe Y, Okamoto I, Subramaniam D, Mok T, Rosell R, Luo J, Salomon DS, Wang Y, Giaccone G. CRIPTO1 expression in EGFR-mutant NSCLC elicits intrinsic EGFR-inhibitor resistance. J Clin Invest. 2014;124:3003–15.
90. Shien K, Toyooka S, Yamamoto H, Soh J, Jida M, Thu KL, Hashida S, Maki Y, Ichihara E, Asano H, Tsukuda K, Takigawa N, Kiura K, Gazdar AF, Lam WL, Miyoshi S. Acquired resistance to EGFR inhibitors is associated with a manifestation of stem cell-like properties in cancer cells. Cancer Res. 2013;73:3051–61.
91. Hershkovitz-Rokah O, Modai S, Pasmanik-Chor M, Toren A, Shomron N, Raanani P, Shpilberg O, Granot G. MiR-30e induces apoptosis and sensitizes K562 cells to imatinib treatment via regulation of the BCR-ABL protein. Cancer Lett. 2014;356(2 Pt B):597–605.
92. Joshi D, Chandrakala S, Korgaonkar S, Ghosh K, Vundinti BR. Down-regulation of miR-199b associated with imatinib drug resistance in 9q34.1 deleted BCR/ABL positive CML patients. Gene. 2014;542:109–12.
93. Li Y, Yuan Y, Tao K, Wang X, Xiao Q, Huang Z, Zhong L, Cao W, Wen J, Feng W. Inhibition of BCR/ABL protein expression by miR-203 sensitizes for imatinib mesylate. PLoS One. 2013;8, e61858.
94. Liu L, Wang S, Chen R, Wu Y, Zhang B, Huang S, Zhang J, Xiao F, Wang M, Liang Y. Myc induced miR-144/451 contributes to the acquired imatinib resistance in chronic myelogenous leukemia cell K562. Biochem Biophys Res Commun. 2012;425:368–73.
95. Lopotova T, Zackova M, Klamova H, Moravcova J. MicroRNA-451 in chronic myeloid leukemia: miR-451-BCR-ABL regulatory loop? Leuk Res. 2011;35:974–7.
96. Shibuta T, Honda E, Shiotsu H, Tanaka Y, Vellasamy S, Shiratsuchi M, Umemura T. Imatinib induces demethylation of miR-203 gene: an epigenetic mechanism of anti-tumor effect of imatinib. Leuk Res. 2013;37:1278–86.
97. Venturini L, Battmer K, Castoldi M, Schultheis B, Hochhaus A, Muckenthaler MU, Ganser A, Eder M, Scherr M. Expression of the miR-17-92 polycistron in chronic myeloid leukemia (CML) CD34+ cells. Blood. 2007;109:4399–405.
98. Xu C, Fu H, Gao L, Wang L, Wang W, Li J, Li Y, Dou L, Gao X, Luo X, Jing Y, Chim CS, Zheng X, Yu L. BCR-ABL/GATA1/miR-138 mini circuitry contributes to the leukemogenesis of chronic myeloid leukemia. Oncogene. 2014;33:44–54.
99. Zimmerman EI, Dollins CM, Crawford M, Grant S, Nana-Sinkam SP, Richards KL, Hammond SM, Graves LM. Lyn kinase-dependent regulation of miR181 and myeloid cell leukemia-1 expression: implications for drug resistance in myelogenous leukemia. Mol Pharmacol. 2010;78:811–7.
100. Fan R, Zhong J, Zheng S, Wang Z, Xu Y, Li S, Zhou J, Yuan F. microRNA-218 increase the sensitivity of gastrointestinal stromal tumor to imatinib through PI3K/AKT pathway. Clin Exp Med. 2014;15(2):137–44.
101. Gensler M, Buschbeck M, Ullrich A. Negative regulation of HER2 signaling by the PEST-type protein-tyrosine phosphatase BDP1. J Biol Chem. 2004;279:12110–6.

102. Rubbi L, Titz B, Brown L, Galvan E, Komisopoulou E, Chen SS, Low T, Tahmasian M, Skaggs B, Muschen M, Pellegrini M, Graeber TG. Global phosphoproteomics reveals crosstalk between Bcr-Abl and negative feedback mechanisms controlling Src signaling. Sci Signal. 2011;4:ra18.
103. 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.
104. Schwarzenbach H, Nishida N, Calin GA, Pantel K. Clinical relevance of circulating cell-free microRNAs in cancer. Nat Rev Clin Oncol. 2014;11:145–56.
105. 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.
106. 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.
107. Li Z, Rana TM. Therapeutic targeting of microRNAs: current status and future challenges. Nat Rev Drug Discov. 2014;13:622–38.

Chapter 5
Insights into the Role of microRNAs in Pancreatic Cancer Pathogenesis: Potential for Diagnosis, Prognosis, and Therapy

Mohammad Aslam Khan, Haseeb Zubair, Sanjeev Kumar Srivastava, Seema Singh, and Ajay Pratap Singh

Abstract Pancreatic cancer is a highly lethal malignancy and a fourth leading cause of cancer-related death in the United States. Poor survival of pancreatic cancer patients is largely because of its asymptomatic progression to advanced stage against which no effective therapy is currently available. Over the years, we have developed significant knowledge of molecular progression of pancreatic cancer and identified several genetic and epigenetic aberrations to be involved in its etiology and aggressive behavior. In that regard, recent lines of evidence have suggested important roles of microRNAs (miRNAs/miRs) in pancreatic cancer pathogenesis. microRNAs belonging to a family of small, noncoding RNAs are able to control diverse biological processes due to their ability to regulate gene expression at the posttranscriptional level. Accordingly, dysregulation of miRNAs can lead to several disease conditions, including cancer. There is a long list of microRNAs that exhibit aberrant expression in pancreatic cancer and serve as key microplayers in its initiation, progression, metastasis, and chemoresistance. These findings have suggested that microRNAs could be exploited as novel biomarkers for diagnostic and prognostic assessments of pancreatic cancer and as targets for therapy. This book chapter describes clinical problems associated with pancreatic cancer, roles that microRNAs play in various aspects of pancreatic cancer pathogenesis, and envision opportunities for potential use of microRNAs in pancreatic cancer management.

M.A. Khan • H. Zubair • S.K. Srivastava • S. Singh
Department of Oncologic Sciences, Mitchell Cancer Institute, University of South Alabama, 1660 Springhill Avenue, Mobile, AL 36604-1405, USA

A.P. Singh, Ph.D. (✉)
Department of Oncologic Sciences, Mitchell Cancer Institute, University of South Alabama, 1660 Springhill Avenue, Mobile, AL 36604-1405, USA

Department of Biochemistry and Molecular Biology, College of Medicine, University of South Alabama, Mobile, AL, USA
e-mail: asingh@health.southalabama.edu

© Springer International Publishing Switzerland 2015

G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5_5

Keywords microRNAs • Pancreatic cancer • Initiation • Metastasis • Chemoresistance • Diagnosis and prognosis • Therapy

Introduction

microRNAs (miRNAs) have received considerable attention because of their potential to regulate diverse biological processes such as cell development, cell differentiation, and survival. Moreover, significance of miRNAs has been recognized in various human cancers within past decade. miRNAs are small (~19–25 nucleotide long) noncoding RNA molecules, which are generated from endogenous hairpin transcripts, and serve as negative regulators of gene expression by binding to the 3′-UTRs of target mRNA with partial or full complementarity. This target mRNA-miRNA binding typically results in either translational repression or causes target mRNA decay [1, 2]. To date, in humans approximately 2469 miRNAs have been identified [3], and it is estimated that around one-third of all the genes are regulated by miRNAs [4]. Majority of the target mRNAs contain multiple binding sites for different miRNAs, which suggest that individual mRNA can be regulated by many miRNAs [5]. On the other hand, a single miRNA can also potentially regulate the expression of multiple genes [5–7].

Pancreatic cancer is a highly lethal malignancy and is the eighth leading cause of cancer-related deaths globally [8]. In the United States, it is currently the fourth leading cause of cancer-related death and predicted to become second on the list by 2030 [9, 10]. Post-diagnosis median survival of pancreatic cancer patients is nearly 2–8 months, and only ~6 % of all patients survive more than 5 years [11]. Such a poor prognosis is largely due to late diagnosis of disease, when it is either locally advanced or has already metastasized to distant organ sites [6, 12]. Moreover, currently, there is a complete lack of an effective therapy to treat metastatic pancreatic cancer. Even small pancreatic tumors at the time of diagnosis harbor several genetic or epigenetic alterations that cooperatively promote their aggressiveness and therapeutic-resistance [13, 14]. In view of this grim scenario, there has been increasing interest to define genetic and epigenetic landscape of pancreatic cancer and understand molecular mechanisms underlying its initiation, aggressive progression, and therapy resistance. Clearly, miRNAs have emerged as important biomolecules regulating pancreatic cancer pathobiology.

This book chapter summarizes currently available information on the significance of miRNAs in pancreatic cancer with major emphasis on their roles in disease initiation, progression, and chemoresistance. We also highlight the diagnostic, prognostic, and therapeutic potential of miRNAs in pancreatic cancer and their prospective utility in clinics.

Pancreatic Cancer: A Major Clinical Problem

Pancreatic cancer has the worst prognosis among all cancers. It is referred as a "silent killer" because of the lack of any symptoms in its early stages of progression and its diagnosis is often considered as "death sentence" to the patient. The appeared symptoms are often indistinguishable and share commonality with other abdominal or gastrointestinal (GI) tract pathologies [15, 16]. Consequently, we are still struggling to develop a sensitive and specific diagnostic for pancreatic cancer that would be clinically feasible [17]. Due to these limitations, specific sets of biomarkers are required which could be potentially used for diagnosis and prognosis of pancreatic cancer. Therapy of pancreatic cancer also remains a major challenge in clinics due to highly advanced and metastatic disease at the time of diagnosis. Thus, surgical resection followed by adjuvant therapy, which could be curative, is not an option in most pancreatic cancer patients. Even in some early stage cases, surgical resection largely fails to manage the disease [18]. Chemotherapeutic options for the advanced stage patients are limited and provide minimal survival benefit at best. Gemcitabine as a single-agent drug for the treatment of pancreatic cancer shows only a meager improvement in the median survival by only few weeks [19]. Erlotinib, an epidermal growth factor receptor (EGFR) inhibitor when given in combination with gemcitabine, modestly prolonged the mean survival rates of pancreatic cancer patients as compared to gemcitabine treatment alone [20]. In a phase III study it was shown that combination treatment of gemcitabine with cisplatin did not increase the survival benefits [21]. Moreover, in a multicenter phase II/III trial on patients with metastatic pancreatic adenocarcinoma, FOLFIRINOX in combination with gemcitabine increased median survival to 11.1 months as compared to 6.8 months in the gemcitabine treatment group [22]. Recently, based on the findings from a phase III clinical trial, albumin-bound paclitaxel (nab-paclitaxel) in combination with gemcitabine was introduced. The median overall survival was increased to 8.5 months in patients treated with nab-paclitaxel and gemcitabine as compared to 6.7 months for gemcitabine treatment alone. Moreover, the median progression-free survival was 5.5 months compared to 3.7 months for patients treated with combination and gemcitabine alone, respectively [23]. These studies suggest that so far there is no major stride made that could effectively treat pancreatic cancer. Therefore, there is an urgent clear need for the identification and development of specific and sensitive biomarkers for diagnosing this asymptomatic disease in its early phase.

Deregulation of miRNAs in Pancreatic Cancer

Mounting evidence suggest that miRNAs are aberrantly expressed and highly deregulated in pancreatic cancer [6]. Poy and colleagues first identified specific miRNA signature for the normal pancreas [24]. Since then, extensive studies on miRNAs have been

conducted and several differentially expressed miRNAs identified in pancreatic cancer. Pancreatic cancer-based miRNA screening was clearly able to distinguish pancreatic ductal adenocarcinoma (PDAC) from normal pancreas and pancreatitis [12]. The study identified 100 miRNAs to be differentially expressed in pancreatic cancer including miR-155, miR-21, miR-221, and miR-222, which are also known to be aberrantly expressed in other human cancers [25]. Furthermore, miRNA profiling identified significantly high levels of miR-196a, miR-186, miR-190, miR-95, miR-221, miR-222, miR-200b, and miR-15b in pancreatic tumors [26]. Moreover, we recently provided the evidence that miR-150 is significantly downregulated in pancreatic cancer [27]. Study carried out in the serum samples of pancreatic cancer patients identified miR-2 to be overexpressed in pancreatic cancer [28]. Microarray-based miRNA study revealed that 21 miRNAs were upregulated, and 4 downregulated in pancreatic cancer [29]. Interestingly, this expression pattern differentiated pancreatic cancer from normal/benign pancreatic tissues. In pancreatic neuroendocrine tumors, expression of miR-155, miR-146a, miR-142-3p, and miR-142-5p were high as compared to its normal counterpart [30]. In 15 different pancreatic cancer cell lines, miR-10a, miR-92, and miR-17-5p were observed to be overexpressed [31]. Furthermore, elevated levels of miR-155 and miR-21 were reported in the clinical specimens of intraductal papillary neoplasms. Another similar study suggested that activation of miR-155 is an "early" event in the pancreatic cancer progression, and the expression level of miR-155 increases with the progression from PanIN-2 to PanIN-3 [32]. Similarly, expression of miR-218 was shown to decrease with the progression of pancreatic cancer [33]. Working on the same line, Yu et al., reported altered expression of miRNAs with the pancreatic cancer progression [34]. In low-grade PanINs (PanIN-1 or PanIN-2), miR-133a, miR-151-5p, miR-148a/b, miR-34c-5p, miR-130b, miR-200c, miR-185, miR-331-3p/5p, miR-330-3p, miR-423-5p, miR-378, and miR-129-3p were significantly elevated, while miR-196b was found to be overexpressed in PanIN-3 lesions [34]. Morimura and colleagues identified a specific miRNA signature in the blood specimen of pancreatic cancer patients. They identified significantly higher levels of miR-20a, miR-18a, miR-155, miR-22, miR-21, miR-99a, miR-24, miR-185, miR-25, miR-885-5p, miR-191, miR-642b, and miR-196a in the blood of pancreatic cancer patients' as compared to that from normal donors [35]. miRNA expression analysis in fine-needle aspirates showed upregulation of miR-196a, miR-217, miR-451, and miR-486-5p with downregulation of let-7c, let-7d, let-7f, and miR-200c in pancreatic cancer [36]. Overall these studies suggest that miRNAs are aberrantly expressed in pancreatic cancer and their deregulation could influence the development and progression of pancreatic cancer.

Role of miRNAs in Pancreatic Cancer

Accumulating data over the past several years have defined important roles of miRNAs in pancreatic cancer pathobiology (Fig. 5.1). A number of miRNAs are known to promote early events of pancreatic carcinogenesis, while several others are involved in its metastatic progression and chemoresistance as discussed below.

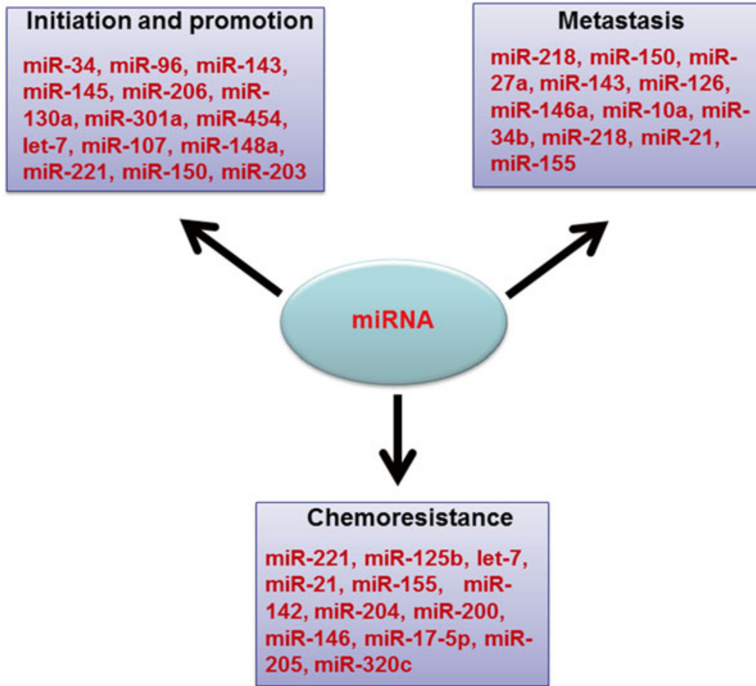

Fig. 5.1 Involvement of miRNAs in pancreatic cancer pathogenesis. Several miRNAs are demonstrated to be involved in pancreatic cancer cell initiation, promotion, metastasis and chemoresistance, and thus greatly impact this malignancy

miRNAs in Pancreatic Cancer Initiation and Promotion

There are several reports indicating a role of miRNAs in pancreatic cancer initiation and promotion. The levels of miR-34 were shown to be significantly downregulated in pancreatic cancer, and its overexpression influenced various processes such as angiogenesis, apoptosis, cell cycle progression, and even metastatic potential [37, 38]. Ji et al. also reported the role of miR-34 in tumor initiation by demonstrating that restoration of miR-34 resulted in the inhibition of tumor-initiating cells [38].

Ample amount of data is available that advocate the significance of miRNAs in controlling the pancreatic cancer growth. For example, Yu and coworkers reported that restoration of miR-96 resulted in K-Ras inhibition followed by pancreatic cancer cell death [39]. Forced expression of miR-143/145 decreased cell proliferation [40]. Furthermore, it was shown that expression level of miR-145 progressively decreased from precursor lesions to late PDAC, and restoration of miR-145 abrogated cell proliferation [41]. Keklikoglou and colleagues reported that restitution of miR-206 was enough to inhibit angiogenesis and tumor growth in pancreatic cancer [42]. Guo et al., reported that overexpression of tumor suppressor miR-410 led to inhibition of angiogenesis in pancreatic tumor model [43].

Moreover, inhibition of miR-21 and miR-210 was also shown to inhibit migration and invasion of pancreatic cancer cells and suppressed angiogenesis [44]. In another report, a role of miR-217 in inhibition of pancreatic cancer growth in vitro and in vivo was demonstrated [45]. Several other miRNAs, such as miR-130a, miR-301a, and miR-454, are also reported to be upregulated in pancreatic cancer and to negatively regulate SMAD4 expression to promote tumor growth [46, 47]. Let-7 is largely known to be either completely lost or downregulated in pancreatic cancer [48] and functional study demonstrated that the overexpression of let-7 caused the inhibition of pancreatic cancer cell proliferation through inhibiting MAPK and K-Ras signaling [48]. Tsuda et al. provided the evidence that synthetic miR-3548 retarded the growth of MiaPaCa-2 cells by targeting Gli-1 [49]. Cell cycle progression is indispensable for cancer cell growth and studies suggest that miRNAs regulate several cell-cycle-related proteins, such as cyclin-dependent kinase, CDK6 by miR-107 [50], CDC25B by miR-148a [51], and CDKN1B by miR-221 [52].

Transfection with inhibitor of miR-221 in pancreatic cancer cells upregulated CDKN1B, and suppressed their growth [53]. miR-424-5p is overexpressed in pancreatic cancer cells and reported to promote the proliferation and apoptosis resistance [54]. Recently, we also identified miR-150 to be a tumor suppressor miRNA in pancreatic cancer cells and demonstrated its tumor suppressor function. Restoration of miR-150 suppressed the growth and malignant potential of pancreatic cancer cells via downregulating MUC4 [27]. Xu et al. demonstrated that restoration of miR-203 caused apoptosis and cell cycle arrest [55]. Moreover, miR-203 also caused significant reduction in tumor growth [55]. Altogether, these reports clearly suggest that miRNAs are of important significance in pancreatic cancer initiation and progression.

miRNAs in Pancreatic Cancer Metastasis

Pancreatic cancer is a highly aggressive malignancy characterized by extensive near and distant metastasis. So far, several molecular targets have been identified that could potentially drive the aggressive nature of this malignancy. Recently, miRNAs have also gained significant attentions for their role in regulating the metastatic processes, and involved miRNAs are often referred as metastamiRs [6]. Two miRNAs, miRNA-218 and miR-155, has been shown to be associated with lymphatic metastasis of pancreatic cancer [33, 56]. Moreover, Mees and colleagues have reported that miR-194, miR-200b, miR-200c, and miR-429, which are overexpressed in highly metastatic pancreatic cancer cells, regulate metastasis by targeting metastatic suppressor gene EP300 [57]. Another study reported that miR-10a is an important mediator in pancreatic cancer metastasis and its repression effectively inhibits the invasion and metastasis of pancreatic tumor cells [58].

Ouyang et al. reported that increased level of miR-10b in pancreatic cancer made pancreatic tumor cells invasive by activating epidermal growth factor (EGF) and transforming growth factor-beta (TGF-β) signaling [59]. Another study identified

overexpression of miR-10b in pancreatic cancer, and suggested its role in the aggressive phenotype associated with metastasis [60]. Giovannetti and coworkers reported that patients exhibiting upregulated level of miR-21 had a shorter survival [61]. Moreover, miR-21 helped in the invasion and metastasis of pancreatic cancer cells by regulating matrix metalloproteinases and vascular endothelial growth factor [61]. Another study showed that deregulated miR-21 expression in pancreatic cancer was associated with high proliferation index and liver metastasis [62].

Kadera et al. also demonstrated the role of miRNAs in pancreatic tumor invasion and metastasis [63]. miRNA expression profile data suggested that levels of miR-100 was significantly higher in metastatic pancreatic cell lines as compared to non-metastatic ones suggesting its role in metastasis of pancreatic cancer [56]. Moreover, miR-146a was also reported to be significantly downregulated in pancreatic cancer, and its restoration inhibited the invasiveness of pancreatic cancer cells by suppressing the expression of EGFR and interleukin-1 receptor-associated kinase 1(IRAK-1) [64].

The expression of tumor suppressor miRNA, miR-143, is lost or downregulated in many cancers, including pancreatic cancer, and its overexpression in pancreatic cancer cells downregulated various genes associated with tumor growth and metastasis. Furthermore, findings suggested that the restoration of miR-143 blocked pancreatic cancer cell migration and invasion in vitro and inhibited liver metastasis and tumor growth in vivo [65]. Downregulation of miR-126 was shown to be a crucial event for attaining an invasive phenotype in PDAC and its re-expression decreased the pancreatic cancer invasiveness [66]. Similarly, Hamada et al. have reported a role of miR-126 in regulating pancreatic tumor invasiveness [67]. Decreased level of miR-34b is associated with tumor-node-metastasis stage and lymph-node metastasis [68]. Taken together, these studies clearly suggest critical roles of miRNAs in the metastatic progression of pancreatic cancer.

miRNAs in Chemoresistance of Pancreatic Cancer

The growing evidence suggests that miRNAs play important role in the chemoresistance of pancreatic cancer. Bhutia et al. demonstrated that overexpression of precursor-let-7 inhibited RRM2 level in pancreatic cancer cells and induced chemosensitization [69]. Similarly, preclinical data demonstrated that increased miR-211 expression in pancreatic cancer cells enhanced therapeutic efficacy of gemcitabine by reducing RRM2 level [70]. Recently, it was shown that miR-1246 promoted pancreatic cancer growth and induced drug resistance [71]. Furthermore, Singh and colleague observed the upregulation of miR-146 and downregulation of miR-205 and let-7 in gemcitabine-resistant pancreatic cancer cell lines and clinical samples, suggesting their involvement in the development of chemoresistance [72]. Another study regarding the role of miRNAs suggested that miR-17-5p was upregulated and imparted gemcitabine resistance to pancreatic cancer cells by targeting pro-apoptotic protein, Bim at the posttranscriptional level [73]. The inhibition of this miRNA

in Panc-1 and BxPC3 cells sensitized them to gemcitabine toxicity by inducing apoptosis [73]. miR-21 is also shown to play an important role in the chemoresistance of cancer cells.

A study carried out by Giovannett and colleagues showed that miR-21 was upregulated in gemcitabine-resistant pancreatic cancer cells, and it modulated the expression of genes associated with survival and invasion and sensitized the cells to gemcitabine therapy [61]. Moreover, this study also revealed that overexpression of miR-21 was associated with poor therapeutic outcome in pancreatic cancer patients treated with gemcitabine. Additional support for the role of miR-21 in chemoresistance came from a study in which it was shown to regulate the expression of anti-apoptotic BCL2 protein [74]. Another study reported that miR-125b was overexpressed in gemcitabine-resistant derivative cell line of BXPC3 (BxPC3-GZR) as well as in advanced PDAC samples and its inhibition sensitized the BxPC3-GZR to gemcitabine [75]. In another recent study, Khan et al. demonstrated that overexpression of miR-145 significantly enhanced the gemcitabine cytotoxicity through downregulation of MUC13 [41]. miR-155 is also reported to be overexpressed in pancreatic cancer, and gets upregulated in pancreatic cancer cells upon gemcitabine treatment further supporting its possible involvement in chemoresistance [25]. Another study revealed that re-expression of miR-200 sensitized pancreatic cancer cells to gemcitabine [28].

Moreover, miR-142-5p and miR-204 were also reported to be significantly downregulated in gemcitabine-resistant PDAC and established cell lines [76]. In a recent report, it was shown that expression level of miR-320c was significantly elevated in gemcitabine-resistant pancreatic cancer lines suggesting its role as a regulator of chemoresistance [77]. Overall, these studies suggest that miRNAs are involved in the chemoresistance in pancreatic cancer.

Opportunities for the Clinical Use of miRNAs in Pancreatic Cancer Management

The widely recognized hurdle in the treatment of pancreatic cancer is its late detection and the lack of effective therapies. Recent studies have provided considerable evidence showing the use of miRNA expression profiles in the diagnosis and prognosis of pancreatic cancer; moreover, miRNAs are also emerging as promising targets for cancer therapy as shown in Fig. 5.2. Below we discuss related data on these aspects to understand the clinical potential of miRNAs in pancreatic cancer.

miRNAs as Diagnostic Markers

It is now becoming evident that miRNAs have the potential to be utilized as biomarkers for cancer diagnosis. For example, certain miRNAs such as miR-376a, miR-301, miR-155, miR-21, miR-221, and miR-222 are overexpressed in pancreatic

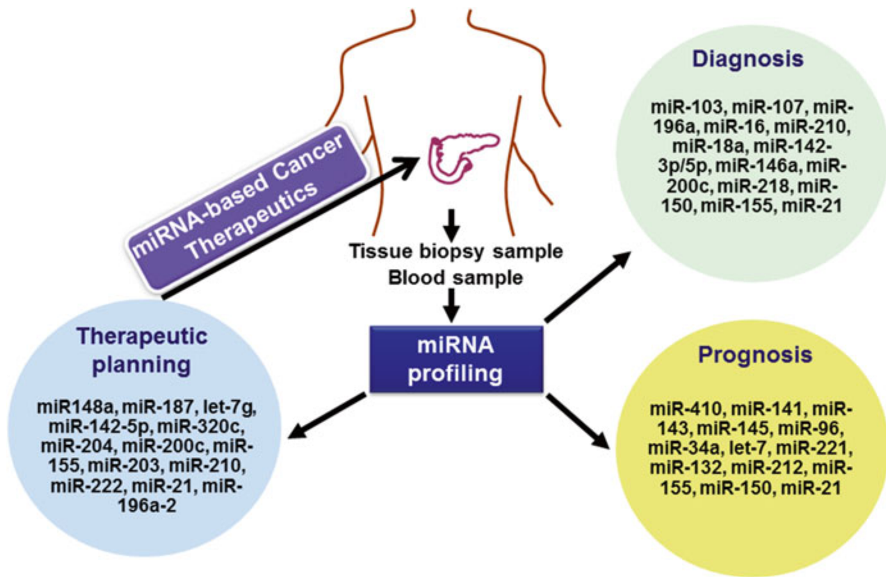

Fig. 5.2 Clinical significance of miRNAs in pancreatic cancer. Specific expression pattern of miRNAs could be used for diagnosis and prognosis of pancreatic cancer. miRNAs could also be used for pancreatic cancer therapy by restoration of tumor suppressor miRNAs or inhibition of oncogenic miRNAs

cancer and their expression is restricted to tumor cells only with no expression in normal acini or ducts [25]. Moreover, differential expression of miR-96 [39], miR-34a [38], and miR-21 [78] was shown to accurately discriminate between pancreatic cancer and normal adjacent tissue. Another expression analysis study between normal pancreas and PDAC suggested that the presence of miR-216 and miR-217 and absence of miR-133a is unique for healthy pancreas [29]. On the other hand, increased expression of miR-103 and miR-107 with low expression of miR-155 is also another signature profile for pancreatic tumors [62].

Bloomston et al. successfully identified 21 overexpressed and 4 downregulated miRNAs in pancreatic cancer, which correctly differentiated 90 % of the malignant cases from the normal tissue. Similarly, using a subset of 15 overexpressed and 8 downregulated miRNAs, they could also accurately discriminate between 93 % of samples as chronic pancreatitis and pancreatic cancer [29].

Furthermore, Szafranska et al. [79] identified 20 additional miRNAs that were able to discriminate pancreatic cancer from chronic pancreatic diseases and normal pancreas. The expression analysis of miR-196a and miR-217 in fine-needle aspirates categorized malignant pancreatic cancer from benign lesions [80]. Later, Kong et al. [81] observed elevated level of miR-196a in the serum samples of pancreatic cancer compared with control groups. This study led to the identification of serum

miR-196a as a potential marker for pancreatic cancer and selection for laparotomy [81]. Yu et al. [34] identified a signature of 35 miRNAs in PanIN-3 lesion, and miR-196b was found to be the best biomarker for detection of these lesions.

Kawaguchi et al. [52] found that pancreatic cancer patients with higher plasma concentration of miR-221 exhibited significant correlation with distant metastasis. In a study measuring 735 circulating miRNAs in pancreatic cancer and control sera, miR-1290 was demonstrated to exhibit the best diagnostic performance among other upregulated circulating miRNAs [82]. Lewis blood group antigen CA19-9 is currently being widely used as standard serum marker for the identification of pancreatic cancer. However, its utilization is limited to monitor response to therapy and it is not a sensitive or specific marker for diagnosis [83, 84].

Interestingly, combination of miR-16 and miR-196a with CA19-9 was demonstrated to be more accurate in discriminating pancreatic cancer from normal tissue with a sensitivity and specificity of 92.0 % and 95.6 %, respectively. Habbe et al. [85] identified miR-155 to be the potential biomarker for detecting early stage of pancreatic cancer. Altogether, these studies highlight the potential of miRNAs to be used as a valuable tool for discriminating pancreatic tumors from normal pancreas and classifying the tumor stage and grade, either alone or in combination with other biomarkers.

miRNAs in Prognostic Assessment

With accumulating data, it is now becoming more obvious that apart from the significance of miRNAs in pancreatic cancer diagnosis, they can also be utilized as potential prognostic biomarkers. An elevated level of miR-21 was shown to be associated with poor therapeutic outcome in patients undergoing gemcitabine therapy [61]. Furthermore, overexpression of miR-21 in PDAC is reported to be correlated with the shorter overall survival in node negative patients and is strongly associated with liver metastasis [78]. Interestingly, patients with low miR-21 expression have been observed to benefit from gemcitabine treatment [86]. Ohuchida et al. [87] demonstrated downregulated expression of miR-204 and miR-142-5p in gemcitabine-resistant pancreatic tumor samples; furthermore, they identified a positive correlation of these miRNAs with prolonged survival of patients with pancreatic cancer. Thus, miR-142-5p was identified to be a predictive marker for gemcitabine response.

Bloomston et al. [29] demonstrated that a set of six miRNAs could distinguish long-term survivors with node-positive disease dying within 2 years. Furthermore, their study suggested that, high miR-196a-2 level could predict poor survival. Others have shown that overexpression of miR-155, miR-200, miR-203, miR-205 [81], miR-212, and miR-675 [31] miR-200c [80], miR-21 [54], and reduced expression of miR-34a, miR-30d [54], miR-130b [88], miR-148a, miR-187 and let-7g [87] in PDAC are associated with poorer survival rate. Ikenaga et al. identified miR-203 as a new prognostic marker of pancreatic adenocarcinoma patients, who underwent

resection [89]. Like miR-21, the expression of miR-155, miR-196a, and miR-10b was also correlated with enhanced invasiveness and poor overall survival of pancreatic cancer patients [60]. Moreover, poor prognosis of pancreatic cancer was also observed in patients expressing high levels of miR-17-5p clusters. Together, these studies highlight the significance of miRNAs in pancreatic cancer prognosis.

miRNAs as Therapeutic Targets

Extensive miRNA-based preclinical studies have been conducted in pancreatic cancer to clearly highlight the role of miRNAs in the initiation, progression, and chemoresistance. Thus, there is wide scope to exploit miRNAs for the development of novel therapeutic strategies against pancreatic cancer. For example, nanoparticle-based delivery of miR-143, miR-145, or miR-34a in mouse model of pancreatic cancer significantly inhibited the tumor growth [90]. Inhibition of miR-21 and miR-221 using antisense approach was also shown to enhance gemcitabine cytotoxicity in pancreatic cancer cells [91]. Similarly, repression of miR-10a in pancreatic cancer cells was able to inhibit tumor growth and metastasis [58]. In another study, it was demonstrated that inhibition of miR-132 and miR-212 by antisense miRNA oligonucleotides decreased the pancreatic tumor growth [92].

Restoration of a tumor suppressor miR-204 resulted in the downregulation of Mcl-1 and caused pancreatic cancer cell death [76]. Similarly, Yan et al. demonstrated that the restoration of miR-20a could potentially downregulate Stat3 at the posttranscriptional level leading to the inhibition of cell proliferation of pancreatic carcinoma [93]. Furthermore, viral vector-mediated delivery of miR-145 or miR-143 effectively inhibited pancreatic cancer development [40]. Hu et al. using adenovirus-mediated delivery of miR-143 demonstrated significant reduction of cancer metastasis [65]. Moreover, we also demonstrated that delivery of miR-150 mimics significantly inhibited pancreatic cancer cell growth and metastatic potential [27]. Similarly, restoration of tumor suppressor let-7 miRNA in cancer-derived cell lines strongly reduced their proliferation via downregulating K-Ras and mitogen-activated protein kinase activation [48]. Yu et al. [39] demonstrated that the delivery of miR-96 mimics inhibited in vivo tumorigenesis [39].

Wang and colleague reported that miR-23b is downregulated in radioresistant pancreatic cancer cells and restoration of miR-23b sensitized pancreatic cancer cells to radiation therapy [94]. Another study demonstrated that re-expression of tumor suppressor miR-141 in pancreatic cancer cells blocked tumor cell growth, invasion, clonogenicity, and increased chemosensitivity [95]. Recently, Guo et al. demonstrated that the delivery of miR-410 mimics inhibited tumor formation in xenograft mouse model [43]. Delivery of anti-miR-21 and anti-miR-221 oligonucleotides inhibited the growth and sensitized pancreatic tumor cells to anticancer drugs, 5-Fluorouracil, and gemcitabine [96]. Thus, these studies provide strong rationale towards the exploitation of miRNAs as effective therapeutic targets for the treatment of pancreatic cancer.

Conclusion and Future Perspectives

microRNAs have undoubtedly established themselves as a novel class of gene regulators, and accumulating data support their roles in multiple biological processes. In fact, what we know today may just be the glimpse of pleiotropic functions that miRNAs can perform, and coming era may unfold many new discoveries. Pancreatic cancer remains a highly lethal malignancy lacking in almost all areas (diagnosis, prognosis, and therapy) of clinical management. Therefore, continued identification of novel, differentially expressed miRNAs and delineation of their important pathobiological functions may open up new possibilities for early and specific diagnosis as well as provide future cancer therapeutics for this devastating malignancy.

We would need to develop clinically feasible, cost-effective systems for sensitive detection of miRNAs to realize miRNA-based cancer diagnosis. Restoration or inhibition of miRNAs involved in malignant cancer phenotypes and chemoresistance though innovative approaches would also be required to enable powerful and effective miRNA-based therapeutic strategies against pancreatic cancer.

Clearly, interest in miRNA research continues to grow in all branches of biological sciences and have encouraged interdisciplinary collaborations to exploit their utility for human health applications. Remarkable progress thus far has built a strong foundation to forthcoming clinical and translational research and the diagnostic/prognostic tools and therapies that will emerge in near future. The expectation is that we will be able to curb pancreatic cancer-related deaths by developing improved understanding of its biology and identification of novel therapeutic targets. The existing data thus far provide great hope from these small, functionally involved biomolecules to prominently impact pancreatic cancer management.

Acknowledgements Grant support: NIH/NCI [CA137513, CA167137, CA175772, CA185490 (to APS) and CA169829 (to SS)], DOD/US Army [PC110545 and PC0739930 (to APS)] and USAMCI. Conflicts of Interest: No potential conflict of interest to disclose.

References

1. Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol Med. 2012;4:143–59.
2. Jansson MD, Lund AH. MicroRNA and cancer. Mol Oncol. 2012;6:590–610.
3. Friedlander MR, Lizano E, Houben AJ, et al. Evidence for the biogenesis of more than 1,000 novel human microRNAs. Genome Biol. 2014;15:R57.
4. Jin Y, Chen Z, Liu X, Zhou X. Evaluating the microRNA targeting sites by luciferase reporter gene assay. Methods Mol Biol. 2013;936:117–27.
5. Saetrom P, Heale BS, Snove Jr O, Aagaard L, Alluin J, Rossi JJ. Distance constraints between microRNA target sites dictate efficacy and cooperativity. Nucleic Acids Res. 2007;35:2333–42.
6. Srivastava SK, Arora S, Singh S, Bhardwaj A, Averett C, Singh AP. MicroRNAs in pancreatic malignancy: progress and promises. Cancer Lett. 2014;347:167–74.

7. Bhardwaj A, Singh S, Singh AP. MicroRNA-based cancer therapeutics: big hope from small RNAs. Mol Cell Pharmacol. 2010;2:213–9.
8. Von Hoff DD, Korn R, Mousses S. Pancreatic cancer--could it be that simple? A different context of vulnerability. Cancer Cell. 2009;16:7–8.
9. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res. 2014;74:2913–21.
10. Ozdemir BC, Pentcheva-Hoang T, Carstens JL, et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. Cancer Cell. 2014;25:719–34.
11. Tyagi N, Bhardwaj A, Singh AP, McClellan S, Carter JE, Singh S. p-21 activated kinase 4 promotes proliferation and survival of pancreatic cancer cells through AKT- and ERK-dependent activation of NF-kappaB pathway. Oncotarget. 2014;5:8778–89.
12. Rachagani S, Macha MA, Heimann N, et al. Clinical implications of miRNAs in the pathogenesis, diagnosis and therapy of pancreatic cancer. Adv Drug Deliv Rev. 2015;81:16–33.
13. Omura N, Goggins M. Epigenetics and epigenetic alterations in pancreatic cancer. Int J Clin Exp Pathol. 2009;2:310–26.
14. Arora S, Bhardwaj A, Singh S, et al. An undesired effect of chemotherapy: gemcitabine promotes pancreatic cancer cell invasiveness through reactive oxygen species-dependent, nuclear factor kappaB- and hypoxia-inducible factor 1alpha-mediated up-regulation of CXCR4. J Biol Chem. 2013;288:21197–207.
15. Hruban RH, Takaori K, Canto M, et al. Clinical importance of precursor lesions in the pancreas. J Hepatobiliary Pancreat Surg. 2007;14:255–63.
16. Hruban RH, Fukushima N. Pancreatic adenocarcinoma: update on the surgical pathology of carcinomas of ductal origin and PanINs. Mod Pathol. 2007;20 Suppl 1:S61–70.
17. Kaur S, Baine MJ, Jain M, Sasson AR, Batra SK. Early diagnosis of pancreatic cancer: challenges and new developments. Biomark Med. 2012;6:597–612.
18. Nentwich MF, Konig A, Izbicki JR. Limits of surgery for pancreatic cancer. Rozhl Chir. 2014;93:445–9.
19. Olive KP, Jacobetz MA, Davidson CJ, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science. 2009;324:1457–61.
20. Moore MJ, Goldstein D, Hamm J, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol. 2007;25:1960–6.
21. Colucci G, Labianca R, Di CF, et al. Randomized phase III trial of gemcitabine plus cisplatin compared with single-agent gemcitabine as first-line treatment of patients with advanced pancreatic cancer: the GIP-1 study. J Clin Oncol. 2010;28:1645–51.
22. Conroy T, Gavoille C, Samalin E, Ychou M, Ducreux M. The role of the FOLFIRINOX regimen for advanced pancreatic cancer. Curr Oncol Rep. 2013;15:182–9.
23. Von Hoff DD, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med. 2013;369:1691–703.
24. Poy MN, Eliasson L, Krutzfeldt J, et al. A pancreatic islet-specific microRNA regulates insulin secretion. Nature. 2004;432:226–30.
25. Lee EJ, Gusev Y, Jiang J, et al. Expression profiling identifies microRNA signature in pancreatic cancer. Int J Cancer. 2007;120:1046–54.
26. Zhang Y, Li M, Wang H, et al. Profiling of 95 microRNAs in pancreatic cancer cell lines and surgical specimens by real-time PCR analysis. World J Surg. 2009;33:698–709.
27. Srivastava SK, Bhardwaj A, Singh S, et al. MicroRNA-150 directly targets MUC4 and suppresses growth and malignant behavior of pancreatic cancer cells. Carcinogenesis. 2011;32:1832–9.
28. Ali S, Ahmad A, Banerjee S, et al. Gemcitabine sensitivity can be induced in pancreatic cancer cells through modulation of miR-200 and miR-21 expression by curcumin or its analogue CDF. Cancer Res. 2010;70:3606–17.

29. Bloomston M, Frankel WL, Petrocca F, et al. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. JAMA. 2007;297:1901–8.
30. Olson P, Lu J, Zhang H, et al. MicroRNA dynamics in the stages of tumorigenesis correlate with hallmark capabilities of cancer. Genes Dev. 2009;23:2152–65.
31. Ohuchida K, Mizumoto K, Lin C, et al. MicroRNA-10a is overexpressed in human pancreatic cancer and involved in its invasiveness partially via suppression of the HOXA1 gene. Ann Surg Oncol. 2012;19:2394–402.
32. Ryu JK, Hong SM, Karikari CA, Hruban RH, Goggins MG, Maitra A. Aberrant MicroRNA-155 expression is an early event in the multistep progression of pancreatic adenocarcinoma. Pancreatology. 2010;10:66–73.
33. He H, Di Y, Liang M, et al. The microRNA-218 and ROBO-1 signaling axis correlates with the lymphatic metastasis of pancreatic cancer. Oncol Rep. 2013;30:651–8.
34. Yu J, Li A, Hong SM, Hruban RH, Goggins M. MicroRNA alterations of pancreatic intraepithelial neoplasias. Clin Cancer Res. 2012;18:981–92.
35. Morimura R, Komatsu S, Ichikawa D, et al. Novel diagnostic value of circulating miR-18a in plasma of patients with pancreatic cancer. Br J Cancer. 2011;105:1733–40.
36. Ali S, Saleh H, Sethi S, Sarkar FH, Philip PA. MicroRNA profiling of diagnostic needle aspirates from patients with pancreatic cancer. Br J Cancer. 2012;107:1354–60.
37. Chang TC, Wentzel EA, Kent OA, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell. 2007;26:745–52.
38. Ji Q, Hao X, Zhang M, et al. MicroRNA miR-34 inhibits human pancreatic cancer tumor-initiating cells. PLoS One. 2009;4, e6816.
39. Yu S, Lu Z, Liu C, et al. miRNA-96 suppresses KRAS and functions as a tumor suppressor gene in pancreatic cancer. Cancer Res. 2010;70:6015–25.
40. Kent OA, Chivukula RR, Mullendore M, et al. Repression of the miR-143/145 cluster by oncogenic Ras initiates a tumor-promoting feed-forward pathway. Genes Dev. 2010;24:2754–9.
41. Khan S, Ebeling MC, Zaman MS, et al. MicroRNA-145 targets MUC13 and suppresses growth and invasion of pancreatic cancer. Oncotarget. 2014;5:7599–609.
42. Keklikoglou I, Hosaka K, Bender C, et al. MicroRNA-206 functions as a pleiotropic modulator of cell proliferation, invasion and lymphangiogenesis in pancreatic adenocarcinoma by targeting ANXA2 and KRAS genes. Oncogene. 2014. doi:10.1038/onc.2014.408.
43. Guo R, Gu J, Zhang Z, Wang Y, Gu C. MicroRNA-410 functions as a tumor suppressor by targeting angiotensin II type 1 receptor in pancreatic cancer. IUBMB Life. 2015;67(1):42–53. doi:10.1002/iub.1342.
44. Bao B, Ali S, Ahmad A, et al. Hypoxia-induced aggressiveness of pancreatic cancer cells is due to increased expression of VEGF, IL-6 and miR-21, which can be attenuated by CDF treatment. PLoS One. 2012;7, e50165.
45. Zhao WG, Yu SN, Lu ZH, Ma YH, Gu YM, Chen J. The miR-217 microRNA functions as a potential tumor suppressor in pancreatic ductal adenocarcinoma by targeting KRAS. Carcinogenesis. 2010;31:1726–33.
46. Lu Z, Li Y, Takwi A, et al. miR-301a as an NF-kappaB activator in pancreatic cancer cells. EMBO J. 2011;30:57–67.
47. Liu L, Nie J, Chen L, et al. The oncogenic role of microRNA-130a/301a/454 in human colorectal cancer via targeting Smad4 expression. PLoS One. 2013;8, e55532.
48. Torrisani J, Bournet B, du Rieu MC, et al. let-7 MicroRNA transfer in pancreatic cancer-derived cells inhibits in vitro cell proliferation but fails to alter tumor progression. Hum Gene Ther. 2009;20:831–44.
49. Tsuda N, Ishiyama S, Li Y, Ioannides CG, Abbruzzese JL, Chang DZ. Synthetic microRNA designed to target glioma-associated antigen 1 transcription factor inhibits division and induces late apoptosis in pancreatic tumor cells. Clin Cancer Res. 2006;12:6557–64.
50. Lee KH, Lotterman C, Karikari C, et al. Epigenetic silencing of MicroRNA miR-107 regulates cyclin-dependent kinase 6 expression in pancreatic cancer. Pancreatology. 2009;9:293–301.

51. Zhang R, Li M, Zang W, et al. MiR-148a regulates the growth and apoptosis in pancreatic cancer by targeting CCKBR and Bcl-2. Tumour Biol. 2014;35:837–44.
52. Kawaguchi T, Komatsu S, Ichikawa D, et al. Clinical impact of circulating miR-221 in plasma of patients with pancreatic cancer. Br J Cancer. 2013;108:361–9.
53. Sarkar S, Dubaybo H, Ali S, et al. Down-regulation of miR-221 inhibits proliferation of pancreatic cancer cells through up-regulation of PTEN, p27(kip1), p57(kip2), and PUMA. Am J Cancer Res. 2013;3:465–77.
54. Wu K, Hu G, He X, et al. MicroRNA-424-5p suppresses the expression of SOCS6 in pancreatic cancer. Pathol Oncol Res. 2013;19:739–48.
55. Xu D, Wang Q, An Y, Xu L. MiR203 regulates the proliferation, apoptosis and cell cycle progression of pancreatic cancer cells by targeting Survivin. Mol Med Rep. 2013;8:379–84.
56. Huang C, Li H, Wu W, Jiang T, Qiu Z. Regulation of miR-155 affects pancreatic cancer cell invasiveness and migration by modulating the STAT3 signaling pathway through SOCS1. Oncol Rep. 2013;30:1223–30.
57. Mees ST, Mardin WA, Wendel C, et al. EP300—a miRNA-regulated metastasis suppressor gene in ductal adenocarcinomas of the pancreas. Int J Cancer. 2010;126:114–24.
58. Weiss FU, Marques IJ, Woltering JM, et al. Retinoic acid receptor antagonists inhibit miR-10a expression and block metastatic behavior of pancreatic cancer. Gastroenterology. 2009;137:2136–45.
59. Ouyang H, Gore J, Deitz S, Korc M. microRNA-10b enhances pancreatic cancer cell invasion by suppressing TIP30 expression and promoting EGF and TGF-beta actions. Oncogene. 2014;33:4664–74.
60. Nakata K, Ohuchida K, Mizumoto K, et al. MicroRNA-10b is overexpressed in pancreatic cancer, promotes its invasiveness, and correlates with a poor prognosis. Surgery. 2011;150:916–22.
61. Giovannetti E, Funel N, Peters GJ, et al. MicroRNA-21 in pancreatic cancer: correlation with clinical outcome and pharmacologic aspects underlying its role in the modulation of gemcitabine activity. Cancer Res. 2010;70:4528–38.
62. Roldo C, Missiaglia E, Hagan JP, et al. MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. J Clin Oncol. 2006;24:4677–84.
63. Kadera BE, Li L, Toste PA, et al. MicroRNA-21 in pancreatic ductal adenocarcinoma tumor-associated fibroblasts promotes metastasis. PLoS One. 2013;8, e71978.
64. Li Y, Vandenboom TG, Wang Z, et al. miR-146a suppresses invasion of pancreatic cancer cells. Cancer Res. 2010;70:1486–95.
65. Hu Y, Ou Y, Wu K, Chen Y, Sun W. miR-143 inhibits the metastasis of pancreatic cancer and an associated signaling pathway. Tumour Biol. 2012;33:1863–70.
66. Frampton AE, Krell J, Jacob J, Stebbing J, Castellano L, Jiao LR. Loss of miR-126 is crucial to pancreatic cancer progression. Expert Rev Anticancer Ther. 2012;12:881–4.
67. Hamada S, Satoh K, Fujibuchi W, et al. MiR-126 acts as a tumor suppressor in pancreatic cancer cells via the regulation of ADAM9. Mol Cancer Res. 2012;10:3–10.
68. Liu C, Cheng H, Shi S, et al. MicroRNA-34b inhibits pancreatic cancer metastasis through repressing Smad3. Curr Mol Med. 2013;13:467–78.
69. Bhutia YD, Hung SW, Krentz M, et al. Differential processing of let-7a precursors influences RRM2 expression and chemosensitivity in pancreatic cancer: role of LIN-28 and SET oncoprotein. PLoS One. 2013;8, e53436.
70. Maftouh M, Avan A, Funel N, et al. miR-211 modulates gemcitabine activity through downregulation of ribonucleotide reductase and inhibits the invasive behavior of pancreatic cancer cells. Nucleosides Nucleotides Nucleic Acids. 2014;33:384–93.
71. Hasegawa S, Eguchi H, Nagano H, et al. MicroRNA-1246 expression associated with CCNG2-mediated chemoresistance and stemness in pancreatic cancer. Br J Cancer. 2014;111:1572–80.
72. Singh S, Chitkara D, Kumar V, Behrman SW, Mahato RI. miRNA profiling in pancreatic cancer and restoration of chemosensitivity. Cancer Lett. 2013;334:211–20.

73. Yan HJ, Liu WS, Sun WH, et al. miR-17-5p inhibitor enhances chemosensitivity to gemcitabine via upregulating Bim expression in pancreatic cancer cells. Dig Dis Sci. 2012;57:3160–7.
74. Dong J, Zhao YP, Zhou L, Zhang TP, Chen G. Bcl-2 upregulation induced by miR-21 via a direct interaction is associated with apoptosis and chemoresistance in MIA PaCa-2 pancreatic cancer cells. Arch Med Res. 2011;42:8–14.
75. Bera A, VenkataSubbaRao K, Manoharan MS, Hill P, Freeman JW. A miRNA signature of chemoresistant mesenchymal phenotype identifies novel molecular targets associated with advanced pancreatic cancer. PLoS One. 2014;9, e106343.
76. Chen Z, Sangwan V, Banerjee S, et al. miR-204 mediated loss of Myeloid cell leukemia-1 results in pancreatic cancer cell death. Mol Cancer. 2013;12:105–12.
77. Iwagami Y, Eguchi H, Nagano H, et al. miR-320c regulates gemcitabine-resistance in pancreatic cancer via SMARCC1. Br J Cancer. 2013;109:502–11.
78. Dillhoff M, Liu J, Frankel W, Croce C, Bloomston M. MicroRNA-21 is overexpressed in pancreatic cancer and a potential predictor of survival. J Gastrointest Surg. 2008;12:2171–6.
79. Szafranska AE, Davison TS, John J, et al. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. Oncogene. 2007;26:4442–52.
80. Szafranska AE, Doleshal M, Edmunds HS, et al. Analysis of microRNAs in pancreatic fine-needle aspirates can classify benign and malignant tissues. Clin Chem. 2008;54:1716–24.
81. Kong X, Du Y, Wang G, et al. Detection of differentially expressed microRNAs in serum of pancreatic ductal adenocarcinoma patients: miR-196a could be a potential marker for poor prognosis. Dig Dis Sci. 2011;56:602–9.
82. Li A, Yu J, Kim H, et al. MicroRNA array analysis finds elevated serum miR-1290 accurately distinguishes patients with low-stage pancreatic cancer from healthy and disease controls. Clin Cancer Res. 2013;19:3600–10.
83. Goggins M. Identifying molecular markers for the early detection of pancreatic neoplasia. Semin Oncol. 2007;34:303–10.
84. Gold DV, Modrak DE, Ying Z, Cardillo TM, Sharkey RM, Goldenberg DM. New MUC1 serum immunoassay differentiates pancreatic cancer from pancreatitis. J Clin Oncol. 2006;24:252–8.
85. Habbe N, Koorstra JB, Mendell JT, et al. MicroRNA miR-155 is a biomarker of early pancreatic neoplasia. Cancer Biol Ther. 2009;8:340–6.
86. Hwang JH, Voortman J, Giovannetti E, et al. Identification of microRNA-21 as a biomarker for chemoresistance and clinical outcome following adjuvant therapy in resectable pancreatic cancer. PLoS One. 2010;5, e10630.
87. Ohuchida K, Mizumoto K, Kayashima T, et al. MicroRNA expression as a predictive marker for gemcitabine response after surgical resection of pancreatic cancer. Ann Surg Oncol. 2011;18:2381–7.
88. Zhang Y, Yang J, Cui X, et al. A novel epigenetic CREB-miR-373 axis mediates ZIP4-induced pancreatic cancer growth. EMBO Mol Med. 2013;5:1322–34.
89. Ikenaga N, Ohuchida K, Mizumoto K, et al. MicroRNA-203 expression as a new prognostic marker of pancreatic adenocarcinoma. Ann Surg Oncol. 2010;17:3120–8.
90. Pramanik D, Campbell NR, Karikari C, et al. Restitution of tumor suppressor microRNAs using a systemic nanovector inhibits pancreatic cancer growth in mice. Mol Cancer Ther. 2011;10:1470–80.
91. Park JK, Lee EJ, Esau C, Schmittgen TD. Antisense inhibition of microRNA-21 or -221 arrests cell cycle, induces apoptosis, and sensitizes the effects of gemcitabine in pancreatic adenocarcinoma. Pancreas. 2009;38:e190–9.
92. Park JK, Henry JC, Jiang J, et al. miR-132 and miR-212 are increased in pancreatic cancer and target the retinoblastoma tumor suppressor. Biochem Biophys Res Commun. 2011;406:518–23.

93. Yan H, Wu J, Liu W, et al. MicroRNA-20a overexpression inhibited proliferation and metastasis of pancreatic carcinoma cells. Hum Gene Ther. 2010;21:1723–34.
94. Wang P, Zhang J, Zhang L, et al. MicroRNA 23b regulates autophagy associated with radioresistance of pancreatic cancer cells. Gastroenterology. 2013;145:1133–43.
95. Zhao G, Wang B, Liu Y, et al. miRNA-141, downregulated in pancreatic cancer, inhibits cell proliferation and invasion by directly targeting MAP4K4. Mol Cancer Ther. 2013;12:2569–80.
96. Zhao Y, Zhao L, Ischenko I, et al. Antisense inhibition of microRNA-21 and microRNA-221 in tumor-initiating stem-like cells modulates tumorigenesis, metastasis, and chemotherapy resistance in pancreatic cancer. Target Oncol. 2015. doi 10.1007/s11523-015-0360-2.

Chapter 6
microRNAs and Colorectal Cancer

Anna Lena Ress, Samantha Perakis, and Martin Pichler

Abstract Colorectal cancer (CRC) is one of the most common types of human cancer with high cancer-related morbidity and mortality rates. The development and clinical validation of novel therapeutic avenues have improved the clinical outcome, but metastatic CRC still remains an incurable disease in most cases. The interest in discovering novel pathophysiological drivers in CRC is intensively ongoing and the search for novel biomarkers for early diagnosis, for patient's stratification for prognostic purposes or for predicting treatment response are warranted. microRNAs are small RNA molecules that regulate the expression of larger messenger RNA species by different mechanisms with the final consequence to provide a fine tuning tool for global gene expression patterns. First discovered in worms, around 15 years ago it became clear that microRNAs are also existing in humans and that they are widely involved in human carcinogenesis. Within the last years, tremendous progress in the understanding of microRNAs and their role in CRC carcinogenesis has been developed. In this book chapter, several examples of previously identified microRNAs and how they influence colorectal carcinogenesis will be discussed. The information starting at the underlying molecular mechanisms towards clinical applications will be depicted and an overview what great potential these small molecules might carry in future colorectal cancer medicine, will be discussed.

Keywords microRNAs • Colorectal cancer • Prognosis • Carcinogenesis

A.L. Ress • S. Perakis
Division of Oncology, Medical University of Graz, Graz, Austria

M. Pichler (✉)
Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
e-mail: mpichler@mdanderson.org

© Springer International Publishing Switzerland 2015

G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5_6

Introduction

In their seminal review paper "The hallmarks of Cancer," D. Hanahan and R. Weinberg described the traits that most forms of cancer have in common [1]. Colorectal cancer (CRC) is determined by these hallmarks, which were later expanded upon in 2011 to include the so-called *emerging hallmark* capabilities. The exact role of the regulatory circuits regulated by epigenetic mechanisms including microRNAs (miRNAs) in the context of the eight hallmarks has yet to be determined. However, at present, dozens of regulatory miRNAs have been explored and discovered and almost all of them demonstrate differential expression and effects that are involved in the pathogenesis of CRC [2].

In general, CRC is the third most commonly diagnosed cancer and the second leading cause of cancer-related death. The high mortality rates are mainly related to the development of metastatic disease, which is associated with only a 12 % 5-year survival rate [3]. Therapeutic decision-making mainly relies on the tumor stage, ranging from surgical treatment alone for localized stages to a multimodal treatment strategy in advanced forms. For several years now, the therapeutic backbone of all current treatment schedules has contained 5-fluorouracil and it is frequently used in combination with oxaliplatin or irinotecan [4], both classical cytotoxic chemotherapeutic drugs. In addition, within the last 10 years monoclonal antibodies against the vascular endothelial growth factor-A (bevacizumab) or the epidermal growth factor receptor (cetuximab and panitumumab), as well as protein-traps (aflibercept) or small molecules (regorafnib) against other soluble or membrane-located angiogenic factors have extended the progression-free and overall survival time for CRC patients [5–7].

In the following pages, we summarize the involvement of miRNAs in the pathogenesis of CRC, relating them to the hallmarks of cancer and highlighting their potential clinical utility in CRC patients. There are different modes of miRNA functioning described in the literature. Apart from the basic approach of a single miRNAs -mRNA interaction, there is the broad approach of one miRNA influencing many mRNAs. Additionally, not only are miRNAs able to target transcripts, but they can also interact with other functionally related miRNAs to obtain certain effects. Adding to their complexity, miRNAs can belong to a cluster of miRNAs which regulates similar transcription factors and target several proteins within a defined pathway [8]. Knowing that miRNAs function in a network explains the involvement of one miRNA in many pathways as well as its ability to cause various effects as demonstrated in the different following topics. In the following sections, our main goal is to exemplify the involvement of miRNAs in CRC; however, these examples are by no means exhaustive.

MiRNAs and Their Relationship to the Hallmarks of Cancer

Regulation of Cell Proliferation and Evasion of Growth Suppressors

The most fundamental characterization of cancer cells is their ability of uncontrolled proliferation. As the growth of normal cells is tightly controlled, healthy cells are able to influence cell growth and antiproliferative signals through strict cell

cycle regulations in order to guarantee a normal tissue architecture and function. Malfunction of this fundamental process can happen when, for example, tumor suppressor genes become deactivated. MiRNAs can function as tumor suppressor-targeting oncomirs. Among other oncogenes, K-ras is the most commonly dysregulated proto-oncogene in CRC patients and influences cell proliferation, but it is also prone as a target for let-7a, mir-143, and mir-133 [9]. Another example of a K-ras-targeting miRNA, which thereby influences cell growth in CRC, is miR-96-5p [10]. Elevated miR-21 expression leads to increased cell proliferation and is one of the most important oncogenic miRNAs that is relevant in most cancer types [11]. DHFR, directly targeted by miR-24, miR-192, and miR-215, is an S-phase-specific enzyme, converting dihydrofolate to tetrahydrofolate. This is essential for the synthesis of purin and thymidylate and partly influences cell proliferation [12]. MiR-145 affects cell growth and its tumor suppressor activity is mediated through different targets. These include the inhibition of the oncogenic insulin receptor substrate-1 (IRS-1), c-Myc, Yamaguchi sarcoma viral oncogene homolog 1 (YES1), signal transducer and activator of transcription (STAT1) and, as recently reported, Friend leukemia integration 1 (FLI1) [13].

MiR-30a-5p suppresses tumor growth in colon carcinoma by affecting denticleless homolog (DTL) [14]. By influencing macrophage migration inhibitory factor (MIF), upregulated miR-451 leads to reduced proliferation in gastrointestinal cancer [15]. MiR-675 influences CRC tumor growth via downregulation of its target pRB [16]. In summary, miRNAs that influence this hallmark can be useful as prognostic factors or as therapeutic targets to improve clinical outcomes in CRC patients.

Evasion of Apoptosis

Apoptosis is the process of programmed cell death that occurs in multicellular organisms, regulated by pro- and anti-apoptotic factors. Cancer cells can interrupt this controlled process of cell death and thereby promote cell survival. The central apoptosis-regulating gene in the miRNA-protein-coding gene network of CRC is the anti-apoptotic factor Bcl-2. Bcl-2 is directly targeted by the tumor suppressors miR-195, miR-129, miR-365, and miR-143 [17–20]. Other suppressors of Bcl-2 include miR-34a and BAG3 (Bcl-2-associated athanogene 3), which is linked with miR-345 and miR-491 and which is also promoted by the frequently overexpressed BCL-xl [21–23]. The central miRNAs for apoptosis are considered to be those of the miR-17-92 cluster, particularly oncomir-1, which has wide influence in CRC apoptosis processes. MiR-17 and miR-92 take part in a negative feedback loop with the E2F1/3 transcription factors, including c-Myc. Also, the BcL-2 like 11 gene, the proapoptotic factor BNIP2 and BIM are putative targets of miR-17-92 and influence apoptosis in CRC [24–26]. The expression of the oncomir miR-21 is supposed to be induced by CD24 via Src signaling and the programmed cell death protein 4 (PDCD4) influences CRC cells in apoptosis-related processes [27, 28]. MiR-30a-5p induces apoptosis by binding to the mRNA of denticleless protein homolog (DTL) [14].

MiR-218 promotes apoptosis by downregulating BMI-1 and was found to be significantly lower in CRC when compared to adjacent normal tissue [29]. In addition, overexpression of miR-429 and miR-96 represses apoptosis by directly targeting SOX2 and the transcription factor CHES1, respectively [30].

Regulating Immortalization or Senescence

Immortalization is one of the first steps a normal cell takes towards malignancy and requires at least two to three genetic events, typically ones that affect the p53 and/or pRb pathway. More specifically, replicative senescence is modulated by key regulatory pathways. These pathways can be categorized as follows: pRb/p53 activity, telomerase maintenance, epigenetic modulation, miRNAs regulation, and oxidative stress response [31]. A direct target of p53 is the activation of transcription of the miR-34 family. Overexpression of miR-34a represses SIRT1, which deacetylates p53 and allows for increased p53 activity. Additionally, during B-RAF-induced senescence, which is independent of p53, a member of E-twenty-six oncogene family of transcription factors (ELK1) activates miR-34a expression. MiR-34 again represses Myc, which provokes senescence [32, 33]. Specific to CRC, transient introduction of miR-34a causes complete suppression of cell proliferation and induces senescence-like growth arrest through modulation of the E2F signaling pathway [34].

Induction of Angiogenesis

Once a primary tumor has reached a certain size or cancer cells have spread to distant organs, they require and therefore induce the generation of new blood vessels to ensure the supply of oxygen and nutrients which are necessary for further tumor growth. In this process, many pro- and antiangiogenic factors are involved, which are again targets of certain miRNAs as several studies have indicated [35]. Hypoxia is one of the dominant drivers for neovascularization in tumor cells and HIF-1 protein plays a central role in hypoxia-activated gene expression. In CRC, miR-145 and miR-107 regulate HIF-1 by targeting p70S6K1 [36, 37]. MiR-17-92 and miR-194 were found to promote angiogenesis by regulating p53 in colon cancer. Both miRNAs repress TSP-1 (thrombospondin-1), which acts as a barrier to neovascularization in CRC [38, 39]. Additionally, the polycistronic miR-17-92 cluster produces six mature miRNAs (mir-17, mir-18a, mir-19a, mir-20a, mir-19b-1, and mir-92-1) and can coordinate multiple functions in tumorigenesis. Among other methods, it is activated by the Myc oncogene and stimulates angiogenesis by inhibiting the TGF-β pathway [40].

MiR-27b plays an important role in angiogenesis and controls the fine balance between stimulators and suppressors of endothelial cell proliferation, migration,

and differentiation by targeting the endogenous angiogenesis inhibitor SEMA6A or by controlling EfnB2, EfnB4, Flt1, and Flt4 [9, 41]. MiR-126, an endothelial cell-restricted miRNA, mediates developmental angiogenesis in vivo and enhances the pro-angiogenic actions of VEGF and FGF. In addition, by repressing the intracellular inhibitor of the angiogenic signaling Spred-1, miR-126 promotes blood vessel formation [42].

Migration Invasion and Metastasis

The major cause of death in CRC patients arises from migration, invasion, and metastasis of the primary tumor cells. This two-step system is followed by proliferation and colonization of the tumor cells into their new secondary site. Leading mechanisms associated with these two steps and the mechanism of metastasis are the epithelial-mesenchymal transition (EMT) and mesenchymal to epithelial transition (MET). Vimentin, β-Catenin, TCF8-ZEB1, E-Cadherin, Snail, and Slug are established EMT markers [43]. In CRC, miR-21 and miR-31 act as downstream effectors in the TGF-β /Wnt signaling pathway, which is one of the prominent pathways in EMT [44] MiR-574-5p and miR-17 downregulate Qki6/7 and P130, which inversely correlate with β-Catenin [45]. In CRC, also a decrease in expression of the miR-200 family (for instance mir-200a and mir-200c), a master regulator of the epithelial phenotype, represses EMT by targeting ZEB1/2. ZEB1/2 upregulates Vimentin and downregulates E-cadherin [46–48]. MiR-147 was found to induce MET through the TGF-β signaling pathway [49]. The elevated expression of miR-103/107 is responsible for local invasion and liver metastasis and these miRNAs induce their effects through DAPK and KLF4 [50]. Prospero Homebox 1 (PROX1) inhibits E-cadherin via miR-9 [51]. Mucin-1, which is frequently upregulated in CRC patients, is a metastasis gene associated with cell invasion and metastasis and is targeted by miR-145 [52].

Further studies which investigate the role of miRNAs in metastasis are supported and are highly desired, as currently about 90 % of patients diagnosed with metastatic CRC die due to metastases [9, 53, 54].

Promotion of Genomic Instability

Calin et al. have shown that miRNAs are frequently clustered in fragile sites of chromosomes or regions of genomic instability. These areas are often associated with various human cancers, including CRC. This association between the location of miRNAs and chromosomal aberrations is significant, as it leads to chromosomal abnormalities, which in turn results in the disruption of miRNA expression. It is currently known that the abnormal expression of miRNAs plays a central role in cancer progression [55–57]. The first described miRNAs in CRC influenced by

chromosomal rearrangements belong to the miR-145/142 cluster, which is located on 5q32 and is affected by a deletion of the 5q32 band [58]. Other changes involve miR-21 (7q23.2, 3′ÚTR of the vacuole membrane protein 1) and mirR-155 (21q21.3) [59]. Besides the fact that miRNAs are frequently affected by chromosomal aberration, they can influence chromosomal instability on their own by targeting different protein-coding genes (see also to an excellent review recently published) [60].

Reprogramming of Energy Metabolism

Particularly in gastrointestinal malignancies, significant malnutrition accompanies malignant processes in approximately 30–50 % of deaths. Cancer cachexia is a complex syndrome characterized by progressive tissue depletion, increased metabolic expenditure, and dysfunctional metabolic processes [61]. "The Warburg effect" may be an approach to explain these dramatic symptoms. This metabolic phenotype in cancer cells allows the cells, even in the presence of oxygen, to shift their ATP generation from oxidative phosphorylation to glycolysis. Contrary to normal cells, cancer cells predominantly generate energy from aerobic glycolysis. Incoming glucose is converted to lactate rather than being metabolized through oxidative phosphorylation. This reprogramming of energy metabolism in the cell allows ATP to be generated more rapidly in comparison to oxidative phosphorylation, but production is less efficient in respect to the amount of ATP produced per unit of glucose consumed. Consequently, cancer cells need an extremely high rate of glucose uptake to fulfill their energy demand [2, 62]. Recent studies have shown that miRNAs play important roles in CRC energy metabolism. MiR-26a regulates glucose metabolism of CRC cells by targeting the pyruvate dehydrogenase protein X component (PDHX), which inhibits the conversion of pyruvate to acetyl CoA in the tricarboxylic acid cycle [63]. The adaptor protein p66Shc, also found in CRC, has the potential to respond to energy status changes and regulate mitogenic signaling [64].

Evasion of Immune System and Inflammation

Inflammation-associated CRC is mainly colitic cancer and develops in patients with inflammatory bowel disease (IBD) or celiac disease [65, 66]. Most of the changes in miRNA expression observed in inflammatory tissues are likely the result of immune cells participating in hematopoietic tumorigenesis. For this reason, it is important to examine changes in miRNA, in immune and epithelial cells when investigating the various miRNA functions in inflammatory-associated cancer development. During inflammation, miRNA expression can be altered in epithelial cells and miRNAs can adopt a tumor suppressor function. In CRC, genomic locations of lethal-7 (let-7) family members, miRNAs targeting the RAS family and c-MYC family, are

frequently deleted [55]. Also, IL-6, a cytokine frequently produced in cancers cells, is directly inhibited by let-7 [67]. Recent studies have shown a direct link between high levels of miR-155 and the development of gastric and colon cancers. MiR-155 is associated with hematopoiesis and the regulation of lymphocyte homeostasis and tolerance, which is substantially influenced by a bic/microRNA-155 interaction in B and T cells. In addition, some genes involved in normal immune functions are regulated by miR-155, including cytokines (IL-4), chemokines (CCL5) and transcription factors (c-Maf) [68, 69].

A positive feedback loop underlying the epigenetic switch which links inflammation to cancer was observed in CRC. STAT3, a transcription factor and downstream target of IL-6, activates miR-21 and miR-181b-1. Both miRNAs inhibit tumor suppressors (PTEN and CYLD), leading to increased NF-κB activity, which is required to support the transformed switch [70]. MiR-31 is associated with the stepwise transformation from IBD to IBD-related neoplasia by regulating the expression of factor inhibiting hypoxia inducible factor 1 [71]. Further studies will be required to improve our understanding of the role of tumor-related miRNAs in inflammation-associated cancer development [72].

Clinical Implications of miRNAs in Colorectal Cancer

MiRNAs as Diagnostics

Circulating miRNAs

The carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are widely-used blood-based biomarkers for CRC detection. Nevertheless, for early-stage CRC, the sensitivity and specificity seems to be insufficient [73]. Therefore, novel noninvasive biomarkers are urgently required for the advancement of diagnostics in CRC. Recent studies have shown a high amount of circulating miRNAs in the blood which are able to withstand adverse and labile conditions (pH, temperature, multiple freeze/thaw cycles) and still show consistent expression levels [74]. It has been hypothesized that most extracellular miRNAs are part of complexes with the Argonaute2 protein, derived from dead or dying cells. In this form, they could serve as a reflection of the underlying disease or help monitor pathological changes during the clinical course of disease. Due to the relatively easy access to plasma and serum, circulating miRNAs are one of the most promising biomarkers for cancer detection and prognosis [75].

Currently, there are several ways of taking advantage of circulating miRNAs. They have been used either alone or in a set of miRNAs as well as in combination with well-established biomarkers to enhance the predictive accuracy of prognostic factors/models. Almost 5 years ago, the overrepresentation of miRNAs in preoperative serum samples of CRC patients was described for the first time and a sensitivity of 89 % and specificity of 70 % was reported for miR-92. One year later, an

independent group confirmed these results of miR-92 as a potential noninvasive biomarker for CRC diagnosis with even higher sensitivity and specificity values [76, 77]. In another study, the levels of miR-23a and miR-1246 in exosomes showed a high sensitivity for stage I CRC samples of 95 % and 90 %, respectively, in comparison to the inferior sensitivities of CA19-9 (10 %) and CEA (15 %) for stage I samples [78]. Biomarkers detecting TNM stage I or II CRC may bear the highest potential for reducing the mortality and overall health burden, as tumors in these stages can be removed by surgery alone and early detection will lead to high cure rates.

Another impressive clinical finding was that miR-200c levels were significantly higher in stage IV than in stage I–III CRCs and showed a positive correlation with lymph node metastases, distant metastases, and tumor recurrence [79]. MiR-29a levels in serum have been proposed as a powerful tool to detect liver metastases, reaching a sensitivity and specificity of 75 % [80].

Wang et al. established a biomarker profile based on six serum miRNAs. In this panel, miR-21 and let-7 g were described as upregulated markers and miR-181b, miR-92a, and miR-203 as downregulated markers. Interestingly, this panel was more accurate for CRC diagnosis (sensitivity and specificity of 93 % and 91 %, respectively) than CEA and CA19-9 (sensitivity and specificity of 35 % and 23 %, respectively) using the same serum samples [81].

Another study showed that in combination with CEA levels, miR-141 enhanced and supplemented the ability to diagnose a subset of stage IV colon cancer patients [82]. One limitation of miRNAs as blood-based diagnostic markers for screening purposes is the high costs and long latency time of serum miRNAs [83].

Mucosal Colon Wash Fluid

Conventional colonoscopy is considered the gold standard for detecting CRC by visual and histological sampling. The wash fluid used in this procedure could possibly serve as an additional specimen for further diagnostic tests. Kamimae et al. collected DNA from the mucosal wash fluid of patients undergoing colonoscopy screening. They assessed methylation levels of miR-34b/c and found that this DNA fragment had the greatest correlation with the invasion depth of tumors (sensitivity 83 %, specificity 76 %). Further studies for optimization will be necessary; however the combination of endoscopy and DNA methylation analysis based on miR-34b/c levels may facilitate accurate preoperative staging of CRC and support the decision-making process to help avoid unnecessary surgery [84].

Feces

The fecal occult blood test (FOBT) detects hidden blood in patient stool. A positive result is not necessarily associated with CRC and may result from any bleeding occurring in the gastrointestinal system. On the other hand, a false negative test

result is frequently the consequence of the low sensitivity of the assay. Although the sensitivity and specificity is not very high, the FOBT is widely used for colorectal tumor screening [85].

To improve the sensitivity, Yamazaki et al. investigated the usefulness of testing fecal miRNAs out of FOBT residuum and evaluated the best technical conditions for sufficient fecal miRNA extraction from FOBT for PCR analysis. They found that storage at 4C for 5 days is feasible without the loss of RNA quality for miRNA analysis [86] In general, it was determined that stool-based miRNAs are relatively stable and show highly reproducible detection rates. Various studies have reported different miRNAs which are differentially expressed in CRC patients in comparison to healthy volunteers. These reports include miR-143, miR-145 [87], miR-34a, miR-34b/c [88], miR-144* [89], and miR-92a [90] among the most promising stool-based biomarkers. Other potential stool-based miRNA biomarkers for the early detection of CRC include miR-4478 and miR-1295b-3p, as their expression levels are significantly lower when compared to healthy controls [91].

In addition to the discrimination power of detecting tumors, miR-135b, miR-221, and miR-18a were increased significantly in stool samples of advanced-stage CRC [92, 93].

Stool-based miRNAs can either be used as individual biomarkers or be integrated into currently existing marker panels. It is most likely the case that the earliest neoplastic changes in the expression pattern of specific miRNAs might be detected in the feces rather than in the blood, considering the increased number of exfoliated cancer cells shed in the colon from CRC patients. Although the listed results need further validation, fecal miRNAs might provide a promising and noninvasive method for the diagnosis of early colorectal neoplasia and thereby serve as diagnostic markers [94, 95].

microRNAs as Therapeutics

The applicability of miRNA-based therapy can be explained as a double-edged sword. On the one hand, modulation of a single miRNA offers the opportunity to target multiple genes and regulatory networks simultaneously. On the other hand, caution and careful design are necessary to prevent any unwanted off-target effects [75]. However, applying miRNAs to anticancer therapy could be efficient and the off-target effects might even be helpful. As in several preclinical models previously shown, there are at least two main strategies for miRNA-based therapy: the restoration of tumor suppressor miRNAs or the inhibition of oncogenic miRNAs. A good example of this, although not in cancer patients, is the anti-miR-122 drug, which has progressed to phase II clinical trials to treat the hepatitis C virus in humans [96].

Drug resistance leads to therapy failure, cancer relapse, and poor prognosis. In this context, miRNAs can also predict individual chemotherapy response and drugs

targeting specific miRNAs can influence chemosensitivity in CRC patients. MiR-20, miR-130, miR-145, miR-216, and miR-372 serum levels are significantly upregulated in oxaliplatin chemotherapy-resistant CRC patients in comparison to chemosensitive patients [97]. MiR-215, miR-99a*, miR-196b, miR-450b-5p, and let-7e are associated with neoadjuvant chemoradiotherapy response [98]. MiR-10b has been involved as a predictor of 5-flourouracil-based chemotherapy resistance [99]. The promising results of preclinical models led to the initiation of the first miRNA-based clinical trial. Recently, it was reported that the data from a phase I clinical trial of a novel drug (MRX34) targeting miR-34 proposed a manageable safety profile in patients with advanced primary liver cancer (hepatocellular carcinoma), other solid tumors with liver metastasis and hematological malignancies (source: www.mirnarx.com).

All the miRNAs described above, as well as many not referred to here, appear to form a network to coordinate and influence the regulation of colorectal carcinogenesis.

Many underlying mechanisms still remain largely unknown. Further research regarding the identification of novel miRNAs, their target genes, new drugs, or CRC characteristics will enhance our knowledge and will be another step towards a more individual and efficient addition to therapy for CRC patients.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57–70.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74.
3. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
4. Schmoll HJ, Van Cutsem E, Stein A, Valentini V, Glimelius B, Haustermans K, et al. ESMO Consensus Guidelines for management of patients with colon and rectal cancer. a personalized approach to clinical decision making. Ann Oncol. 2012;23:2479–516.
5. Stiegelbauer V, Perakis S, Deutsch A, Ling H, Gerger A, Pichler M. MicroRNAs as novel predictive biomarkers and therapeutic targets in colorectal cancer. World J Gastroenterol. 2014;20:11727–35.
6. Linnekamp JF, Wang X, Medema JP, Vermeulen L. Colorectal cancer heterogeneity and targeted therapy: a case for molecular disease subtypes. Cancer Res. 2015;75:245–9.
7. Ahmed S, Johnson K, Ahmed O, Iqbal N. Advances in the management of colorectal cancer: from biology to treatment. Int J Colorectal Dis. 2014;29:1031–42.
8. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med. 2014;20:460–9.
9. Xuan Y, Yang H, Zhao L, Lau WB, Lau B, Ren N, et al. MicroRNAs in colorectal cancer: small molecules with big functions. Cancer Lett. 2014;360(2):89–105.
10. Ress AL, Stiegelbauer V, Winter E, Schwarzenbacher D, Kiesslich T, Lax S, et al. MiR-96-5p influences cellular growth and is associated with poor survival in colorectal cancer patients. Mol Carcinog. 2014. doi:10.1002/mc.22218.
11. Selcuklu SD, Donoghue MT, Spillane C. miR-21 as a key regulator of oncogenic processes. Biochem Soc Trans. 2009;37:918–25.
12. McGuire JJ. Anticancer antifolates: current status and future directions. Curr Pharm Des. 2003;9:2593–613.
13. Schetter AJ, Okayama H, Harris CC. The role of microRNAs in colorectal cancer. Cancer J. 2012;18:244–52.

14. Baraniskin A, Birkenkamp-Demtroder K, Maghnouj A, Zollner H, Munding J, Klein-Scory S, et al. MiR-30a-5p suppresses tumor growth in colon carcinoma by targeting DTL. Carcinogenesis. 2012;33:732–9.
15. Bandres E, Bitarte N, Arias F, Agorreta J, Fortes P, Agirre X, et al. microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. Clin Cancer Res. 2009;15:2281–90.
16. Tsang WP, Ng EK, Ng SS, Jin H, Yu J, Sung JJ, et al. Oncofetal H19-derived miR-675 regulates tumor suppressor RB in human colorectal cancer. Carcinogenesis. 2010;31:350–8.
17. Karaayvaz M, Zhai H, Ju J. miR-129 promotes apoptosis and enhances chemosensitivity to 5-fluorouracil in colorectal cancer. Cell Death Dis. 2013;4:659.
18. Liu L, Chen L, Xu Y, Li R, Du X. microRNA-195 promotes apoptosis and suppresses tumorigenicity of human colorectal cancer cells. Biochem Biophys Res Commun. 2010;400:236–40.
19. Nie J, Liu L, Zheng W, Chen L, Wu X, Xu Y, et al. microRNA-365, down-regulated in colon cancer, inhibits cell cycle progression and promotes apoptosis of colon cancer cells by probably targeting Cyclin D1 and Bcl-2. Carcinogenesis. 2012;33:220–5.
20. Akao Y, Nakagawa Y, Hirata I, Iio A, Itoh T, Kojima K, et al. Role of anti-oncomirs miR-143 and -145 in human colorectal tumors. Cancer Gene Ther. 2010;17:398–408.
21. Tang JT, Wang JL, Du W, Hong J, Zhao SL, Wang YC, et al. MicroRNA 345, a methylation-sensitive microRNA is involved in cell proliferation and invasion in human colorectal cancer. Carcinogenesis. 2011;32:1207–15.
22. Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell. 2007;26:745–52.
23. Nakano H, Miyazawa T, Kinoshita K, Yamada Y, Yoshida T. Functional screening identifies a microRNA, miR-491 that induces apoptosis by targeting Bcl-X(L) in colorectal cancer cells. Int J Cancer. 2010;127:1072–80.
24. Woods K, Thomson JM, Hammond SM. Direct regulation of an oncogenic micro-RNA cluster by E2F transcription factors. J Biol Chem. 2007;282:2130–4.
25. Diosdado B, van de Wiel MA, Terhaar Sive Droste JS, Mongera S, Postma C, Meijerink WJ, et al. MiR-17-92 cluster is associated with 13q gain and c-myc expression during colorectal adenoma to adenocarcinoma progression. Br J Cancer. 2009;101:707–14.
26. Chai H, Liu M, Tian R, Li X, Tang H. miR-20a targets BNIP2 and contributes chemotherapeutic resistance in colorectal adenocarcinoma SW480 and SW620 cell lines. Acta Biochim Biophys Sin. 2011;43:217–25.
27. Muppala S, Mudduluru G, Leupold JH, Buergy D, Sleeman JP, Allgayer H. CD24 induces expression of the oncomir miR-21 via Src, and CD24 and Src are both post-transcriptionally downregulated by the tumor suppressor miR-34a. PLoS One. 2013;8, e59563.
28. Leupold JH, Yang HS, Colburn NH, Asangani I, Post S, Allgayer H. Tumor suppressor Pdcd4 inhibits invasion/intravasation and regulates urokinase receptor (u-PAR) gene expression via Sp-transcription factors. Oncogene. 2007;26:4550–62.
29. He X, Dong Y, Wu CW, Zhao Z, Ng SS, Chan FK, et al. MicroRNA-218 inhibits cell cycle progression and promotes apoptosis in colon cancer by downregulating BMI1 polycomb ring finger oncogene. Mol Med. 2012;18:1491–8.
30. Li J, Du L, Yang Y, Wang C, Liu H, Wang L, et al. MiR-429 is an independent prognostic factor in colorectal cancer and exerts its anti-apoptotic function by targeting SOX2. Cancer Lett. 2013;329:84–90.
31. Leal JA, Feliciano A, Lleonart ME. Stem cell microRNAs in senescence and immortalization: novel players in cancer therapy. Med Res Rev. 2013;33:112–38.
32. Christoffersen NR, Shalgi R, Frankel LB, Leucci E, Lees M, Klausen M, et al. p53-independent upregulation of miR-34a during oncogene-induced senescence represses MYC. Cell Death Differ. 2010;17:236–45.
33. Yamakuchi M, Ferlito M, Lowenstein CJ. miR-34a repression of SIRT1 regulates apoptosis. Proc Natl Acad Sci U S A. 2008;105:13421–6.

34. Tazawa H, Tsuchiya N, Izumiya M, Nakagama H. Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells. Proc Natl Acad Sci U S A. 2007;104:15472–7.
35. Hur K. MicroRNAs: promising biomarkers for diagnosis and therapeutic targets in human colorectal cancer metastasis. BMB Rep. 2015;48(4):217–22.
36. Xu Q, Liu LZ, Qian X, Chen Q, Jiang Y, Li D, et al. MiR-145 directly targets p70S6K1 in cancer cells to inhibit tumor growth and angiogenesis. Nucleic Acids Res. 2012;40:761–74.
37. Yamakuchi M, Lotterman CD, Bao C, Hruban RH, Karim B, Mendell JT, et al. P53-induced microRNA-107 inhibits HIF-1 and tumor angiogenesis. Proc Natl Acad Sci U S A. 2010;107:6334–9.
38. Lawler J. Thrombospondin-1 as an endogenous inhibitor of angiogenesis and tumor growth. J Cell Mol Med. 2002;6:1–12.
39. Sundaram P, Hultine S, Smith LM, Dews M, Fox JL, Biyashev D, et al. p53-responsive miR-194 inhibits thrombospondin-1 and promotes angiogenesis in colon cancers. Cancer Res. 2011;71:7490–501.
40. Dews M, Fox JL, Hultine S, Sundaram P, Wang W, Liu YY, et al. The myc-miR-17 ~ 92 axis blunts TGF{beta} signaling and production of multiple TGF{beta}-dependent antiangiogenic factors. Cancer Res. 2010;70:8233–46.
41. Melo SA, Kalluri R. Angiogenesis is controlled by miR-27b associated with endothelial tip cells. Blood. 2012;119:2439–40.
42. Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell. 2008;15:261–71.
43. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119:1420–8.
44. Cottonham CL, Kaneko S, Xu L. miR-21 and miR-31 converge on TIAM1 to regulate migration and invasion of colon carcinoma cells. J Biol Chem. 2010;285:35293–302.
45. Ji S, Ye G, Zhang J, Wang L, Wang T, Wang Z, et al. miR-574-5p negatively regulates Qki6/7 to impact beta-catenin/Wnt signalling and the development of colorectal cancer. Gut. 2013;62:716–26.
46. Davalos V, Moutinho C, Villanueva A, Boque R, Silva P, Carneiro F, et al. Dynamic epigenetic regulation of the microRNA-200 family mediates epithelial and mesenchymal transitions in human tumorigenesis. Oncogene. 2012;31:2062–74.
47. Pichler M, Ress AL, Winter E, Stiegelbauer V, Karbiener M, Schwarzenbacher D, et al. MiR-200a regulates epithelial to mesenchymal transition-related gene expression and determines prognosis in colorectal cancer patients. Br J Cancer. 2014;110:1614–21.
48. Chen ML, Liang LS, Wang XK. miR-200c inhibits invasion and migration in human colon cancer cells SW480/620 by targeting ZEB1. Clin Exp Metastasis. 2012;29:457–69.
49. Lee CG, McCarthy S, Gruidl M, Timme C, Yeatman TJ. MicroRNA-147 induces a mesenchymal-to-epithelial transition (MET) and reverses EGFR inhibitor resistance. PLoS One. 2014;9, e84597.
50. Chen HY, Lin YM, Chung HC, Lang YD, Lin CJ, Huang J, et al. miR-103/107 promote metastasis of colorectal cancer by targeting the metastasis suppressors DAPK and KLF4. Cancer Res. 2012;72:3631–41.
51. Lu MH, Huang CC, Pan MR, Chen HH, Hung WC. Prospero homeobox 1 promotes epithelial-mesenchymal transition in colon cancer cells by inhibiting E-cadherin via miR-9. Clin Cancer Res. 2012;18:6416–25.
52. Sachdeva M, Mo YY. MicroRNA-145 suppresses cell invasion and metastasis by directly targeting mucin 1. Cancer Res. 2010;70:378–87.
53. Muhammad S, Kaur K, Huang R, Zhang Q, Kaur P, Yazdani HO, et al. MicroRNAs in colorectal cancer: role in metastasis and clinical perspectives. World J Gastroenterol. 2014;20:17011–9.
54. Zhou JJ, Zheng S, Sun LF, Zheng L. MicroRNA regulation network in colorectal cancer metastasis. World J Biol Chem. 2014;5:301–7.

55. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A. 2004;101:2999–3004.
56. Calin GA, Croce CM. MicroRNAs and chromosomal abnormalities in cancer cells. Oncogene. 2006;25:6202–10.
57. Calin GA, Croce CM. Chromosomal rearrangements and microRNAs: a new cancer link with clinical implications. J Clin Invest. 2007;117:2059–66.
58. Michael MZ, O'Connor SM, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res. 2003;1:882–91.
59. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A. 2006;103:2257–61.
60. Vincent K, Pichler M, Lee GW, Ling H. MicroRNAs, genomic instability and cancer. Int J Mol Sci. 2014;15:14475–91.
61. Palesty JA, Dudrick SJ. What we have learned about cachexia in gastrointestinal cancer. Digestive Dis. 2003;21:198–213.
62. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. Nat Rev Cancer. 2011;11:85–95.
63. Chen B, Liu Y, Jin X, Lu W, Liu J, Xia Z, et al. MicroRNA-26a regulates glucose metabolism by direct targeting PDHX in colorectal cancer cells. BMC Cancer. 2014;14:443.
64. Lebiedzinska-Arciszewska M, Oparka M, Vega-Naredo I, Karkucinska-Wieckowska A, Pinton P, Duszynski J, et al. The interplay between p66Shc, reactive oxygen species and cancer cell metabolism. Eur J Clin Invest. 2015;45 Suppl 1:25–31.
65. Bernstein CN, Blanchard JF, Kliewer E, Wajda A. Cancer risk in patients with inflammatory bowel disease: a population-based study. Cancer. 2001;91:854–62.
66. Elfstrom P, Granath F, Ye W, Ludvigsson JF. Low risk of gastrointestinal cancer among patients with celiac disease, inflammation, or latent celiac disease. Clin Gastroenterol Hepatol. 2012;10:30–6.
67. Iliopoulos D, Hirsch HA, Struhl K. An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. Cell. 2009;139:693–706.
68. Tili E, Croce CM, Michaille JJ. miR-155: on the crosstalk between inflammation and cancer. Int Rev Immunol. 2009;28:264–84.
69. Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, et al. Requirement of bic/microRNA-155 for normal immune function. Science. 2007;316:608–11.
70. Iliopoulos D, Jaeger SA, Hirsch HA, Bulyk ML, Struhl K. STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. Mol Cell. 2010;39:493–506.
71. Olaru AV, Selaru FM, Mori Y, Vazquez C, David S, Paun B, et al. Dynamic changes in the expression of MicroRNA-31 during inflammatory bowel disease-associated neoplastic transformation. Inflamm Bowel Dis. 2011;17:221–31.
72. Chiba T, Marusawa H, Ushijima T. Inflammation-associated cancer development in digestive organs: mechanisms and roles for genetic and epigenetic modulation. Gastroenterology. 2012;143:550–63.
73. Duffy MJ, van Dalen A, Haglund C, Hansson L, Holinski-Feder E, Klapdor R, et al. Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. Eur J Cancer. 2007;43:1348–60.
74. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008;105:10513–8.
75. Ajit SK. Circulating microRNAs as biomarkers, therapeutic targets, and signaling molecules. Sensors. 2012;12:3359–69.
76. Ng EK, Chong WW, Jin H, Lam EK, Shin VY, Yu J, et al. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. Gut. 2009;58:1375–81.

77. Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. Int J Cancer. 2010;127:118–26.
78. Ogata-Kawata H, Izumiya M, Kurioka D, Honma Y, Yamada Y, Furuta K, et al. Circulating exosomal microRNAs as biomarkers of colon cancer. PLoS One. 2014;9, e92921.
79. Toiyama Y, Hur K, Tanaka K, Inoue Y, Kusunoki M, Boland CR, et al. Serum miR-200c is a novel prognostic and metastasis-predictive biomarker in patients with colorectal cancer. Ann Surg. 2014;259:735–43.
80. Wang LG, Gu J. Serum microRNA-29a is a promising novel marker for early detection of colorectal liver metastasis. Cancer Epidemiol. 2012;36:e61–7.
81. Wang J, Huang SK, Zhao M, Yang M, Zhong JL, Gu YY, et al. Identification of a circulating microRNA signature for colorectal cancer detection. PLoS One. 2014;9, e87451.
82. Cheng H, Zhang L, Cogdell DE, Zheng H, Schetter AJ, Nykter M, et al. Circulating plasma MiR-141 is a novel biomarker for metastatic colon cancer and predicts poor prognosis. PLoS One. 2011;6, e17745.
83. Brase JC, Wuttig D, Kuner R, Sultmann H. Serum microRNAs as non-invasive biomarkers for cancer. Mol Cancer. 2010;9:306.
84. Kamimae S, Yamamoto E, Yamano HO, Nojima M, Suzuki H, Ashida M, et al. Epigenetic alteration of DNA in mucosal wash fluid predicts invasiveness of colorectal tumors. Cancer Prev Res (Phila). 2011;4:674–83.
85. Burch JA, Soares-Weiser K, St John DJ, Duffy S, Smith S, Kleijnen J, et al. Diagnostic accuracy of faecal occult blood tests used in screening for colorectal cancer: a systematic review. J Med Screen. 2007;14:132–7.
86. Yamazaki N, Koga Y, Yamamoto S, Kakugawa Y, Otake Y, Hayashi R, et al. Application of the fecal microRNA test to the residuum from the fecal occult blood test. Jpn J Clin Oncol. 2013;43:726–33.
87. Li JM, Zhao RH, Li ST, Xie CX, Jiang HH, Ding WJ, et al. Down-regulation of fecal miR-143 and miR-145 as potential markers for colorectal cancer. Saudi Med J. 2012;33:24–9.
88. Wu XD, Song YC, Cao PL, Zhang H, Guo Q, Yan R, et al. Detection of miR-34a and miR-34b/c in stool sample as potential screening biomarkers for noninvasive diagnosis of colorectal cancer. Med Oncol. 2014;31:894.
89. Kalimutho M, Del Vecchio BG, Di Cecilia S, Sileri P, Cretella M, Pallone F, et al. Differential expression of miR-144* as a novel fecal-based diagnostic marker for colorectal cancer. J Gastroenterol. 2011;46:1391–402.
90. Wu CW, Ng SS, Dong YJ, Ng SC, Leung WW, Lee CW, et al. Detection of miR-92a and miR-21 in stool samples as potential screening biomarkers for colorectal cancer and polyps. Gut. 2012;61:739–45.
91. Ghanbari R, Mosakhani N, Asadi J, Nouraee N, Mowla SJ, Poustchi H, et al. Decreased expression of fecal miR-4478 and miR-1295b-3p in early-stage colorectal cancer. Cancer Biomark. 2014;15(2):189–95.
92. Yau TO, Wu CW, Dong Y, Tang CM, Ng SS, Chan FK, et al. microRNA-221 and microRNA-18a identification in stool as potential biomarkers for the non-invasive diagnosis of colorectal carcinoma. Br J Cancer. 2014;111:1765–71.
93. Wu CW, Ng SC, Dong Y, Tian L, Ng SS, Leung WW, et al. Identification of microRNA-135b in stool as a potential noninvasive biomarker for colorectal cancer and adenoma. Clin Cancer Res. 2014;20:2994–3002.
94. Link A, Balaguer F, Shen Y, Nagasaka T, Lozano JJ, Boland CR, et al. Fecal MicroRNAs as novel biomarkers for colon cancer screening. Cancer Epidemiol Biomarkers Prev. 2010;19:1766–74.
95. Menendez P, Villarejo P, Padilla D, Menendez JM, Montes JA. Diagnostic and prognostic significance of serum microRNAs in colorectal cancer. J Surg Oncol. 2013;107:217–20.
96. Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science. 2010;327:198–201.

97. Zhang J, Zhang K, Bi M, Jiao X, Zhang D, Dong Q. Circulating microRNA expressions in colorectal cancer as predictors of response to chemotherapy. Anticancer Drugs. 2014;25:346–52.
98. Svoboda M, Sana J, Fabian P, Kocakova I, Gombosova J, Nekvindova J, et al. MicroRNA expression profile associated with response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer patients. Radiat Oncol. 2012;7:195.
99. Nishida N, Yamashita S, Mimori K, Sudo T, Tanaka F, Shibata K, et al. MicroRNA-10b is a prognostic indicator in colorectal cancer and confers resistance to the chemotherapeutic agent 5-fluorouracil in colorectal cancer cells. Ann Surg Oncol. 2012;19:3065–71.

Chapter 7
microRNAs and Prostate Cancer

Sajni Josson, Leland W.K. Chung, and Murali Gururajan

Abstract microRNAs are noncoding RNAs that are important for embryonic stem cell development and epithelial to mesenchymal transition (EMT). Tumor cells hijack EMT and stemness to grow and metastasize to distant organs including bone. In the tumor microenvironment, tumor cells interact with the stromal fibroblasts at the primary and metastatic sites and this interaction leads to tumor growth, EMT, and bone metastasis. Tumor-stromal interactions are a dynamic process that involves both cell–cell communications and extracellular vesicles and soluble factors. Growing body of evidence suggests that microRNAs are part of the payload that comprises the extracellular vesicles. microRNAs induce reactive stroma and thus convert normal stroma into tumor-associated stroma to promote aggressive tumorigenicity in vitro and in vivo. Landmark published studies demonstrate that expression of specific microRNAs of DLK1-DIO3 stem cell cluster correlates with patient survival in metastatic prostate cancer. Thus, microRNAs mediate tumor growth, EMT, and metastasis through cell intrinsic mechanisms and extracellular communications and could be novel biomarkers and therapeutic targets in bone metastatic prostate cancer.

Keywords microRNA • Prostate cancer • DLK1-DIO3 cluster • EMT • Extracellular vesicles

---

S. Josson
Uro-Oncology Research Program, Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

Neostrata Inc., Princeton, NJ 08540, USA
e-mail: sajni.j@gmail.com

L.W.K. Chung
Uro-Oncology Research Program, Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA
e-mail: Leland.Chung@cshs.org

M. Gururajan (✉)
Uro-Oncology Research Program, Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

Bristol-Myers Squibb Inc., Princeton, NJ 08543, USA
e-mail: gururajanmurali@gmail.com

© Springer International Publishing Switzerland 2015
G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5_7

Introduction

Cancer cells metastasize to distant organs by hijacking of embryonic processes such as EMT and through intercellular communication via secretomes. The role of noncoding RNAs in EMT and metastasis of cancer cells remains poorly understood. Recent studies highlight the directive functions of noncoding RNAs including microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) in modulating cancer cell growth, survival, EMT, and metastasis [1–5]. In contrast to lncRNAs, miRNAs are small (~20–23 nucleotides) noncoding ribonucleic acid (RNA) molecules that bind to complementary sites in the messenger RNAs (mRNAs) of their target genes, thereby inducing the posttranscriptional silencing of genes [6]. It is predicted that miRNAs might regulate up to one-third of all genes. The presumed number of unidentified miRNAs is large. Currently, about 1881 miRNAs are annotated in *Homo sapiens* (human) based on miRBase. MiRNAs are located throughout the genome including intergenic regions and in the introns of both protein-coding and noncoding genes. Intronic miRNAs are primarily expressed with their host gene expression. The nonintronic miRNA encoding sequences are clustered at distinct genomic positions and are often coexpressed as a single polycistronic transcript.

Role of miRNA in the Epigenetic Reprogramming of Prostate Cancer Metastasis

DLK1-DIO3 cluster in prostate cancer bone metastasis: One of the largest miRNA clusters in the genome is on human chromosome 14q32 [7]. The delta-like 1 homolog-deiodinase, iodothyronine 3 (DLK1-DIO3) contains about 10 % of the miRNAs currently known in mouse and human. This cluster is located within a well-known maternally imprinted region that is characterized by mono-allelic expression of the encompassed genes [7].

Evidence suggests deregulated expression of DLK1-DIO3 miRNAs and lncRNAs in cancer progression and metastasis [8, 9]. Key miRNAs in this cluster including miR-409-3p/-5p, miR-154*, and miR-379 have been shown to be upregulated in prostate cancer and play a critical role in bone metastasis [10–12]. The DLK1-DIO3 miRNA members, known to affect embryonic development, were shown to be expressed by clinical prostate cancer specimens and actively participated in tumor-stromal interactions in cell and animal models of prostate cancer bone metastasis [8, 9, 13, 14].

The DLK1-DIO3 gene cluster was previously shown to be aberrantly silenced in human- and mouse-induced pluripotent stem cells (iPSCs) but not in fully pluripotent embryonic stem cells, indicating the importance in the generation of fully functional iPSCs [13, 14]. In these studies, miR-409-3p and miR-379 were expressed in embryonic cells and silenced in iPSC, which were not functional. In other studies compared mouse germline competent and incompetent cells and showed elevated

levels of miR-379 and miR-409-5p in the germline competent cells compared to incompetent cells [13]. This suggests that these miRNAs are involved in embryogenesis and totipotency. The DLK1-DIO3 members appear to be activated during the metastatic process of cancer development. Several transgenic mouse models of prostate, liver and lung cancer also exhibit deregulated levels of DLK1-DIO3 cluster miRNA members [15–19]. Interestingly, miRNA members of the DLK1-DIO3 cluster have been shown to be upregulated in the serum of cancer patients. Members of this cluster, miR-379, miR-154*, and miR-409-3p, show increased levels in the circulating exosomes of patients with prostate cancer [20], breast cancers [21], and lung adenocarcinomas [22].

Specifically, in prostate cancer, miR-409-3p has been shown to be upregulated in the serum of high-risk prostate cancer patients compared to low-risk prostate cancer patients [20]. Using MSKCC database it was demonstrated that miR-409-3p and miR-379 expression is strongly associated with progression-free survival of prostate cancer patients [10, 11]. It was also shown that miR-409-3p/-5p and miR-154* have higher expression in human prostate cancer tissues with higher Gleason (≥7) score compared to benign prostatic hyperplasia using in situ hybridization and quantum dot analysis. Interestingly, increased staining of miR-409-3p/-5p and miR-154* expression was observed in prostate cancer bone metastatic tissue specimens. miR-379 expression was elevated in the tissues of metastatic prostate cancer compared to localized prostate cancer [10, 11]. These studies demonstrate that some of the members of the DLK1-DIO3 cluster are elevated in both localized prostate cancer tissues, and metastatic prostate cancer tissues and in the serum of prostate cancer patients, and could be potential biomarkers for predicting the transition of indolent to aggressive form of prostate cancer.

miR-409-3p/-5p: miR-409-3p/-5p were shown to be elevated in prostate cancer tissues [11]. Previous studies demonstrated that miR-409 is tumorigenic in mouse models of prostate cancer. miR-409 was oncogenic when delivered orthotopically into intact mouse prostate gland using lentiviral miR-409 expressing plasmids. Overexpression of miR-409 led to transformation of normal mouse epithelial cells and prostatic hyperplasia and adenocarcinoma [11]. Thus, remarkably a single miRNA was shown to induce prostate cancer in mouse models. In prostate cancer cell models, overexpression of miR-409 led to decrease in expression of several tumor suppressor genes (Fig. 7.1).

Interestingly, miR-409-3p and miR-409-5p have distinct and shared target genes. Both miR-409-3p and miR-409-5p target Ras suppressor protein 1 (RSU1). RSU1 protein blocks the oncogenic Ras/MAPK pathway and integrin-linked kinase (ILK) pathway in prostate cancer [23–25]. miR-409-5p also targets stromal antigen 2 (STAG2). In the tumor cells, STAG2 is part of the cohesion complex. In cancer cells STAG2 is decreased and results in the deregulation of the cohesion complex, which is thought to cause aneuploidy, cancer initiation, and progression [26, 27]. miR-409-3p also targets key proteins such as polyhometic complex 3 (PHC3) and Von-Hippel–Lindau protein (VHL). PHC3 is part of the polycomb group complexes, involved in epigenetic reprogramming [28, 29]. Through analysis of MSKCC database, we observed that high miR-409-3p and low PHC3 expression correlated with

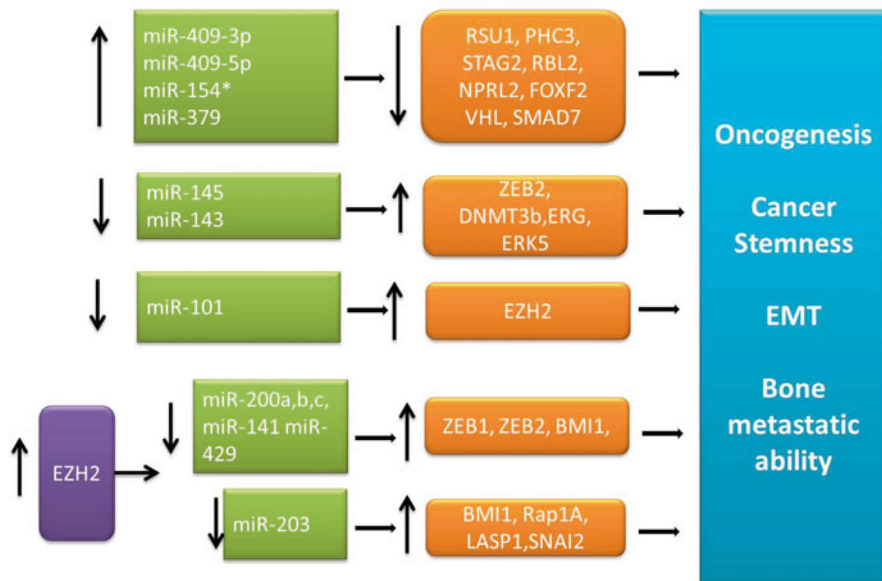

Fig. 7.1 miRNA-mediated pathways in prostate cancer

disease-free survival of prostate cancer patients. Since PHC3 in the polycomb repressive complex 1 (PRC1) maintains the transcriptional repressive state of many genes and is critical for stem cell renewal, inhibition of PHC3 through miR-409-3p resulted in the reactivation of genes that promote cellular reprogramming and induce cancer stemness [28, 30].

The VHL protein is an ubiquitin ligase which ubiquitinates hypoxia-inducible factor-1α (HIF-1α) and marks it for proteasome degradation [31]. Thus, inhibition of VHL by miR-409-3p leads to activation of HIF-1α pathway. Interestingly, The DLK1-DIO3 cluster in embryogenesis is thought to be modulated by hypoxia [32]. The DLK1-DIO3 miRNA cluster is active in the early embryonic stages and is silenced subsequently [32]. Xie et al., compared initial-passage embryonic stem cells (ihESCs, <10 passages) and early passage ESC (ehESCs, 20–30 passages) from diverse cell types and demonstrated that in most of the cells DLK1-DIO3 cluster is silenced in ehESCs but not in ihESCs. They concluded that silencing of DLK1-DIO3 is triggered by high oxygen conditions (20 %) and this cluster is active only under low (5 %) oxygen conditions [32]. Additionally, miR-409 overexpression in prostate cancer cells also increased mitochondrial superoxide production by inhibition of mitochondrial manganese superoxide dismutase (Josson et al., unpublished observations).

Since combined miR-409-3p/-5p promote aneuploidy, activate oncogenic Ras, and hypoxia-induced signaling network through increased HIF-1α and elevated reactive oxygen species, this leads to the induction of EMT and cancer cell invasion and metastasis [10]. Consistent with these observations, inhibition of miR-409-5p

in a human prostate cancer ARCaP EMT model we observed a reversal of $ARCaP_M$ to $ARCaP_E$, or mesenchymal to epithelial transition (MET) and decreased cancer cell growth, invasion, and metastasis [11]. We demonstrated whereas in immune-deficient SCID mice, after inoculated with $ARCaP_M$ cells, 100 % incidence of bone metastasis was observed, mice inoculated with $ARCaP_M$ cells with miR-409-5p knockdown prevented both tumor formation and tumor metastasis [11]. These results together suggest miR-409 plays a directive role conferring the development of aggressive prostate cancer and bone metastasis.

miR-154 and miR-379:* miR-154* is located adjacent to miR-409 within the long noncoding RNA, MEG9. miR-379 lies upstream of miR-154* in the DLK1-DIO3 cluster. Interestingly, consistent with the expression pattern of miR-409, miR-379 and miR-154* was also found to be elevated in prostate cancer human tissues [10]. Expression levels of miR-379 correlated with prostate cancer patient progression-free survival. miR-154* and miR-379 also promote EMT of prostate cancer cells. miR-154* was elevated in several isogenic prostate cancer ARCaP and LNCaP lines driving EMT and bone metastasis in mice. Additionally, miR-154* was elevated in embryonic stem cells. Inhibition of miR-154* in aggressive $ARCaP_M$ prostate cancer cells led to reversal of EMT with increased E-cadherin and decreased invasion in vitro [10]. Consistently, miR-154* inhibition also lead to reduced bone metastasis and increased survival in mice [10]. Mechanistically, miR-154* targets include tumor suppressors such as STAG2 and SMAD7. Decrease in STAG2 has been shown to induce aneuploidy in cancer cells [26, 27]. SMAD7 plays a critical role in inhibiting TGF-β pathways [33] that lead to EMT [34]. miR-379 is also elevated in metastatic prostate cancer patients when compared to patients with localized disease [35].

In summary, overexpression of all four miRNA members (miR-409-3p, miR-409-5p, miR-154*, and miR-379) in nonmetastatic prostate cancer cells led to induction of EMT whereas inhibition of these cluster members led to the reversal of EMT in metastatic prostate cancer cells. Several targets such as STAG2 which is repressed by miR-409-5p and miR-154* and RSU1, targeted by miR-409-3p and miR-409-5p work synergistically to mediate the downstream effects. The oncogenic pathways that are activated in response to expression of this mega-cluster in cancer cells include, chromosomal aneuploidy, altered Ras-, HIF-1α-, TGF-β-, E2F-, Akt-, and polycomb-mediated signaling pathways [10]. Thus, members of this mega-cluster primarily function together to repress tumor suppressors and modulate the expression of genes involved in oncogenesis, EMT, and stemness to mediate downstream convergent signal axes.

miR-145 and miR-143: Unlike the imprinted clusters of miRNAs, miR-145 and miR-143 are significantly *decreased* in prostate cancer and in bone metastatic patients compared to normal prostate tissues [36]. miR-145 plays a critical role in EMT by targeting a master transcription regulator, ZEB2 [37]. Additionally, ZEB2 inhibits miR-145 forming a negative-feedback loop [37]. Other studies demonstrate that miR-145 targets mRNAs of ERG [38] and DNMT3b [39], whereas miR-143 targets ERK5 [40]. miR-145 is regulated through DNA methylation and p53 mutation pathways [41]. Overexpression of miR-145 and -143 decreased invasion and migration, with increased E-cadherin and decreased fibronectin. These cells underwent MET and had

decreased prostate cancer bone metastasis [36]. Thus, downregulation of these miRNA are critical for EMT and prostate cancer bone metastasis and strongly predict disease-free survival [36, 37].

Epigenetic Silencing by EZH2 Regulate miRNA

Cao et al. demonstrated that several miRNAs were downregulated in prostate cancer, resulting in the activation of members of the polycomb group complexes [42]. The polycomb repressive complexes (PRC) include PRC1 and PRC2. These are involved in silencing of genes through histone modification and compaction of chromatin [43, 44]. The PRC2 complex methylates histones and allows for the binding of the PRC1 complex to the methylated site. PRC1 complex further ubiquinates histones and leads to compaction of chromatin. This prevents RNA Pol II activity and SWI-SNF accessibility to promoters leading to gene silencing [44].

miR-200 cluster: miR-200 cluster includes miR-200a, miR-200b, miR-200c, miR-141, and miR-429. Several of these miRNA are shown to be downregulated in human prostate cancer tissues compared to normal tissues [45, 46]. While the steady-state levels of these miRNAs are upregulated in normal epithelial cells, downregulation of miR-200 members has shown to induce EMT and cancer metastasis in several cancer types [4, 47]. These miRNAs repress EMT master regulators such as ZEB1 and ZEB2 [48]. MiR-200 cluster members target PRC1 members of the epigenetic machinery [42]. Both, miR-200b and miR-200c repress RING2 of PRC1 complex and miR-200a, b target BMI1 [42]. miR-200b and miR-200c are also negatively regulated by PRC2 protein EZH2 (a histone methyl transferase). EZH2 is elevated in human prostate cancer tissues [49]. EZH2 protein downregulates miR-200b and miR-200c. Decreased miR-200 in turn derepresses PRC1 members resulting in activation of PRC1 members and transcriptionally represses their target genes [42]. Overexpression of these miRNA in prostate cancer cells lead to decreased cell growth, invasion, decreased growth soft agar, and prostatosphere formation [42].

miR-181a and miR-181b: Similar to miR-200b,c, these miRNAs are also significantly downregulated in human prostate cancer tissue compared to normal [42]. These miRNA are also downregulated by EZH2 leading to activation of PRC1 member (RING2) and transcription repression of genes. Overexpression of these miRNA in prostate cancer cells blocked growth invasion and self-renewal capacity of cancer cells [42].

miR-203: miR-203 expression is decreased in prostate cancer tissues compared to normal prostate. Several studies demonstrate the role of miR-203 in EMT and cancer metastasis [50, 51]. miR-203 target genes include, BMI1, Ras inhibitor (Rap1A) [52], LIM and SH3 domain protein 1 (LASP1, involved in dynamic actin-based cytoskeletal activities) [50] and SNAI2 [53]. These proteins are regulators of EMT and cancer metastasis. Overexpression of miR-203, as expected, suppressed prostate cancer bone metastasis through inhibition of invasion and migration and EMT. miR-203 was shown to downregulate pro-metastatic genes such as ZEB2,

survivin, and Runx2, which are believed to be key master regulators of bone metastasis [51]. LASP, a target of miR-203 has been linked to metastatic disease in breast, colon, liver, and bladder cancer [50]. Negative-feedback action was shown between miR-203 and EZH2 [42]. Increased EZH2 in prostate cancer cells downregulated miR-203, which could potentially drive the migratory, invasive, and metastatic programs in prostate cancer cells [42].

miR-101: miR-101 was shown to be significantly downregulated in human prostate cancer tissues and serum compared to normal prostate [49, 54]. It has been shown that one or two of the genomic loci encoding miR-101 is lost in one-third of the localized prostate cancer cells and in two-third of the metastatic disease cells [49]. miR-101 targets EZH2, and downregulation of miR-101 lead to activation of EZH2 expression levels in prostate cancer tissues. miR-101 appears to be upstream of miR-200b,c and miR-181a,b signaling [42, 55]. Several studies in different prostate cancer cells demonstrated that overexpression of miR-101 decreased cell proliferation in vitro [42, 56, 57]. Prostate cancer cells expressing miR-101 also had decreased tumor growth in xenograft mouse models [57, 58]. Upstream regulators of miR-101 include androgen signaling and HIF1α signaling [56].

Role of Stromal miRNA in Prostate Cancer Development

Aberrant tumor-stromal interactions in the tumor microenvironment could trigger cancer progression [59]. The stromal fibroblasts have been shown to drive cancer growth and metastasis [60]. In prostate cancer, the cancer-associated "reactive" stromal fibroblasts obtained from prostate cancer patients at the primary site and bone metastatic site (i.e., marrow stromal or osteosarcoma) accelerated prostate cancer growth, invasion, and metastasis in a highly cell contact-dependent manner, readily demonstrable in 3-D in vitro coculture or in vivo tumor models [59]. Tumor-stromal interactions resulted in the *co-evolution of* prostate cancer *and stroma* which occurred under 3-D cocultured conditions. As a consequence of this co-evolution, the epithelium and stroma express shared biomarkers, which offer predictive value for prostate cancer invasion and metastasis [59, 61]. Several stroma-derived miRNA have been described to promote prostate tumor growth, EMT, stemness, and bone metastasis [12, 62, 63].

miRNA from the DLK1-DIO3 cluster: Interestingly, members of the DLK1-DIO3 cluster were highly expressed in cancer-associated stromal (CAS) fibroblasts derived from human prostate cancer patients. These miRNAs include miR-409-3p/-5p, miR-379, and miR-154* [12] (Fig. 7.2).

miR-409 was highly elevated in the CAS from both the prostate and the bone. MiR-409-3p and miR-409-5p expression analysis was performed using in situ hybridization and quantum dots multiplex labeling by employing human prostate cancer and normal prostate tissues [12]. Results of these studies demonstrated that miR-409-3p was significantly elevated in the stroma of patients with high Gleason score (≥7) compared to low Gleason (<7). miR-409 was transduced and overexpressed in normal prostatic stromal cells. Interestingly, these stromal cells had morphologic features of myofibroblasts and became highly secretory [12].

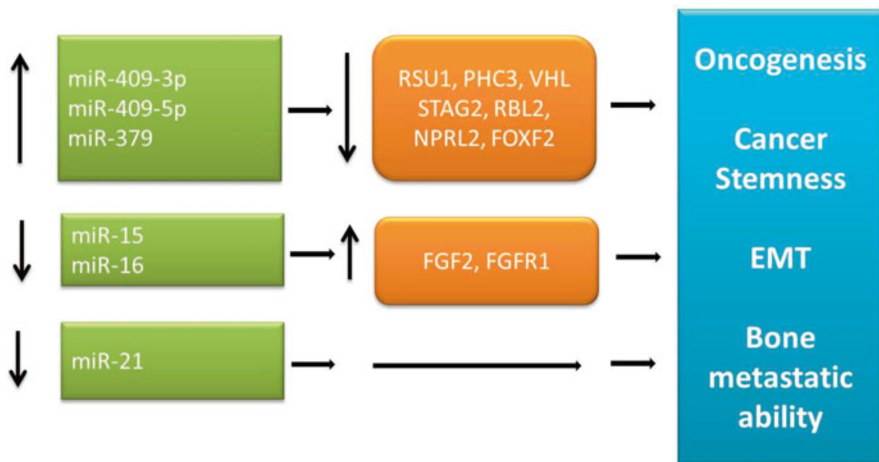

Fig. 7.2 Stromal miRNA-mediated pathways in prostate cancer

The secretory microvesicles had elevated levels of miR-409-3p and miR-409-5p. miR-409-3p was significantly more abundant than miR-409-5p. Cancer cells, maintained in conditioned media collected from normal and miR-409 expressing prostate stromal cells, underwent EMT with an increase in miR-409-3p and miR-409-5p. These miRNA were taken up by the cancer cells, and downregulated miR-409-3p and miR-409-5p target genes such as STAG2, RSU1, and PHC3 in the cancer cells; decreased function of these tumor suppressors could be the underlying molecular basis leading to activation of EMT and oncogenic pathways in the cancer cells [12]. When the stroma cells expressing miR-409 were co-inoculated with prostate cancer cells (not expressing miR-409) in nude mice, they developed tumors with explosive growths. Stromal miR-409 was shown to be secreted into the microenvironment, taken up by the adjacent cancer cells, and directed cancer migration, invasion, and spread through the tumor areas. miR-409 release through extracellular vesicles resulted in tumor growth by inhibiting tumor suppressors such as RSU1 and STAG2 both in vitro and in vivo. Stromal miR-409 expression orchestrates secretion of miR-409-3p/-5p and other soluble factors, such as β2-M which induces EMT and increases metastatic ability of prostate cancer cells [12].

Since miR-409 is activated during embryogenesis, we observed some of the stem cell markers (SOX2) upregulated in miR-409-expressing stromal cells. Stroma-derived miR-409 is capable of promoting tumor growth and EMT of adjacent tumor epithelia. Thus, miR-409 may be a new therapeutic target to break away the vicious cycle between stromal-epithelial interaction leading to EMT and prostate cancer bone and visceral organ metastases [12].

Recently, it was reported that breast cancer-associated exosomes contain pre-miRNAs, along with Dicer, argonaute protein (AGO2), and TAR (HIV-1) RNA Binding Protein (TRBP) [64, 65]. This process was mediated by CD43 that promoted accumulation of Dicer specifically in cancer exosomes. Melo et al. demonstrated

that cancer exosomes mediated an efficient and rapid silencing of mRNAs in the target cells to reprogram the target cell transcriptome [64]. Exosomes derived from cancer cells and serum of patients with breast cancer provoked nontumorigenic epithelial cells to form tumors in a Dicer-dependent manner [64]. It will be interesting to see if the miRNAs payload from exosomes can alter permanently the phenotype of nontumorigenic or bystander tumor cells and whether these communications, mediated by miRNAs, are targetable.

Musumeci et al., demonstrated that miR-15 and miR-16 are dowregulated in CAS. Decreased miR-15 and miR-16 resulted in increased tumor growth and progression through repression of FGF2 and FGFR1 signaling known to mediate tumor-stroma interaction in prostate cancer [66]. Stromal-derived miR-21 was shown to predict biochemical recurrence in prostate cancer patients with Gleason grade 6 [67].

Pathophysiological Relevance of DLK1-DIO3 Cluster microRNAs in Prostate Cancer and Its Implications for Prostate Cancer Biology, Biomarker Studies, and Therapy

Since imprinted cluster of miRNAs are upregulated, the knockdown studies generally reveal more pathophysiologic relevant information than the downregulated miRNAs which has to be overexpressed to establish their role in cancer biology and metastasis (Table 7.1). In this context, the recent findings on the oncogenic and metastatic role for DLK1-DIO3 cluster miRNAs utilizing knockdown approaches establish the functions of specific miRNAs in the pathphysiology of prostate cancer and thus uncover potential biomarker and therapeutic targets for treating lethal bone metastatic prostate cancer.

Recent studies suggest another class of noncoding RNAs called lncRNAs in cancer development and progression. LncRNAs are more than 200 nucleotides long and regulate mRNAs by multiple mechanisms. However, unlike miRNAs, lncRNAs

Table 7.1 Summary of microRNAs with a mechanistic role in prostate cancer

microRNAs in prostate cancer	Up/Downregulated	References
miR-200a, b, c	Downregulated (tumor)	[45, 46]
miR-181a,b	Downregulated (tumor)	[53]
miR-145	Downregulated (tumor)	[38]
miR-143	Downregulated (tumor)	[36]
miR-101	Downregulated (tumor)	[56]
miR-203	Downregulated (tumor)	[50]
miR-15, miR-16	Downregulated (tumor/stroma)	[60]
miR-21	Upregulated (tumor/stroma)	[57]
miR-409	Upregulated (tumor/stroma)	[11, 12]
miR-379	Upregulated (tumor/stroma)	[10, 12]
miR-154*	Upregulated (tumor/stroma)	[10, 12]

are not processed by the Dicer machinery and hence are not generated at equal lengths (unlike miRNAs which are 22 nucleotides in length) and have different mechanisms of target repression. However, increasing evidence suggests miRNAs and potentially lncRNAs helped pathologists diagnose and distinguish indolent from aggressive cancer. In addition to its role in biomarker evaluation, therapeutic targeting of miRNAs should sensitize prostate cancer cells to radiation therapy and chemotherapy.

Conclusions

Accumulating evidence suggest oncogenic and metastatic role for specific miRNAs of DLK1-DIO3 cluster in prostate cancer and that inhibition of cluster members led to reversal of EMT and reduced bone metastasis of prostate cancer. More importantly, elevated levels of miR-409 and miR-379 predict progression-free survival of patients, and thus promising potential biomarker for prostate cancer patients. DLK1-DIO3 cluster miRNAs have prognostic value in other cancer types including lung cancer. Future studies will need to explore large cohorts of patients for biomarker use of DLK1-DIO3 cluster miRNAs and small molecule approaches to target specific miRNAs of DLK1-DIO3 cluster to treat prostate cancer bone metastasis utilizing preclinical animal or 3-D growth models.

Acknowledgement Grant support from PO1 CA098912.

References

1. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature. 2007;449(7163):682–8.
2. Tavazoie SF, Alarcon C, Oskarsson T, Padua D, Wang Q, Bos PD, et al. Endogenous human microRNAs that suppress breast cancer metastasis. Nature. 2008;451(7175):147–52.
3. Zhang Y, Yang P, Sun T, Li D, Xu X, Rui Y, et al. miR-126 and miR-126* repress recruitment of mesenchymal stem cells and inflammatory monocytes to inhibit breast cancer metastasis. Nat Cell Biol. 2013;15(3):284–94.
4. Korpal M, Ell BJ, Buffa FM, Ibrahim T, Blanco MA, Celia-Terrassa T, et al. Direct targeting of Sec23a by miR-200s influences cancer cell secretome and promotes metastatic colonization. Nat Med. 2011;17(9):1101–8.
5. Chou J, Lin JH, Brenot A, Kim JW, Provot S, Werb Z. GATA3 suppresses metastasis and modulates the tumour microenvironment by regulating microRNA-29b expression. Nat Cell Biol. 2013;15(2):201–13.
6. Ambros V. microRNAs: tiny regulators with great potential. Cell. 2001;107(7):823–6.
7. Kircher M, Bock C, Paulsen M. Structural conservation versus functional divergence of maternally expressed microRNAs in the Dlk1/Gtl2 imprinting region. BMC Genomics. 2008;9:346.
8. Hagan JP, O'Neill BL, Stewart CL, Kozlov SV, Croce CM. At least ten genes define the imprinted Dlk1-Dio3 cluster on mouse chromosome 12qF1. PLoS One. 2009;4(2):e4352.

9. Formosa A, Markert EK, Lena AM, Italiano D, Finazzi-Agro E, Levine AJ, et al. MicroRNAs, miR-154, miR-299-5p, miR-376a, miR-376c, miR-377, miR-381, miR-487b, miR-485-3p, miR-495 and miR-654-3p, mapped to the 14q32.31 locus, regulate proliferation, apoptosis, migration and invasion in metastatic prostate cancer cells. Oncogene. 2013;33(44):5173–82.
10. Gururajan M, Josson S, Chu GC, Lu CL, Lu YT, Haga CL, et al. miR-154* and miR-379 in the DLK1-DIO3 microRNA mega-cluster regulate epithelial to mesenchymal transition and bone metastasis of prostate cancer. Clin Cancer Res. 2014;20(24):6559–69.
11. Josson S, Gururajan M, Hu P, Shao C, Chu CY, Zhau HE, et al. miR-409-3p/-5p promotes tumorigenesis, epithelial to mesenchymal transition and bone metastasis of human prostate cancer. Clin Cancer Res. 2014;20(17):4636–46.
12. Josson S, Gururajan M, Sung SY, Hu P, Shao C, Zhau HE, et al. Stromal fibroblast-derived miR-409 promotes epithelial-to-mesenchymal transition and prostate tumorigenesis. Oncogene. 2015;34(21):2690–9.
13. Liu L, Luo GZ, Yang W, Zhao X, Zheng Q, Lv Z, et al. Activation of the imprinted Dlk1-Dio3 region correlates with pluripotency levels of mouse stem cells. J Biol Chem. 2010; 285(25):19483–90.
14. Stadtfeld M, Apostolou E, Akutsu H, Fukuda A, Follett P, Natesan S, et al. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. Nature. 2010;465(7295):175–81.
15. Lim L, Balakrishnan A, Huskey N, Jones KD, Jodari M, Ng R, et al. MiR-494 within an oncogenic MicroRNA megacluster regulates G1/S transition in liver tumorigenesis through suppression of MCC. Hepatology. 2014;59(1):202–15.
16. Luk JM, Burchard J, Zhang C, Liu AM, Wong KF, Shek FH, et al. DLK1-DIO3 genomic imprinted microRNA cluster at 14q32.2 defines a stemlike subtype of hepatocellular carcinoma associated with poor survival. J Biol Chem. 2011;286(35):30706–13.
17. Haga CL, Phinney DG. MicroRNAs in the imprinted DLK1-DIO3 region repress the epithelial-to-mesenchymal transition by targeting the TWIST1 protein signaling network. J Biol Chem. 2012;287(51):42695–707.
18. Lempiainen H, Couttet P, Bolognani F, Muller A, Dubost V, Luisier R, et al. Identification of Dlk1-Dio3 imprinted gene cluster noncoding RNAs as novel candidate biomarkers for liver tumor promotion. Toxicol Sci. 2013;131(2):375–86.
19. Valdmanis PN, Roy-Chaudhuri B, Kim HK, Sayles LC, Zheng Y, Chuang CH, et al. Upregulation of the microRNA cluster at the Dlk1-Dio3 locus in lung adenocarcinoma. Oncogene. 2015; 34(1):94–103.
20. Nguyen HC, Xie W, Yang M, Hsieh CL, Drouin S, Lee GS, et al. Expression differences of circulating microRNAs in metastatic castration resistant prostate cancer and low-risk, localized prostate cancer. Prostate. 2013;73(4):346–54.
21. Li S, Meng H, Zhou F, Zhai L, Zhang L, Gu F, et al. MicroRNA-132 is frequently down-regulated in ductal carcinoma in situ (DCIS) of breast and acts as a tumor suppressor by inhibiting cell proliferation. Pathol Res Pract. 2013;209(3):179–83.
22. Cazzoli R, Buttitta F, Di Nicola M, Malatesta S, Marchetti A, Rom WN, et al. MicroRNAs derived from circulating exosomes as noninvasive biomarkers for screening and diagnosing lung cancer. J Thorac Oncol. 2013;8(9):1156–62.
23. Gonzalez-Nieves R, Desantis AI, Cutler ML. Rsu1 contributes to regulation of cell adhesion and spreading by PINCH1-dependent and - independent mechanisms. J Cell Commun Signal. 2013;7(4):273–79.
24. Dougherty GW, Jose C, Gimona M, Cutler ML. The Rsu-1-PINCH1-ILK complex is regulated by Ras activation in tumor cells. Eur J Cell Biol. 2008;87(8-9):721–34.
25. Becker-Santos DD, Guo Y, Ghaffari M, Vickers ED, Lehman M, Altamirano-Dimas M, et al. Integrin-linked kinase as a target for ERG-mediated invasive properties in prostate cancer models. Carcinogenesis. 2012;33(12):2558–67.
26. Solomon DA, Kim T, Diaz-Martinez LA, Fair J, Elkahloun AG, Harris BT, et al. Mutational inactivation of STAG2 causes aneuploidy in human cancer. Science. 2011;333(6045):1039–43.

27. Kim MS, Kim SS, Je EM, Yoo NJ, Lee SH. Mutational and expressional analyses of STAG2 gene in solid cancers. Neoplasma. 2012;59(5):524–9.
28. Deshpande AM, Akunowicz JD, Reveles XT, Patel BB, Saria EA, Gorlick RG, et al. PHC3, a component of the hPRC-H complex, associates with E2F6 during G0 and is lost in osteosarcoma tumors. Oncogene. 2007;26(12):1714–22.
29. Iwata S, Takenobu H, Kageyama H, Koseki H, Ishii T, Nakazawa A, et al. Polycomb group molecule PHC3 regulates polycomb complex composition and prognosis of osteosarcoma. Cancer Sci. 2010;101(7):1646–52.
30. Isono K, Fujimura Y, Shinga J, Yamaki M, O-Wang J, Takihara Y, et al. Mammalian polyhomeotic homologues Phc2 and Phc1 act in synergy to mediate polycomb repression of Hox genes. Mol Cell Biol. 2005;25(15):6694–706.
31. Palayoor ST, Burgos MA, Shoaibi A, Tofilon PJ, Coleman CN. Effect of radiation and ibuprofen on normoxic renal carcinoma cells overexpressing hypoxia-inducible factors by loss of von Hippel-Lindau tumor suppressor gene function. Clin Cancer Res. 2004;10(12 Pt 1):4158–64.
32. Xie P, Sun Y, Ouyang Q, Hu L, Tan Y, Zhou X, et al. Physiological oxygen prevents frequent silencing of the DLK1-DIO3 cluster during human embryonic stem cells culture. Stem Cells. 2014;32(2):391–401.
33. Edlund S, Bu S, Schuster N, Aspenstrom P, Heuchel R, Heldin NE, et al. Transforming growth factor-beta1 (TGF-beta)-induced apoptosis of prostate cancer cells involves Smad7-dependent activation of p38 by TGF-beta-activated kinase 1 and mitogen-activated protein kinase 3. Mol Biol Cell. 2003;14(2):529–44.
34. Lenferink AE, Cantin C, Nantel A, Wang E, Durocher Y, Banville M, et al. Transcriptome profiling of a TGF-beta-induced epithelial-to-mesenchymal transition reveals extracellular clusterin as a target for therapeutic antibodies. Oncogene. 2010;29(6):831–44.
35. Bryant RJ, Pawlowski T, Catto JW, Marsden G, Vessella RL, Rhees B, et al. Changes in circulating microRNA levels associated with prostate cancer. Br J Cancer. 2012;106(4):768–74.
36. Peng X, Guo W, Liu T, Wang X, Tu X, Xiong D, et al. Identification of miRs-143 and -145 that is associated with bone metastasis of prostate cancer and involved in the regulation of EMT. PLoS One. 2011;6(5), e20341.
37. Ren D, Wang M, Guo W, Huang S, Wang Z, Zhao X, et al. Double-negative feedback loop between ZEB2 and miR-145 regulates epithelial-mesenchymal transition and stem cell properties in prostate cancer cells. Cell Tissue Res. 2014;358(3):763–78.
38. Hart M, Wach S, Nolte E, Szczyrba J, Menon R, Taubert H, et al. The proto-oncogene ERG is a target of microRNA miR-145 in prostate cancer. FEBS J. 2013;280(9):2105–16.
39. Xue G, Ren Z, Chen Y, Zhu J, Du Y, Pan D, et al. A feedback regulation between miR-145 and DNA methyltransferase 3b in prostate cancer cell and their responses to irradiation. Cancer Lett. 2015;5.
40. Clape C, Fritz V, Henriquet C, Apparailly F, Fernandez PL, Iborra F, et al. miR-143 interferes with ERK5 signaling, and abrogates prostate cancer progression in mice. PLoS One. 2009;4(10):7542.
41. Suh SO, Chen Y, Zaman MS, Hirata H, Yamamura S, Shahryari V, et al. MicroRNA-145 is regulated by DNA methylation and p53 gene mutation in prostate cancer. Carcinogenesis. 2011;32(5):772–8.
42. Cao Q, Mani RS, Ateeq B, Dhanasekaran SM, Asangani IA, Prensner JR, et al. Coordinated regulation of polycomb group complexes through microRNAs in cancer. Cancer Cell. 2011; 20(2):187–99.
43. Bracken AP, Helin K. Polycomb group proteins: navigators of lineage pathways led astray in cancer. Nat Rev Cancer. 2009;9(11):773–84.
44. Di Croce L, Helin K. Transcriptional regulation by Polycomb group proteins. Nat Struct Mol Biol. 2013;20(10):1147–55.
45. Barron N, Keenan J, Gammell P, Martinez VG, Freeman A, Masters JR, et al. Biochemical relapse following radical prostatectomy and miR-200a levels in prostate cancer. Prostate. 2012;72(11):1193–9.

46. Liu YN, Yin JJ, Abou-Kheir W, Hynes PG, Casey OM, Fang L, et al. MiR-1 and miR-200 inhibit EMT via Slug-dependent and tumorigenesis via Slug-independent mechanisms. Oncogene. 2013;32(3):296–306.
47. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol. 2008;10(5):593–601.
48. Guo F, Parker Kerrigan BC, Yang D, Hu L, Shmulevich I, Sood AK, et al. Post-transcriptional regulatory network of epithelial-to-mesenchymal and mesenchymal-to-epithelial transitions. J Hematol Oncol. 2014;7:19.
49. Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, et al. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science. 2008;322(5908):1695–9.
50. Hailer A, Grunewald TG, Orth M, Reiss C, Kneitz B, Spahn M, et al. Loss of tumor suppressor mir-203 mediates overexpression of LIM and SH3 Protein 1 (LASP1) in high-risk prostate cancer thereby increasing cell proliferation and migration. Oncotarget. 2014;5(12): 4144–53.
51. Saini S, Majid S, Yamamura S, Tabatabai L, Suh SO, Shahryari V, et al. Regulatory role of mir-203 in prostate cancer progression and metastasis. Clin Cancer Res. 2011;17(16):5287–98.
52. Xiang J, Bian C, Wang H, Huang S, Wu D. MiR-203 down-regulates Rap1A and suppresses cell proliferation, adhesion and invasion in prostate cancer. J Exp Clin Cancer Res. 2015;34(1):8.
53. Qu Y, Li WC, Hellem MR, Rostad K, Popa M, McCormack E, et al. MiR-182 and miR-203 induce mesenchymal to epithelial transition and self-sufficiency of growth signals via repressing SNAI2 in prostate cells. Int J Cancer. 2013;133(3):544–55.
54. Srivastava A, Goldberger H, Dimtchev A, Marian C, Soldin O, Li X, et al. Circulatory miR-628-5p is downregulated in prostate cancer patients. Tumour Biol. 2014;35(5):4867–73.
55. Banerjee R, Mani RS, Russo N, Scanlon CS, Tsodikov A, Jing X, et al. The tumor suppressor gene rap1GAP is silenced by miR-101-mediated EZH2 overexpression in invasive squamous cell carcinoma. Oncogene. 2011;30(42):4339–49.
56. Cao P, Deng Z, Wan M, Huang W, Cramer SD, Xu J, et al. MicroRNA-101 negatively regulates Ezh2 and its expression is modulated by androgen receptor and HIF-1alpha/HIF-1beta. Mol Cancer. 2010;9:108.
57. Hao Y, Gu X, Zhao Y, Greene S, Sha W, Smoot DT, et al. Enforced expression of miR-101 inhibits prostate cancer cell growth by modulating the COX-2 pathway in vivo. Cancer Prev Res. 2011;4(7):1073–83.
58. Ren G, Baritaki S, Marathe H, Feng J, Park S, Beach S, et al. Polycomb protein EZH2 regulates tumor invasion via the transcriptional repression of the metastasis suppressor RKIP in breast and prostate cancer. Cancer Res. 2012;72(12):3091–104.
59. Sung SY, Hsieh CL, Law A, Zhau HE, Pathak S, Multani AS, et al. Coevolution of prostate cancer and bone stroma in three-dimensional coculture: implications for cancer growth and metastasis. Cancer Res. 2008;68(23):9996–10003.
60. Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S, et al. TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science. 2004; 303(5659):848–51.
61. Sung SY, Chung LW. Prostate tumor-stroma interaction: molecular mechanisms and opportunities for therapeutic targeting. Differentiation. 2002;70(9–10):506–21.
62. Gandellini P, Giannoni E, Casamichele A, Taddei ML, Callari M, Piovan C, et al. miR-205 hinders the malignant interplay between prostate cancer cells and associated fibroblasts. Antioxid Redox Signal. 2014;20(7):1045–59.
63. Ren Q, Liang J, Wei J, Basturk O, Wang J, Daniels G, et al. Epithelial and stromal expression of miRNAs during prostate cancer progression. Am J Transl Res. 2014;6(4):329–39.
64. Melo SA, Sugimoto H, O'Connell JT, Kato N, Villanueva A, Vidal A, et al. Cancer exosomes perform cell-independent microRNA biogenesis and promote tumorigenesis. Cancer Cell. 2014;26(5):707–21.

65. Sato-Kuwabara Y, Melo SA, Soares FA, Calin GA. The fusion of two worlds: non-coding RNAs and extracellular vesicles—diagnostic and therapeutic implications (Review). Int J Oncol. 2015;46(1):17–27.
66. Musumeci M, Coppola V, Addario A, Patrizii M, Maugeri-Sacca M, Memeo L, et al. Control of tumor and microenvironment cross-talk by miR-15a and miR-16 in prostate cancer. Oncogene. 2011;30(41):4231–42.
67. Melbo-Jorgensen C, Ness N, Andersen S, Valkov A, Donnem T, Al-Saad S, et al. Stromal expression of MiR-21 predicts biochemical failure in prostate cancer patients with Gleason score 6. PLoS One. 2014;9(11):e113039.

Chapter 8
microRNA and Ovarian Cancer

Ream Langhe

Abstract Ovarian cancer is the fifth most common cancer in women and the leading cause of death from gynaecological malignancy in the Western world. The majority of ovarian cancers are diagnosed at an advanced stage and this is due to lack of a reliable screening test and the vagueness of symptoms. Early diagnosis is key as the 5-year survival rate for women diagnosed with late-stage disease is less than 20 % compared to up to 90 % for women diagnosed at early-stage disease. Early-stage disease that has a good prognosis cannot be detected easily.

Currently, no standardized reliable screening test exists. Lack of a reliable screening test is due to the fact that the underlying molecular biology of oncogenesis in ovarian cancer is a complex pathway. Once the molecular biology of the ovarian cancer is known, more reliable and sensitive screening tests can be established and a better and effective treatment can be found. Current diagnostic tools include imaging and CA125 have their limitations in terms of accuracy.

There is a strong need for prognostic and predictive markers to diagnose it early and to help optimize and personalize treatment. microRNAs were recently found to be involved in the pathophysiology of all types of analyzed human cancers mainly by aberrant gene expression. microRNA profiling has allowed the identification of signatures associated with diagnosis, prognosis, and response to treatment of human tumors. Several studies showed that microRNAs are deregulated in ovarian cancer. This chapter reviews the role of microRNAs in ovarian cancer and their utility of microRNAs as diagnostic and prognostic markers for ovarian cancer

Keywords Ovarian cancer • microRNA • Biomarkers

R. Langhe (✉)
Department of Obstetrics and Gynaecology, Trinity Centre for Health Sciences, St. James's Hospital, Dublin, Ireland
e-mail: reamlanghe@yahoo.co.uk

© Springer International Publishing Switzerland 2015

G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5_8

Introduction

Incidence of Ovarian Cancer

Ovarian cancer is the fifth most common cause of cancer death in women, and the leading cause of death from gynaecological malignancy in the western world [1]. The incidence rates of the disease vary significantly across different regions with world age-standardized rates in more developed areas being almost twice as high as those in less developed countries. In 2008, the estimated world age-standardized incidence rate for the more developed regions of the world was 9 per 100,000 and 5 per 100,000 for the less developed countries [2]. Figures 8.1 and 8.2 display the worldwide age distribution of incidence and mortality of ovarian cancer. Ovarian cancer is associated with poor long-term survival, which is due to the insidious asymptomatic nature of this disease in its early onset, the lack of robust and minimally invasive methods for early detection, and development of chemoresistance.

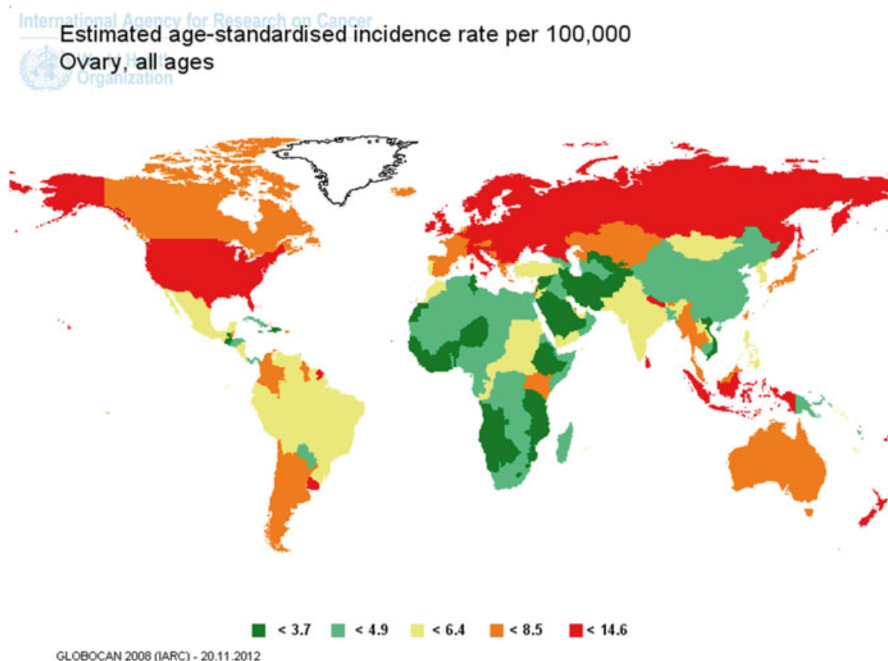

Fig. 8.1 Estimated age standardized worldwide incidence rate of ovarian cancer. *Source*: Globocan 2008 http://globocan.iarc.fr/

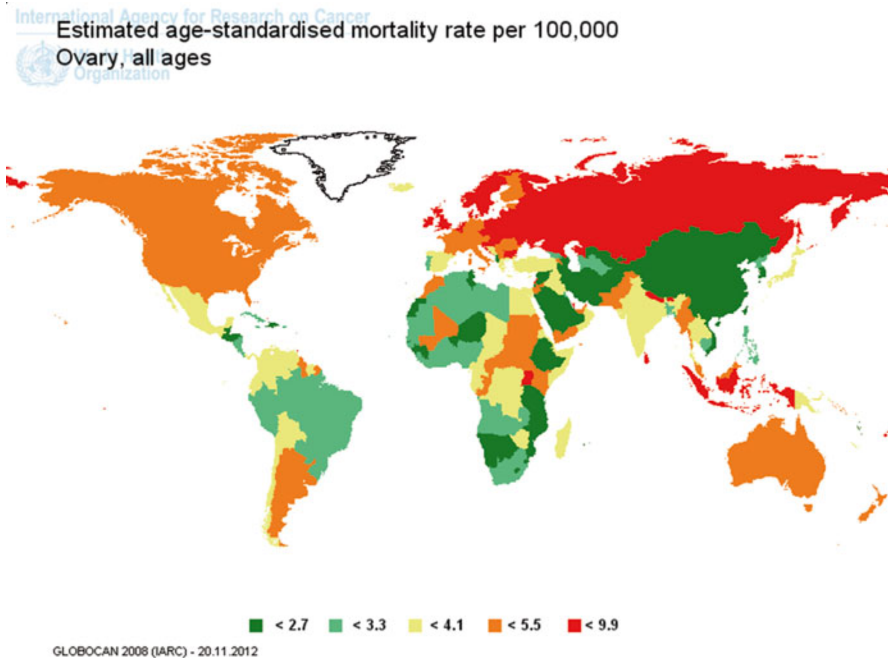

Fig. 8.2 Estimated age standardized worldwide mortality rate of ovarian cancer. *Source*: Globocan 2008 http://globocan.iarc.fr/

Etiology of Ovarian Cancer

The cause of ovarian cancer is still unknown although there are several factors associated with the development of ovarian cancer. Age is the strongest risk factor, the incidence of ovarian cancer increases with each additional year of life [3]. In addition early menarche and late menopause have also been linked to an increased risk of ovarian cancer [4]. Conditions such as polycystic ovarian syndrome (PCOS) and endometriosis have all been linked to increased ovarian cancer risk due to an imbalance of reproduction hormones [5, 6].

Factors such as chronic anovulation, breastfeeding and multiparity, use of contraceptive pills, and childbirth are found to be protective for ovarian cancer [7]. Published data showed that pregnancy is associated with decreased risk of developing the disease.

The greatest protection is afforded by the first pregnancy with a 20 % reduction in the risk is conferred by each additional child [8]. Lactation also reduces the risk [9–11]; however, uncertainties remain in the evidence [9, 10]. Long-term oral contraceptive use has been linked to reduced risk of ovarian cancer [12, 13]. Protection increases with increasing duration of use, and remains following cessation of use. The reduction in risk continues for more than 30 years after use has stopped [9].

Risk is also reduced by mechanical sterilization and by hysterectomy [14–16]. Bilateral salpingo-oophorectomy and hysterectomy are shown to reduce risk of ovarian cancer [17]. Recent evidence suggests that the majority of ovarian tumors actually arise in the fallopian tube, and it has been suggested that removal of fallopian tubes post-menopause could be beneficial in terms of reducing risk with virtually no side-effects [18].

Histological Classification of Ovarian Cancer

Ovarian cancer comprises a heterogeneous group of neoplasms. The histological classification of ovarian cancer as presented by World Health Organization (WHO), is based on histogenesis of the normal ovary, which categorizes ovarian neoplasms with regard to their derivation (Figs. 8.3 and 8.4) from coelomic surface epithelial cells, germ cells, and mesenchyme (the stroma and the sex cord) [19, 20]. Approximately 90 % of primary malignant ovarian tumors are epithelial and arise from the ovarian surface epithelium (OSE) or more likely from surface epithelial inclusion cysts [21–23]. The four major types of epithelial tumors (serous, endometrioid, clear cell, and mucinous) (Fig. 8.5) bear strong likeliness to the normal cells lining different organs in the female genital tract [23].

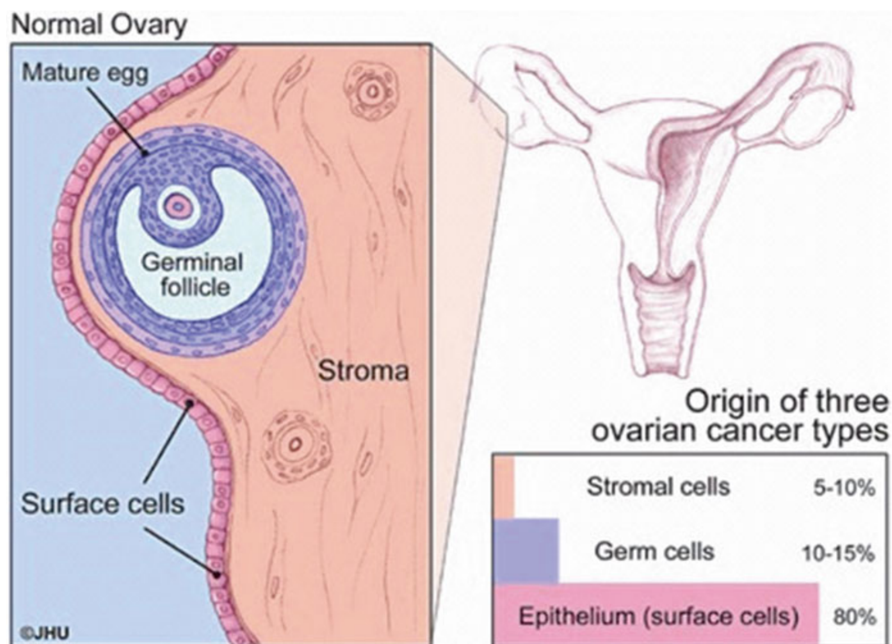

Fig. 8.3 Origin of three types of ovarian cancer

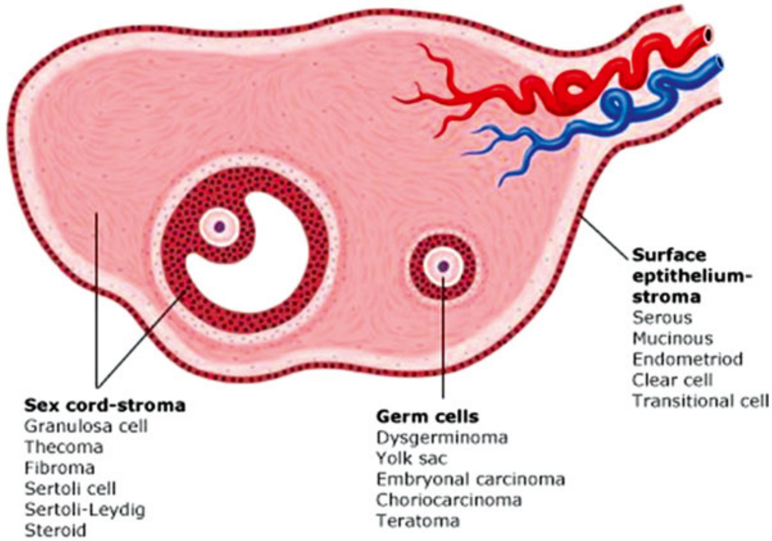

Fig. 8.4 Derivation of ovarian cancer

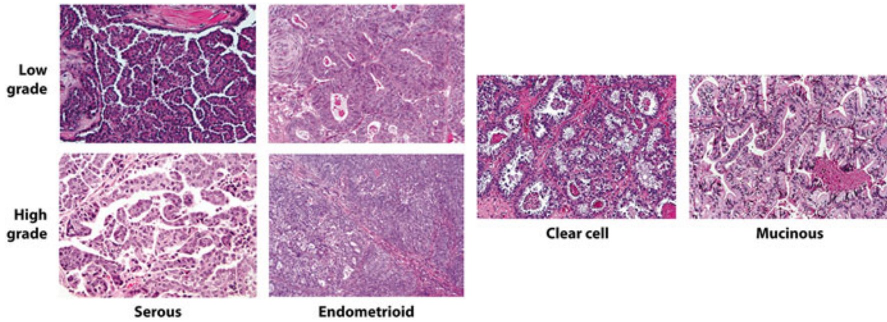

Fig. 8.5 Representative examples of the major histological types of ovarian carcinoma

Recently, epithelial ovarian tumors have been broadly classified into two distinct groups Type I and Type II [24, 25]. Type I tumors include low-grade serous, mucinous, endometrioid, clear cell, and transitional cell carcinomas and are less responsive to chemotherapy. Type II tumors comprise high-grade serous carcinomas, undifferentiated carcinomas, and carcinosarcomas responsive to chemotherapy [24, 25].

The etiology of epithelial ovarian cancer is still unclear. A number of hypotheses regarding ovarian cancer etiology have been described in one study [26]. All of these hypotheses can be related to the risk factors identified for ovarian cancer, e.g., incessant ovulation—early menarche and late menopause confer increased risk. Ovarian cancer is thought to occur as a result of transformation of the surface

epithelium [27], although recent studies have postulated that ovarian cancer might have developed in the fallopian tube and metastasized [28–30]. Two major theories, the incessant ovulation [31] and the gonadotropin hypotheses [32] have been proposed to explain the development of the disease.

Symptoms of Ovarian Cancer

Ovarian cancer symptoms develop insidiously and are nonspecific. These symptoms occur when the disease is widely disseminated throughout the abdominal cavity [33] and include abdominal bloating, discomfort or vague pain, bowel habit changes, abdominal fullness early satiety, dyspepsia are frequent presenting symptoms [34, 35].

Occasionally some patients may present with bowel obstruction, or shortness of breath that is due to pleural effusion. Early-stage disease, when the tumor is confined to the ovary is usually asymptomatic. However, such patients may present with pressure symptoms (urinary frequency, constipation, pelvic pain/pressure, dyspareunia). Less frequent symptoms include abnormal vaginal bleeding in premenopausal and postmenopausal women.

Treatment of Ovarian Cancer

Debulking surgery followed by platinum-based chemotherapy schemes is considered standard care for patients diagnosed with ovarian cancer. When ovarian cancer is diagnosed in stage 1 more than 90 % of patients can be cured with conventional surgery and chemotherapy. At present, however, only 25 % of ovarian cancers are detected in stage 1. Neoadjuvant chemotherapy, a preoperative chemotherapy that is given to reduce the tumor size before debulking is considered the optimum treatment for patients, who present with late-stage ovarian cancer [36].

While the majority of patients (80 %) initially respond well to chemotherapy, a large percentage relapse, and become resistant to chemotherapy [37]. Recently, the addition of bevacizumab; an antiangiogenic monoclonal antibody targeted against VEGF to standard chemotherapy has been explored [38]. The VEGF inhibitor bevacizumab has been shown to prolong progression-free survival but not overall survival; however, in another study of recurrent ovarian cancer, it was found that treatment with bevacizumab in platinum-sensitive cancers improved overall survival [39].

Diagnosis of Ovarian Cancer

Diagnosis of ovarian cancer is based usually on a pelvic examination for palpation of abdominal masses and radiological imaging such as transvaginal ultrasound and/or computed tomography (CT) scanning and magnetic resonance imaging (MRI) [40, 41].

Screening of Ovarian Cancer

Currently diagnosis of early-stage disease is very limited as there have been no standardized tests or screening mechanisms approved for this purpose. Pelvic examination is practiced widely, but it is not sensitive enough to be used as a reliable screening tool for diagnosis of ovarian cancer. Women at high risk of developing ovarian cancer might undergo screening with transvaginal ultrasound and CA125 [42]. Recently, the Prostate, Lung, Colorectal and Ovarian (PLCO), a screening randomized trial in the USA designed to determine whether ovarian cancer screening improves survival has reported its results. In this trial women were randomly assigned to undergo either annual CA125 testing plus Transvaginal Ultrasound (TVU) or to receive the usual care [43]. The trial reported no mortality benefit for annual screening with CA125/TVU compared to the usual care, however, it results in an increase in invasive medical procedures performed [44].

Another trial in the UK (UKCTOCS), assessing screening with ultrasound and CA125. Here, women randomly assigned to undergo annual pelvic examination, annual TVU or annual measurement of CA125, where CA125 was evaluated over time using the Risk of Ovarian Cancer Algorithm (ROCA) to try to predict the woman's risk of having the disease [41]. The results of this trial will be available in the latter part of 2015 (http://www.ukctocs.org.uk/).

Current Diagnostic Biomarkers

Currently the only biomarker that is widely used in clinical practice is cancer antigen 125 (CA125) [45]. This high MW glycoprotein CA125 is elevated in 90 % of patients with advanced stage disease. The use of CA125 for early diagnosis is limited as only 50 % of symptomatic stage 1 cases have elevated CA125 levels in serum. Also, monitoring of CA-125 blood serum levels is useful to determine how ovarian cancer is responding to treatment and to predict the prognosis after treatment and as a strong predictor of the recurrence of ovarian cancer [46–48].

A number of false positive results could also occur since levels of CA125 could naturally be elevated during ovulation and may also be elevated due to a range of benign gynaecologic causes such as fibroids, endometriosis, and pelvic inflammatory disease among others. CA125 can also be elevated in a variety of cancers other than ovarian such as pancreatic, lung, and breast cancer [49, 50].

In addition to CA125, other biomarkers are used routinely in medical practice and these include CA 19-9, CA 15-3, CA 72-4, and CEA. CA 19-9 has the advantage of high sensitivity for mucinous ovarian cancers that fail to express CA125 [51]. Serum levels of CA 19-9 are elevated in 68–83 % of mucinous ovarian cancers but in only 28–29 % of non-mucinous types, whereas CA-125 is elevated in 80 % of non-mucinous ovarian tumors [52–55]. CA 15-3, CA 72-4, and CEA levels are found to be elevated in 50–56 %, 63–71 %, and 25–50 % of patients with ovarian cancer [52, 56–64]. However, serial measurement of these tumor markers still plays a vital role in the management of patients with a CA125 negative tumor [51].

One study shows that CA125 correlates with the clinical course of disease better than the other antigens, and in patients with positive CA125 assay at diagnosis the concomitant evaluation of CA 19.9 or CA 72.4 or CA 15.3 does not offer any additional benefit for monitoring ovarian carcinoma [51]. However serial measurement of the other markers could be useful in the monitoring of patients with normal CA125 values prior to initial surgery [65].

Recently, addition of a new biomarker such as HE4 a secreted glycoprotein product of the WFDC2 gene [66] has shown great promise as a diagnostic biomarker for ovarian cancer. The US Food and Drug Administration have recently approved it for monitoring recurrence and progression in patients with epithelial ovarian cancer (EOC) [67–69]. The diagnostic and prognostic potential of serum HE4 levels has been examined in numerous ovarian cancer studies. HE4 has been detected at high concentration in serum of patients of ovarian cancer particularly women with serous and endometrioid adenocarcinoma and furthermore was found to be increased in more than half of the ovarian cancers, which do not express CA125 [69–73]. Several studies found that high preoperative HE4 measurements was associated with advanced stage disease and poor prognosis in patients with ovarian cancer [72, 74, 75]. Therefore HE4 could act as a potential prognostic marker in patients with ovarian cancer.

microRNAs

In recent years new biomarkers with diagnostic implications have been identified. Such biomarkers are microRNAs [76, 77]. microRNAs have been found to be differentially expressed in tumor versus normal tissues in a range of solid and hematopoietic tumors [78, 79]. The expression of microRNA may act as a novel class of tumor suppresser genes or oncogenes [76, 80–84]. The first possible link between microRNAs and cancer was seen in B-cell chronic lymphocytic leukemia, where miR-15 and miR-16, located on chromosome 13q14.3, were found to be downregulated or deleted in the majority of tumors [80, 85]. A second study showed that the expressions of miR-143 and miR-145 were reduced in adenomatous and cancer stages of colorectal neoplasia [86].

microRNAs in Ovarian Cancer

An increasing number of studies have revealed the vital role microRNAs play in ovarian cancer carcinogenesis [16, 87–89]. The first study, which suggested that microRNAs might play a role in ovarian cancer, showed that around 40 % of microRNAs display changed DNA copy number [84]. Thereafter two more studies demonstrated that high expression levels of Dicer, Drosha, and eIF6, proteins, which are involved in the biogenesis of microRNAs, are associated with a favorable prognosis of ovarian cancer [90, 91].

Furthermore other studies reported that the deregulated microRNAs seen in ovarian cancer are associated with histological subtypes, stage/grade of the tumor, BRCA mutated/epigenetically changed, primary or recurrent tumors and survival [76, 92–98]. Most of these studies have been performed on ovarian tissue samples. Besides diagnosis, microRNAs have the potential to be used as biomarkers for monitoring of the disease and to predict the chemosensitivity so treatment can be best tailored [95, 99, 100].

miR-200 Family

One example of microRNAs, which have been found highly expressed in ovarian cancer, is miR-200 family [76, 93, 96, 98, 101]. The miR-200 family contains miR-200a, miR-200b, miR-200c, miR-141, and miR-429. High levels of expression of miR-200 family are also found to be associated with decreased progression-free survival and decrease overall survival rate [93]. One study reported that miR-200a is upregulated in a significant proportion of advanced ovarian carcinomas. The elevated expression level of miR-200a facilitates tumor progression. These findings support the notion that miR-200a acts as oncogene for ovarian cancer, and its elevated level is a useful potential diagnostic indicator [102].

Another study found that upregulation of miR-200a in advanced disease was correlated with poor ovarian cancer outcome [103]. Furthermore miR-200c was found in one study to be downregulated in stage 1 epithelial ovarian cancer (EOC) relapsers compared to nonrelapsers and it was associated with PFS, overall survival, in multivariate analysis [104]. Moreover, one study suggested that expression of miR-200 might increase the sensitivity to microtubule-targeting chemotherapeutic agents; therefore upregulation of miR-200c levels in an ovarian cancer cell line increased the sensitivity for these drugs up to 85 %. However, low levels of miR-200c have been associated with chemoresistance [105].

Moreover, one recent study assessed the 200 family members which were highly expressed in serous epithelial ovarian cancer cell lines (SEOC) as candidate biomarkers [106]. They found that miR-200a, miR-200b and miR-200c and miR-103 were significantly higher in the SEOC cohort with the best predictive classifier of SEOC being the combination of miR-200b, miR-200c, and miR-103 [106].

let-7 Family

The let-7 family was frequently downregulated in ovarian cancer [76, 93, 94, 100, 107]. Low expression levels of let-7b were found in one study to be associated with poor prognosis in patients with serous ovarian carcinomas [93]. Furthermore, high expression levels of let-7 miRNA inhibitors (lin28, lin28b) [108] coordinate with shorter progression-free survival and overall survival in ovarian cancer patients

[109]. However, another study, which investigated the potential roles, clinicopathological functions, and prognostic values of let-7 miRNA family in high-grade serous ovarian cancer (HGSOC), showed that elevated expression of let-7b is associated with poor survival rates [110].

Low level of let-7i expression was significantly associated with the shorter progression-free survival of patients with advanced ovarian cancer. This might suggest that let-7i might be used as a potential biomarker to predict the response to chemotherapy and survival in ovarian cancer patients [100]. Despite most members of the let-7 family being reported as suppressors [111], isoform let-7a-3 was thought to act as an oncogene and enhance carcinogenesis [112], therefore epigenetic silencing of the let-7a-3 locus in ovarian cancer is associated with a good prognosis [113].

In addition to that, let-7 and miR-200 families are reported to be linked with regulation of "epithelial to mesenchymal transition" (EMT), where differentiated cells of epithelial phenotype separate from each other, gain mesenchymal properties, and become mobile. EMT, which plays a vital role during embryo development and wound healing, was found to be involved in carcinogenesis [114–116]. Tumors, which have undergone EMT, are less invasive, more aggressive, and more advanced. EMT process is similar to cancer progression, where cells downregulate E-cadherin and upregulate Vimentin expression.

One study reported miR-200 family as a powerful marker for cells that express E-cadherin but lack expression of Vimentin. In this study high level of miR-200 expression caused upregulation of E-cadherin in cancer cell lines and reduced their motility through direct targeting of the mRNA of the E-cadherin transcriptional repressors ZEB1 (TCF8/_EF1) and ZEB2 (SMAD-interacting protein 1 [SIP1]/ZFXH1B). Inhibition of miR-200 decreased expression of E-cadherin and increased expression of Vimentin and induced EMT [117].

Another study reported a positive correlation between miR-200a and E-cadherin in EOC, where both miR-200a and E-cadherin were upregulated in EOC compared to benign epithelial ovarian cysts and normal ovarian tissues [118]. Furthermore, miR-200a was downregulated in advanced disease compared with early-stage and grade disease. The expression of E-cadherin was low or absent in late-stage disease but was frequently expressed in benign disease. Taken together, these results indicate that miR-200 may act as a potential marker for diagnosis of early-stage disease [118].

miR-200 family showed different expression levels in different ovarian cancer tissue types [119]. Boyerinas et al. showed that miR-200a and miR-200c are expressed in serous adenocarcinoma, clear cell adenocarcinoma, and endometrioid adenocarcinoma, and miR-200b and miR-141 occur in endometrioid adenocarcinoma and mucinous adenocarcinoma [120].

EMT in cancers has been linked with chemoresistance [121]. Low expression levels of miR-200c are reported to be associated with a mesenchymal phenotype and therefore associated with chemoresistance. Conversely miR-200 expression may increase the sensitivity to microtubule-targeting drugs [105].

miR-7

miR-7 was downregulated in metastatic epithelial ovarian cancer tissues compared with primary EOC tissues. Furthermore, miR-7 was found to reverse EMT through AKT and ERK1/2 pathway inactivation by reducing the expression of EGFR in cell lines. These results suggest that miR-7 might be a potential prognostic biomarker and therapeutic target for ovarian cancer metastasis intervention [122].

miR-9

miR-9 was also downregulated in serous ovarian cancer cells compared to normal ovarian cells. miR-9 acts as a tumor suppressor inhibiting cell growth and invasion by downregulating the expression of talin 1 (TLN1), a focal adhesion protein, which is overexpressed in cancer tissues compared to normal tissues and highly expressed in metastatic tumors compared to primary tumor [123].

miR-15a and miR-16

One study investigated whether microRNA deregulation influences the regulation of Bmi-1, an oncogene that is found widely in epithelial malignancy including ovarian cancer [124]. In this study two microRNAs, miR-15a and miR-16 were identified and found to be underexpressed in ovarian cancer cell lines and primary ovarian tissues compared to normal ovarian epithelial cells and normal ovarian tissues. Transfection of cell lines with miR-15 and miR-16 downregulated Bmi-protein level and caused a significant reduction in ovarian cancer cell proliferation and clonal growth. These findings suggest that miR-15a and miR-16 may be used as potential therapeutic intervention in ovarian cancer and in other cancers that involve upregulation of Bmi-1 [124]. Moreover, miR-15 was associated with cellular resistance to many drugs through targeting the BCL2 gene [125].

miR-22

miR-22 is another microRNA, which was downregulated in EOC tissues compared to normal ovarian tissues [126, 127]. Li et al. reported a negative correlation between miR-22 and the metastatic potential in ovarian cancer cells, which suggests that miR-22 may be involved in inhibiting metastasis in ovarian cancer [126]. Low expression of miR-22 correlated with FIGO stage, tumor grade, and lymph node metastases indicating that miR-22 might be involved in the carcinogenesis and metastasis of EOC. Low miR-22 was also associated with poor overall survival and progression-free survival of EOC patients. These findings indicate that low miR-22 could be served as a promising prognostic biomarker for EOC patients [127].

miR-29b

Flavin et al. showed that miR-29b was downregulated in a significant proportion of ovarian serous carcinomas [128]. Furthermore, high expression levels of miR-29b were associated with reduced disease-free survival [128]. miR-29 family and predicted target genes were found in one study among the strongest negative correlated miRNA:mRNA pairs; overexpression of miR-29a in vitro repressed several anti-correlated genes (including DNMT3A and DNMT3B) and substantially reduced the viability of ovarian cancer cells [129]. miR-29 family members have been reported to act as tumor suppressors in acute myeloid leukemia and lung cancer, partly by reverting aberrant methylation patterns via its targeting of DNA methyltransferases (DNMT) and methylation-silenced tumor suppressors [130, 131].

miR-34

miR-34 family expression was significantly reduced in human EOC, particularly in patients with p53 mutations in several studies [132, 133]. Reduced miR-34b*/c expression might be particularly important for progression to the most advanced stages. Thus inactivation of miR-34 family contributes to the carcinogenesis and progression of serous ovarian carcinomas [132].

miR-152

miR-152 has shown potential in ovarian cancer and was significantly reduced in ovarian cancer tissues compared to normal ovarian epithelium tissues [134]. Cell proliferation was significantly inhibited following transfection of miR-152 and miR-148a into ovarian cancer cell lines. These findings suggest that miR-152 and miR-148a is involved in the carcinogenesis of ovarian cancer through deregulation of cell proliferation [134].

miR-124

miR-124 was downregulated in ovarian cancer cell lines and tumor tissues compared with normal ovarian surface epithelial cells and normal ovarian tissues [135]. miR-124 overexpression inhibited migration and invasion in EOC cells through targeting SphK1 an invasion and metastasis-related gene in human cancers. Therefore loss of miR-124 may contribute to the invasion and migration in EOC cells [135].

miR-497

Wand et al. reported that miR-497 expression was underexpressed in human ovarian cancer tissues. Low level of miR-497 expression was significantly associated with increased angiogenesis. Exogenous miR-497 was found to suppress the formation

of capillary endothelial cells in ovarian cells. miR-497 inhibited angiogenesis by suppressing expression of VEGFA in ovarian cancer cells and thus impairing the VEGFR2-mediated PI3K/AKT and MAPK/ERK pathways. These findings suggest that downregulation of miR-497 may contribute to angiogenesis in ovarian cancer. Taken together miR-497 may be a potential target for prevention and treatment of ovarian cancer [136].

miR-100

Several studies showed that miR-100 was found to be downregulated in most of the ovarian cancer cell lines, particularly clear cell ovarian carcinoma cell lines and ovarian cancer tissues [137–139]. The level of miR-100 expression in 98 EOC tissues was significantly lower compared to 15 adjacent normal epithelial tissues. Low expression of miR-100 was associated with advanced stage, higher serum CA125, lymph node involvement, and shorter overall survival. This may indicate that low miR-100 expression might act as an independent poor prognostic factor for ovarian cancer [138]. Moreover miR-100 represses mTOR signaling and increases sensitivity to the cancer drug everolimus in clear cell carcinomas cell lines [140].

miR-133a

Luo et al. showed that miR-133a was downregulated in EOC. Low miR-133a expression was associated with advanced clinical stage, poor histological differentiation, and lymph node metastasis [141]. Moreover, miR-133a was found to act on the cell viability, apoptosis, invasion, and migration of ovarian cancer OVCAR-3 cells. These findings suggest that miR-133a may act as a potential biomarker for the prediction of ovarian cancer progression and a useful promising target for gene therapy [141]. Guo et al. found that the suppressive effect of miR-133a on cell proliferation was through targeting insulin-like growth factor 1 receptor (IGF1R) [142].

miR-145

miR-145 was downregulated in ovarian cancer [143, 144]. Zhang et al. found that upregulation of miR-145 in ovarian cancer cell lines inhibited cell proliferation and promoted cell apoptosis by targeting c-Myc 3′-UTR [144]. Wu et al. showed that overexpression of p70S6K1 and MUC1, which are negatively regulated by miR-145 could lead to restoration of colony formation and cell invasion. These findings indicated that miR-145 could be used as a potential therapeutic target in ovarian cancer [143, 144]. Furthermore, miR-145 was identified as a tumor suppressor by indirectly downregulating the expression of hypoxia-inducible factor 1 (HIF-1) and vascular endothelial growth factor (VEGF) by targeting p70S6K1, which leads to inhibition of tumor growth and angiogenesis [145].

miR-203

Wang et al. investigated whether the increased expression of miR-203 can be used as a potential diagnostic and prognostic biomarker in EOC. They found that miR-203 was significantly upregulated in EOC tissues compared to adjacent normal ovarian tissues [146]. In addition, high miR-203 expression was closely correlated with advanced FIGO stage, undifferentiated tumor, lymph node involvement, and recurrence. Furthermore, miR-203 upregulation associated with shorter overall survival and progression-free survival. These findings prove that the high expression levels of miR-203 may serve as a novel biomarker to predict the progression of aggressive tumor and unfavorable prognosis of EOC patients [146].

miR-508-3p, miR-509-5p, and miR-510

YU et al. validated a microRNA expression profile that could discriminate the early-stage ovarian serous carcinoma (OSC) from advanced stages, also correlated the resulted microRNA expression with prognosis of OSC. miR-510, miR-509-5p, and miR-508-3p were significantly downregulated while miR-483-5p was upregulated in stage III OSC compared with stage I. Low miR-510 expression, low miR-509-5p expression, and advanced FIGO stage, and chemoresistance were significantly associated with poorer overall survival. These results suggested that microRNAs may play a role in the progression of OSC, and miR-510 and miR-509-5p may be served as promising biomarkers for predicting the outcome of OSC [147].

miR-21

miR-21 was significantly upregulated in EOC tissue specimens compared with benign ovarian cysts and normal ovarian tissues [148]. Overexpression of miR-21 correlated well with clinicopathological features such as advanced clinical stage, high-grade, and lymph node metastases suggesting that miR-21 deregulation might be involved in the development, progression, and metastasis of EOC. Expression of PTEN, a tumor suppressor gene for ovarian cancer expression negatively correlated with the expression of miR-21 in EOC tissues [148]. These findings suggest that miR-21 promotes tumor invasion and metastasis through downregulating expression of PTEN [148].

miR-148b

Chang et al. compared miR-148b expression levels in ovarian cancer and normal tissues [149]. miR-148b was overexpressed in the vast majority of ovarian cancer samples; however, overexpression of miR-148b was not associated with poor prognosis of patients with ovarian cancer. Taken together, miR-148b may play an

important role in the early stage of ovarian carcinogenesis; therefore miR-148b could be used as an efficient diagnostic biomarker for ovarian cancer [149].

miR-25

The expression level of miR-25 in EOC tissue was significantly higher than in adjacent normal tissue [150–152]. Apoptosis was induced by downregulation of miR-25 in ovarian cancer cells, while overexpression of miR-25 enhanced cell proliferation. Also there was an inverse relationship between the pro-apoptotic protein Bim and expression of miR-25 expression in ovarian cancer tissues. These results suggested that miR-25 directly regulates apoptosis through targeting Bim in ovarian cancer and miR-25 may serve as a potential therapeutic target for ovarian cancer [150]. Higher levels of miR-25 had significantly poorer survival than those with lower expression of this microRNA [151]. Contradicting these findings miR-25 was found to be downregulated in cancer patients compared to their benign controls [153]. These results might suggest that miR-25 may serve as a potential biomarker to monitor targeted therapies.

miR-378

Chan et al. examined the utility of miR-378 as a biomarker in ovarian cancer patients receiving to antiangiogenic therapy. They found that that miR-378 was overexpressed in ovarian cancer cells and tumors compared to normal ovarian epithelial cells [154]. Furthermore, overexpressing miR-378 in ovarian cancer cells changed expression of genes associated with angiogenesis, apoptosis, and cell cycle regulation. miR-378 expression was found as an independent predictor for PFS after antiangiogenic treatment. These results suggest that miR-378 and its downstream targets may serve as markers for response to antiangiogenic therapy [154].

microRNAs as Noninvasive Biomarkers in Serum, Plasma, and Blood of Ovarian Cancer

Several studies have demonstrated that microRNAs are circulating freely in serum and other body fluids in a highly stable, cell-free form [155–161]. In addition, microRNAs are reported to be released into the circulation from tumor cells [155]. These circulating miRNAs are highly tissue-specific; therefore they can identify the origin of metastasis [155, 162]. microRNAs have been profiled in serum and plasma in cancer and other diseases [155, 156, 159, 160]. This indicates the feasibility of using microRNAs as novel noninvasive diagnostic and prognostic biomarkers. The first study reported extraction and reliably determined microRNAs in serum was in 2008. This study showed that sera level of miR-21 was associated with relapse-free survival in patients diagnosed with diffuse large B-cell lymphoma [159].

Recently, the feasibility of profiling microRNAs from serum of ovarian cancer patients was described in one study [163]. In this study three overexpressed microRNAs were identified as potential oncomirs; miR-21, 92 and 93 with miR-92 being the most consistent overexpressed microRNA in serum from ovarian cancer patients compared to healthy controls. High expression level of miR-93 was associated with shorter progression-free and overall survival in ovarian cancer patients [163]. Recent studies showed that the level of miR-21 expression was significantly higher in serum of patients with epithelial ovarian cancer (EOC) compared to healthy controls. Moreover, increased serum miR-21 expression was associated with advanced stage, high tumor grade, and shortened overall survival. This in turn might indicate that serum miR-21 could represent a diagnostic and prognostic marker for EOC [164].

miR-221 is another microRNA which was reported to be upregulated in serum of patients with ovarian cancer. The expression of miR-221 was measured using real-time reverse polymerase reaction (RT-PCR) in serum from patients with ovarian cancer before having their surgery and compared to those of healthy control. miR-221 was elevated in ovarian cancer patients compared to normal controls, furthermore miR-221 expression correlated with advanced stage and high-grade tumor and was associated with a poor outcome [165].

Kan et al. investigated whether serum levels of microRNA could discriminate women with high-grade SEOC from healthy controls. In this study serum were significantly elevated levels of miR-200a, b and c in SEOC cell lines were also elevated in serum from patients with SEOC compared to healthy group, which indicates that circulating microRNAs were shed from cancer tissues and could be used as noninvasive diagnostic biomarkers for ovarian cancer [106].

Chung et al. found that serum miR-132, miR-26a, let-7b, and miR-145 were significantly underexpressed in epithelial ovarian cancer compared to healthy controls [87]. Furthermore miR-127, 155 and 99b were underexpressed in the serum of ovarian cancer patients. Interestingly, miR-127 was identified previously as potent suppressor in ovarian cell lines [94], which supports the underexpression in this study.

miR-99a was underexpressed in EOC tissues, serums, and cell lines SKOV-3 [166]. miR-99a targets fibroblast growth factor receptor 3 (FGFR3) directly. FGFR3 expression was higher in EOC cells. Moreover, FGFR3 expression at both mRNA and protein levels was decreased dramatically by overexpression of miR-99a These findings proved that FGFR3 is inversely correlated with miR-99a. In this study overexpression of miR-99a could significantly inhibit proliferation of EOC cell by decreasing the expression of FGFR3 which also reduced the EOC cell growth after siRNA knockdown. In summary, miR-99a expression was obviously downregulated in serums, tissues, and cell and suppresses EOC cell proliferation by targeting FGFR3, suggesting miR-99a as a potential promising prognostic biomarker and tumor suppressor for EOC therapeutics [166].

Langhe et al. found a panel of four microRNAs; let-7i-5p, miR-152, miR-122-5p, and miR-25-3p that were significantly downregulated in the serum of ovarian cancer patients compared to patients with benign ovarian neoplasm [153]. Bioinformatic analysis of the validated targets for these microRNAs revealed numerous pathways

involved in cancer such as WNT signaling, AKT/mTOR, and TLR-4/MyD88. These miRNAs have shown some potential as serum-based biomarkers in other cancers and some have already shown important roles in the biology of ovarian cancer including their invasive potential which is one of the key hallmarks that segregates benign and malignant tumors. Further work will need to be done to validate this signature in a clinical trial setting [153].

In another study the expression levels of microRNAs that showed a consistent regulation tendency through serum, ascites, and tissue specimens from ovarian cancer patients were compared to those of healthy controls with more than a twofold difference in serum. Five microRNAs, which include miR-132, miR-26a, let-7b, miR-145, and miR-143, were markedly downregulated in the serum from ovarian cancer compared to those of healthy controls. Of those microRNAs, four microRNAs were significantly underexpressed in the serum of ovarian cancer patients [87].

More recently, the utility of serum miR-145 as potential biomarker was examined in one study. Serum level of miR-145 was significantly downregulated in patients with ovarian cancer compared to healthy controls. Low levels of serum miR-145 were associated with a significant shorter median overall survival rate [167].

Another study showed that miR-200c and miR-141 were significantly higher in the serum of patient with ovarian cancer compared to healthy controls. miR-200c expression level showed a descending trend from early stages to advanced stages, while the level of miR-141 had an ascending pattern. Two-year survival rate was significantly higher in patients with high miR-200c level compared to the other group while low miR-141 group showed a significantly higher survival rate. These findings suggest that serumnmiR-200c and miR-141 as prognostic biomarkers for ovarian cancer [168].

The feasibility of circulating plasma microRNAs as suitable biomarkers for ovarian cancer was examined in few studies [169–172]. One study performed microRNA profiles on presurgical plasma samples from 42 with confirmed serous epithelial ovarian cancer, 36 women diagnosed with a benign disease, and 23 healthy controls. 19 microRNAs were significantly downregulated in cancer patients compared to healthy group. Six microRNAs were underexpressed in cancer group compared to women diagnosed with a benign disease. The utility of microRNAs in predicting the outcome in women confirmed with ovarian cancer was examined as well. Furthermore, there was a significant difference in microRNA expression in presurgical plasma from women diagnosed with ovarian cancer that had short overall survival compared to women with long overall survival [170].

Zheng et al. found that lower plasma level of let-7f expression in EOC patients compared with healthy controls. These lower levels were correlated with poor prognosis. Moreover, plasma miR-205 was overexpressed in EOC patients than in healthy controls. No difference in miR-205 expression between EOC tumor tissues and adjacent healthy tissues. miR-483-5p was upregulated in late stages (III and IV) compared with stages I and II, which in turn was consistent with its expression pattern in tumor tissues [171].

miR-26a was overexpressed in plasma in EOC patients compared to the healthy controls and the expression level in EOC specimens was much higher in EOC samples than in normal ovary samples. miR-26a was found to promote the proliferation of EOC cells through targeting ERa. Inhibition of miR-26a decreased the growth of EOC cells in culture and in nude mice. These findings suggest that overexpression of miR-26a might contribute to the development of ovarian cancer. Patients with positive ERa were found to have a significant better progression-free survival than patients with no expression. Taken together these results indicate that miR-26a could act as a potential target for therapeutic intervention in ovarian cancer [172].

Profiling of whole blood microRNAs in ovarian cancer patients was examined in one study. However, the pattern of profiling was not sensitive enough to be used for screening or monitoring of progression of ovarian cancer [173]. Profiling of peripheral blood microRNA could be combined with other serum biomarkers such as CA125 and transvaginal ultrasound to improve the overall screening of ovarian cancer [173].

Exosomes as Potential Biomarkers for Ovarian Cancer

Exosomes are small (30-100 nm in diameter) extracellular membrane-enclosed vesicles released by different cell types [174] and are formed by inward budding of late endosomes, producing multivesicular bodies (MVBs). Exosomes are released into the environment by fusion of the MVBs with the plasma membrane [20, 175]. Exosomes are thought to contribute in T-cell activation and immunosuppressive function. Therefore drugs interfere with exosome secretion can restore immunity and reduce progression of the disease [175].

A wide range of cells has been shown to release these vesicles such as B cells, T cells, epithelial cells, mast cells, tumor cells, dendritic cells, lymphoid cells, embryonic cells or cells from different tissues or organs. However, their accumulation in the peripheral circulation appears to be unique to pregnancy and cancer [21–23].

Exosomes have been detected to date in several biological fluids, such as urine, serum, CSF, plasma, blood, saliva, ascites, and breast milk [176]. Exosomes have been found to carry internal cargo representative of their cell of origin, such as membrane and cytoplasmic proteins, mRNA and miRNAs [160, 177, 178]. A variety of functions have been demonstrated for exosomes, such as cellular communication, immune system modulation and tumor progression, and the transfer of RNA [179–181].

The presence of exosomes in ovarian cancer cell cultures and plasma/serum or ascites of ovarian cancer patients has been demonstrated in several studies [160, 182–192] and other cancers. Diagnostic microRNAs were reported from exosomes from ovarian cancer patients' serum using magnetic beads and anti-EpCAM antibody [160]. In this study specific microRNAs have been profiled from tumor tissue samples and compared to microRNAs from exosomes from the peripheral circulation. Eight microRNAs (miR-21, 141, 200a/b/c, 203, 205, and 214) were upregu-

lated in cancer exosomes [160]. Profiles of circulating tumor exosomal microRNAs from ovarian cancer patients are closely related with microRNA expression in primary tumors and could be used to distinguish cancer patients from patients with benign ovarian disease and from normal control [160].

These results suggest that microRNA profiling of circulating tumor exosomes could be used as potential diagnostic markers for diagnosing and monitoring of disease and also predicting response to treatment.

Furthermore, one study showed that the level of circulating tumor-derived exosomes in the serum of women with invasive ovarian cancer was higher than their level in women with benign ovarian disease and normal controls. In addition to that the level of the exosomes was significantly greater in women with advanced disease [193]. These results suggest that profiling of microRNAs of the circulating tumor exosomes could be used as potential diagnostic markers for screening of asymptomatic women and monitoring of the disease recurrence [160, 188].

microRNAs and Chemoresistance

Chemoresistance is one of the most important obstacles to the successful treatment of ovarian cancer. microRNAs play important roles in disease processes, including the development of drug resistance. Several microRNAs have been involved in chemosensitivity.

miR-21 was overexpressed in the resistant ovarian cell line and its inhibition induced apoptosis. knockdown of miR-21 was found to increase the expression of PDCD4, a tumor suppressor and attenuate apoptosis inhibitor c-IAP2. Interestingly, women with tumors that overexpressed miR-21 were associated with a shorter progression-free survival [194].

Frederick et al. evaluated the differential expression of microRNAs among tumor cells in ascites and matched omental metastasis in patients with EOC, who were undergoing primary surgical cytoreduction. Cell viability and microRNA profiling were evaluated in patients' samples following treatment with carboplatin, paclitaxel, and combination chemotherapy. Malignant cells in ascites showed greater cell viability when treated with carboplatin compared to omental metastasis. miR-21 and miR-214 were significantly upregulated in malignant cells from ascites compared to omental metastasis [195].

Another microRNA which showed potential in chemosensitivity is miR-29. Cisplatin-resistant cells expressed a lower level of miR-29a/b/c. Ectopic expression of miR-29 alone or in combination with cisplatin treatment efficaciously reduced the tumorigenicity of ovarian cancer cell lines in vivo. Downregulation of miR-29 increases cisplatin resistance in ovarian cancer cells. This suggests that miR-29 overexpression is a potential sensitizer to cisplatin treatment, which might have therapeutic implications [196].

Low levels of miR-199a might act as a reliable predictor for chemoresistance in patients with recurrent tumors [93] by targeting IKKB, which induces a pro-

inflammatory environment that is associated with cancer progression and chemoresistance. This might indicate miR-199a as a biomarker to monitor the response to chemotherapy [197]. Furthermore, one study reported that cisplatin resistance could be reversed by miR-199a through the inhibition of mammalian target of rapamycin (mTOR) and that mTOR may be the target of miR-199a during this process [127].

miR-199a and miR-125b were found to be downregulated in ovarian cancer tissues and cell lines. Overexpression of miR-199a and miR-125b inhibited tumor angiogenesis associated with the decrease of expression of HIF-1a and VEGF in ovarian cancer cells. Forced expression of HER2 and HER3, which are direct targets of miR199a and miR-125 retrieve miR-199a- and miR-125b-inhibiting angiogenesis responses and Akt/p70S6K1/HIF-1a pathway. These results indicate that miR-199a, miR-125b could be used as potential therapeutic markers to suppress tumor angiogenesis ovarian cancer treatment in the future [89].

Yang et al. found that miR-214 was frequently expressed in ovarian cancer tissues and that let-7i, which enhances re-sensitization to platinum resistance, was expressed less in the same tissues [100]. miR-214 has been found to induce cell survival and cisplatin resistance by targeting PTEN, which regulates cell proliferation [101]. Knockdown of miR-214 was found to decrease cisplatin resistance in cisplatin-resistant cell line, while exogenous expression of miR-214 makes cisplatin-sensitive cell lines resistant to cisplatin-induced apoptosis. miR-214 was found to activate the Akt pathway, which leads to chemoresistance by targeting PTEN. Therefore, miR-214 may play a critical role in cisplatin resistance by targeting the PTEN/Akt pathway [137, 198].

Other microRNAs, which might have a role in chemoresistance, are miR-130a, which targets the prometastatic and chemoresistance-associated M-CSF [99] and miR-27a and miR-451, which regulate the expression of a protein involved in drug resistance in ovarian cancer [199–201]. Overexpression levels of miR-130a correlated with cisplatin resistance in ovarian cancer. Accordingly inhibition of expression of miR-130a might counteract cisplatin resistance of ovarian cancer [202]. miR-27a and miR-451, which regulate the expression of a protein involved in drug resistance in ovarian cancer [199–201].

miR-449a was significantly underexpressed in the cisplatin-resistant ovarian cell lines compared with their sensitive parent line. Overexpression of miR-449a inhibits proliferation and promotes apoptosis thus increasing cisplatin sensitivity of cell lines. miR-449a was found to function through suppressing NOTCH1 directly. These results suggest that the ectopic expression of miR-449a may be a potential therapeutic candidate for the management of cisplatin resistance in ovarian cancer [203].

miR-106a was upregulated in the CDDP-resistant ovarian cancer cell line compared with the parental OVCAR3 cell line. Overexpression of miR-106a contributes to the generation of CDDP-resistant ovarian cancer cells, partly by targeting PDCD4, which enhance CDDP-induced apoptosis via death of receptor-mediated pathway [204].

Huh et al. compared microRNA expression in paclitaxel-resistant cell lines with that of Paclitaxel-sensitive, parental ovarian cancer cell line. They found that upregulation of miR-106a and downregulation of miR-591 was associated with Paclitaxel

resistance [205]. Modulation of miR-106a and miR-591 in paclitaxel-resistant cancer cells re-sensitized the cells to paclitaxel through the increase of several pro-apoptotic genes of TNF ligand/receptor and caspase families, and by directly targeting ZEB1, BCL10, and caspase-7. Therefore, suggesting that the modulation of these microRNAs may be a potential therapeutic strategy to overcome Paclitaxel resistance in ovarian cancer [205].

Kim et al. compared microRNA expression in taxol-resistant patients compared to taxol-sensitive group. microRNA profiling showed that miR-663 and miR-622 were identified as significant prognostic biomarkers of the chemoresistant patient group. Downregulation of these microRNAs increased the sensitivity of cancer cells to Taxol and were associated with better survival. In the chemosensitive patient group, only miR-647 was identified as a potential prognostic biomarker. Taken together, the data indicate that the three miRNAs are closely associated with Taxol resistance and potentially better prognosis factors, therefore would be used in the development of microRNA therapies in treating ovarian cancer [206].

Hong et al. showed that miR-376c reduces signaling of Nodal/activin receptor-like kinase 7 (ALK7), which is involved in drug sensitivity, and reduces the effects of cisplatin and carboplatin [119, 207]. Ectopic expression of miR-376c significantly promoted cell proliferation, spheroid formation, and survival through targeting ALK7. Knockdown of ALK7 could lead to an increase in cell proliferation, viability, and spheroid formation. Overexpression of miR-376c blocked cisplatin-induced cell death, whereas anti-miR-376c enhanced the effect of cisplatin. Moreover, in serous carcinoma samples taken from ovarian cancer patients who responded well to chemotherapy, ALK7 levels were higher and miR-376c levels were lower in chemosensitive tumors than in their chemoresistant counterparts, By contrast, ALK7 expression was weak and miR-376c levels were high in samples from patients who responded poorly to chemotherapy [208].

The effect of let-7a expression on survival outcomes of EOC patients treated with different chemotherapy was investigated by Lu et al. [209]. The expression of Let-7a was not correlated with the stage of the disease, tumor grade, histology, and debulking status. Let-7a expression was significantly lower in patients who responded to platinum with paclitaxel compared to those who did not. High let-7a associated with better survival compared to Low let-7a when patients were treated with platinum without paclitaxel. However, when platinum was added to paclitaxel, high let-7a was associated with worse progression-free and overall survival. Patients treated with paclitaxel in addition to platinum with low let-7a, had a better survival than those treated without paclitaxel. There was no difference in survival among patients with high let-7a, who had two types of treatment [209]. Let-7 g was found to be involved in drug resistance. Boyerinas et al. showed that let-7 g suppressed IMP-1, which is involved in multidrug resistance and increased sensitivity to taxanes [119, 120].

Liu et al. identified a panel of microRNAs-related drug resistance. miR-152 and miR-381 expressions were significantly higher in drug-resistant OC tissue compared with those in drug-sensitive tissue. While miR-200a-3p and miR-429 were downregulated in drug-resistant tissues. miR-152, miR-200a-3p, miR-381, and

miR-429 may contribute in the formation of chemoresistance in EOC through the target genes predicted [210].

The expression of miR-125b in cisplatin-sensitive ovarian cancer cell line was compared with the expression in its resistant variant. miR-125b was upregulated in cisplatin-resistant cells. The upregulation of miR-125b led to marked inhibition of cisplatin-induced cytotoxicity and apoptosis and a subsequent increase in the resistance to cisplatin in resistant and sensitive cells by suppressing Bak1. These results have important impact on the development of targeted therapeutics for overcoming cisplatin resistance in ovarian cancer [211].

Expression of miR-128 was significantly decreased in the cisplatin-resistant ovarian cancer cell line compared with parental cells and reduced following treatment with cisplatin. miR-128 overexpression was found to resensitize resistant cells to cisplatin and reduced the expression of cisplatin-resistant-related proteins ABCC5 and Bmi-1, while miR-128 inhibitors increased cisplatin resistance in parent cells. These findings suggest that miR-128 may act as a promising potential therapeutic target for improvement of chemosensitivity in ovarian cancer [212].

The expression of miR-197 was measured in normal ovarian cells, ovarian cancer cells, and Taxol-resistant ovarian cancer cells. miR-197 was significantly increased in Taxol-resistant ovarian cancer cells. Overexpression of miR-197 can enhance Taxol resistance, cell proliferation, and invasion of ovarian cancer cells through downregulating NLK, a negative regulator of WNT signaling pathway. Furthermore, of miR-197 repression in ovarian cancer cells can sensitize response of the cells to Taxol and promote attenuated cell proliferation and invasion ability [213].

Mitamura et al. found that lower level of miR-31 expressions and higher expressions of MET (also known as c-Met or hepatocyte growth factor receptor) were significantly correlated with paclitaxel resistance and poor prognosis in ovarian cancer patients [214]. Creighton et al. showed that miR-31 was underexpressed in both serous ovarian cancer cell lines and tissues compared to normal ovarian epithelium. miR-31 was found to act as tumor suppressor in ovarian cancer and inhibits the expression of cell cycle regulators, e.g., E2F2 and STK40 thus inhibited proliferation, and induced apoptosis. Deficiency of miR-31 was associated with mutation in TP53 pathway and functions in serous ovarian cancer. These results suggest that miR-31 might be beneficial for patients with cancers that are deficient in TP53 activity [215].

Vecchione et al. identified a panel of microRNAs (miR-217, miR-484, and miR-617) that could predict chemoresistance in 198 serous ovarian cancer samples. miR-484 found to have a potential to improve chemosensitivity through the modulation of tumor angiogenesis, by directly targeting VEGFB and KDR [216].

Conclusion

Currently no established ovarian cancer screening programs exist, early diagnosis of ovarian cancer and successful optimal debulking surgery are the key determinants in the overall outcome for the patient. A key focus of current studies is to

diagnose the disease early and to define which patients are likely to respond to particular chemotherapeutics or biological agents, by identifying molecular markers in the cancer tissue or serum in a move towards personalized cancer treatment.

An enormous number of studies reports that microRNAs are aberrantly expressed in human cancer. Several microRNA signatures from tumor cells/tissues or serum/plasma have been described in ovarian cancer. microRNAs expression profiles are quite different in ovarian cancer compared to normal control tissue. Profiling of microRNA using arrays might contribute to the detection of tissue type, stage, and prognosis. Accumulating body of evidence supports the rationale of suppression of upregulated microRNAs, or substitution of downregulated microRNAs could be novel therapeutic approaches for ovarian cancer.

However, despite all the advances there is still a long way to understand and implement microRNA in the management of cancer and ovarian cancer in particular. We hope that increased understanding of the role of microRNAs in cancer development will give a new input to the management of ovarian cancer.

References

1. Clarke DL. Screening for ovarian cancer. N Engl J Med. 2009;361:170–7.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127(12):2893–917.
3. Hensley M, Alektiar D, Chi D. Ovarian and fallopian-tube cancer. In: Barakat RR, Bevers MW, Gershenson D, Hoskins W, editors. Handbook of gynecologic oncology. London: Martin Dunitz; 2002.
4. Sueblinvong T, Carney ME. Current understanding of risk factors for ovarian cancer. Curr Treat Options Oncol. 2009;10:67–81.
5. Salehi F, Dunfield L, Phillips KP, Krewski D, Vanderhyden BC. Risk factors for ovarian cancer: an overview with emphasis on hormonal factors. J Toxicol Environ Health B Crit Rev. 2008;11(3-4):301–21.
6. Van Gorp T, Amant F, Neven P, Vergote I, Moerman P. Endometriosis and the development of malignant tumors of the pelvis. A review of literature. Best Pract Res Clin Obstet Gynaecol. 2004;18(2):349–71.
7. DeCherney AH, Nathan L, Laufer N. Premalignant and malignant disorders of the ovaries and oviducts. 9th ed. New York: Springer; 2003. p. 933–46.
8. Riman T, Nilsson S, Persson IR. Review of epidemiological evidence for reproductive and hormonal factors in relation to the risk of epithelial ovarian malignancies. Acta Obstet Gynecol Scand. 2004;83(9):783–95.
9. National Cancer Irish Registry. http://www.ncri.ie/.
10. Reynolds K. Benign and malignant ovarian masses. In: Luesley DM, Baker PN, editors. Obstetrics and gynaecology. 1st ed. London: Hodder Arnold; 2004.
11. Tsai WC, Hsu SD, Hsu CS, Lai TC, Chen SJ, Shen R, et al. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. J Clin Invest. 2012;122(8):2884–97.
12. Yu M, Stott S, Toner M, Maheswaran S, Haber DA. Circulating tumor cells: approaches to isolation and characterization. J Cell Biol. 2011;192(3):373–82.
13. Clarke-Pearson DL. Clinical practice. Screening for ovarian cancer. N Engl J Med. 2009;361(2):170–7.
14. Santulli G, D'Ascia C. Atrial remodelling in echocardiographic super-responders to cardiac resynchronization therapy. Heart. 2012;98(6):517.

15. Moyer VA. Screening for ovarian cancer: U.S. Preventive Services Task Force reaffirmation recommendation statement. Ann Intern Med. 2012;157(12):900–4.
16. Vang S, Wu HT, Fischer A, Miller DH, Maclaughlan S, Douglass E, et al. Identification of ovarian cancer metastatic miRNAs. PLoS One. 2013;8(3), e58226.
17. Hankinson SHD, Colditz G, Willett W, Stampfer M, Rosner B, Hennekens C, Speizer F. Tubal ligation, hysterectomy, and risk of ovarian cancer. JAMA. 1993;270:2813–8.
18. Dietl J, Wischhusen J, Hausler SF. The post-reproductive Fallopian tube: better removed? Hum Reprod. 2011;26(11):2918–24.
19. Kahlert C, Kalluri R. Exosomes in tumor microenvironment influence cancer progression and metastasis. J Mol Med. 2013;91(4):431–7.
20. Couzin J. Cell biology: the ins and outs of exosomes. Science. 2005;308(5730):1862–3.
21. Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. Nat Rev Immunol. 2009;9(8):581–93.
22. Taylor DD, Black PH. Shedding of plasma membrane fragments. Neoplastic and developmental importance. Dev Biol. 1986;3:33–57.
23. Taylor DD, Bohler HC, Gercel-Taylor C. Pregnancy-linked suppression of TcR signaling pathways by a circulating factor absent in recurrent spontaneous pregnancy loss (RPL). Mol Immunol. 2006;43(11):1872–80.
24. Landen Jr CN, Birrer MJ, Sood AK. Early events in the pathogenesis of epithelial ovarian cancer. J Clin Oncol. 2008;26(6):995–1005.
25. Shih IM, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. Am J Pathol. 2004;164(5):1511–8.
26. Schorge JO, Modesitt SC, Coleman RL, Cohn DE, Kauff ND, Duska LR, et al. SGO White Paper on ovarian cancer: etiology, screening and surveillance. Gynecol Oncol. 2010;119(1):7–17.
27. Zhu C, Li J, Ding Q, Cheng G, Zhou H, Tao L, et al. miR-152 controls migration and invasive potential by targeting TGFalpha in prostate cancer cell lines. Prostate. 2013;73(10):1082–9.
28. Ginsberg SD. RNA amplification strategies for small sample populations. Methods. 2005;37(3):229–37.
29. Kacharmina JE, Crino PB, Eberwine J. Preparation of cDNA from single cells and subcellular regions. Methods Enzymol. 1999;303:3–18.
30. Che S, Ginesberg SD. Trends in RNA research. New York: Nova Science; 2006.
31. Fathalla MF. Incessant ovulation—a factor in ovarian neoplasia? Lancet. 1971;2(7716):163.
32. Cramer DW, Welch WR. Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis. J Natl Cancer Inst. 1983;71(4):717–21.
33. Qiao C, Yuan Z, Li J, He B, Zheng H, Mayer C, et al. Liver-specific microRNA-122 target sequences incorporated in AAV vectors efficiently inhibits transgene expression in the liver. Gene Ther. 2011;18(4):403–10.
34. Qi R, Weiland M, Gao XH, Zhou L, Mi QS. Identification of endogenous normalizers for serum microRNAs by microarray profiling: U6 small nuclear RNA is not a reliable normalizer. Hepatology. 2012;55(5):1640–2; author reply 2–3.
35. Debernardi S, Skoulakis S, Molloy G, Chaplin T, Dixon-McIver A, Young BD. MicroRNA miR-181a correlates with morphological sub-class of acute myeloid leukaemia and the expression of its target genes in global genome-wide analysis. Leukemia. 2007;21(5):912–6.
36. Huober J, Meyer A, Wagner U, Wallwiener D. The role of neoadjuvant chemotherapy and interval laparotomy in advanced ovarian cancer. J Cancer Res Clin Oncol. 2002;128(3): 153–60.
37. Chien JR, Aletti G, Bell DA, Keeney GL, Shridhar V, Hartmann LC. Molecular pathogenesis and therapeutic targets in epithelial ovarian cancer. J Cell Biochem. 2007;102(5):1117–29.
38. Teoh DG, Secord AA. Antiangiogenic therapies in epithelial ovarian cancer. Cancer Control. 2011;18(1):31–43.
39. Suh DH, Kim K, Kim JW. Major clinical research advances in gynecologic cancer in 2011. J Gynecol Oncol. 2012;23(1):53–64.

40. Fishman DA, Cohen L, Blank SV, Shulman L, Singh D, Bozorgi K, et al. The role of ultrasound evaluation in the detection of early-stage epithelial ovarian cancer. Am J Obstet Gynecol. 2005;192(4):1214–21; discussion 21–2.
41. Menon U, Gentry-Maharaj A, Hallett R, Ryan A, Burnell M, Sharma A, et al. Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). Lancet Oncol. 2009;10(4):327–40.
42. Jacobs IJ, Menon U. Progress and challenges in screening for early detection of ovarian cancer. Mol Cell Proteomics. 2004;3(4):355–66.
43. Partridge E, Kreimer AR, Greenlee RT, Williams C, Xu JL, Church TR, et al. Results from four rounds of ovarian cancer screening in a randomized trial. Obstet Gynecol. 2009;113(4):775–82.
44. Buys SS, Partridge E, Black A, Johnson CC, Lamerato L, Isaacs C, et al. Effect of screening on ovarian cancer mortality: the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Randomized Controlled Trial. JAMA. 2011;305(22):2295–303.
45. Skates SJ, Jacobs IJ, Knapp RC. Tumor markers in screening for ovarian cancer. Methods Mol Med. 2001;39:61–73.
46. Bast Jr RC, Klug TL, St John E, Jenison E, Niloff JM, Lazarus H, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med. 1983;309(15):883–7.
47. Gocze P, Vahrson H. [Ovarian carcinoma antigen (CA 125) and ovarian cancer (clinical follow-up and prognostic studies)]. Orv Hetil. 1993;134(17):915–8.
48. Santillan A, Garg R, Zahurak ML, Gardner GJ, Giuntoli 2nd RL, Armstrong DK, et al. Risk of epithelial ovarian cancer recurrence in patients with rising serum CA-125 levels within the normal range. J Clin Oncol. 2005;23(36):9338–43.
49. Gupta D, Lis CG. Role of CA125 in predicting ovarian cancer survival—a review of the epidemiological literature. J Ovarian Res. 2009;2:13.
50. Helzlsouer KJ, Bush TL, Alberg AJ, Bass KM, Zacur H, Comstock GW. Prospective study of serum CA-125 levels as markers of ovarian cancer. JAMA. 1993;269(9):1123–6.
51. Gadducci A, Cosio S, Carpi A, Nicolini A, Genazzani AR. Serum tumor markers in the management of ovarian, endometrial and cervical cancer. Biomed Pharmacother. 2004;58(1):24–38.
52. Gadducci A, Ferdeghini M, Prontera C, Moretti L, Mariani G, Bianchi R, et al. The concomitant determination of different tumor markers in patients with epithelial ovarian cancer and benign ovarian masses: relevance for differential diagnosis. Gynecol Oncol. 1992;44(2):147–54.
53. Koelma IA, Nap M, Rodenburg CJ, Fleuren GJ. The value of tumor marker CA 125 in surgical pathology. Histopathology. 1987;11(3):287–94.
54. Kudoh K, Kikuchi Y, Kita T, Tode T, Takano M, Hirata J, et al. Preoperative determination of several serum tumor markers in patients with primary epithelial ovarian carcinoma. Gynecol Obstet Invest. 1999;47(1):52–7.
55. Negishi Y, Furukawa T, Oka T, Sakamoto M, Hirata T, Okabe K, et al. Clinical use of CA 125 and its combination assay with other tumor marker in patients with ovarian carcinoma. Gynecol Obstet Invest. 1987;23(3):200–7.
56. Castelli M, Battaglia F, Scambia G, Panici PB, Ferrandina G, Mileo AM, et al. Immunosuppressive acidic protein and CA 125 levels in patients with ovarian cancer. Oncology. 1991;48(1):13–7.
57. Castelli M, Romano P, Atlante G, Pozzi M, Ferrini U. Immunosuppressive acidic protein (IAP) and CA 125 assays in detection of human ovarian cancer: preliminary results. Int J Biol Markers. 1987;2(3):187–90.
58. Fioretti P, Gadducci A, Ferdeghini M, Prontera C, Malagnino G, Facchini V, et al. The concomitant determination of different serum tumor markers in epithelial ovarian cancer: relevance for monitoring the response to chemotherapy and follow-up of patients. Gynecol Oncol. 1992;44(2):155–60.

59. Negishi Y, Iwabuchi H, Sakunaga H, Sakamoto M, Okabe K, Sato H, et al. Serum and tissue measurements of CA72-4 in ovarian cancer patients. Gynecol Oncol. 1993;48(2):148–54.
60. Sawada M, Okudaira Y, Matsui Y, Shimizu Y. Immunosuppressive acidic protein in patients with ovarian cancer. Cancer. 1983;52(11):2081–5.
61. Yedema C, Massuger L, Hilgers J, Servaas J, Poels L, Thomas C, et al. Pre-operative discrimination between benign and malignant ovarian tumors using a combination of CA125 and CA15.3 serum assays. Int J Cancer Suppl. 1988;3:61–7.
62. Onsrud M. Tumor markers in gynaecologic oncology. Scand J Clin Lab Invest Suppl. 1991;206:60–70.
63. Roman LD, Muderspach LI, Burnett AF, Morrow CP. Carcinoembryonic antigen in women with isolated pelvic masses. Clinical utility? J Reprod Med. 1998;43(5):403–7.
64. Kobayashi E, Ueda Y, Matsuzaki S, Yokoyama T, Kimura T, Yoshino K, et al. Biomarkers for screening, diagnosis, and monitoring of ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2012;21(11):1902–12.
65. Gadducci A, Ferdeghini M, Ceccarini T, Prontera C, Facchini V, Bianchi R, et al. A comparative evaluation of the ability of serum CA 125, CA 19-9, CA 15-3, CA 50, CA 72-4 and TATI assays in reflecting the course of disease in patients with ovarian carcinoma. Eur J Gynaecol Oncol. 1990;11(2):127–33.
66. Gao L, Cheng HY, Dong L, Ye X, Liu YN, Chang XH, et al. The role of HE4 in ovarian cancer: inhibiting tumor cell proliferation and metastasis. J Int Med Res. 2011;39(5): 1645–60.
67. Andersen MR, Goff BA, Lowe KA, Scholler N, Bergan L, Drescher CW, et al. Use of a Symptom Index, CA125, and HE4 to predict ovarian cancer. Gynecol Oncol. 2010;116(3): 378–83.
68. Montagnana M, Danese E, Giudici S, Franchi M, Guidi GC, Plebani M, et al. HE4 in ovarian cancer: from discovery to clinical application. Adv Clin Chem. 2011;55:1–20.
69. Drapkin R, von Horsten HH, Lin Y, Mok SC, Crum CP, Welch WR, et al. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. Cancer Res. 2005;65(6):2162–9.
70. Van Gorp T, Cadron I, Despierre E, Daemen A, Leunen K, Amant F, et al. HE4 and CA125 as a diagnostic test in ovarian cancer: prospective validation of the Risk of Ovarian Malignancy Algorithm. Br J Cancer. 2011;104(5):863–70.
71. Moore RG, Brown AK, Miller MC, Skates S, Allard WJ, Verch T, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. Gynecol Oncol. 2008;108(2):402–8.
72. Kalapotharakos G, Asciutto C, Henic E, Casslen B, Borgfeldt C. High preoperative blood levels of HE4 predicts poor prognosis in patients with ovarian cancer. J Ovarian Res. 2012;5(1):20.
73. Angioli R, Plotti F, Capriglione S, Aloisi A, Montera R, Luvero D, et al. Can the preoperative HE4 level predict optimal cytoreduction in patients with advanced ovarian carcinoma? Gynecol Oncol. 2013;128(3):579–83.
74. Paek J, Lee SH, Yim GW, Lee M, Kim YJ, Nam EJ, et al. Prognostic significance of human epididymis protein 4 in epithelial ovarian cancer. Eur J Obstet Gynecol Reprod Biol. 2011;158(2):338–42.
75. Kong SY, Han MH, Yoo HJ, Hwang JH, Lim MC, Seo SS, et al. Serum HE4 level is an independent prognostic factor in epithelial ovarian cancer. Ann Surg Oncol. 2012;19(5):1707–12.
76. Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, et al. MicroRNA signatures in human ovarian cancer. Cancer Res. 2007;67(18):8699–707.
77. Calin GA, Croce CM. MicroRNA-cancer connection: the beginning of a new tale. Cancer Res. 2006;66(15):7390–4.
78. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer. 2006;6(11): 857–66.

79. Esquela-Kerscher A, Slack FJ. Oncomirs—microRNAs with a role in cancer. Nat Rev Cancer. 2006;6(4):259–69.
80. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. 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(24):15524–9.
81. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res. 2005;65(14):6029–33.
82. He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, et al. The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci U S A. 2005;102(52):19075–80.
83. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, et al. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res. 2004;64(11):3753–6.
84. Zhang L, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A, et al. microRNAs exhibit high frequency genomic alterations in human cancer. Proc Natl Acad Sci U S A. 2006;103(24):9136–41.
85. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A. 2005;102(39):13944–9.
86. Michael MZ, O'Connor SM, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res. 2003;1(12):882–91.
87. Chung YW, Bae HS, Song JY, Lee JK, Lee NW, Kim T, et al. Detection of MicroRNA as novel biomarkers of epithelial ovarian cancer from the serum of ovarian cancer patient. Int J Gynecol Cancer. 2013;23:673–9.
88. Qin W, Ren Q, Liu T, Huang Y, Wang J. MicroRNA-155 is a novel suppressor of ovarian cancer-initiating cells that targets CLDN1. FEBS Lett. 2013;587(9):1434–9.
89. He J, Jing Y, Li W, Qian X, Xu Q, Li FS, et al. Roles and mechanism of miR-199a and miR-125b in tumor angiogenesis. PLoS One. 2013;8(2):e56647.
90. Flavin RJ, Smyth PC, Finn SP, Laios A, O'Toole SA, Barrett C, et al. Altered eIF6 and Dicer expression is associated with clinicopathological features in ovarian serous carcinoma patients. Mod Pathol. 2008;21(6):676–84.
91. Merritt WM, Lin YG, Han LY, Kamat AA, Spannuth WA, Schmandt R, et al. Dicer, Drosha, and outcomes in patients with ovarian cancer. N Engl J Med. 2008;359(25):2641–50.
92. Laios A, O'Toole S, Flavin R, Martin C, Kelly L, Ring M, et al. Potential role of miR-9 and miR-223 in recurrent ovarian cancer. Mol Cancer. 2008;7:35.
93. Nam EJ, Yoon H, Kim SW, Kim H, Kim YT, Kim JH, et al. MicroRNA expression profiles in serous ovarian carcinoma. Clin Cancer Res. 2008;14(9):2690–5.
94. Zhang L, Volinia S, Bonome T, Calin GA, Greshock J, Yang N, et al. Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer. Proc Natl Acad Sci U S A. 2008;105(19):7004–9.
95. Eitan R, Kushnir M, Lithwick-Yanai G, David MB, Hoshen M, Glezerman M, et al. Tumor microRNA expression patterns associated with resistance to platinum based chemotherapy and survival in ovarian cancer patients. Gynecol Oncol. 2009;114(2):253–9.
96. Gallagher MF, Flavin RJ, Elbaruni SA, McInerney JK, Smyth PC, Salley YM, et al. Regulation of microRNA biosynthesis and expression in 2102Ep embryonal carcinoma stem cells is mirrored in ovarian serous adenocarcinoma patients. J Ovarian Res. 2009;2:19.
97. Lee CH, Subramanian S, Beck AH, Espinosa I, Senz J, Zhu SX, et al. MicroRNA profiling of BRCA1/2 mutation-carrying and non-mutation-carrying high-grade serous carcinomas of ovary. PLoS One. 2009;4(10), e7314.
98. Wyman SK, Parkin RK, Mitchell PS, Fritz BR, O'Briant K, Godwin AK, et al. Repertoire of microRNAs in epithelial ovarian cancer as determined by next generation sequencing of small RNA cDNA libraries. PLoS One. 2009;4(4), e5311.
99. Sorrentino A, Liu CG, Addario A, Peschle C, Scambia G, Ferlini C. Role of microRNAs in drug-resistant ovarian cancer cells. Gynecol Oncol. 2008;111(3):478–86.

100. Yang N, Kaur S, Volinia S, Greshock J, Lassus H, Hasegawa K, et al. MicroRNA microarray identifies Let-7i as a novel biomarker and therapeutic target in human epithelial ovarian cancer. Cancer Res. 2008;68(24):10307–14.
101. Yang H, Kong W, He L, Zhao JJ, O'Donnell JD, Wang J, et al. MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. Cancer Res. 2008;68(2):425–33.
102. Zhu CL, Gao GS. miR-200a overexpression in advanced ovarian carcinomas as a prognostic indicator. Asian Pac J Cancer Prev. 2014;15(20):8595–601.
103. Hu X, Macdonald DM, Huettner PC, Feng Z, El Naqa IM, Schwarz JK, et al. A miR-200 microRNA cluster as prognostic marker in advanced ovarian cancer. Gynecol Oncol. 2009;114(3):457–64.
104. Marchini S, Cavalieri D, Fruscio R, Calura E, Garavaglia D, Nerini IF, et al. Association between miR-200c and the survival of patients with stage I epithelial ovarian cancer: a retrospective study of two independent tumor tissue collections. Lancet Oncol. 2011;12(3):273–85.
105. Cochrane DR, Spoelstra NS, Howe EN, Nordeen SK, Richer JK. MicroRNA-200c mitigates invasiveness and restores sensitivity to microtubule-targeting chemotherapeutic agents. Mol Cancer Ther. 2009;8(5):1055–66.
106. Kan CW, Hahn MA, Gard GB, Maidens J, Huh JY, Marsh DJ, et al. Elevated levels of circulating microRNA-200 family members correlate with serous epithelial ovarian cancer. BMC Cancer. 2012;12:627.
107. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih Ie M, Zhang Y, et al. MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One. 2008;3(6), e2436.
108. Viswanathan SR, Daley GQ, Gregory RI. Selective blockade of microRNA processing by Lin28. Science. 2008;320(5872):97–100.
109. Lu L, Katsaros D, Shaverdashvili K, Qian B, Wu Y, de la Longrais IA, et al. Pluripotent factor lin-28 and its homologue lin-28b in epithelial ovarian cancer and their associations with disease outcomes and expression of let-7a and IGF-II. Eur J Cancer. 2009;45(12):2212–8.
110. Tang Z, Ow GS, Thiery JP, Ivshina AV, Kuznetsov VA. Meta-analysis of transcriptome reveals let-7b as an unfavorable prognostic biomarker and predicts molecular and clinical subclasses in high-grade serous ovarian carcinoma. Int J Cancer. 2014;134(2):306–18.
111. Bussing I, Slack FJ, Grosshans H. let-7 microRNAs in development, stem cells and cancer. Trends Mol Med. 2008;14(9):400–9.
112. Brueckner B, Stresemann C, Kuner R, Mund C, Musch T, Meister M, et al. The human let-7a-3 locus contains an epigenetically regulated microRNA gene with oncogenic function. Cancer Res. 2007;67(4):1419–23.
113. Lu L, Katsaros D, de la Longrais IA, Sochirca O, Yu H. Hypermethylation of let-7a-3 in epithelial ovarian cancer is associated with low insulin-like growth factor-II expression and favorable prognosis. Cancer Res. 2007;67(21):10117–22.
114. van Jaarsveld MT, Helleman J, Berns EM, Wiemer EA. MicroRNAs in ovarian cancer biology and therapy resistance. Int J Biochem Cell Biol. 2010;42(8):1282–90.
115. Koutsaki M, Spandidos DA, Zaravinos A. Epithelial-mesenchymal transition-associated miRNAs in ovarian carcinoma, with highlight on the miR-200 family: prognostic value and prospective role in ovarian cancer therapeutics. Cancer Lett. 2014;351(2):173–81.
116. Chen J, Wang L, Matyunina LV, Hill CG, McDonald JF. Overexpression of miR-429 induces mesenchymal-to-epithelial transition (MET) in metastatic ovarian cancer cells. Gynecol Oncol. 2011;121(1):200–5.
117. Park SM, Gaur AB, Lengyel E, Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev. 2008;22(7):894–907.
118. Xu S, Xu P, Wu W, Ou Y, Xu J, Zhang G, et al. The biphasic expression pattern of miR-200a and E-cadherin in epithelial ovarian cancer and its correlation with clinicopathological features. Curr Pharm Des. 2014;20(11):1888–95.

119. Banno K, Yanokura M, Iida M, Adachi M, Nakamura K, Nogami Y, et al. Application of microRNA in diagnosis and treatment of ovarian cancer. BioMed Res Int. 2014; 2014:232817.
120. Boyerinas B, Park SM, Murmann AE, Gwin K, Montag AG, Zillhardt M, et al. Let-7 modulates acquired resistance of ovarian cancer to Taxanes via IMP-1-mediated stabilization of multidrug resistance 1. Int J Cancer. 2012;130(8):1787–97.
121. Voulgari A, Pintzas A. Epithelial-mesenchymal transition in cancer metastasis: mechanisms, markers and strategies to overcome drug resistance in the clinic. Biochim Biophys Acta. 2009;1796(2):75–90.
122. Zhou X, Hu Y, Dai L, Wang Y, Zhou J, Wang W, et al. MicroRNA-7 inhibits tumor metastasis and reverses epithelial-mesenchymal transition through AKT/ERK1/2 inactivation by targeting EGFR in epithelial ovarian cancer. PLoS One. 2014;9(5), e96718.
123. Tang H, Yao L, Tao X, Yu Y, Chen M, Zhang R, et al. miR-9 functions as a tumor suppressor in ovarian serous carcinoma by targeting TLN1. Int J Mol Med. 2013;32(2):381–8.
124. Bhattacharya R, Nicoloso M, Arvizo R, Wang E, Cortez A, Rossi S, et al. MiR-15a and MiR-16 control Bmi-1 expression in ovarian cancer. Cancer Res. 2009;69(23):9090–5.
125. Aqeilan RI, Calin GA, Croce CM. miR-15a and miR-16-1 in cancer: discovery, function and future perspectives. Cell Death Differ. 2010;17(2):215–20.
126. Li J, Liang S, Yu H, Zhang J, Ma D, Lu X. An inhibitory effect of miR-22 on cell migration and invasion in ovarian cancer. Gynecol Oncol. 2010;119(3):543–8.
127. Wang Z, Ting Z, Li Y, Chen G, Lu Y, Hao X. microRNA-199a is able to reverse cisplatin resistance in human ovarian cancer cells through the inhibition of mammalian target of rapamycin. Oncol Lett. 2013;6(3):789–94.
128. Flavin R, Smyth P, Barrett C, Russell S, Wen H, Wei J, et al. miR-29b expression is associated with disease-free survival in patients with ovarian serous carcinoma. Int J Gynecol Cancer. 2009;19(4):641–7.
129. Creighton CJ, Hernandez-Herrera A, Jacobsen A, Levine DA, Mankoo P, Schultz N, et al. Integrated analyses of microRNAs demonstrate their widespread influence on gene expression in high-grade serous ovarian carcinoma. PLoS One. 2012;7(3), e34546.
130. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, et al. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci U S A. 2007;104(40):15805–10.
131. Garzon R, Liu S, Fabbri M, Liu Z, Heaphy CE, Callegari E, et al. MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1. Blood. 2009;113(25): 6411–8.
132. Zhang Q, He XJ, Ma LP, Li N, Yang J, Cheng YX, et al. Expression and significance of microRNAs in the p53 pathway in ovarian cancer cells and serous ovarian cancer tissues. Zhonghua Zhong Liu ZA Zhi. 2011;33(12):885–90 [Chinese Journal of Oncology].
133. Corney DC, Hwang CI, Matoso A, Vogt M, Flesken-Nikitin A, Godwin AK, et al. Frequent downregulation of miR-34 family in human ovarian cancers. Clin Cancer Res. 2010;16(4):1119–28.
134. Zhou X, Zhao F, Wang ZN, Song YX, Chang H, Chiang Y, et al. Altered expression of miR-152 and miR-148a in ovarian cancer is related to cell proliferation. Oncol Rep. 2012;27(2): 447–54.
135. Zhang H, Wang Q, Zhao Q, Di W. MiR-124 inhibits the migration and invasion of ovarian cancer cells by targeting SphK1. J Ovarian Res. 2013;6(1):84.
136. Wang W, Ren F, Wu Q, Jiang D, Li H, Shi H. MicroRNA-497 suppresses angiogenesis by targeting vascular endothelial growth factor A through the PI3K/AKT and MAPK/ERK pathways in ovarian cancer. Oncol Rep. 2014;32(5):2127–33.
137. Zaman MS, Maher DM, Khan S, Jaggi M, Chauhan SC. Current status and implications of microRNAs in ovarian cancer diagnosis and therapy. J Ovarian Res. 2012;5(1):44.
138. Peng DX, Luo M, Qiu LW, He YL, Wang XF. Prognostic implications of microRNA-100 and its functional roles in human epithelial ovarian cancer. Oncol Rep. 2012;27(4):1238–44.

139. Nagaraja AK, Creighton CJ, Yu Z, Zhu H, Gunaratne PH, Reid JG, et al. A link between mir-100 and FRAP1/mTOR in clear cell ovarian cancer. Mol Endocrinol. 2010;24(2):447–63.
140. Mabuchi S, Kawase C, Altomare DA, Morishige K, Sawada K, Hayashi M, et al. mTOR is a promising therapeutic target both in cisplatin-sensitive and cisplatin-resistant clear cell carcinoma of the ovary. Clin Cancer Res. 2009;15(17):5404–13.
141. Luo J, Zhou J, Cheng Q, Zhou C, Ding Z. Role of microRNA-133a in epithelial ovarian cancer pathogenesis and progression. Oncol Lett. 2014;7(4):1043–8.
142. Guo J, Xia B, Meng F, Lou G. miR-133a suppresses ovarian cancer cell proliferation by directly targeting insulin-like growth factor 1 receptor. Tumor Biol. 2014;35(2):1557–64.
143. Wu H, Xiao Z, Wang K, Liu W, Hao Q. MiR-145 is downregulated in human ovarian cancer and modulates cell growth and invasion by targeting p70S6K1 and MUC1. Biochem Biophys Res Commun. 2013;441(4):693–700.
144. Zhang W, Wang Q, Yu M, Wu N, Wang H. MicroRNA-145 function as a cell growth repressor by directly targeting c-Myc in human ovarian cancer. Technol Cancer Res Treat. 2014;13(2):161–8.
145. Xu Q, Liu LZ, Qian X, Chen Q, Jiang Y, Li D, et al. MiR-145 directly targets p70S6K1 in cancer cells to inhibit tumor growth and angiogenesis. Nucleic Acids Res. 2012;40(2):761–74.
146. Wang S, Zhao X, Wang J, Wen Y, Zhang L, Wang D, et al. Upregulation of microRNA-203 is associated with advanced tumor progression and poor prognosis in epithelial ovarian cancer. Med Oncol. 2013;30(3):681.
147. Yu X, Zhang X, Bi T, Ding Y, Zhao J, Wang C, et al. MiRNA expression signature for potentially predicting the prognosis of ovarian serous carcinoma. Tumor Biol. 2013;34(6): 3501–8.
148. Lou Y, Yang X, Wang F, Cui Z, Huang Y. MicroRNA-21 promotes the cell proliferation, invasion and migration abilities in ovarian epithelial carcinomas through inhibiting the expression of PTEN protein. Int J Mol Med. 2010;26(6):819–27.
149. Chang H, Zhou X, Wang ZN, Song YX, Zhao F, Gao P, et al. Increased expression of miR-148b in ovarian carcinoma and its clinical significance. Mol Med Rep. 2012;5(5):1277–80.
150. Zhang H, Zuo Z, Lu X, Wang L, Wang H, Zhu Z. MiR-25 regulates apoptosis by targeting Bim in human ovarian cancer. Oncol Rep. 2012;27(2):594–8.
151. Wang X, Meng X, Li H, Liu W, Shen S, Gao Z. MicroRNA-25 expression level is an independent prognostic factor in epithelial ovarian cancer. Clin Transl Oncol. 2014;16(11): 954–8.
152. Feng S, Pan W, Jin Y, Zheng J. MiR-25 promotes ovarian cancer proliferation and motility by targeting LATS2. Tumor Biol. 2014;35(12):12339–44.
153. Langhe R, Norris L, Saadeh FA, Blackshields G, Varley R, Harrison A, et al. A novel serum microRNA panel to discriminate benign from malignant ovarian disease. Cancer Lett. 2015;356(2 Pt B):628–36.
154. Chan JK, Kiet TK, Blansit K, Ramasubbaiah R, Hilton JF, Kapp DS, et al. MiR-378 as a biomarker for response to anti-angiogenic treatment in ovarian cancer. Gynecol Oncol. 2014;133(3):568–74.
155. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008;105(30):10513–8.
156. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 2008;18(10): 997–1006.
157. Chim SS, Shing TK, Hung EC, Leung TY, Lau TK, Chiu RW, et al. Detection and characterization of placental microRNAs in maternal plasma. Clin Chem. 2008;54(3):482–90.
158. Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, et al. Serum microRNAs are promising novel biomarkers. PLoS One. 2008;3(9), e3148.
159. Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, et al. Detection of elevated levels of tumor-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol. 2008;141(5):672–5.

160. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol. 2008;110(1):13–21.
161. Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, et al. Detection of microRNA expression in human peripheral blood microvesicles. PLoS One. 2008;3(11), e3694.
162. Cheng HH, Mitchell PS, Kroh EM, Dowell AE, Chery L, Siddiqui J, et al. Circulating microRNA profiling identifies a subset of metastatic prostate cancer patients with evidence of cancer-associated hypoxia. PLoS One. 2013;8(7), e69239.
163. Resnick TD, McCulloch KA, Rougvie AE. miRNAs give worms the time of their lives: small RNAs and temporal control in Caenorhabditis elegans. Dev Dyn. 2010;239(5):1477–89.
164. Xu YZ, Xi QH, Ge WL, Zhang XQ. Identification of serum MicroRNA-21 as a biomarker for early detection and prognosis in human epithelial ovarian cancer. Asian Pac J Cancer Prev. 2013;14(2):1057–60.
165. Hong F, Li Y, Xu Y, Zhu L. Prognostic significance of serum microRNA-221 expression in human epithelial ovarian cancer. J Int Med Res. 2013;41(1):64–71.
166. Jiang H, Qu L, Wang Y, Cong J, Wang W, Yang X. miR-99a promotes proliferation targeting FGFR3 in human epithelial ovarian cancer cells. Biomed Pharmacother. 2014;68(2):163–9.
167. Liang H, Jiang Z, Xie G, Lu Y. Serum microRNA-145 as a novel biomarker in human ovarian cancer. Tumor Biol. 2015;36(7):5305–13.
168. Gao YC, Wu J. MicroRNA-200c and microRNA-141 as potential diagnostic and prognostic biomarkers for ovarian cancer. Tumor Biol. 2015;36(6):4843–50.
169. Ayaz L, Cayan F, Balci S, Gorur A, Akbayir S, Yildirim Yaroglu H, et al. Circulating microRNA expression profiles in ovarian cancer. J Obstet Gynaecol. 2014;34(7):620–4.
170. Shapira I, Oswald M, Lovecchio J, Khalili H, Menzin A, Whyte J, et al. Circulating biomarkers for detection of ovarian cancer and predicting cancer outcomes. Br J Cancer. 2014;110(4):976–83.
171. Zheng H, Zhang L, Zhao Y, Yang D, Song F, Wen Y, et al. Plasma miRNAs as diagnostic and prognostic biomarkers for ovarian cancer. PLoS One. 2013;8(11), e77853.
172. Shen W, Song M, Liu J, Qiu G, Li T, Hu Y, et al. MiR-26a promotes ovarian cancer proliferation and tumorigenesis. PLoS One. 2014;9(1), e86871.
173. Hausler SF, Keller A, Chandran PA, Ziegler K, Zipp K, Heuer S, et al. Whole blood-derived miRNA profiles as potential new tools for ovarian cancer screening. Br J Cancer. 2010;103(5):693–700.
174. Masyuk AI, Masyuk TV, Larusso NF. Exosomes in the pathogenesis, diagnostics and therapeutics of liver diseases. J Hepatol. 2013;59(3):621–5.
175. Iero M, Valenti R, Huber V, Filipazzi P, Parmiani G, Fais S, et al. Tumor-released exosomes and their implications in cancer immunity. Cell Death Differ. 2008;15(1):80–8.
176. Simpson RJ, Lim JW, Moritz RL, Mathivanan S. Exosomes: proteomic insights and diagnostic potential. Expert Rev Proteomics. 2009;6(3):267–83.
177. 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(6):654–9.
178. Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, et al. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumor cells. Nat Cell Biol. 2008;10(5):619–24.
179. Stoorvogel W, Kleijmeer MJ, Geuze HJ, Raposo G. The biogenesis and functions of exosomes. Traffic. 2002;3(5):321–30.
180. Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol. 2002;2(8):569–79.
181. Al-Nedawi K, Meehan B, Rak J. Microvesicles: messengers and mediators of tumor progression. Cell Cycle. 2009;8(13):2014–8.
182. Keller S, Rupp C, Stoeck A, Runz S, Fogel M, Lugert S, et al. CD24 is a marker of exosomes secreted into urine and amniotic fluid. Kidney Int. 2007;72(9):1095–102.
183. Li QL, Bu N, Yu YC, Hua W, Xin XY. Exvivo experiments of human ovarian cancer ascites-derived exosomes presented by dendritic cells derived from umbilical cord blood for immunotherapy treatment. Clin Med Oncol. 2008;2:461–7.

184. Runz S, Keller S, Rupp C, Stoeck A, Issa Y, Koensgen D, et al. Malignant ascites-derived exosomes of ovarian carcinoma patients contain CD24 and EpCAM. Gynecol Oncol. 2007;107(3):563–71.
185. Keller S, Konig AK, Marme F, Runz S, Wolterink S, Koensgen D, et al. Systemic presence and tumor-growth promoting effect of ovarian carcinoma released exosomes. Cancer Lett. 2009;278(1):73–81.
186. Cho JA, Yeo DJ, Son HY, Kim HW, Jung DS, Ko JK, et al. Exosomes: a new delivery system for tumor antigens in cancer immunotherapy. Int J Cancer. 2005;114(4):613–22.
187. Hegmans JP, Bard MP, Hemmes A, Luider TM, Kleijmeer MJ, Prins JB, et al. Proteomic analysis of exosomes secreted by human mesothelioma cells. Am J Pathol. 2004;164(5):1807–15.
188. Liang B, Peng P, Chen S, Li L, Zhang M, Cao D, et al. Characterization and proteomic analysis of ovarian cancer-derived exosomes. J Proteomics. 2013;80:171–82.
189. Taylor DD, Gercel-Taylor C. Tumor-derived exosomes and their role in cancer-associated T-cell signalling defects. Br J Cancer. 2005;92(2):305–11.
190. Peng P, Yan Y, Keng S. Exosomes in the ascites of ovarian cancer patients: origin and effects on anti-tumor immunity. Oncol Rep. 2011;25(3):749–62.
191. Taylor DD, Gercel-Taylor C. The origin, function, and diagnostic potential of RNA within extracellular vesicles present in human biological fluids. Front Genet. 2013;4:142.
192. Beach A, Zhang HG, Ratajczak MZ, Kakar SS. Exosomes: an overview of biogenesis, composition and role in ovarian cancer. J Ovarian Res. 2014;7(1):14.
193. Shih KK, Levine DA. Exosomal microRNAs step into the biomarker arena. Gynecol Oncol. 2008;110(1):1–2.
194. Chan JK, Blansit K, Kiet T, Sherman A, Wong G, Earle C, et al. The inhibition of miR-21 promotes apoptosis and chemosensitivity in ovarian cancer. Gynecol Oncol. 2014;132(3):739–44.
195. Frederick PJ, Green HN, Huang JS, Egger ME, Frieboes HB, Grizzle WE, et al. Chemoresistance in ovarian cancer linked to expression of microRNAs. Biotech Histochem. 2013;88(7):403–9.
196. Yu PN, Yan MD, Lai HC, Huang RL, Chou YC, Lin WC, et al. Downregulation of miR-29 contributes to cisplatin resistance of ovarian cancer cells. Int J Cancer. 2014;134(3):542–51.
197. Chen R, Alvero AB, Silasi DA, Kelly MG, Fest S, Visintin I, et al. Regulation of IKKbeta by miR-199a affects NF-kappaB activity in ovarian cancer cells. Oncogene. 2008;27(34):4712–23.
198. Testa JR, Bellacosa A. AKT plays a central role in tumorigenesis. Proc Natl Acad Sci U S A. 2001;98(20):10983–5.
199. Kamazawa S, Kigawa J, Kanamori Y, Itamochi H, Sato S, Iba T, et al. Multidrug resistance gene-1 is a useful predictor of Paclitaxel-based chemotherapy for patients with ovarian cancer. Gynecol Oncol. 2002;86(2):171–6.
200. Penson RT, Oliva E, Skates SJ, Glyptis T, Fuller Jr AF, Goodman A, et al. Expression of multidrug resistance-1 protein inversely correlates with paclitaxel response and survival in ovarian cancer patients: a study in serial samples. Gynecol Oncol. 2004;93(1):98–106.
201. Zhu H, Wu H, Liu X, Evans BR, Medina DJ, Liu CG, et al. Role of MicroRNA miR-27a and miR-451 in the regulation of MDR1/P-glycoprotein expression in human cancer cells. Biochem Pharmacol. 2008;76(5):582–8.
202. Yang LY, Wang HJ, Jia XB, Wang X, Luo J, Zhang XY. [Expression of miR-130a in cisplatin resistant cell lines of ovarian cancer]. Sichuan Da Xue Xue Bao Yi Xue Ban. 2012;43(1):60–4.
203. Zhou Y, Chen Q, Qin R, Zhang K, Li H. MicroRNA-449a reduces cell survival and enhances cisplatin-induced cytotoxicity via downregulation of NOTCH1 in ovarian cancer cells. Tumor Biol. 2014;35(12):12369–78.
204. Li H, Xu H, Shen H, Li H. microRNA-106a modulates cisplatin sensitivity by targeting PDCD4 in human ovarian cancer cells. Oncol Lett. 2014;7(1):183–8.

205. Huh JH, Kim TH, Kim K, Song JA, Jung YJ, Jeong JY, et al. Dysregulation of miR-106a and miR-591 confers paclitaxel resistance to ovarian cancer. Br J Cancer. 2013;109(2):452–61.
206. Kim YW, Kim EY, Jeon D, Liu JL, Kim HS, Choi JW, et al. Differential microRNA expression signatures and cell type-specific association with Taxol resistance in ovarian cancer cells. Drug Des Devel Ther. 2014;8:293–314.
207. Hong L, Yang Z, Ma J, Fan D. Function of miRNA in controlling drug resistance of human cancers. Curr Drug Targets. 2013;14(10):1118–27.
208. Ye G, Fu G, Cui S, Zhao S, Bernaudo S, Bai Y, et al. MicroRNA 376c enhances ovarian cancer cell survival by targeting activin receptor-like kinase 7: implications for chemoresistance. J Cell Sci. 2011;124(Pt 3):359–68.
209. Lu L, Schwartz P, Scarampi L, Rutherford T, Canuto EM, Yu H, et al. MicroRNA let-7a: a potential marker for selection of paclitaxel in ovarian cancer management. Gynecol Oncol. 2011;122(2):366–71.
210. Liu L, Zou J, Wang Q, Yin FQ, Zhang W, Li L. Novel microRNAs expression of patients with chemotherapy drug-resistant and chemotherapy-sensitive epithelial ovarian cancer. Tumor Biol. 2014;35(8):7713–7.
211. Kong F, Sun C, Wang Z, Han L, Weng D, Lu Y, et al. miR-125b confers resistance of ovarian cancer cells to cisplatin by targeting pro-apoptotic Bcl-2 antagonist killer 1. J Huazhong Univ Sci Technolog Med Sci. 2011;31(4):543–9.
212. Li B, Chen H, Wu N, Zhang WJ, Shang LX. Deregulation of miR-128 in ovarian cancer promotes cisplatin resistance. Int J Gynecol Cancer. 2014;24(8):1381–8.
213. Zou D, Wang D, Li R, Tang Y, Yuan L, Long X, et al. MiR-197 induces Taxol resistance in human ovarian cancer cells by regulating NLK. Tumor Biology. 2015
214. Mitamura T, Watari H, Wang L, Kanno H, Hassan MK, Miyazaki M, et al. Downregulation of miRNA-31 induces taxane resistance in ovarian cancer cells through increase of receptor tyrosine kinase MET. Oncogenesis. 2013;2, e40.
215. Creighton CJ, Fountain MD, Yu Z, Nagaraja AK, Zhu H, Khan M, et al. Molecular profiling uncovers a p53-associated role for microRNA-31 in inhibiting the proliferation of serous ovarian carcinomas and other cancers. Cancer Res. 2010;70(5):1906–15.
216. Vecchione A, Belletti B, Lovat F, Volinia S, Chiappetta G, Giglio S, et al. A microRNA signature defines chemoresistance in ovarian cancer through modulation of angiogenesis. Proc Natl Acad Sci U S A. 2013;110(24):9845–50.

Chapter 9
microRNA and Lung Cancer

Valerio Del Vescovo and Michela A. Denti

Abstract Lung cancer is the leading cause of cancer mortality worldwide. microRNAs (miRNAs) have been established as players with a relevant role in lung cancer development, epithelial–mesenchymal transition and response to therapy. Additionally, in the last decade, miRNAs, measured in resected tumor samples or in fine-needle aspirate samples have emerged as compelling biomarkers for tumor diagnosis, prognosis, and prediction of response to treatment, due to the ease of their detection and in their extreme specificity. Moreover, miRNAs present in sputum, in plasma, in serum or in whole-blood have increasingly been explored in the last 5 years as less invasive biomarkers for the early detection of cancers.

Keywords miRNA • Lung cancer • Biomarker • Diagnosis • Circulating miRNA

Introduction

Lung cancer is the leading cause of cancer mortality worldwide [1]. About 270,000 individuals were predicted to die of lung cancer in the European Union in 2013 [2]. The poor prognosis of the disease caused by a late disease presentation, tumor heterogeneities within histological subtypes, and the relatively limited understanding of tumor biology are responsible for this high mortality. Most patients with lung cancer are diagnosed at an advanced stage of the disease, and, a small subset of these patients can be treated with new drugs offering improved survival and tolerant quality of life, otherwise the majority of patients can only be treated with palliative chemotherapy. In general survival remains poor, and many patients die within a few months of diagnosis.

Lung cancer is not a unique condition, but indeed a group of diseases: small-cell lung carcinomas (SCLCs) are high-grade neuroendocrine tumors (NET), metastasize earlier and are initially more chemosensitive than the so called non-small-cell

V. Del Vescovo • M.A. Denti (✉)
Laboratory of RNA Biology and Biotechnology, Centre for Integrative Biology, University of Trento, via Sommarive 9, Trento 38123, Italy
e-mail: michela.denti@unitn.it

© Springer International Publishing Switzerland 2015

G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5_9

lung carcinomas (NSCLC). The two main subgroups of NSCLC are adenocarcinoma (AD) and squamous cell carcinoma (SCC), with a remaining third class of carcinomas devoid of histological features of adeno- or squamous- differentiation, named LCC (large-cell carcinoma) [3]. The appearance of these tumors at light microscopy is substantially different, suggesting that their biology and etiology diverge as well. Importantly, the histological subgroups of NSCLC respond differently to some chemotherapeutic substances [4–7] and side effects of some therapies appear to vary among subgroups [8].

As well as SCLC, NETs include a spectrum of tumors from the low-grade typical carcinoid (TC) and intermediate-grade atypical carcinoid (AC) to the high-grade large-cell NE carcinoma (LCNEC). The distinction among these various entities may be occasionally difficult on histological grounds, but is of great therapeutic importance.

Biomarkers that are able to stratify for the specific subtype of lung cancer, prognosticate the course of disease, or predict the treatment response are in rising demand. Lung cancer subtyping has traditionally relied on the histopathological observation of resected specimens, bronchoscopic biopsies, fine-needle aspirations or sputum, which represent samples with decreasing invasiveness for the patient, but also of increasing challenge for the pathologist, as proportionally fewer tumor cells are captured [9–11]. Recently, the introduction of immunohistochemical markers has rendered lung cancer subtyping more accurate and clinically useful [12].

In the last decade, microRNAs (miRNAs), measured either from tumor samples or in biofluids, have emerged as biomarkers for diagnosis, prognosis, and prediction of response to treatment.

miRNAs and Lung Cancer

As far as lung cancers are concerned, the role of miRNAs in lung carcinogenesis was indicated as early as 2004, when the Croce lab demonstrated that more than half of the miRNA genes then known were located in cancer-associated genomic regions or in fragile sites and that several miRNAs located in these deleted regions have low expression levels in lung cancer cell lines as well as in chronic lymphocytic leukemia samples [13]. Takamizawa and colleagues, during the same year, reported reduced expression of the let-7 microRNA in human NSCLC lung cancers [14], followed by distinct independent reports [15–17]. The let-7 family was later shown to have an oncosuppressor activity in NSCLC tumor development in mice xenografts [18]. One of the consequences of let-7a downregulation in lung cancer has been demonstrated to be the upregulation of RAS protein [15]. A single nucleotide polymorphism (SNP)in a let-7 complementary site of KRAS mRNA was found to be associated with increased risk of NSCLC in moderate smokers [19]. Based on in vitro experiments and analyses of patient samples the authors concluded that this SNP alters the ability of let-7 to regulate translation of KRAS, leading to overexpression of KRAS and increased lung cancer risk.

Other miRNAs may also interact with RAS. For instance, Wang and colleagues [20] found that miR-451 is downregulated in NSCLC, and that low expression correlated with poor survival. The authors were able to show that miR-451 inhibits the expression of ras-related protein 14 (RAB14), suggesting that lower expression of miR-451 may allow this oncogene to escape regulation.

There are evidences that other miRNAs can act as oncosuppressor agents. The overexpression of miR-126 in NSCLC cell lines is able to decrease cell proliferation in vitro, and tumor growth in the nude mouse xenograft model, by targeting PI3KR2 mRNA, and repressing its protein product, and consequently regulating the PI3K-Akt pathway [21]. miR-145 can target c-myc and suppress the c-Myc/eIF4E pathway, inhibiting the G1/S transition, influencing cell proliferation in A549 and H23, NSCLC cell line models [22]. Additionally, in NSCLC patient samples, decreased level of tumor suppressive miRNA-16 lead the inhibition of tumor cell lines growth and motility: this miRNA target and suppress hepatoma-derived growth factor (HDGF) [23]. Most recently, miR-340 expression was shown to be inversely correlated with the four clinical stages in a small cohort of NSCLC patients. miR-340 target multiple negative regulators of p27, i.e., PUM1, PUM2, and SKP2 [24].

Additionally, also epigenetic changes are mechanisms of transcriptional silencing of miRNAs in cancer. In a novel research it was reported that the expression level of miR-373 was enhanced by suberoylanilide hydroxamic acid (SAHA), a histone deacetylase (HDAC) inhibitor, and importantly, miR-373 was found to be downregulated in NSCLC tissues and cell lines. Transfection of miR-373 into A549 and Calu-6 cells attenuated cell proliferation, migration, and invasion and reduced the expression of mesenchymal markers. Microarray analysis of miR-373-transfected cells and computational predictions identified IRAK2 and LAMP1 as targets of miR-373 and the knockdown of these two genes showed similar biological effects to those of miR-373 overexpression. In clinical samples, overexpression of IRAK2 correlated with decreased disease-free survival of patients with non-adenocarcinoma. The purposed mechanism indicate that miR-373 is silenced by histone modification in lung cancer cells and it functions as a tumor suppressor and negative regulator of the mesenchymal phenotype through downstream IRAK2 and LAMP1 target genes [25].

Researchers have reported that miRNAs whose expression is altered in tumors, may function also as a novel class of oncogenes. The oncogenic miR-17-92 cluster is markedly overexpressed in lung cancers, especially with SCLC histology and enhances cell proliferation in vitro, therefore possibly playing a role in the development of lung cancers [26]. On the other hand, deletion of the miR-17-92 cluster, in mice, is lethal and causes lung and lymphoid cell developmental defects [27]. The increased expression of oncogenic miRNA-221/miRNA-222 was shown to be more aggressive in NSCLC compared with less invasive or normal tissues, those miRNAs target PTEN and metalloproteinase inhibitors 3 (TIMP3), to induce TNF-related apoptosis-inducing ligand (TRAIL) resistance and lead to cell migration by the activation of AKT signaling pathway [28].

Overexpression of miR-21 augmented tumorigenesis by repression of negative regulators of the Ras/MEK/ERK pathway and inhibition of apoptosis too [29].

miR-21 was observed additionally to inhibit (PTEN) and stimulate growth and invasion in NSCLC derived cell lines [30].

Recently, miR-1271 and its predicted targets were identified differentially expressed in NSCLC samples. Specifically, the study indicated that miR-1271 regulates the cell proliferation and invasion, via the downregulation of HOXA5 [31].

The tumor suppressor protein p53 is mutated in a large number of lung cancer-derived cell lines and tumor specimens from patients with lung cancer [32, 33]. There is growing evidence that p53 regulates the expression of several miRNAs [34–39]. p53 directly regulates the expression of miR-34 family members, and the upregulation of these miRNAs result in the downregulation of genes associated with cell cycle control [39] and promotion of apoptosis [37] in cultured lung cancer cells. Further miRNAs, including miR-125a, have more recently also been linked to p53-regulated apoptosis in lung cancer cells [38].

miRNAs and Resistance

The treatments against lung cancer use routinely chemotherapy in combination with radiotherapy, especially for NSCLC, being favorable to improved disease control and reduction of metastasis events. Chemoresistance and radiation resistance are common, precluding successful long-term therapy. Researchers are continually engaged for the developing new therapies by investigating the mechanisms behind resistance. The development and progression of tumors has been correlated with misregulation of several miRNAs, and the reversal of their expression is able to modulate the cancer phenotype, suggesting the potential of targeting miRNAs with anticancer drugs.

Chemotherapy Resistance

There are evidences that miRNA have been implicated in cisplatin resistance. In 2010 it was demonstrated that a specific panel of miRNAs is upregulated by NSCLC cells in response to CDDP (cisplatin), and some of these miRNAs also strongly modulate this cytotoxic response. Specifically, miR-181a sensitized A549 cell line to the lethal action of CDDP Conversely, miR-630 conferred robust cytoprotection against CDDP, resulting from decreased proliferation coupled to upstream inhibition of the signaling cascades that are caused from damaged DNA and gather on p53 activation [40].

Using the same cell line model, it was demonstrated that ectopic expression of miR-451 could sensitize A549 cells to cisplatin by inducing apoptosis by the inactivation of Akt signaling pathway [41]. In the same year it was showed that transfection with miR-98/miR-453 was able to inhibit p53 expression, and upon treatment with cisplatin, the expression of miR-98 decreases, while p53 increases, so it was

speculated that regulation of p53 pathway might play an important role in the action of cisplatin on A549 cell growth [42]. It was observed that miR-155 inversely correlate to Apaf-1 (apoptotic protease activating factor-1) in lung cancer tissues, then the silencing of miR-155 or overexpressing Apaf-1 in A549 cells, considerably increased the sensitivity to cisplatin treatment, by the interplay between miR and Apaf-1-mediated pathway, involving an increased expression of Bax and caspase-9 [43]. Overexpression of miR-513a-3p could enhance cisplatin-induced apoptosis in human lung adenocarcinoma cell lines, A549/CDDP and SPC-A-1, by targeting and repressing Glutathione S-transferase P1 (GSTP1) [44].

Other miR are shown to be able to regulate the resistance to CDDP in cell lines: miR-503 by targeting Bcl-2 [45], miR-135a/b by targeting MCL1 [46], miR-495 by the modulation of copper-transporting P-type adenosine triphosphatase A (ATP7A) [47], miR-31 by regulating the drug transporter ABCB9 [48] and miR-15b by targeting phosphatidylethanolamine-binding protein 4 (PEBP4) [49]. As additional level of genetic control, silencing of tumor-suppressive miRs in cancer can occur through epigenetic modifications, recently was shown that the re-expression of miR-512-5p and niR-373 by treatment with 5'aza-deoxycytidine plus Trichostatin A to reverse DNA methylation and histone deacetylation, is able to augment cisplatin-induced apoptosis and then inhibit cell migration, by the targeting of TEAD4 mRNA, by miR-512-5p and RelA and PIK3CA mRNA by miR-373 [50].

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) can induce apoptosis in certain tumor cells. However, a significant proportion of human cancer cells are resistant to TRAIL-induced apoptosis, and the mechanisms of sensitization vary among cell types. A miRNA signature was identified in 2008 by Garofalo and colleagues about TRAIL resistance in human NSCLC. An increased level of miR-221 and miR-222 was found in TRAIL-resistant CALU-1 cells and moderately resistant A459 and A549 NSCLC cells, compared to TRAIL-sensitive H460 cells. An induced repression of miR-221 and miR-222 by inhibitors, sensitized TRAIL-resistant cells to TRAIL-induced apoptosis, while overexpression of TRAIL-sensitive cells with pre-miRs led to a TRAIL-resistant phenotype. miR-221 and miR-222 inhibit TRAIL function by downregulating the expression of key cell cycle regulatory proteins, such as p27kip1 blocking progression at the G1 phase of the cell cycle. Overexpression of these miRNAs has previously been reported also in aggressive NSCLC tumors [51]. It was further demonstrated that miR-130a, expressed at low level in lung cancer cell lines, can target MET, and through this inhibition is able to reduce TRAIL resistance in NSCLC cells, by a c-Jun-mediated downregulation of miR-221 and miR-222 [52].

Radiotherapy Resistance

Ionizing radiation (IR) plays an important role in the treatment of epithelial tumors. The mechanisms of radioresistance remain unclear, however, recent evidences support that miRNAs can have a role in the modulation of key cellular pathways mediating the response to this treatment.

Among the IR-responsive genes, activation of NFκB1 following genotoxic stress allows time for DNA-damage repair and ensures cell survival accounting for acquired chemoresistance, an impediment to effective cancer therapy [53] and it was demonstrated that its inhibition promote the sensitivity of cancer cells to chemotherapeutic agents and radiation exposure [54]. The induced overexpression of miR-9 can downregulate the level of NFκB1 in γ-irradiated H1299 human lung cancer cell line and decrease the surviving fraction of γ-irradiated cells, moreover let-7g also is able to suppress the expression of NFκB1, by targeting a noncanonical target in NFκB1 3'-UTR [55]. It has also been reported that expression of miR-214 is upregulated in radioresistant NSCLC cell lines compared to their radiosensitive counterparts.

Knockdown of miR-214 augmented the sensitivity to radiotherapy of previously radioresistant NSCLC cell line, U-1810, and stimulated senescence by the upregulation of a senescence regulator, p27Kip1 following irradiation, leading to a prolongation of the G2 arrest. On the contrary, induction of miR-214 in the radiosensitive NSCLC cell line, H23, protected cells from radiotherapy-induced apoptosis, by decreasing the number of cells with active caspase-3 [56]. On the one hand studies have suggested a connection between expression of several miRNAs and radiotherapy, on the other hand, more recent reports have emerged demonstrating the upregulation of a specific set of miRNAs by hypoxia. Recently, investigators reported for the first time that several normoxic cancer cell lines expressing the "hypoxamir" miR-210 exhibit radioresistance similar to that found in hypoxic control cells [57]. All the information reported are summarized in Table 9.1.

Table 9.1 miRNA in resistance

miRNAs	Target	Drug/treatment	Resistance/sensitivity	References
miR-181	N/A	Cisplatin	S	[40]
miR-451	N/A	Cisplatin	S	[41]
miR-98	TP53	Cisplatin	R	[42]
miR-21	PTEN, BCL2	Cisplatin	R	[105]
miR-221, miR-222	P27kip1	TRAIL	R	[51]
miR-130a	MET	TRAIL	S	[52]
miR-9, let-7g	NFκB1	Radiotherapy	S	[53]
miR-214	N/A; PTEN	Radiotherapy; gefitinib	R	[55]
miR-210	Stabilizes HIF-1A in normoxia	Radiotherapy	R	[56]

N/A non available, *R* resistance, *S* sensitivity

miRNAs as Biomarkers

In 2005, the Horvitz and Golub labs used a bead-based flow cytometric miRNA expression method to identify complex profiles consisting of approximately a hundred of dysregulated miRNAs, able to classify 11 different tumor types, among which lung tumors [58]. Subsequently, the Croce lab used a custom-made oligonucleotide miRNA microarray to compare lung carcinomas to normal tissue, and identified a group of three downregulated and 35 upregulated miRNAs. Among these, miR-21 was commonly upregulated in the six cancer types analyzed (lung, breast, stomach, prostate, colon, and pancreatic tumors) and miR-17-5p, miR-128b, miR-155, miR-191, and miR-199a-1 were upregulated in at least other two cancer types [59].

miRNAs as Biomarkers for the Diagnosis of Lung Cancer and for Stratifying Lung Cancer Subtypes

Yanaihara and colleagues in 2006 [16] performed a comparison of miRNAs expression profiles in a list of 104 pairs of lung cancer tissues and corresponding noncancerous lung tissue, identifying a unique profile made of 43 differently expressed miRNAs (Table 9.2), this allowed the distinction of lung cancer from the noncancerous lung tissue. Of the 15 upregulated and 28 downregulated miRNAs, miR-21 and miR-205 are specifically located in a chromosomal region amplified and miR-32, miR-126-5p, and miR-126-3p in a region deleted in lung cancers, respectively; then they validated the profiling results by qRT-PCR, confirming the upregulation of miR-21 and miR-205 and miR-126-5p downregulation in lung cancer tissues when compared with the corresponding healthy lung tissues.

Comparison analyses between ADC vs. noncancerous tissues and SCC vs. noncancerous tissues revealed 17 and 16 miRNAs with statistically different expression, respectively (Table 9.2). A panel of six distinct miRNAs (miR-21, miR-155, miR-191, mir-126-5p, miR-210, and miR-224) were shared in both histological types of NSCLC. Yanaihara and colleagues also directly compared the two most common histological types of NSCLC, identifying two miRNAs (miR-99b and miR-102) that were higher in ADC and 4 miRNAs (miR-202, miR-203, miR-205, and the precursor of miR-204) that were higher in SCC. However, the authors do not explore further the issue of distinguishing ADCs from SCCs, in this paper.

It was observed by qRT-PCR in lung cancer tissues and cell lines a decreased expression of miR-107, miR-185, and let7a, and the overexpression of miR-31a, compared to normal lung tissue [60].

Landi and colleagues showed a panel of 34 miRNAs that significantly differentiated SCCs from ADs in male smoker patients, of which two were downregulated and 32 upregulated in AD vs. SCC [61]. Raponi and colleagues [62] used Ambion microarrays to profile total RNA from a number of 61 SCC samples and 10 reference normal lung samples, and identified 15 miRNAs that were differen-

Table 9.2 miRNAs from resected samples as biomarkers for the diagnosis of lung cancer

miRNA	Scope	Sample	References
mir-21, mir-191, mir-210, mir-155, mir-205, mir-24-2, mir-212, mir-214, mir-17-3p, mir-106a, mir-197, mir-192, mir-146, mir-203, mir-150, (**UP**) mir-126-5p, mir-143, mir-192-prec, mir-224, mir-126, mir-30a-5p, mir-140, mir-9, mir-124a-1, mir-218-2, mir-95, mir-145, mir-198, mir-216-prec, mir-219-1, mir-125a-prec, mir-26a-1-prec, mir-199b-prec, let-7a-2-prec, mir-27b, mir-32, mir-29b-2, mir-220, mir-33, mir-181c-prec, mir-101-1, mir-124a-3, mir-125a (**DOWN**)	Lung cancer vs. Normal	Solid (not specified)	[16]
mir-21, mir-191, mir-155, mir-210, mir-24-2 (UP) mir-126-5p, mir-126-3p, mir-219-1, mir-95, mir-192-prec, mir-220, mir-216-prec, mir-204-prec, mir-188, mir-198, mir-145, mir-224 (**DOWN**)	ADs vs. normal	Solid (Not specified)	[16]
mir-205, mir-191, mir-210, mir-17-3p, mir-203, mir-155, mir-21, mir-214, mir-212, mir-197 (**UP**) mir-224, mir-126*, mir-140, mir-29b, mir-143, mir-30a-5p (**DOWN**)	SCC vs. normal	Solid (not specified)	[16]
miR-31 (**UP**) miR-107, miR-185, let-7a (**DOWN**)	Lung cancer tissue vs. normal	Solid (not specified)	[60]
miR-26a, let-7g, let-7f, miR-98, miR-29a, let-7c, miR-30b, let-7i, let-7b, miR-29b, miR-26b, let-7a, miR-146b-5p, miR-195, miR-29c, miR-30d, miR-20a, miR-17, miR-19b, miR-106a, miR-16, let-7d, miR-106b, miR-181a, miR-498, miR-103, miR-107, miR-191, mir-663, miR-491-5p, let-7e, mir-654-5p (**UP**) miR-453, miR-509-3p (**DOWN**)	AD vs. SCC; male smoker patients	Solid, formalin-fixed, paraffin-embedded (FFPE)	[61]
miR-17-5p, miR-20a, miR-20b, miR-93, miR-106a, miR-106b, miR-182, miR-183, miR-200a, miR-200c, miR-203, miR-210, miR-224 (**UP**) miR-125a, let7e (**DOWN**)	SCC vs. normal	Solid, snap-frozen	[62]
miR-30a, miR-140-3p, miR-182, miR-210, miR-486-5p	Stage I-III vs. normal	Solid, snap-frozen	[63]
miR-182, miR-200c, miR-141, miR-375, miR-7, miR-429, miR-200a, miR-370, miR-200b, miR-382 (**UP**) miR-126, miR-451, miR-195, miR-486-5p, miR-214, miR-199a-5p (**DOWN**)	Primary lung tumors vs. metastases	Solid, formalin-fixed, paraffin-embedded (FFPE)	[64]

(continued)

Table 9.2 (continued)

miRNA	Scope	Sample	References
miR-205, miR-21 (**Relative Expression**)	AD vs. SCC	Solid, formalin-fixed, paraffin-embedded (FFPE)	[65]
miR-21, miR-155 (**UP**)	LCNECs and SCLCs vs. TCs and Acs	Solid, formalin-fixed, paraffin-embedded (FFPE)	[69]
miR-205, miR-27a, miR-29a, miR-29b, miR-34a (DOWN in NSCLC) miR-25, miR-375 (**UP** in NSCLC)	SCLC vs. NSCLC	Solid, formalin-fixed, paraffin-embedded (FFPE)	[70]
miR-29a, miR-29b, miR-34a, miR-375 (DOWN in SQ) miR-205, miR-25, miR-27a (**UP** in SQ)	SQ vs. AC	Solid, formalin-fixed, paraffin-embedded (FFPE)	[70]
miR-7, miR-21, miR-29b, miR-106a, miR-125a-5p, miR-129-3p, miR-205, miR-375 (**Relative Expression**)	Carcinoid, SCLC, and squamous and nonsquamous NSCLC	Solid, Fresh Biopsy	[71]
miR-21, miR-155, miR-7 (**UP**)	Tumor vs. normal	Solid, fine-needle aspirate (FNA)	[72]
miR-21, miR-155 (**UP**)	NSCLC vs. normal	Sputum	[81]
miR-205, miR-210, miR-708 (**Relative Expression**)	SCC vs. normal	Sputum	[82]
miR-21, miR-200b, miR-375 and miR-486 (**Relative Expression**)	AD vs. normal	Sputum	[83]
miR-31, miR-210 (**Relative Expression**)	Stage I NSCLC vs. normal	Sputum	[84]
miR-31, miR-210 (**Relative Expression**) + Computed Tomography	Stage I NSCLC vs. normal	Sputum	[85]
miR-21, miR-31, miR-210 (**Relative Expression**)	Malignant solitary pulmonary nodules (SPNs)	Sputum	[87]

AD adenocarcinoma, *SCC* squamous cell carcinoma, *LCNEC* large-cell neuroendocrine carcinoma, *SCLC* small-cell lung carcinoma, *TC* typical carcinoid, *AC* atypical carcinoid, *NSCLC* non-small-cell lung cancer

tially expressed between normal lung and SCC (Table 9.2). Two miRNAs were downregulated in SCCs (miR-125a and let7e) while the remaining 13 miRNAs were upregulated (miR-17-5p, miR-20a, miR-20b, miR-93, miR-106a, miR-106b, miR-182, miR-183, miR-200a, miR-200c, miR-203, miR-210, miR-224). More recently, a classifier based on five miRNA, was built by microarray analysis (miR-30a, miR-140-3p, miR-182, miR-210, miR-486-5p,) that could distinguish stage I-III SCC from normal lung tissues [63]. This classifier performs with an accuracy of 94.1 % in a training cohort (34 patients) and 96.2 % in a test cohort (26 patients).

16 miRNAs were reported to differentiate among primary lung tumors and metastases to the lung of various origin (Table 9.2) [64]. This panel involves miR-182, which was most strongly overexpressed in the lung primary tumors, and miR-126, which was overexpressed in the metastatic tumors.

One of the most ambitious aim for researchers, is also to define one or few miRNAs that can be used as a convenient tool for lung cancer diagnosis. Lebanony and colleagues [65] used a microarray to measure miRNA levels in AD and SCC FFPE samples, verifying the results by qRT-PCR. They identified miR-205 as a highly specific marker for SCC, when combined with the measured miR21 levels. The finding was confirmed by other papers [66, 67]. In addition, an algorithm for accurate classification of NSCLC cases, diagnosed as LCC on purely morphologic grounds, was proposed by integrating immune-histochemical markers (Δnp63, DSC3, and napsin A) with miR-205 and miR-21 measurement [68].

Lee and coauthors [69], by the evaluation of FPPE specimens from NETs, found that the levels of miR-21 and miR-155 were significantly higher in high-grade NET carcinomas (LCNECs and SCLCs) compared to carcinoid tumors (TCs and ACs).

An high diagnostic accuracy in discriminating SCLC from NSCLC was achieved by two miRNA panels (miR-29a and miR-375) and in differentiating SCC from AD (miR-205 and miR-34a) starting from FFPE surgical lung samples [70]. In addition, the same miRNA panels accurately discriminated SCLC from NSCLC and SCC from AD in bronchial brushing specimens.

Gilad and colleagues provided a single assay useful for the classification of the four main types of lung cancer (Table 9.2): carcinoid, SCLC, and squamous and nonsquamous NSCLC. This test is based on the measurement of eight miRNAs (miR-7, miR-21, miR-29b, miR-106a, miR-125a-5p, miR-129-3p, miR-205, miR-375) [71]. Such assay has an efficient outcome both on resected and on cytologic (fine-needle aspiration (FNA) and bronchial brushing and washing) lung cancer specimens.

In 2013, a study has also evaluated the diagnostic efficacy of miRNAs measurements in FNA NSCLC biopsies [72], results showed that miR-21, miR-155, and miR-7 showed a higher level in tumoral FNA compared to normal FNA specimens, while let-7a showed a lower level. The direct comparison of FNAs with resected specimens, from the same patients, indicated that the measured miRNAs had the same trend in the two types of specimens.

miRNAs as Biomarkers for Lung Cancer Prognosis

Reduced expression of the miRNAs comprised in the let-7 family has been correlated with poor postoperative survival in NSCLC [14]. Later, it was shown that AD patients with an increased expression of either mir-155, mir-17-3p, mir-106a, mir-93, or mir-21 and low expression of either let-7a-2, let-7b, or mir-145 have notably a worse prognosis (Table 9.3) [16]. It was also demonstrated as predictive of poor survival the overexpression of the precursor of miR-155 and reduced expression of let-7-a.

The analysis of frozen resected specimens derived from NSCLC patients [73], yielded to the identification of a five-microRNA signature that can predict the survival and relapse of patients with lung cancer (Table 9.3). miR-221 and let-7a were protective (i.e., their downregulation correlated with poor survival and high relapse probability), while miR-137, miR-182-3p, and miR-372 were related to a risk, and their upregulation was predictive of poor survival and high relapse probability. It was also demonstrated that miR-221, miR-137, miR-182-3p, and miR-372 can alter the invasive ability of lung cancer cell lines.

In the already described work by Raponi and colleagues [62] 20 miRNAs were described as having a significant association with overall survival in lung SCC patients (Table 9.3). Among these miRNAs, miR-146b specifically was identified to have the strongest prediction accuracy as the group with high miR-146b expression had significantly worse overall survival.

The miR-34 family, p53-dependent, was observed downregulated in surgically resected NSCLC tumor samples compared with normal tissue, and low levels of miR-34a expression were correlated with a high probability of relapse [74]. miRNA expression profiles, that included mir-124-5p, mir-146b-3p, mir-200b-5p, mir-30c-1-3p, mir-510, mir-585, mir-630, mir-657, and mir-70, were also identified that may predict recurrence of localized stage I NSCLC after surgical resection [75] (Table 9.3).

High levels of miR-16, measured in resected NSCLC samples, were disclosed as a prognostic factor for poor disease-free survival and poor overall survival [76]. Low miR-145 and high miR-367 are associated with shorter time to relapse (TTR) in resected NSCLC specimens [77]. Remarkably, p53 regulates miR-145 expression, which, in turn, inhibits the translation of SRY-related HMG box (SOX)2 and octamer-binding transcription factor (OCT)4, then these transcription factors control the expression of the miR-302-367 cluster.

A microarray analysis depicted a panel of 27 miRNAs which were observed to be deregulated in NSCLC resected samples, compared to normal lung tissue, identifying three miRNAs whose levels were related to clinicopathologic characteristics or patient prognosis: low levels of miR-143 were significantly correlated with smoking status, high miR-21 expression, and low miR-181a expression were associated with poor survival [78].

A signature based on 5 miRNA with lower expression level (miR-25, miR-34c-5p, miR-191, let-7e, and miR-34a) was correlated with poor overall survival among SCC patients (Table 9.3) [61], and high expression of miR-31 was associated with poor survival in Chinese SCC patients [63].

Table 9.3 miRNAs as biomarkers for the prognosis and to predict response to therapy

miRNA	Experiment	Scope	Sample	References
let-7a family (**DOWN**)	lung cancer tissue vs. normal	NSCLC poor postoperative survival	Solid, (not specified)	[14]
mir-155, mir-17-3p, mir-106a, mir-93, mir-21 (UP) let-7a-2, let-7b (**DOWN**)	AD vs. SQ	AD poor survival	Solid, (not specified)	[16]
miR-221, let-7a (**DOWN**) miR-137, miR-182-3p, miR-372 (**UP**)	NSCLC vs. normal	NSCLC poor survival	Solid, snap-frozen	[73]
miR-146b, miR-191, miR-155, miR-15a, miR-511, miR-100, miR-10a, miR-21, miR-126 (**UP**) miR-206, miR-299-3p, miR-122a, miR-513, miR-184, miR-453, miR-379, miR-202, miR-494, miR-432, miR-370 (**DOWN**)	SCC vs. normal	SCC Overall Survival	Solid, snap-frozen	[62]
miR-34a (**DOWN**)	NSCLC vs. normal	NSCLC Probability of relapse	Solid, (not specified)	[74]
miR-16 (**UP**)	NSCLC vs. normal	NSCLC poor survival	Solid, (not specified)	[76]
miR-143 (**DOWN**)	lung cancer tissue vs. normal	smoking status	Solid, snap-frozen	[78]
miR-21 (**UP**) miR-181a (**DOWN**)	lung cancer tissue vs. normal	NSCLC poor survival	Solid, snap-frozen	[78]
miR-25, miR-34c-5p, miR-191, let-7e, miR-34a (**DOWN**)	AD vs. SQ	SCC survival	Solid, formalin-fixed, paraffin-embedded (FFPE)	[61]
miR-31 (**UP**)	SCC vs. normal	SCC poor survival	Solid, snap-frozen	[63]
miR-325, miR-326, miR-328, miR-329-2-pre, miR-330-3p, miR-500a-3p, miR-370, miR-650-pre (**UP**)	BM (Brain Metastasis) – NSCLC vs. BM + NSCLC	NSCLC, risk for brain metastasis	Solid, formalin-fixed, paraffin-embedded (FFPE)	[79]

NSCLC non-small-cell lung cancer, *AD* adenocarcinoma, *SCC* squamous cell carcinoma, *BM* brain metastasis

An analysis performed in NSCLC FFPE samples from patients with brain metastases compared with patients without brain metastases (Table 9.3) [79] showed eight miRNAs significantly differentially expressed. Notably, the miR-328 and miR-330-3p overexpression was indicated as a marker for patients at risk for brain metastases, and a role for miR-328 in conferring migratory potential to NSCLC cells was suggested.

miRNA in Sputum *as Noninvasive Lung Cancer Biomarkers*

An early diagnosis of cancer remains a challenge and, in this context, it is important to find a noninvasive and sensitive tool able to detect early neoplastic changes. One relatively noninvasive source of miRNAs for the diagnosis of lung cancers is sputum [80]. Endogenous miRNAs are stably present in sputum specimens. Differential level of miR-21 and miR-155 were detected by qRT–PCR, of which miR-21 was significantly overexpressed in sputum of NSCLC patients as compared with cancer-free subjects [81]. Additionally, elevated miR-21 expression was more sensitive (70 %) than conventional sputum cytology (48 %) in diagnosing lung cancer. The same research group defined miRNA signatures for different histologic types of lung cancer in studies of similar design [82, 83].

For the diagnosis of SCC, the combination of miR-205, miR-210, and miR-708 produced classifier with 73 % sensitivity and 96 % specificity. A panel consisting of miR-21, miR-200b, miR-375, and miR-486 yielded 81 % sensitivity and 92 % specificity in discriminating sputum of AD patients from controls. The investigators found no association between miRNA expression and stage of lung cancer, suggesting that the miRNA signatures can be used as a tool in the detection of early lung cancer. The same research group stated that combined quantification of miR-31 and miR-210 copy number by digital PCR in sputum of the cases and controls provided 65.71 % sensitivity and 85.00 % specificity for stage I NSCLC diagnosis [84]. In addition they also reported that combining miR-31 and miR-210 detection by qRT-PCR and Computed Tomography there is an improvement in NSCLC diagnosis specificity [85].

In an independent study, a profile comprising five miRNAs (miR-21, miR-143, miR-155, miR-210, miR-372) measured by qRT-PCR on sputum samples detected NSCLC with 83.3 % sensitivity and 100 % specificity [86].

Recently, a panel of three miRNA biomarkers (miR-21, miR-31, and miR-210), obtained evaluating sputum miRNA signatures, was developed, producing 82.93 % sensitivity and 87.84 % specificity for identifying malignant solitary pulmonary nodules (SPNs) [87].

Circulating miRNAs as Lung Cancer Biomarkers

miRNAs measured in body fluids can reflect altered physiological conditions, representing new efficacious biomarkers [88]. miR-155, miR-197, and miR-182 can be potential biomarkers for early detection of lung cancer with 81.33 % sensitivity and

86.76 % specificity. The levels of these miRNAs in plasma of NSCLC patients are higher compared with healthy controls [89]. But also another set of plasma miRNAs (miR-21, miR-126, miR-210, and miR-486-5p), had 86.22 % sensitivity and 96.55 % specificity in discriminating NSCLC patients from the healthy controls. In addition, the panel of four miRNAs produced 73.33 % sensitivity and 96.55 % specificity in identifying stage I NSCLC patients. The miR panel had higher sensitivity (91.67 %) in diagnosis of AD compared with SCC (82.35 %) [90]. Authors from the same lab reported also that quantification of miR-21–5p and miR-335–3p extracted from plasma blood, by digital PCR, provided 71.8 % sensitivity and 80.6 % specificity in distinguishing lung cancer patients from cancer-free subjects [91].

Higher plasma miR-21 and miR-155 and lower plasma miR-145 expression levels distinguish lung cancer patients from healthy smokers with 69.4 % sensitivity and 78.3 % specificity [92]. Levels of miR-361-3p and miR-625-3p might have a protective influence on the development of NSCLC, and the measurement of these miRNAs in serum could be useful for the diagnosis of NSCLC, particularly in smoker patients [93].

It was provided evidence that some serum-circulating miRNAs are important to identify asymptomatic high-risk individuals with early stage lung cancer among the others, there was highlighted the importance of let-7 family, members of miR-17-92 cluster, miR-126 and miR-486 in NSCLC patients derived sera [94].

A recent study was based on the use of qRT-PCR to assess miR-205-5p, miR-205-3p, and miR-21-3p expressions in serum and tissue samples [95]. The relative expressions of miR-205-5p and miR-205-3p were significantly higher in NSCLC tissues compared with cancer-adjacent paired specimens. Specifically, in the serum, higher miR-205-5p, miR-205-3p, and miR-21-3p relative expressions were observed in the NSCLC group, compared with healthy volunteers or patients diagnosed with a benign lung disease (pneumonia, pulmonary tuberculosis, chronic obstructive pulmonary disease, or interstitial pneumonia). The relative expressions of miR-205-5p and miR-21-3p in NSCLC tissues and serum were significantly correlated, although there was no significant correlation for miR-205-3p. Expressions of miR-205-5p and miR-205-3p in SCC specimens were significantly higher than in lung adenocarcinoma specimens. In a similar way, higher serum miR-205-5p and miR-205-3p levels were measured in SCC patients.

miRNAs expression profiling preformed in whole-blood depicted the presence of miR-190b, miR-630, miR-942, and miR-1284 in a majority of classifiers generated during the analyses to distinguish lung cancer cases from controls [96]. In a different study, miR-22, miR-24, and miR-34a were found more expressed in RNA extracted from whole-blood of NSCLC patients vs. healthy controls [97].

Recently, a specific panel of miRNAs (miR-205, -19a, -19b, -30b, and -20a) is decreasing in plasma of patients after SCC surgery. Interestingly, high levels of these miRNA are found in tumor-specific exosomes [98].

The introduction and the use of Next-Generation Sequencing (NGS) has been used to describe the differential expression of miRNAs in peripheral blood of lung cancer patients discovering 76 previously unknown miRNAs and 41 novel mature forms of known precursors. Additionally, the authors of this research identified 32

annotated and seven unknown miRNAs that were significantly altered in NSCLC patients [99].

A classifier-based 24-miRNA signature derived by plasma blood with predictive, diagnostic, and prognostic value was described, whose use could reduce the false-positive rate of low-dose computed tomography (LDCT), improving then the efficacy of lung cancer screening [100, 101].

Concerning the potential role of circulating miRNAs as prognostic factors, the levels of miR-155 and miR-197 have been found higher in plasma from lung cancer patients with metastasis compared to those without metastasis [89].

Adopting NGS approach, it was described that serum levels of miR-486, miR-30d, miR-1, and miR-499 are significantly associated with overall survival [102]. Patients with NSCLC and healthy controls differ in vesicle-related miRNAs extracted from plasma: let-7f and miR-30e-3p levels decreased in plasma vesicles of NSCLC patients and the expression of these miRNAs is associated with poor outcome [103]. Furthermore, serum miR-125b may represent a biomarker in NSCLC with an independent prognostic potential for overall survival [104].

The ability to predict response to treatment was explored for circulating miRNA too. miR-21 expression has trends similar in plasma and matched resected samples, and was significantly overexpressed in platinum-based chemotherapy-resistant patients, in which induction of miR-21 was associated with the shorter disease-free survival [105].

The expression of miR-21 and miR-10b was much higher in plasma samples derived from patients with NSCLCs with EGFR mutation than without mutation [106]. Patients who had upregulated miR-21 expression had shorter overall survival, but a better response to gefitinib than patients who had low expression of the microRNA. Moreover, miR-10b is described to be highly expressed in progressive disease compared with complete remission or stable disease.

Another scientific article, reported a correlation between overexpression of miR-22 in whole-blood and the lack of response in NSCLC patients treated with pemetrexed [97].

Investigators also measured miR-138 and one of its target mRNA (PDK1) levels in serum of NSCLC and their associations with patients prognosis. miR-138 downregulation and PDK1 upregulation were both significantly associated with advanced tumor-node-metastasis (TNM) stage and positive lymph node metastasis of NSCLC patients. Moreover, the overall survival of NSCLC patients with low miR-138 expression or high PDK1 mRNA expression was shorter than those with high miR-138 expression or low PDK1 mRNA expression. Notably, NSCLC patients with combined miR-138 downregulation and PDK1 upregulation (miR-138-low/PDK1-high) had shortest overall survival [107].

miR-125a-5p, miR-145, and miR-146a measured from serum, were found to be overexpressed in NSCLC patients compared with healthy controls. Classifiers obtained from this analysis yielded a sensitivity and specificity of 73.53 % and 55.71 %, 92.75 % and 61.43 %, 84.06 %, and 58.57 %, respectively in differentiating NSCLC patients from healthy controls [108].

Another study, comprising 152 NSCLC patients and 300 healthy controls, found serum miR-148a, miR-148b, and miR-152 significantly downregulated in NSCLC

patients, while miR-21 was measured as overexpressed. The combination of these four candidate miRNAs exhibited the highest predictive accuracy in NSCLC screening compared with individual miRNAs (AUC = 0.97). Low level of miRNA-148/152 members may associate with advanced stage, large tumor size, malignant cell differentiation, and metastasis. High expression of miR-21 was possibly correlated with large size tumor and advanced cancer stage [109].

Recently, investigators identified a serum microRNA signature (called "miR-Test"), based on the expression of 13 miR (miR-92a-3p, miR-30b-5p, miR-191-5p, miR-484, miR-328-3p, miR-30c-5p, miR-374a-5p, let-7d-5p, miR-331-3p, miR-29a-3p, miR-148a-3p, miR-223-3p, miR-140-5p) that could identify the optimal target population that needs to be screened by low-dose computed tomography screening (LDCT). It was validated by a large-scale study in high-risk individuals ($n = 1115$). The overall accuracy, sensitivity, and specificity of the miR-Test were 74.9 %, 77.8 %, and 74.8 %, respectively [110].

A resume of all researches reported is summarized in Table 9.4.

Table 9.4 Circulating miRNAs as biomarkers in lung cancer

miRNA	Function	Scope	Sample	References
miR-155, miR-197, miR-182 (**UP**)	Diagnostic	Lung cancer patients vs. healthy controls	Plasma	[89]
miR-21, miR-210, miR-126, miR-486-5p (**Relative Expression**)	Diagnostic	NSCLC patients vs. healthy controls	Plasma	[90]
miR-21–5p (UP) and miR-335–3p (**DOWN**)	Diagnostic	Lung cancer patients vs. healthy controls	Plasma	[91]
miR-21, miR-155 (UP), miR-145 (**DOWN**)	Diagnostic	Lung cancer patient vs. healthy smokers	Plasma	[92]
miR-361-3p, miR-625* (**DOWN**)	Diagnostic	Lung cancer patients vs. healthy controls	Serum	[93]
miR-92a, miR-484, miR-486-5p, miR-328, miR-191, miR-376a, miR-342, miR-331-3p, miR-30c, miR-28-5p, miR-98, miR-17-5p, miR-26b, miR-374, miR-30b, miR-26a, miR-142-3p, miR-103, miR-126, let-7a, let-7d, let-7b, miR-22, miR-148b, miR-139 (**DOWN**), miR-32, miR-133b, miR-566, miR-432-3p, miR-223, miR-29a, miR-148a, miR-142-5p, miR-140-5p (**UP**)	Diagnostic	Asymptomatic NSCLC patients vs. healthy smokers	Serum	[94]

(continued)

Table 9.4 (continued)

miRNA	Function	Scope	Sample	References
miR-205-5p, miR-205-3p, and miR-21-3p (**UP**)	Diagnostic	NSCLC patients vs. benign lung disease and healthy controls	Serum	[95]
miR-190b, miR-630, miR-942 and miR-1284 (**Relative Expression**)	Diagnostic	Lung cancer patients vs. healthy controls	Whole-blood	[75]
miR-22, miR-24, and miR-34a (**UP**)	Diagnostic	NSCLC patients vs. healthy controls	Whole-blood	[97]
miR-205, miR-19a, miR-19b, miR-30b, miR-20a (**DOWN**)	Diagnostic	Patients after lung cancer surgery vs. healthy controls	Plasma	[98]
miR-7, miR-21, miR-200b, miR-210, miR-219-1, miR-324 (**UP**), miR-126, miR-451, miR-30a, miR-486 (**DOWN**)	Diagnostic	NSCLC patients vs. healthy controls	Plasma	[100]
miR-101, miR-106a miR-126, miR-133a, miR-140-3p, miR-140-5p, miR-142-3p, miR-145, miR-148a, miR-15b, miR-16, miR-17, miR-197, miR-19b, miR-21, miR-221, miR-28-3p, miR-30b, miR-30c, miR-320, miR-451, miR-486-5p, miR-660, and miR-92a (**Relative Expression**)	Diagnostic	NSCLC patients vs. healthy controls	Plasma	[101]
miR-155, miR-197 (**UP**)	Prognostic	Lung cancer patients with metastasis vs. patients without metastasis	Plasma	[89]
miR-486, miR-30d, miR-1, miR-499 (**Relative Expression**)	Prognostic	NSCLC patients vs. healthy controls	Serum	[102]
let-7f, miR-30e-3p (**DOWN**)	Prognostic	NSCLC patients vs. healthy controls	Plasma	[103]
miR-125b (**Relative Expression**)	Prognostic	NSCLC patients vs. healthy controls	Serum	[104]
miR-21 and miR-10b (**UP**)	Response to treatment	NSCLC patients with EGFR mutation vs. patients without mutation	Plasma	[106]
miR-22 (**UP**)	Response to treatment	NSCLC patients vs. healthy controls	Whole-blood	[97]

(continued)

Table 9.4 (continued)

miRNA	Function	Scope	Sample	References
miR-138 (**DOWN**)	Prognostic	advanced tumor-node-metastasis (TNM) stage and positive lymph node metastasis of NSCLC patients vs. healthy controls	Serum	[107]
miR-125a-5p, miR-145 and miR-146a (**UP**)	Diagnostic	NSCLC patients vs. healthy controls	Serum	[108]
miR-148a, miR-148b, and miR-152 (**DOWN**) miR-21 (**UP**)	Diagnostic	NSCLC patients vs. healthy controls	Serum	[109]
miR-92a-3p, miR-30b-5p, miR-191-5p, miR-484, miR-328-3p, miR-30c-5p, miR-374a-5p, let-7d-5p, miR-331-3p, miR-29a-3p, miR-148a-3p, miR-223-3p, miR-140-5p (**Relative Expression**)	Diagnostic	NSCLC high-risk individuals	Serum	[110]

NSCLC non-small-cell lung cancer

Conclusion

miRNAs have increasingly been pointed as important players in carcinogenesis, cancer progression, chemo- and radioresistance but also as potential diagnostic and prognostic biomarkers.

Circulating miRNAs could open new opportunities in the field of diagnosis and prognosis in various types of human cancers. The ease, specificity, and sensitivity of determining body fluid miRNA profiles paves the way for several applications and provides hope to accomplish this task. However, from the technical and applicative point of view, there are still several limitations to consider. Further studies are necessary to establish panels of miRNAs distinct to each tumor type, taking into account early or advanced cancer stages, response to treatment, patient outcome, and recurrence.

References

1. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer. 2010;46:765–81.
2. Malvezzi M, Bertuccio P, Levi F, La Vecchia C, Negri E. European cancer mortality predictions for the year 2013. Ann Oncol. 2013;24:792–800.

3. Rossi G, Mengoli MC, Cavazza A, Nicoli D, Barbareschi M, Cantaloni C, Papotti M, Tironi A, Graziano P, Paci M, Stefani A, Migaldi M, Sartori G, Pelosi G. Large cell carcinoma of the lung: clinically oriented classification integrating immunohistochemistry and molecular biology. Virchows Arch. 2014;464:61–8.
4. Scagliotti GV, Selvaggi G. New data integrating multitargeted antifolates into treatment of first-line and relapsed non-small-cell lung cancer. Clin Lung Cancer. 2008;3:S122–8.
5. Hanna N, Shepherd FA, Fossella FV, Pereira JR, De Marinis F, von Pawel J, Gatzemeier U, Tsao TC, Pless M, Muller T, Lim HL, Desch C, Szondy K, Gervais R, Shaharyar A, Manegold C, Paul S, Paoletti P, Einhorn L, Bunn Jr PA. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncol. 2004;22:1589–97.
6. Ciuleanu T, Brodowicz T, Zielinski C, Kim JH, Krzakowski M, Laack E, Wu YL, Bover I, Begbie S, Tzekova V, Cucevic B, Pereira JR, Yang SH, Madhavan J, Sugarman KP, Peterson P, John WJ, Krejcy K, Belani CP. Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomised, double-blind, phase 3 study. Lancet. 2009;374:1432–40.
7. Ardizzoni A, Boni L, Tiseo M, Fossella FV, Schiller JH, Paesmans M, Radosavljevic D, Paccagnella A, Zatloukal P, Mazzanti P, Bisset D, Rosell R, CISCA (CISplatin versus CArboplatin) Meta-analysis Group. Cisplatin- versus carboplatin-based chemotherapy in first-line treatment of advanced non-small-cell lung cancer: an individual patient data meta-analysis. J Natl Cancer Inst. 2007;99:847–57.
8. Sandler AB, Schiller JH, Gray R, Dimery I, Brahmer J, Samant M, et al. Retrospective evaluation of the clinical and radiographic risk factors associated with severe pulmonary hemorrhage in first-line advanced, unresectable non-small-cell lung cancer treated with carboplatin and paclitaxel plus Bevacizumab. J Clin Oncol. 2009;27:1405–12.
9. Rekhtman N, Brandt SM, Sigel CS, Friedlander MA, Riely GJ, Travis WD, Zakowski MF, Moreira AL. Suitability of thoracic cytology for new therapeutic paradigms in non-small cell lung carcinoma: high accuracy of tumor subtyping and feasibility of EGFR and KRAS molecular testing. J Thorac Oncol. 2011;6:451–8.
10. Nizzoli R, Tiseo M, Gelsomino F, Bartolotti M, Majori M, Ferrari L, De Filippo M, Rindi G, Silini EM, Guazzi A, Ardizzoni A. Accuracy of fine needle aspiration cytology in the pathological typing of non-small cell lung cancer. J Thorac Oncol. 2011;6:489–93.
11. Travis WD, Rekhtman N. Pathological diagnosis and classification of lung cancer in small biopsies and cytology: strategic management of tissue for molecular testing. Semin Respir Crit Care Med. 2011;32:22–31.
12. Rossi G, Pelosi G, Barbareschi M, Graziano P, Cavazza A, Papotti M. Subtyping non-small cell lung cancer: relevant issues and operative recommendations for the best pathology practice. Int J Surg Pathol. 2013;21:326–36.
13. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A. 2004;101:2999–3004.
14. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, Mitsudomi T, Takahashi T. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res. 2004;64:3753–6.
15. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. RAS is regulated by the let-7 microRNA family. Cell. 2005;120:635–47.
16. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM, Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell. 2006;9:189–98.
17. Inamura K, Togashi Y, Nomura K, Ninomiya H, Hiramatsu M, Satoh Y, Okumura S, Nakagawa K, Ishikawa Y. let-7 microRNA expression is reduced in bronchioloalveolar car-

cinoma, a non-invasive carcinoma, and is not correlated with prognosis. Lung Cancer. 2007;58:392–6.
18. Kumar MS, Erkeland SJ, Pester RE, Chen CY, Ebert MS, Sharp PA, Jacks T. Suppression of non-small cell lung tumor development by the let- 7 microRNA family. Proc Natl Acad Sci U S A. 2008;105:3903–8.
19. Chin LJ, Ratner E, Leng S, Zhai R, Nallur S, Babar I, Muller RU, Straka E, Su L, Burki EA, Crowell RE, Patel R, Kulkarni T, Homer R, Zelterman D, Kidd KK, Zhu Y, Christiani DC, Belinsky SA, Slack FJ, Weidhaas JB. A SNP in a let-7 microRNA complementary site in the KRAS 3' untranslated region increases non-small cell lung cancer risk. Cancer Res. 2008;68:8535–40.
20. Wang R, Wang ZX, Yang JS, Pan X, De W, Chen LB. MicroRNA-451 functions as a tumor suppressor in human non-small cell lung cancer by targeting ras-related protein 14 (RAB14). Oncogene. 2011;30:2644–58.
21. Yang J, Lan H, Huang X, Liu B, Tong Y. MicroRNA-126 inhibits tumor cell growth and its expression level correlates with poor survival in non-small cell lung cancer patients. PLoS One. 2012;7, e42978.
22. Chen Z, Zeng H, Guo Y, Liu P, Pan H, Deng A, Hu J. miRNA-145 inhibits non-small cell lung cancer cell proliferation by targeting c-Myc. J Exp Clin Cancer Res. 2010;29:151.
23. Ke Y, Zhao W, Xiong J, Cao R. Downregulation of miR-16 promotes growth and motility by targeting HDGF in non-small cell lung cancer cells. FEBS Lett. 2013;587:3153–7.
24. Fernandez S, Risolino M, Mandia N, Talotta F, Soini T, Incoronato M, Condorelli G, Banfi S, Verde P. miR-340 inhibits tumor cell proliferation and induces apoptosis by targeting multiple negative regulators of p27 in non-small cell lung cancer. Oncogene. 2015;35(25):3240–50.
25. Seol HS, Akiyama Y, Shimada S, Lee HJ, Kim TI, Chun SM, Singh SR, Jang SJ. Epigenetic silencing of microRNA-373 to epithelial-mesenchymal transition in non-small cell lung cancer through IRAK2 and LAMP1 axes. Cancer Lett. 2014;353(2):232–41.
26. Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, Yatabe Y, Kawahara K, Sekido Y, Takahashi T. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res. 2005;65: 9628–32.
27. Ventura A, Young AG, Winslow MM, Lintault L, Meissner A, Erkeland SJ, Newman J, Bronson RT, Crowley D, Stone JR, et al. Targeted deletion reveals essential and overlapping functions of the miR-17–92 family of miRNA clusters. Cell. 2008;132(5):875–86.
28. Garofalo M, Di Leva G, Romano G, Nuovo G, Suh SS, Ngankeu A, Taccioli C, Pichiorri F, Alder H, Secchiero P, Gasparini P, Gonelli A, Costinean S, Acunzo M, Condorelli G, Croce CM. miR- 221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. Cancer Cell. 2009;16:498–509.
29. Hatley ME, Patrick DM, Garcia MR, Richardson JA, Bassel-Duby R, van Rooij E, Olson EN. Modulation of K-Ras-dependent lung tumorigenesis by MicroRNA-21. Cancer Cell. 2010;18:282–93.
30. Zhang JG, Wang JJ, Zhao F, Liu Q, Jiang K, Yang GH. MicroRNA-21 (miR-21) represses tumor suppressor PTEN and promotes growth and invasion in non-small cell lung cancer (NSCLC). Clin Chim Acta. 2010;411:846–52.
31. Wang Y, Xu L, Jiang L. miR-1271 promotes non-small-cell lung cancer cell proliferation and invasion via targeting HOXA5. Biochem Biophys Res Commun. 2015;458(3):714–9.
32. Takahashi T, Nau MM, Chiba I, Birrer MJ, Rosenberg RK, Vinocour M, Levitt M, Pass H, Gazdar AF, Minna JD. p53: a frequent target for genetic abnormalities in lung cancer. Science. 1989;246:491–4.
33. Ding L, Getz G, Wheeler D, Mardis E, McLellan M, Cibulskis K, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature. 2008;455:1069–75.
34. He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, Xue W, Zender L, Magnus J, Ridzon D, Jackson AL, Linsley PS, Chen C, Lowe SW, Cleary MA, Hannon GJ. A microRNA component of the p53 tumour suppressor network. Nature. 2007;447:1130–4.
35. 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.
36. Corney DC, Flesken-Nikitin A, Godwin AK, Wang W, Nikitin AY. MicroRNA-34b and MicroRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. Cancer Res. 2007;67:8433–8.
37. Raver-Shapira N, Marciano E, Meiri E, Spector Y, Rosenfeld N, Moskovits N, Bentwich Z, Oren M. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. Mol Cell. 2007;26:731–43.
38. Jiang L, Huang Q, Chang J, Wang E, Qiu X. MicroRNA HSA-miR-125a-5p induces apoptosis by activating p53 in lung cancer cells. Exp Lung Res. 2011;37:387–98.
39. Bisio A, De Sanctis V, Del Vescovo V, Denti MA, Jegga AG, Inga A, Ciribilli Y. Identification of new p53 target microRNAs by bioinformatics and functional analysis. BMC Cancer. 2013;13:552.
40. Galluzzi L, Morselli E, Vitale I, Kepp O, Senovilla L, Criollo A, Servant N, Paccard C, Hupé P, Robert T, Ripoche H, Lazar V, Harel-Bellan A, Dessen P, Barillot E, Kroemer G. miR-181a and miR-630 regulate cisplatin-induced cancer cell death. Cancer Res. 2010;70:1793–803.
41. Bian HB, Pan X, Yang JS, Wang ZX, De W. Upregulation of microRNA-451 increases cisplatin sensitivity of non-small cell lung cancer cell line (A549). J Exp Clin Cancer Res. 2011;30:20.
42. Zhang S, Zhang C, Li Y, Wang P, Yue Z, Xie S. miR-98 regulates cisplatin-induced A549 cell death by inhibiting TP53 pathway. Biomed Pharmacother. 2011;65:436–42.
43. Zang YS, Zhong YF, Fang Z, Li B, An J. MiR-155 inhibits the sensitivity of lung cancer cells to cisplatin via negative regulation of Apaf-1 expression. Cancer Gene Ther. 2012;19:773–8.
44. Zhang X, Zhu J, Xing R, Tie Y, Fu H, Zheng X, Yu B. miR-513a-3p sensitizes human lung adenocarcinoma cells to chemotherapy by targeting GSTP1. Lung Cancer. 2012;77(3): 488–94.
45. Qiu T, Zhou L, Wang T, Xu J, Wang J, Chen W, Zhou X, Huang Z, Zhu W, Shu Y, Liu P. miR-503 regulates the resistance of non-small cell lung cancer cells to cisplatin by targeting Bcl-2. Int J Mol Med. 2013;32(3):593–8.
46. Zhou L, Qiu T, Xu J, Wang T, Wang J, Zhou X, Huang Z, Zhu W, Shu Y, Liu P. miR-135a/b modulate cisplatin resistance of human lung cancer cell line by targeting MCL1. Pathol Oncol Res. 2013;19(4):677–83.
47. Song L, Li Y, Li W, Wu S, Li Z. miR-495 enhances the sensitivity of non-small cell lung cancer cells to platinum by modulation of copper-transporting P-type adenosine triphosphatase A (ATP7A). J Cell Biochem. 2014;115(7):1234–42.
48. Dong Z, Zhong Z, Yang L, Wang S, Gong Z. MicroRNA-31 inhibits cisplatin-induced apoptosis in non-small cell lung cancer cells by regulating the drug transporter ABCB9. Cancer Lett. 2014;343(2):249–57.
49. Zhao Z, Zhang L, Yao Q, Tao Z. miR-15b regulates cisplatin resistance and metastasis by targeting PEBP4 in human lung adenocarcinoma cells. Cancer Gene Ther. 2015;22(3): 108–14.
50. Adi Harel S, Bossel Ben-Moshe N, Aylon Y, Bublik DR, Moskovits N, Toperoff G, Azaiza D, Biagoni F, Fuchs G, Wilder S, Hellman A, Blandino G, Domany E, Oren M. Reactivation of epigenetically silenced miR-512 and miR-373 sensitizes lung cancer cells to cisplatin and restricts tumor growth. Cell Death Differ. 2015;22(8):1328–40.
51. Garofalo M, Quintavalle C, Di Leva G, Zanca C, Romano G, Taccioli C, Liu CG, Croce CM, Condorelli G. MicroRNA signatures of TRAIL resistance in human non-small cell lung cancer. Oncogene. 2008;27:3845–55.
52. Acunzo M, Visone R, Romano G, Veronese A, Lovat F, Palmieri D, Bottoni A, Garofalo M, Gasparini P, Condorelli G, Chiariello M, Croce CM. miR-130a targets MET and induces TRAIL-sensitivity in NSCLC by downregulating miR-221 and 222. Oncogene. 2012;31:634–42.
53. Janssens S, Tinel A, Lippens S, Tschopp J. PIDD mediates NF-kappaB activation in response to DNA damage. Cell. 2005;123:1079–92.

54. Li F, Sethi G. Targeting transcription factor NF-kappaB to overcome chemoresistance and radioresistance in cancer therapy. Biochim Biophys Acta. 1805;2010:167–80.
55. Arora H, Qureshi R, Jin S, Park AK, Park WY. miR-9 and let-7g enhance the sensitivity to ionizing radiation by suppression of NFκB1. Exp Mol Med. 2011;43:298–304.
56. Salim H, Akbar NS, Zong D, Vaculova AH, Lewensohn R, Moshfegh A, Viktorsson K, Zhivotovsky B. MiRNA-214 modulates radiotherapy response of non-small cell lung cancer cells through regulation of p38MAPK, apoptosis and senescence. Br J Cancer. 2012;107(8):1361–73.
57. Grosso S, Doyen J, Parks SK, Bertero T, Paye A, Cardinaud B, Gounon P, Lacas-Gervais S, Noël A, Pouysségur J, Barbry P, Mazure NM, Mari B. MiR-210 promotes a hypoxic phenotype and increases radioresistance in human lung cancer cell lines. Cell Death Dis. 2013;4, e544.
58. 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.
59. 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.
60. Takahashi Y, Forrest AR, Maeno E, Hashimoto T, Daub CO, Yasuda J. MiR-107 and MiR-185 can induce cell cycle arrest in human non-small cell lung cancer cell lines. PLoS One. 2009;4, e6677.
61. Landi MT, Zhao Y, Rotunno M, Koshiol J, Liu H, Bergen AW, Rubagotti M, Goldstein AM, Linnoila I, Marincola FM, Tucker MA, Bertazzi PA, Pesatori AC, Caporaso NE, McShane LM, Wang E. MicroRNA expression differentiates histology and predicts survival of lung cancer. Clin Cancer Res. 2010;16:430–41.
62. Raponi M, Dossey L, Jatkoe T, Wu X, Chen G, Fan H, Beer DG. MicroRNA classifiers for predicting prognosis of squamous cell lung cancer. Cancer Res. 2009;69:5776–83.
63. Tan X, Qin W, Zhang L, Hang J, Li B, Zhang C, Wan J, Zhou F, Shao K, Sun Y, Wu J, Zhang X, Qiu B, Li N, Shi S, Feng X, Zhao S, Wang Z, Zhao X, Chen Z, Mitchelson K, Cheng J, Guo Y, He J. A 5-microRNA signature for lung squamous cell carcinoma diagnosis and hsa-miR-31 for prognosis. Clin Cancer Res. 2011;17:6802–11.
64. Barshack I, Lithwick-Yanai G, Afek A, Rosenblatt K, Tabibian-Keissar H, Zepeniuk M, Cohen L, Dan H, Zion O, Strenov Y, Polak-Charcon S, Perelman M. MicroRNA expression differentiates between primary lung tumors and metastases to the lung. Pathol Res Pract. 2010;206:578–84.
65. Lebanony D, Benjamin H, Gilad S, Ezagouri M, Dov A, Ashkenazi K, Gefen N, Izraeli S, Rechavi G, Pass H, Nonaka D, Li J, Spector Y, Rosenfeld N, Chajut A, Cohen D, Aharonov R, Mansukhani M. Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma. J Clin Oncol. 2009;27:2030–7.
66. Bishop JA, Benjamin H, Cholakh H, Chajut A, Clark DP, Westra WH. Accurate classification of non-small cell lung carcinoma using a novel microRNA-based approach. Clin Cancer Res. 2010;16:610–9.
67. Del Vescovo V, Cantaloni C, Cucino A, Girlando S, Silvestri M, Bragantini E, Fasanella S, Cuorvo LV, Palma PD, Rossi G, Papotti M, Pelosi G, Graziano P, Cavazza A, Denti MA, Barbareschi M. miR-205 Expression levels in nonsmall cell lung cancer do not always distinguish adenocarcinomas from squamous cell carcinomas. Am J Surg Pathol. 2011;35:268–75.
68. Barbareschi M, Cantaloni C, Del Vescovo V, Cavazza A, Monica V, Carella R, Rossi G, Morelli L, Cucino A, Silvestri M, Tirone G, Pelosi G, Graziano P, Papotti M, Dalla Palma P, Doglioni C, Denti MA. Heterogeneity of large cell carcinoma of the lung: an immunophenotypic and miRNA-based analysis. Am J Clin Pathol. 2011;136:773–82.
69. Lee HW, Lee EH, Ha SY, Lee CH, Chang HK, Chang S, Kwon KY, Hwang IS, Roh MS, Seo JW. Altered expression of microRNA miR-21, miR-155, and let-7a and their roles in pulmonary neuroendocrine tumors. Pathol Int. 2012;62:583–91.

70. Huang W, Hu J, Yang DW, Fan XT, Jin Y, Hou YY, Wang JP, Yuan YF, Tan YS, Zhu XZ, Bai CX, Wu Y, Zhu HG, Lu SH. Two microRNA panels to discriminate three subtypes of lung carcinoma in bronchial brushing specimens. Am J Respir Crit Care Med. 2012;186:1160–7.
71. Gilad S, Lithwick-Yanai G, Barshack I, Benjamin S, Krivitsky I, Edmonston TB, Bibbo M, Thurm C, Horowitz L, Huang Y, Feinmesser M, Hou JS, St Cyr B, Burnstein I, Gibori H, Dromi N, Sanden M, Kushnir M, Aharonov R. Classification of the four main types of lung cancer using a microRNA-based diagnostic assay. J Mol Diagn. 2012;14:510–7.
72. Petriella D, Galetta D, Rubini V, Savino E, Paradiso A, Simone G, Tommasi S. Molecular profiling of thin-prep FNA samples in assisting clinical management of non-small-cell lung cancer. Mol Biotechnol. 2013;54:913–9.
73. Yu SL, Chen HY, Chang GC, Chen CY, Chen HW, Singh S, Cheng CL, Yu CJ, Lee YC, Chen HS, Su TJ, Chiang CC, Li HN, Hong QS, Su HY, Chen CC, Chen WJ, Liu CC, Chan WK, Chen WJ, Li KC, Chen JJ, Yang PC. MicroRNA signature predicts survival and relapse in lung cancer. Cancer Cell. 2008;13:48–57.
74. Gallardo E, Navarro A, Viñolas N, Marrades RM, Diaz T, Gel B, Quera A, Bandres E, Garcia-Foncillas J, Ramirez J, Monzo M. miR-34a as a prognostic marker of relapse in surgically resected non-small-cell lung cancer. Carcinogenesis. 2009;30:1903–9.
75. Patnaik SK, Yendamuri S, Kannisto E, Kucharczuk JC, Singhal S, Vachani A. MicroRNA expression profiles of whole blood in lung adenocarcinoma. PLoS One. 2012;7, e46045.
76. Navarro A, Diaz T, Gallardo E, Viñolas N, Marrades RM, Gel B, Campayo M, Quera A, Bandres E, Garcia-Foncillas J, Ramirez J, Monzo M. Prognostic implications of miR-16 expression levels in resected non-small-cell lung cancer. J Surg Oncol. 2011;103:411–5.
77. Campayo M, Navarro A, Viñolas N, Diaz T, Tejero R, Gimferrer JM, Molins L, Cabanas ML, Ramirez J, Monzo M, Marrades R. Low miR-145 and high miR-367 are associated with unfavourable prognosis in resected nonsmall cell lung cancer. Eur Respir J. 2013;41: 1172–8.
78. Gao W, Yu Y, Cao H, Shen H, Li X, Pan S, Shu Y. Deregulated expression of miR-21, miR-143 and miR-181a in non small cell lung cancer is related to clinicopathologic characteristics or patient prognosis. Biomed Pharmacother. 2010;64:399–408.
79. Arora S, Ranade AR, Tran NL, Nasser S, Sridhar S, Korn RL, Ross JT, Dhruv H, Foss KM, Sibenaller Z, Ryken T, Gotway MB, Kim S, Weiss GJ. MicroRNA-328 is associated with (non-small) cell lung cancer (NSCLC) brain metastasis and mediates NSCLC migration. Int J Cancer. 2011;129:2621–31.
80. Hubers AJ, Prinsen CF, Sozzi G, Witte BI, Thunnissen E. Molecular sputum analysis for the diagnosis of lung cancer. Br J Cancer. 2013;109:530–7.
81. Xie Y, Todd NW, Liu Z, Zhan M, Fang H, Peng H, Alattar M, Deepak J, Stass SA, Jiang F. Altered miRNA expression in sputum for diagnosis of non-small cell lung cancer. Lung Cancer. 2010;67:170–6.
82. Xing L, Todd NW, Yu L, Fang H, Jiang F. Early detection of squamous cell lung cancer in sputum by a panel of microRNA markers. Mod Pathol. 2010;23:1157–64.
83. Yu L, Todd NW, Xing L, Xie Y, Zhang H, Liu Z, Fang H, Zhang J, Katz RL, Jiang F. Early detection of lung adenocarcinoma in sputum by a panel of microRNA markers. Int J Cancer. 2010;127:2870–8.
84. Li N, Ma J, Guarnera MA, Fang H, Cai L, Jiang F. Digital PCR quantification of miRNAs in sputum for diagnosis of lung cancer. J Cancer Res Clin Oncol. 2014;140:145–50.
85. Shen J, Liao J, Guarnera MA, Fang H, Cai L, Stass SA, Jiang F. Analysis of MicroRNAs in Sputum to Improve Computed Tomography for Lung Cancer Diagnosis. J Thorac Oncol. 2014;9:33–40.
86. Roa WH, Kim JO, Razzak R, Du H, Guo L, Singh R, Gazala S, Ghosh S, Wong E, Joy AA, Xing JZ, Bedard EL. Sputum microRNA profiling: a novel approach for the early detection of non-small cell lung cancer. Clin Invest Med. 2012;35, E271.
87. Xing L, Su J, Guarnera MA, Zhang H, Cai L, Zhou R, Stass SA, Jiang F. Sputum microRNA biomarkers for identifying lung cancer in indeterminate solitary pulmonary nodules. Clin Cancer Res. 2015;21(2):484–9.

88. Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholakh H, Melamed N, Bentwich Z, Hod M, Goren Y, Chajut A. Serum MicroRNAs are promising novel biomarkers. PLoS One. 2008;3, e3148.
89. Zheng D, Haddadin S, Wang Y, Gu L, Perry MC, Freter CE, Wang MX. Plasma microRNAs as novel biomarkers for early detection of lung cancer. Int J Clin Exp Pathol. 2011;4:575–86.
90. Shen J, Todd NW, Zhang H, Yu L, Lingxiao X, Mei Y, Guarnera M, Liao J, Chou A, Lu CL, Jiang Z, Fang H, Katz RL, Jiang F. Plasma microRNAs as potential biomarkers for non-small-cell lung cancer. Lab Invest. 2011;91:579–87.
91. Ma J, Li N, Guarnera M, Jiang F. Quantification of plasma miRNAs by digital PCR for cancer diagnosis. Biomark Insights. 2013;8:127–36.
92. Tang D, Shena Y, Wanga M, Yanga R, Wanga Z, Suib A, Jiaoa W, Wang Y. Identification of plasma microRNAs as novel non-invasive biomarkers for early detection of lung cancer. Eur J Cancer Prev. 2013;22:540–8.
93. Roth C, Stuckrath I, Pantel K, Izbicki JR, Tachezy M, Schwarzenbach H. Low levels of cell-free circulating miR-361-3p and miR-625* as blood-based markers for discriminating malignant from benign lung tumors. PLoS One. 2012;7, e38248.
94. Bianchi F, Nicassio F, Marzi M, Belloni E, Dall'olio V, Bernard L, Pelosi G, Maisonneuve P, Veronesi G, Di Fiore PP. A serum circulating miRNA diagnostic test to identify asymptomatic high-risk individuals with early stage lung cancer. EMBO Mol Med. 2011;3:495–503.
95. Jiang M, Zhang P, Hu G, Xiao Z, Xu F, Zhong T, Huang F, Kuang H, Zhang W. Relative expressions of miR-205-5p, miR-205-3p, and miR-21 in tissues and serum of non-small cell lung cancer patients. Mol Cell Biochem. 2013;383:67–75.
96. Patnaik SK, Kannisto E, Knudsen S, Yendamuri S. Evaluation of microRNA expression profiles that may predict recurrence of localized stage I non-small cell lung cancer after surgical resection. Cancer Res. 2010;70:36–45.
97. Franchina T, Amodeo V, Bronte G, Savio G, Ricciardi GR, Picciotto M, Russo A, Giordano A, Adamo V. Circulating miR-22, miR-24 and miR-34a as novel predictive biomarkers to pemetrexed-based chemotherapy in advanced non-small cell lung cancer. J Cell Physiol. 2014;229:97–9.
98. Aushev VN, Zborovskaya IB, Laktionov KK, Girard N, Cros MP, Herceg Z, Krutovskikh V. Comparisons of microRNA patterns in plasma before and after tumor removal reveal new biomarkers of lung squamous cell carcinoma. PLoS One. 2013;8, e78649.
99. Keller A, Backes C, Leidinger P, Kefer N, Boisguerin V, Barbacioru C, Vogel B, Matzas M, Huwer H, Katus HA, Stähler C, Meder B, Meese E. Next-generation sequencing identifies novel microRNAs in peripheral blood of lung cancer patients. Mol Biosyst. 2011;7: 3187–99.
100. Boeri M, Verri C, Conte D, Roz L, Modena P, Facchinetti F, Calabrò E, Croce CM, Pastorino U, Sozzi G. MicroRNA signatures in tissues and plasma predict development and prognosis of computed tomography detected lung cancer. Proc Natl Acad Sci U S A. 2011;108:3713–8.
101. Sozzi G, Boeri M, Rossi M, Verri C, Suatoni P, Bravi F, Roz L, Conte D, Grassi M, Sverzellati N, Marchiano A, Negri E, La Vecchia C, Pastorino U. Clinical utility of a plasma-based miRNA signature classifier within computed tomography lung cancer screening: a correlative MILD trial study. J Clin Oncol. 2014;32:768–73.
102. Hu Z, Chen X, Zhao Y, Tian T, Jin G, Shu Y, Chen Y, Xu L, Zen K, Zhang C, Shen H. Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non-small-cell lung cancer. J Clin Oncol. 2010;28:1721–6.
103. Silva J, Garcia V, Zaballos A, Provencio M, Lombardia L, Almonacid L, Garcia JM, Dominguez G, Pena C, Diaz R, Herrera M, Varela A, Bonilla F. Vesicle-related microRNAs in plasma of non small cell lung cancer patients and correlation with survival. Eur Respir J. 2011;37:617–23.
104. Yuxia M, Zhennan T, Zhang W. Circulating miR-125b is a novel biomarker for screening non-small-cell lung cancer and predicts poor prognosis. J Cancer Res Clin Oncol. 2012;138: 2045–50.

105. Gao W, Lu X, Liu L, Xu J, Feng D, Shu Y. MiRNA-21: a biomarker predictive for platinum-based adjuvant chemotherapy response in patients with non-small cell lung cancer. Cancer Biol Ther. 2012;13:330–40.
106. Shen Y, Tang D, Yao R, Wang M, Wang Y, Yao Y, Li X. Zhang H.microRNA expression profiles associated with survival, disease progression, and response to gefitinib in completely resected non-small-cell lung cancer with EGFR mutation. Med Oncol. 2013;30:750.
107. Han L, Zhang G, Zhang N, Li H, Liu Y, Fu A, Zheng Y. Prognostic potential of microRNA-138 and its target mRNA PDK1 in sera for patients with non-small cell lung cancer. Med Oncol. 2014;31(9):129.
108. Wang RJ, Zheng YH, Wang P, Zhang JZ. Serum miR-125a-5p, miR-145 and miR-146a as diagnostic biomarkers in non-small cell lung cancer. Int J Clin Exp Pathol. 2015;8(1):765–71.
109. Yang JS, Li BJ, Lu HW, Chen Y, Lu C, Zhu RX, Liu SH, Yi QT, Li J, Song CH. Serum miR-152, miR-148a, miR-148b, and miR-21 as novel biomarkers in non-small cell lung cancer screening. Tumour Biol. 2015;36(4):3035–42.
110. Montani F, Marzi MJ, Dezi F, Dama E, Carletti RM, Bonizzi G, Bertolotti R, Bellomi M, Rampinelli C, Maisonneuve P, Spaggiari L, Veronesi G, Nicassio F, Di Fiore PP, Bianchi F. miR-Test: a blood test for lung cancer early detection. J Natl Cancer Inst. 2015;107:6.

Chapter 10
microRNAs and Soft Tissue Sarcomas

Tomohiro Fujiwara, Toshiyuki Kunisada, Koji Uotani, Aki Yoshida, Ken Takeda, and Toshifumi Ozaki

Abstract Soft tissue sarcomas are a highly heterogenous group of malignant tumors that originate from mesenchymal tissues including muscle, adipose and fibrous tissues, blood vessels, and peripheral nerves. A large variety of histological subtypes that current diagnostic approaches recognize present a diagnostic challenge because their clinical and histopathological characteristics are not always distinct. One of the important clinical problems is a lack of useful biomarkers; therefore, the discovery of biomarkers that can be used to detect tumors or predict tumor response to chemotherapy or radiotherapy could help clinicians provide more effective clinical management. Recent reports on microRNAs (miRNAs) in soft tissue sarcomas have provided clues to solve the problem. Evidence for miRNAs in tumor tissues as well as circulating miRNAs in patients' blood is accelerating the potential to transform clinical applications. In this chapter, we summarize the emerging

T. Fujiwara (✉)
Center for Innovative Clinical Medicine, Okayama University Hospital, Okayama, Japan

Department of Orthopaedic Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan
e-mail: tomomedvn@gmail.com

T. Kunisada
Department of Orthopaedic Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

Department of Medical Materials for Musculoskeletal Reconstruction, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

K. Uotani • A. Yoshida
Department of Orthopaedic Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

K. Takeda
Department of Intelligent Orthopaedic System, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Department of Orthopaedic Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

T. Ozaki
Department of Orthopaedic Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

© Springer International Publishing Switzerland 2015

G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5_10

evidence of dysregulated miRNAs in tumor tissues and patients' blood and discuss the potential of miRNAs as novel biomarkers and therapeutic targets.

Keywords microRNA • Soft tissue sarcoma • Biomarker • Therapeutics

Introduction

Soft tissues are defined as nonepithelial extraskeletal tissues of the body, exclusive of the reticuloendothelial system, glia, and supporting tissues of various organs [1]. Soft tissues are represented by fibrous and adipose tissues, voluntary muscles, blood vessels, and peripheral nerves. Soft tissue sarcomas are malignant tumors of soft tissues, from which the name "sarcoma" is derived from the Greek word "sarkoma," which means fleshy outgrowth [2]. The World Health Organization (WHO) classification system is generally accepted as the basis for classification of soft tissue sarcomas. According to the data from the Surveillance, Epidemiology, and End Results (SEER), which included 26,758 cases from 1978 to 2001, leiomyosarcomas (LMSs) were the most common form, accounting for 23.9 % of all cases. Other major histological types were malignant fibrous histiocytomas (MFHs; 17.1 %), liposarcomas (11.5 %), dermatofibrosarcomas (10.5 %), rhabdomyosarcomas (RMSs; 4.6 %), angiosarcomas (4.1 %), malignant peripheral nerve sheath tumors (MPNSTs; 4.0 %), fibrosarcomas (3.6 %), synovial sarcomas (2.3 %), and clear cell sarcomas (0.4 %) [3]. Notably, the diagnostic term "MFH" was removed from the 2002 edition of the WHO classification, in which it was synonymous with undifferentiated pleomorphic sarcoma (UPS). UPS, now includes the separate and new category of undifferentiated/unclassified sarcomas in the 2013 edition of WHO classifications.

Surgical resection and adjuvant chemotherapy and radiotherapy are the mainstays of the treatment for most patients with soft tissue sarcomas. Although treatment outcomes have gradually improved in recent years, a significant proportion of patients with sarcomas poorly respond to chemotherapy, leading to local recurrences or distant metastases. The main cause of death among patients is lung metastases [4, 5]. Therefore, early detection of recurrent or metastatic diseases or early decision making according to tumor responses to chemotherapy is important and could improve prognoses. However, there are no useful biomarkers for early diagnoses or the ability to predict drug responses. Although imaging methods including computed tomography (CT) or magnetic resonance imaging (MRI) are available, it is not until tumors grow up to several millimeters that they can be detected using these methods. Therefore, the discovery of novel biomarkers to detect tumors, predict their drug sensitivities, and monitor tumor growth is one of the most important challenges that must be overcome.

In these recent years, a growing amount of evidence in favor of utilizing microRNA (miRNA) profiling in the diagnosis of soft tissue sarcomas has emerged.

In this chapter, we review the accumulating evidence for miRNAs in soft tissue sarcomas, highlighting their function in each histological type of soft tissue sarcoma as well as their clinical relevance. In addition, we update the clinical trials on the basis of miRNA profiling using patients' blood as well as address the potential of miRNAs as novel biomarkers and therapeutic targets for soft tissue sarcomas.

Aberrant miRNA Expression in Soft Tissue Sarcomas

The importance of miRNA in malignant diseases was suggested in 2004 when miRNA genes were found to be specifically deleted in leukemia [6, 7]. Subsequent reports have demonstrated that miRNAs are dysregulated in many malignant tumors, and these dysregulated miRNAs can initiate carcinogenesis or drive progression [7]. miRNA dysregulation can arise from either genetic or epigenetic means. Many miRNAs are located within chromosomal fragile sites, and these are often deleted or rearranged in malignant tumors [8].

Mutations in miRNAs have been found in rare cases [9, 10]. The first systematic profiling report detailing miRNA expression in soft tissue sarcomas was published in 2008. Subramanian et al. performed global miRNA expression profiling on a series of 27 sarcomas, five normal smooth and two normal skeletal muscle tissues using microarrays and individual molecule sequencing. Their data showed that different histological types of sarcomas have distinct miRNA expression patterns, reflecting the apparent lineage and differentiation status of the soft tissue tumors [11]. To date, more than 50 reports have examined miRNA expression in soft tissue sarcomas (Tables 10.1 and 10.2) [12].

Leiomyosarcoma

LMSsare malignant sarcomas showing smooth muscle differentiation. LMS of soft tissues usually occurs in middle-aged or older individuals, although it may develop in young adults and even in children [13]. It arises in retroperitoneal lesions (40–45 %), extremities (30–35 %), skin (15–20 %), and larger blood vessels (5 %). Surgical resection is the most reliable treatment, and the effectiveness of chemo- and radiotherapy is uncertain, while a clear survival benefit of chemo- or radiotherapy is evident if surgical margins are not adequate. The 5-year survival rate is 60–70 % for patients with LMS in the extremities, which is much better than those with LMS in the retroperitoneum [14].

Emerging evidence from miRNA profiling of LMS has focused on those of sarcomas originating from the extremities as well as the uterus. All studies have identified up- or downregulation of miRNAs in LMS in comparison with those in leiomyomas, benign counterparts, or other soft tissue sarcomas. Subramanian et al. [11] reported that miR-1, -133a, and -133b, which play major roles in myogenesis

Table 10.1 Aberrant miRNAs associated with leiomyosarcoma, undifferentiated pleomorphic sarcoma, and liposarcoma

Histology	Subtypes	Dysregulated miRNAs		Function	miRNA target	Reference
		Upregulated	Downregulated			
Leiomyosarcoma	(Not specified)	miR-1, -133a, -133b		N/D	N/D	[11]
		miR-1, 133a, -449a				[16]
		miR-320a				[15]
			miR-483-5p, -656, -323-3p			[16]
	Uterine LMS	let-7		Cell proliferation	*HMGA2*	[18]
		miR-17-92 cluster		Smooth muscle differentiation	N/D	[17]
		miR-221		N/D		[19]
UPS		miR-126, -223, -451, -1274b				[15]
			miR-100, -886-3p, -1260, -1274a, -1274b		*IMP3*	[15]
Liposarcoma	DDLS	miR-21, -26a			N/D	[23]
		miR-26a-2		Clonogenicity, adipocyte differentiation, cell apoptosis	*RCBTB1*	[28]
		miR-155		Cell proliferation, colony formation, tumor growth	*CK1α*	[24]
		miR-218-1*		N/D	N/D	[16]
			miR-143, -145	Cell proliferation, apoptosis	*BCL2, Topoisomerase 2A, PRC1, PLK1*	[23]
			miR-144, -1238	N/D	N/D	[16]
			miR-193b	N/D		[25]
			miR-1257	N/D	*CALR*	[26]
	MLS	miR-9, -891a, -888			N/D	[16]
		miR-26a-2		Clonogenicity, adipocyte differentiation, cell apoptosis	*RCBTB1*	[28]
			miR-486	Cell proliferation	*PAI-1*	[27]
			miR-486-3p, -1290	N/D	N/D	[16]
	PLS	miR-296-5p, -455-5p, -1249				[16]
			miR-200b*, -200, -139-3p			[16]

LMS leiomyosarcoma, *UPS* undifferentiated pleomorphic sarcoma, *DDLS* dedifferentiated liposarcoma, *MLS* myxoid liposarcoma, *PLS* pleomorphic liposarcoma, *N/D* no data

Table 10.2 Aberrant miRNAs associated with rhabdomyosarcoma, synovial sarcoma, MPNST, angiosarcoma, fibrosarcoma, and epithelioid sarcoma

Histology	Subtypes	Dysregulated miRNAs		Function	miRNA Target	Reference
		Upregulated	Downregulated			
Rhabdomyo-sarcoma	(Not specified)	miR-9*		Cell migration	*E-cadherin*	[44]
		miR-183		Cell migration, cell invasion	*EGR1*, *PTEN*	[43, 57]
		miR-485-3p (N/D)		Drug resistance	*NF-YB*	[46]
			miR-1, -133a/b	Myogenic differentiation, cell proliferation	*SRF*, *Cyclin D2*	[34]
			miR-26a	N/D	*Ezh2*	[40]
			miR-29	Cell-cycle arrest, muscle differentiation, tumor growth	*YY1*	[42] [37]
			miR-200c	Cell migration	N/D	[44]
			miR-203	Myogenic differentiation, cell proliferation, cell migration, tumor growth	*p63*, *LIFR*	[41]
			miR-206	Myogenic differentiation, cell growth, cell migration, tumor growth, correlation with prognosis	*c-Met*, *PAX3*, *PAX7*, *CCDN2*, *HDAC4*	[35] [36] [37] [39]
	alveolar RMS	miR-335		N/D	*CHFR*, *HAND1*, *SP1*	[11]
		miR-17-92 cluster		Correlation with prognosis in 13q31 amplified ARMS	N/D	[45]

(continued)

Table 10.2 (continued)

Histology	Subtypes	Dysregulated miRNAs		Function	miRNA Target	Reference
		Upregulated	Downregulated			
Synovial sarcoma		let-7e, miR-99b, miR-125a-3p		Cell proliferation	*HMGA2*, *SMARCA5*	[49]
		miR-183		Cell migration, cell invasion	*EGR1*	[43, 57]
		miR-183, 200b*, -375		N/D	N/D	[16]
		miR-17-5p		Cell proliferation, colony formation, tumor growth	*CDKN1A*	[50]
			miR-34b*, -142-5p, -34c-3p	N/D	N/D	[16]
			miR-143	N/D	*SSX1*	[11]
MPNST		miR-10b		Cell proliferation, migration, and invasion	*NF1*	[55]
		miR-21		Apoptosis	*PDCD4*	[52]
		miR-204		Cell proliferation, migration, and invasion	*HMGA2*	[54]
		miR-210, -339-5p		N/D	N/D	[53]
			miR-29c	Cell invasion	*MMP2*	[53]
			miR-30d	Apoptosis	*KPNB1*	[56]
			miR-34a	Apoptosis	*MYCN*, *E2F2*, *CDK4*	[51]
Angiosarcoma	(Not specified)	miR-520c-3p, -519a, -520h		Not analyzed	N/D	[43, 57]
	MYC-amplified AS	miR-17-92 cluster		Not analyzed	*THBS1*	[58]
Fibrosarcoma		miR-520c, 373		Cell growth, cell migration	*mTOR*, *SIRT1*	[60]
		miR-409-3p		Cell proliferation, tumor growth, vascularization, metastasis	*ANG*	[61]
Epithelioid sarcoma		miR-206, -381, -671-5p		N/D	*SMARCB1*	[69]

RMS rhabdomyosarcoma, *MPNST* malignant peripheral nerve sheath tumor, *AS* angiosarcoma, *N/D* no data

and myoblast proliferation, are significantly upregulated in LMS relative to normal smooth muscle. miR-206, highly expressed in normal skeletal muscle, was downregulated in both LMS and normal smooth muscle [11].

Two recent reports have demonstrated miRNA dysregulation in LMS compared with the other soft tissue sarcomas. Guled et al. [15] profiled 10 high-grade LMS and 10 high-grade UPS samples with miRNA microarrays and identified upregulated miR-320a in LMS relative to UPS. Similarly, Renner et al. [16] reported that miR-133a, -1, and -449a were upregulated, while miR-483-5p, -656, and -323-3p were downregulated, when LMS was compared with the other sarcoma subtypes. These results partially agreed with those of Subramanian et al. [11].

Several researchers have investigated miRNA profiling of uterine LMS. Danielson et al. [17] demonstrated that the miR-17-92 cluster was upregulated in uterine LMS compared with myometrium. Shi et al. [18] focused on the downregulation of HMGA2 in uterine LMS and found that it was caused by let-7 repression. Nuovo et al. [19] performed in situ hybridization and found that miR-221 was upregulated in uterine LMS but was not detected in leiomyomas or benign metastasizing leiomyomas.

Undifferentiated Pleomorphic Sarcoma

MFHs were first described in 1963 and became widely accepted as a specific soft tissue sarcoma type in the 1970s. The term “MFH” implies that the tumor cells are of fibroblastic and histiocytic origin. However, the precise origin of MFH cells has been disputed, and the concept of fibrohistiocytic differentiation has been changed. In 2002, WHO declassified MFH as a formal diagnosed entity and renamed it an UPS not otherwise specified [20]. In 2013, UPS/MFH was classified as undifferentiated/unclassified sarcomas [21]. Undifferentiated/unclassified sarcomas account for up to 20 % of all sarcomas and have no clinical or morphological characteristics that would otherwise place them under specific types of sarcomas.

Since it remains a challenge to differentiate between LMS and UPS, Guled and colleagues conducted miRNA profiling on a series of both samples to identify specific signatures useful for differential diagnoses. Profiling of 10 LMS and 10 UPS samples, using two cultured human mesenchymal stem cell samples as controls, revealed that 38 and 46 human miRNAs were determined to be significantly differentially expressed in UPS and LMS, respectively, compared with control samples [15]. In UPS samples, miR-126, -223, -451, and -1274b were significantly upregulated, and miR-100, -886-3p, -1260, -1274a, and -1274b were significantly downregulated compared with controls [15]. In the comparison of the profiles of LMS and UPS, miR-199-5p was highly expressed in UPS, while miR-320a was highly expressed in LMS [15]. In addition, several genes, including *IMP3, ROR2, MDM2, CDK4,* and *UPA,* were revealed to be the targets of differentially expressed miR-

NAs, and their expression was verified by immunohistochemistry in both sarcomas.

Liposarcoma

Liposarcomas are subdivided into the following four major types: atypical lipomatous tumor/well-differentiated liposarcomas (WDLSs), myxoid liposarcomas (MLSs), pleomorphic liposarcomas (PLSs), and dedifferentiated liposarcomas (DDLSs). The definition of DDLS is described as a WDLS that shows an abrupt transition to a nonlipogenic sarcoma. MLS is relatively sensitive to chemotherapy and radiotherapy in comparison with the other types [22]. The prognosis of WDLS is good, while that of DDLS is much worse with a survival rate of approximately 28–30 % at the 5-year follow-up [13].

Among the various histological subtypes of liposarcomas, DDLS has been mostly analyzed. Upregulated miRNAs include miR-21, -26a, -155, and -218-1*, while downregulated miRNAs include miR-143, -144, -145, -193b, -1238, and -1257. Ugras et al., who performed deep sequencing of small RNA libraries and hybridization-based microarrays, was the first to report miRNA dysregulation. More than 40 miRNAs were dysregulated in DDLS (not in normal adipose tissues) and WDLS, which included upregulated miR-21 and -26, and downregulated miR-143, and -145 [23]. miR-143 re-expression in DDLS cell lines inhibited cell proliferation and induced apoptosis through downregulation of BCL2, topoisomerase 2A, protein regulator of cytokinesis 1, and polo-like kinase 1 [23]. A similar approach was adopted by Zhang et al., who determined that miR-155 was upregulated in DDLS, which had not been identified by Ugras et al. [24]. miR-155 silencing in DDLS cells inhibited cell growth and colony formation, induced G1-S cell-cycle arrest in vitro and blocked tumor growth in vivo. One of the miR-155 direct targets was *casein kinase 1α*, which enhanced β-catenin signaling [24]. Renner et al. [16] identified miR-218-1* as an upregulated miRNA and miR-144 and -1238 as downregulated miRNAs relative to that in normal adipose tissues. Taylor et al. performed unbiased genome-wide methylation sequencing and identified that miR-193b was downregulated in DDLS relative to normal adipose tissues through the methylation of miR-193b promoters [25]. Hisaoka et al. [26] identified a decreased expression of miR-1257, which targets calreticulin, an inhibitor of adipocyte differentiation.

MLS, the second most common type of liposarcoma representing 30–40 % of all liposarcomas, has a unique genomic abnormality characterized by t(12;16)(q13;p11) translocation, which creates the TLS-CHOP chimeric oncoprotein. The investigation of the molecular functions of *TLS-CHOP* by Borijigin et al. [27] revealed that miR-486 was downregulated in both TLS-CHOP-expressing fibroblasts and MLS. The *plasminogen activator inhibitor-1* (*PAI-1*) was identified as a target of miR-486; therefore, TLS-CHOP–miR-486–PAI-1 could be critical for MLS tumorigenesis and development [27]. Renner et al. investigated miRNA profiling of MLS relative to normal adipose tissue and determined that miR-9, -891a, and -888 were

upregulated, and miR-486-3p and -1290 were downregulated. Notably, these results were partially consistent with the report by Borijigin et al. [16], who also reported that miR-296-5p, -455-5p, and -1249 were upregulated, and miR-139-3p, -200, and -200b* were downregulated.

A clinical correlation of miRNA dysregulation of liposarcomas has been recently demonstrated. A single SNP array of 75 liposarcoma samples by Lee et al. [28] identified frequent amplification of miR-26a-2c, which was upregulated in WDLS/DDLS and MLS. Furthermore, high miR-26a-2 expression significantly correlated with poor patient survival, regardless of histological subtypes. The regulator of chromosome condensation and BTB domain-containing protein 1 was revealed to be one of the targets of miR-26a-2, which regulates cellular apoptosis [28].

Rhabdomyosarcoma

RMSs are the most common soft tissue sarcomas in children under 15 years of age, representing 5–8 % of all pediatric malignancies [29]. RMS is histopathologically classified into the following four subtypes: embryonal RMS (eRMS), alveolar RMS (aRMS), pleomorphic RMS (pRMS), and spindle cell/sclerosing RMS. Multiagent chemotherapy is performed for most patients with RMS, and most will also undergo either radiotherapy or surgery depending on the size and location of the primary tumor. Adult patients with RMS who showed complete response to chemotherapy have a 5-year survival rate of 57 % compared with only 7 % for poor responders [30].

The muscle-specific miRNAs, involved in skeletal muscle development, have been focused on in most studies, since RMS has been predicted to originate from mesenchymal progenitor cells located in muscle tissue [31–33]. Subramanian et al. [11] performed global miRNA expression analyses and demonstrated that muscle-specific miRNAs (miR-1 and -133) were relatively downregulated in pRMS relative to normal skeletal muscle, while miR-335 was upregulated in aRMS relative to normal skeletal muscle. miR-335 resides in intron 2 of *MEST*, which has been indicated to play a role in muscle differentiation. Importantly, *MEST* is a downstream target of PAX3, the gene involved in the *PAX3–FKHR* fusion that is typical for aRMS. Rao et al. [34] also reported that miR-1 and -133a were drastically reduced in both eRMS and aRMS cell lines. Taulli et al. and Yan et al. [35, 36] examined the role of the muscle-specific miR-1 and -206 in RMS and showed that their re-expression in RMS cells targeted *c-Met* mRNA to promote myogenic differentiation, decreased cell growth and migration, and inhibited tumor growth in xenografted mice. In addition, Li et al. [37] showed that miR-1, -206, and also miR-29 could regulate *PAX3* and *CCND2* expression. Taulli et al. recently further investigate a miR-206 target, BAF53a, a subunit of the SWI/SNF chromatin remodeling complex, which is important for chromatin remodeling in the transcriptional changes occurring during myogenic differentiation. BAF53a silencing in RMS cells inhibited cell proliferation and anchorage-independent growth in vitro, inhibited eRMS

and aRMS tumor growth, and induced myogenic differentiation in vivo [38]. The clinical relevance of these muscle-specific miRNAs was demonstrated by Missiaglia et al. [39], who analyzed miR-1, -206, -133a, and -133b expression in 163 primary RMS samples.

A clinical correlation between overall survival and miR-206 expression was identified, whereas no correlation was observed with miR-1 or -133a/b. In particular, low miR-206 expression correlated with poor overall survival in metastatic eRMS and aRMS cases without *PAX3/7–FOXO1* fusion genes [39]. Among the muscle-specific miRNAs, Ciarapica et al. [40] found that miR-26a was also downregulated in RMS cells via regulation of the expression of *Ezh2*. miR-203 was found to be downregulated in RMS by Diao et al. [41], which was due to promoter hypermethylation and could be re-expressed after DNA demethylation. Re-expression of miR-203 suppressed tumor growth by directly targeting *p63* and *LIFR* and inhibited the Notch and JAK1/STAT1/STAT3 pathways [41].

Non-muscle-specific miRNAs have also been reported as key molecules of RMS. miR-29 was downregulated in RMS and acted as a tumor suppressor [11, 37, 42]. Wang et al. reported that NF-κB and YY1 downregulation caused derepression of miR-29 during myogenesis, whereas in RMS, miR-29 was epigenetically silenced by an activated NF-κB-YY1 pathway. Sarver et al. [43] reported that *EGR1* is regulated by miR-183 in multiple tumor types in addition to RMS. miR-183 silencing in RMS cells revealed deregulation of an miRNA network composed of miR-183–EGR1–PTEN [43]. Armeanu-Ebinger et al. [44] demonstrated that miR-9* was overexpressed in aRMS, whereas miR-200c was expressed at lower levels in aRMS but not in malignant rhabdoid tumors (MRTs). Another important study on ARMS was reported by Reichek et al. [45], who investigated the 13q31 amplicon that contains the miR-17-92 cluster gene. The 13q31 amplicon was present in 23 % of ARMS cases, particularly in *PAX7–FKHR*-positive cases compared with *PAX3–FKHR*-positive and fusion-negative cases. Importantly, high expression of the miR-17-91 cluster significantly correlated with poor prognosis in the 13q31-amplified group of patients, most of whom represented *PAX7–FKHR*-positive cases [45].

miRNA associated with drug-resistant RMS includes miR-485-3p, expressed at lower levels in drug-resistant lymphoblastic leukemia cells than in parental cells [46]. miR-485-3p targets NF-YB, which may be a mediator of topoisomerase 2α. Chen et al. [46] replicated these results in drug-sensitive and -resistant RMS cells and found that the miR-485-3p-Top2α-NF-YB pathway represented a general phenomenon associated with drug sensitivity.

Synovial Sarcoma

Synovial sarcomas account for up to 5 % of soft tissue sarcomas and can occur anywhere in the body. They feature local invasiveness and a propensity to metastasize [47]. More than half of patients with these sarcomas are teenagers and young adults. The two major histological subtypes are biphasic and monophasic [48]. This

tumor has a specific chromosomal translocation t(X;18)(p11;q11) that leads to formation of an *SS18–SSX* fusion gene. Although treatment is mainly based on the surgical resection, adjuvant radiotherapy or chemotherapy may be beneficial, particularly in high-risk patients. The reported 5-year overall survival is 55 % for axial synovial sarcoma and 84 % for synovial sarcomas of the extremities [47].

The first miRNA profiling of synovial sarcomas was performed by Subramanian and colleagues in 2008. They utilized microarrays, cloning, and northern blot analyses and demonstrated that miR-143 was downregulated in synovial sarcomas relative to the other sarcomas, gastrointestinal stromal tumors (GIST), and LMS [11]. Since *SSX1* is predicted to be a target for miR-143 in computer-simulated (*in silico*) databases, it is speculated that decreased miR-143 expression could enable the production of the SS18–SSX1 oncoprotein. Sarver et al. focused on the molecular features of synovial sarcomas in which the SS18–SSX oncoprotein repressed *EGR1* expression through a direct association with the *EGR1* promoter. They identified that miR-183 is significantly overexpressed in synovial sarcomas and investigated the correlation between *EGR1* and miR-183 [43]. In their analyses, miR-183 could target *EGR1* mRNA, which contributed to cell migration and invasion in synovial sarcoma cells and was found to have an oncogenic role through the miR-183-EGR1-PTEN pathway in synovial sarcomas, RMS, and colon cancers [43]. Notably, Renner et al. also indicated that miR-183 is upregulated in synovial sarcomas relative to other sarcomas. Additional upregulated miRNAs were miR-200b* and -375, while the downregulated miRNAs included miR-34b*, -142-5p, and -34c-3p [16].

The global miRNA expression in synovial sarcomas compared with Ewing sarcomas and normal skeletal muscle was examined by Hisaoka et al. [49], which revealed 21 significantly upregulated miRNAs, including let-7e, miR-99b, and -125-3p. Functional analyses showed that the silencing of let-7e and miR-99b in synovial sarcoma cell lines resulted in the suppression of cell proliferation. The expression of *HMGA2* and *SMARCA5*, the putative targets of these miRNAs, was modulated by these miRNAs [49]. A recent investigation, based on functional analyses using the OncomiR mina Precursor Virus Library, was reported by Minami et al. Consequently, miR-17-5p was identified from the large colonies of Fuji cells that were transfected by the virus library. Overexpression of miR-17-5 into the other cell lines resulted in increased cell growth and colony formation and increased tumor size in mice [50]. *CDKN1A (p21)* was identified as a miR-17 target, evoked by doxorubicin treatment, suggesting that miR-17 rescued doxorubicin-induced cell growth suppression [50].

Malignant Peripheral Nerve Sheath Tumor

MPNSTs are malignant nerve sheath tumors arising from peripheral nerves, from a preexisting benign nerve sheath tumor (usually neurofibroma), or in a patient with neurofibromatosis type 1 (NF1). Approximately 50 % of MPNSTs occur spontaneously, with the remaining originating in patients with NF1 [13]. Patients with NF1

have higher risk of developing MPNSTs, which are aggressive tumors with poor prognoses for patients.

Most reports have performed the global miRNA profiling of MPNSTs in comparison with benign counterparts (neurofibromas). Using these methods, Subramanian et al. found a relative downregulation of miR-34a expression in most MPNSTs. They also determined the gene expression signatures for benign tumors and MPNSTs, which indicated that *p53* inactivation occurred in the majority of MPNSTs [51]. Consequently, they concluded that *p53* inactivation and the subsequent loss of miR-34a expression may significantly contribute to MPNST development [51]. Our group also utilized a similar approach and identified the overexpression of miR-21 in MPNSTs compared with neurofibromas. *In silico* research predicted *programmed cell death protein 4* as a putative target of miR-21 [52]. Silencing of miR-21 in an MPNST cell line could induce apoptosis of MPNST cells [52]. Presneau et al. [53] also compared miRNA profiling of MPNSTs with NFs and identified two upregulated (miR-210 and -339-5p) and 14 downregulated miRNAs (miR-29c, -30c, -139-5p, 195, -151-5p, 342-5p, 146a, -150, and -223). Among the downregulated miRNAs, enforced expression of miR-29c reduced cell invasion of MPNST cells, thus regulating the expression of *MMP2* [53]. Using a similar approach, Gong et al. [54] identified downregulated miR-204 expression in MPNSTs and its targets, *Ras* and *HMGA2*. A different approach was used by Chai et al. [55] miR-10b was found to be upregulated in primary Schwann cells isolated from NF1 neurofibromas as well as in the cell lines and tumor tissues from MPNSTs. Importantly, *NF1* mRNA appears to be the target for miR-10b [55]. Zhang et al. [56] focused on the expression of the polycomb group protein enhancer of zeste homolog 2 (Ezh2), an important regulator for various human malignancies, and identified significant overexpression in MPNSTs. Ezh2 inhibited miR-30d expression by binding to its promoter, and *KPNB1* was a miR-30d target, which was identified in an *in silico* database. Therefore, EZH2-miR-30d-KPNB1 signaling could be critical for MPNST survival and tumorigenicity [56].

Angiosarcoma

Angiosarcomas are malignant tumors that retain many of the morphological and functional characteristics of normal endothelium [13]. They comprise less than 1 % of all sarcomas and usually originate in the deep muscles of the lower extremities [1]. A high rate of tumor-related deaths was reported, and more than half of patients die within the first year [13].

In the Sarcoma miRNA Expression Database generated by Sarver et al. (http://www.oncomir.umn.edu/) [57], miRNAs that are significantly dysregulated in angiosarcomas compared with other sarcomas (>80-fold change) included miR-520c-3p, -519a, and -520h. Italiano et al. investigated miRNA profiling based on *MYC* abnormalities in angiosarcomas. These abnormalities were identified in 3 of 6 primary and 8 of 12 secondary angiosarcomas by array-comparative genomic hybridization

and fluorescence in situ hybridization analyses. By comparing the miRNA profile of *MYC*-amplified and *MYC*-unamplified angiosarcomas using deep sequencing of small RNA libraries, the miR-17-92 cluster was identified to be preferentially overexpressed in *MYC*-amplified angiosarcomas. *MYC*-amplified angiosarcomas were associated with lower expression of thrombospondin-1 (THBS1), the first endogenous inhibitor of angiogenesis. *MYC* amplification may be important in the angiogenic phenotype of angiosarcomas through upregulation of the miR-17-92 cluster, which appears to downregulate *THBS1* expression [58].

Fibrosarcoma

Fibrosarcomas of soft tissue are classified into infantile and adult fibrosarcomas. The infantile fibrosarcoma has a distinctive *ETV6–NTRK3* gene fusion and favorable outcome, although histologically it is similar to classic adult fibrosarcoma. Most of adult fibrosarcoma cases (>80 %) were reported to be high-grade in the recent series of strictly defined cases [59].

To date, miRNA profiling has not been investigated for fibrosarcoma tissue specimens but has been limited to the fibrosarcoma cell line, HT1080. Liu and Wilson [60] investigated the correlation between matrix metalloproteinases (MMPs) and miR-520c and -373 using HT1080, which have been reported to play important roles in cancer cell metastasis as oncomiRNAs. Their data showed that miR-520c and -373 suppressed the translation of *mTOR* and *SIRT1* by directly targeting the 3′-untranslated region (UTR). Since mTOR and SIRT1 are negative regulators of MMP9 via inactivation of the Ras/Raf/MEK/Erk signaling pathway, these miRNAs increased MMP9 expression by directly targeting *mTOR* and *SIRT1*, which stimulated cell growth and migration [60]. Another investigation using HT1080 cells was performed by Weng et al., who focused on the regulatory mechanism of angiogenin (ANG) expression. In their *in silico* analysis, they found that *ANG* mRNA was targeted by miR-409-3p via its 3′UTR, and overexpression of miR-409-3p in HT1080 cells silenced *ANG* expression [61]. Their in vitro analyses demonstrated that ectopic expression of miR-409-3p inhibited tumor growth, vascularization, and metastasis through downregulation of *ANG* expression [61].

Epithelioid Sarcoma

Epithelioid sarcomas represent about 1 % of all sarcomas and are most prevalent in adolescents and young adults between 10 and 35 years [62, 63]. These are the most common soft tissue sarcomas in the hands and wrists, followed by aRMS and synovial sarcomas [1]. There are two clinicopathological subtypes: (1) the conventional or classic (distal) form, characterized by its proclivity for acral sites and pseudo granulomatous growth patterns and (2) the proximal-type (large-cell) variant, which

Table 10.3 Circulating miRNAs in the serum of patients with soft tissue sarcomas

Histology	Promising circulating miRNAs	Study design	Samples	Procedure	No. of miRNAs examined	Normalization	Reference
Rhabdomyosarcoma	miR-206	RMS vs. non-RMS vs. healthy volunteer	Serum	qRT-PCR	4	miR-16	[76]
MPNST	miR-24, 801, 214	Sporadic MPNST vs. NF1 MPNST vs. NF1	Serum	Solexa sequencing, qRT-PCR	Genome-wide profiling	cell miR-39	[77]

RMS rhabdomyosarcoma, *MPNST* malignant peripheral nerve sheath tumor, *NF1* neurofibromatosis 1

originates mainly in proximal/truncal regions and consists of nests and sheets of large epithelioid cells. The reported 5- and 10-year overall survival rates are 60–80 % and 42–62 %, respectively [64–66]. The prognoses for patients with the proximal-type are worse than that for patients with the classic form [66–68].

Proximal-type epithelioid sarcomas have genetic similarities with MRTs, including the lack of nuclear immunoreactivity of *SMARCB1* (also known as *INI1*, *BAF47*, or *hSNF5*). Papp et al. investigated the miRNAs that regulate *SMARCB1* expression and analyzed eight candidate miRNAs selected by *in silico* analysis. Quantitative PCR using epithelioid sarcomas and MRT specimens identified the overexpression of miR-206, -381, -671-5p, and -765 in epithelioid sarcomas [69]. Among them, three upregulated miRNAs (miR-206, miR-381, and miR-671-5p) could silence *SMARCB1* mRNA expression by transfection into cell cultures, and the most effective miRNA was miR-206. Therefore, the loss of *SMARCB1* expression in epithelioid sarcomas might be due to an epigenetic mechanism of gene silencing by these miRNAs [69].

Circulating miRNA AS a Possible Novel Biomarker of Soft Tissue Sarcomas

Tumor cells have been recently shown to secrete miRNAs into the circulation [70]. Analysis of circulating miRNA levels in serum or plasma presents a novel approach for diagnostic cancer screening or monitoring. Lawrie et al. [71] were the first to report that tumor-associated miRNA levels in the patient sera were higher than those in healthy individuals, indicating that circulating miRNAs can be used as biomarkers to monitor cancer cells. This group also demonstrated that high miR-21 expression was associated with poor prognoses for patients with large B-cell lymphomas [71]. Expression of other circulating miRNAs in blood has been widely reported and, to date, differential expression of circulating miRNA has been reported in cancers of the breast, lung, stomach, liver, kidney, bladder, prostate, and ovaries, among others [70, 72–74]. To date, the studies of circulating miRNAs of soft tissue sarcomas have been limited to those of RMS and MPNST (Table 10.3) [75].

Rhabdomyosarcoma

In 2010, Miyachi et al. [76] performed the first clinical trial of circulating miRNAs as novel biomarkers in soft tissue sarcomas using serum samples derived from patients with RMS. They focused on muscle-specific miRNAs (miR-1, -133a, -133b, and -206) that were highly expressed in RMS. Expression levels of miR-206, one of the muscle-specific miRNAs, were abundantly expressed in RMS cell lines compared with those in neuroblastoma, Ewing sarcoma, and MRT cell lines. Notably, these results were dependent on the culture medium used for these cell

lines. In addition, muscle-specific miRNAs (miR-1, -133a, -133b, and -206) were significantly upregulated in RMS tumor specimens, and serum levels of these miRNAs were also significantly higher in patients with RMS compared with those without RMS. Among these muscle-specific miRNAs, serum miR-206 showed the highest sensitivity and specificity [76].

These results in the RMS patient serum was consistent with results from previous studies using RMS tissues [35, 36, 39], indicating that miRNA deregulation in patient tissue specimens could reflect those in patients' sera.

Malignant Peripheral Nerve Sheath Tumor

Weng et al. have shown the possibility of miRNAs representing noninvasive biomarkers for the diagnosis of MPNST. Genome-wide serum miRNA expression analysis was performed to distinguish MPNST patients with and without NF1. Patients with NF1 have a high lifetime risk of developing MPNST. Solexa sequencing was applied to screen for differentially expressed miRNAs in the serum from 10 patients with NF1, 10 patients with spontaneous MPNST, and 10 patients with NF1 MPNST. Based on the large studies on more patient sets, miR-214 and -801 showed higher expression in patients with MPNST (both sporadic and NF1 cases) than NF1 patients [77]. In addition, miR-24 was significantly upregulated only in MPNST patients with NF1. Thus, the combination of the three miRNAs (miR-24, -214, and -801) could differentiate patients with sporadic MPNST from those with NF1 MPNST [77].

Conclusions

To date, there are few useful biomarkers to diagnose and monitor soft tissue sarcomas. In the past three decades, genetic investigations, such as identification of fusion genes, have greatly improved the diagnoses for soft tissue sarcomas. However, these findings are applicable to the limited histological subtypes including synovial sarcoma, MLS, aRMS, or clear cell sarcoma. Therefore, the identification of miRNAs specific to histological subtypes may be a novel breakthrough for sarcoma research.

Recent reports have indicated various types of miRNA dysregulation in soft tissue sarcoma tissues as shown in Tables 10.1 and 10.2. However, these investigations could be clinically applicable only after tissue specimens are resected by invasive procedures, and such investigations have less diagnostic impact compared with currently used fusion gene analysis. A significant step forward would be achieved by development of noninvasive "liquid biopsy." Since these trials have been limited to a few histological subtypes (Table 10.3), further investigations to cover a variety of subtypes are expected. In addition, identification of circulating miRNAs serving as biomarkers that reflect drug resistance would help clinicians to determine optimal

individual treatment options, thus leading to the improvement of the prognoses of the patients.

Recent studies also suggested the possibility of miRNA therapeutics in sarcomas. For example, supplementary administration of miR-143 mimics or miR-133a inhibitors into osteosarcoma-bearing mice has been shown to inhibit lung metastases [78, 79]. However, no preclinical evaluation has been performed against soft tissue sarcomas, despite various information about dysregulated miRNAs in tumor tissues. Some researchers have done in vivo trials for soft tissue sarcomas, most of which utilize viral transduction into cells before xenografting into mice, while few trials have utilized systemic administration of oligonucleotides. Since the high number of mRNAs could be targeted by a single miRNA, miRNA therapeutics might represent a therapeutic advantage compared with specific gene silencing by siRNAs. Identification of the miRNAs that are critical and specific to each sarcoma subtype would be an important step to the clinical application of miRNA therapeutics.

While some issues remain unresolved regarding the methods of evaluation of the expression levels of circulating miRNAs or tissue miRNAs, novel trials of miRNA-based diagnoses and therapeutics will be an important step for patients with soft tissue sarcomas.

Acknowledgments The authors acknowledge a grant-in-aid for Management Express Grants from Okayama University Hospital and Japan Orthopaedics and Traumatology Research Foundation, Inc. No. 311.

References

1. Weiss SW, Goldblum JR, Enzinger FM. Enzinger and Weiss's soft tissue tumors. 5th ed. Philadelphia: Mosby Elsevier; 2008.
2. Misra A, Mistry N, Grimer R, Peart F. The management of soft tissue sarcoma. J Plast Reconstr Aesthet Surg. 2009;62(2):161–74.
3. Toro JR, Travis LB, Wu HJ, Zhu K, Fletcher CD, Devesa SS. Incidence patterns of soft tissue sarcomas, regardless of primary site, in the surveillance, epidemiology and end results program, 1978–2001: an analysis of 26,758 cases. Int J Cancer. 2006;119(12):2922–30.
4. Fujiwara T, Kawai A, Yoshida A, Ozaki T, Ochiya T. Cancer stem cells of sarcoma. New Hampshire: CRC Press; 2013;23–72.
5. Tsuchiya H. Effect of timing of pulmonary metastases identification on prognosis of patients with osteosarcoma: the Japanese musculoskeletal oncology group study. J Clin Oncol. 2002;20(16):3470–7.
6. Calin GA, Liu C-G, Sevignani C, Ferracin M, Felli N, Dumitru CD, Shimizu M, Cimmino A, Zupo S, Dono M. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci U S A. 2004;101(32):11755–60.
7. Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet. 2009;10(10):704–14.
8. Lamy P, Andersen C, Dyrskjøt L, Tørring N, Ørntoft T, Wiuf C. Are microRNAs located in genomic regions associated with cancer? Br J Cancer. 2006;95(10):1415–8.
9. Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med. 2005;353(17):1793–801.

10. Catto JW, Alcaraz A, Bjartell AS, De Vere WR, Evans CP, Fussel S, Hamdy FC, Kallioniemi O, Mengual L, Schlomm T, Visakorpi T. MicroRNA in prostate, bladder, and kidney cancer: a systematic review. Eur Urol. 2011;59(5):671–81.
11. Subramanian S, Lui WO, Lee CH, Espinosa I, Nielsen TO, Heinrich MC, Corless CL, Fire AZ, van de Rijn M. MicroRNA expression signature of human sarcomas. Oncogene. 2008;27(14):2015–26.
12. Fujiwara T, Kunisada T, Takeda K, Uotani K, Yoshida A, Ochiya T, Ozaki T. MicroRNAs in soft tissue sarcomas: overview of the accumulating evidence and importance as novel biomarkers. BioMed Res Int. 2014;2014:15.
13. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F. WHO classification of tumours of soft tissue and bone. 4th ed. Lyon: IARC Press; 2013.
14. Farshid G, Pradhan M, Goldblum J, Weiss SW. Leiomyosarcoma of somatic soft tissues: a tumor of vascular origin with multivariate analysis of outcome in 42 cases. Am J Surg Pathol. 2002;26(1):14–24.
15. Guled M, Pazzaglia L, Borze I, Mosakhani N, Novello C, Benassi MS, Knuutila S. Differentiating soft tissue leiomyosarcoma and undifferentiated pleomorphic sarcoma: a miRNA analysis. Genes Chromosomes Cancer. 2014;53(8):693–702.
16. Renner M, Czwan E, Hartmann W, Penzel R, Brors B, Eils R, Wardelmann E, Büttner R, Lichter P, Schirmacher P. MicroRNA profiling of primary high-grade soft tissue sarcomas. Genes Chromosomes Cancer. 2012;51(11):982–96.
17. Danielson LS, Menendez S, Attolini CS-O, Guijarro MV, Bisogna M, Wei J, Socci ND, Levine DA, Michor F, Hernando E. A differentiation-based microRNA signature identifies leiomyosarcoma as a mesenchymal stem cell-related malignancy. Am J Pathol. 2010;177(2):908–17.
18. Shi G, Perle MA, Mittal K, Chen H, Zou X, Narita M, Hernando E, Lee P, Wei JJ. Let-7 repression leads to HMGA2 overexpression in uterine leiomyosarcoma. J Cell Mol Med. 2009;13(9b):3898–905.
19. Nuovo GJ, Schmittgen TD. Benign metastasizing leiomyoma of the lung: clinicopathologic, immunohistochemical, and micro-RNA analyses. Diagn Mol Pathol. 2008;17(3):145.
20. Fletcher CD. The evolving classification of soft tissue tumours: an update based on the new WHO classification. Histopathology. 2006;48(1):3–12.
21. Fletcher CD. The evolving classification of soft tissue tumours—an update based on the new 2013 WHO classification. Histopathology. 2014;64(1):2–11.
22. Jones RL, Fisher C, Al-Muderis O, Judson IR. Differential sensitivity of liposarcoma subtypes to chemotherapy. Eur J Cancer. 2005;41(18):2853–60.
23. Ugras S, Brill E, Jacobsen A, Hafner M, Socci ND, Decarolis PL, Khanin R, O'Connor R, Mihailovic A, Taylor BS, Sheridan R, Gimble JM, Viale A, Crago A, Antonescu CR, Sander C, Tuschl T, Singer S. Small RNA sequencing and functional characterization reveals MicroRNA-143 tumor suppressor activity in liposarcoma. Cancer Res. 2011;71(17):5659–69.
24. Zhang P, Bill K, Liu J, Young E, Peng T, Bolshakov S, Hoffman A, Song Y, Demicco EG, Terrada DL. MiR-155 is a liposarcoma oncogene that targets casein kinase-1α and enhances β-catenin signaling. Cancer Res. 2012;72(7):1751–62.
25. Taylor BS, DeCarolis PL, Angeles CV, Brenet F, Schultz N, Antonescu CR, Scandura JM, Sander C, Viale AJ, Socci ND. Frequent alterations and epigenetic silencing of differentiation pathway genes in structurally rearranged liposarcomas. Cancer Dis. 2011;1(7):587–97.
26. Hisaoka M, Matsuyama A, Nakamoto M. Aberrant calreticulin expression is involved in the dedifferentiation of dedifferentiated liposarcoma. Am J Pathol. 2012;180(5):2076–83.
27. Borjigin N, Ohno S, Wu W, Tanaka M, Suzuki R, Fujita K, Takanashi M, Oikawa K, Goto T, Motoi T. TLS-CHOP represses miR-486 expression, inducing upregulation of a metastasis regulator PAI-1 in human myxoid liposarcoma. Biochem Biophys Res Commun. 2012;427(2):355–60.
28. Lee DH, Amanat S, Goff C, Weiss LM, Said JW, Doan NB, Sato-Otsubo A, Ogawa S, Forscher C, Koeffler HP. Overexpression of miR-26a-2 in human liposarcoma is correlated with poor patient survival. Oncogenesis. 2013;2, e47.
29. De Giovanni C, Landuzzi L, Nicoletti G, Lollini P-L, Nanni P. Molecular and cellular biology of rhabdomyosarcoma. Future Oncol. 2009;5(9):1449–75.

30. Esnaola NF, Rubin BP, Baldini EH, Vasudevan N, Demetri GD, Fletcher CD, Singer S. Response to chemotherapy and predictors of survival in adult rhabdomyosarcoma. Ann Surg. 2001;234(2):215.
31. Cieśla M, Dulak J, Józkowicz A. MicroRNAs and epigenetic mechanisms of rhabdomyosarcoma development. Int J Biochem Cell Biol. 2014;16(2):113–27.
32. Novák J, Vinklárek J, Bienertová Vašků J, Slabý O. MicroRNAs involved in skeletal muscle development and their roles in rhabdomyosarcoma pathogenesis. Pediatr Blood Cancer. 2013;60(11):1739–46.
33. Rota R, Ciarapica R, Giordano A, Miele L, Locatelli F. MicroRNAs in rhabdomyosarcoma: pathogenetic implications and translational potentiality. Mol Cancer. 2011;10:120.
34. Rao PK, Missiaglia E, Shields L, Hyde G, Yuan B, Shepherd CJ, Shipley J, Lodish HF. Distinct roles for miR-1 and miR-133a in the proliferation and differentiation of rhabdomyosarcoma cells. FASEB J. 2010;24(9):3427–37.
35. Taulli R, Bersani F, Foglizzo V, Linari A, Vigna E, Ladanyi M, Tuschl T, Ponzetto C. The muscle-specific microRNA miR-206 blocks human rhabdomyosarcoma growth in xenotransplanted mice by promoting myogenic differentiation. J Clin Invest. 2009;119(8):2366–78.
36. Yan D, Dong Xda E, Chen X, Wang L, Lu C, Wang J, Qu J, Tu L. MicroRNA-1/206 targets c-Met and inhibits Rhabdomyosarcoma development. J Biol Chem. 2009;284(43):29596–604.
37. Li L, Sarver AL, Alamgir S, Subramanian S. Downregulation of microRNAs miR-1, -206 and -29 stabilizes PAX3 and CCND2 expression in rhabdomyosarcoma. Lab Invest. 2012;92(4): 571–83.
38. Taulli R, Foglizzo V, Morena D, Coda D, Ala U, Bersani F, Maestro N, Ponzetto C. Failure to downregulate the BAF53a subunit of the SWI/SNF chromatin remodeling complex contributes to the differentiation block in Rhabdomyosarcoma. Oncogene. 2014;33(18):2354–62.
39. Missiaglia E, Shepherd C, Patel S, Thway K, Pierron G, Pritchard-Jones K, Renard M, Sciot R, Rao P, Oberlin O. MicroRNA-206 expression levels correlate with clinical behaviour of rhabdomyosarcomas. Br J Cancer. 2010;102(12):1769–77.
40. Ciarapica R, Russo G, Verginelli F, Raimondi L, Donfrancesco A, Rota R, Giordano A. Deregulated expression of miR-26a and Ezh2 in Rhabdomyosarcoma. Cell Cycle. 2009;8(1):172–5.
41. Diao Y, Guo X, Jiang L, Wang G, Zhang C, Wan J, Jin Y, Wu Z. miR-203, a tumor suppressor frequently down-regulated by promoter hypermethylation in Rhabdomyosarcoma. J Biol Chem. 2014;289(1):529–39.
42. Wang H, Garzon R, Sun H, Ladner KJ, Singh R, Dahlman J, Cheng A, Hall BM, Qualman SJ, Chandler DS. NF-κB-YY1-miR-29 regulatory circuitry in skeletal myogenesis and rhabdomyosarcoma. Cancer Cell. 2008;14(5):369–81.
43. Sarver AL, Li L, Subramanian S. MicroRNA miR-183 functions as an oncogene by targeting the transcription factor EGR1 and promoting tumor cell migration. Cancer Res. 2010;70(23): 9570–80.
44. Armeanu-Ebinger S, Herrmann D, Bonin M, Leuschner I, Warmann SW, Fuchs J, Seitz G. Differential expression of miRNAs in Rhabdomyosarcoma and malignant rhabdoid tumor. Exp Cell Res. 2012;318(20):2567–77.
45. Reichek JL, Duan F, Smith LM, Gustafson DM, O'Connor RS, Zhang C, Dunlevy MJ, Gastier-Foster JM, Barr FG. Genomic and clinical analysis of amplification of the 13q31 chromosomal region in alveolar Rhabdomyosarcoma: a report from the Children's Oncology Group. Clin Cancer Res. 2011;17(6):1463–73.
46. Chen CF, He X, Arslan AD, Mo YY, Reinhold WC, Pommier Y, Beck WT. Novel regulation of nuclear factor-YB by miR-485-3p affects the expression of DNA topoisomerase IIα and drug responsiveness. Mol Pharmacol. 2011;79(4):735–41.
47. Ferrari A, Bisogno G, Alaggio R, Cecchetto G, Collini P, Rosolen A, Meazza C, Indolfi P, Garaventa A, De Sio L. Synovial sarcoma of children and adolescents: the prognostic role of axial sites. Eur J Cancer. 2008;44(9):1202–9.
48. Kawai A, Healey JH, Boland PJ, Lin PP, Huvos AG, Meyers PA. Prognostic factors for patients with sarcomas of the pelvic bones. Cancer. 1998;82(5):851–9.

49. Hisaoka M, Matsuyama A, Nagao Y, Luan L, Kuroda T, Akiyama H, Kondo S, Hashimoto H. Identification of altered MicroRNA expression patterns in synovial sarcoma. Genes Chromosomes Cancer. 2011;50(3):137–45.
50. Minami Y, Kohsaka S, Tsuda M, Yachi K, Hatori N, Tanino M, Kimura T, Nishihara H, Minami A, Iwasaki N, Tanaka S. SS18-SSX-regulated miR-17 promotes tumor growth of synovial sarcoma by inhibiting p21WAF1/CIP1. Cancer Sci. 2014;105(9):1152–9.
51. Subramanian S, Thayanithy V, West RB, Lee CH, Beck AH, Zhu S, Downs-Kelly E, Montgomery K, Goldblum JR, Hogendoorn PCW. Genome-wide transcriptome analyses reveal p53 inactivation mediated loss of miR 34a expression in malignant peripheral nerve sheath tumours. J Pathol. 2010;220(1):58–70.
52. Itani S, Kunisada T, Morimoto Y, Yoshida A, Sasaki T, Ito S, Ouchida M, Sugihara S, Shimizu K, Ozaki T. MicroRNA-21 correlates with tumorigenesis in malignant peripheral nerve sheath tumor (MPNST) via programmed cell death protein 4 (PDCD4). J Cancer Res Clin Oncol. 2012;1–9.
53. Presneau N, Eskandarpour M, Shemais T, Henderson S, Halai D, Tirabosco R, Flanagan A. MicroRNA profiling of peripheral nerve sheath tumours identifies miR-29c as a tumour suppressor gene involved in tumour progression. Br J Cancer. 2013;108(4):964–72.
54. Gong M, Ma J, Li M, Zhou M, Hock JM, Yu X. MicroRNA-204 critically regulates carcinogenesis in malignant peripheral nerve sheath tumors. Neuro Oncol. 2012;14(8):1007–17.
55. Chai G, Liu N, Ma J, Li H, Oblinger JL, Prahalad AK, Gong M, Chang LS, Wallace M, Muir D. MicroRNA 10b regulates tumorigenesis in neurofibromatosis type 1. Cancer Sci. 2010;101(9):1997–2004.
56. Zhang P, Garnett J, Creighton CJ, Al Sannaa GA, Igram DR, Lazar A, Liu X, Liu C, Pollock RE. EZH2-miR-30d-KPNB1 pathway regulates malignant peripheral nerve sheath tumour cell survival and tumourigenesis. J Pathol. 2014;232(3):308–18.
57. Sarver AL, Phalak R, Thayanithy V, Subramanian S. S-MED: sarcoma microRNA expression database. Lab Invest. 2010;90(5):753–61.
58. Italiano A, Thomas R, Breen M, Zhang L, Crago AM, Singer S, Khanin R, Maki RG, Mihailovic A, Hafner M. The miR-17-92 cluster and its target THBS1 are differentially expressed in angiosarcomas dependent on MYC amplification. Genes Chromosomes Cancer. 2012;51(6):569–78.
59. Bahrami A, Folpe AL. Adult-type fibrosarcoma: a reevaluation of 163 putative cases diagnosed at a single institution over a 48-year period. Am J Surg Pathol. 2010;34(10):1504–13.
60. Liu P, Wilson MJ. miR-520c and miR-373 upregulate MMP9 expression by targeting mTOR and SIRT1, and activate the Ras/Raf/MEK/Erk signaling pathway and NF-κB factor in human fibrosarcoma cells. J Cell Physiol. 2012;227(2):867–76.
61. Weng C, Dong H, Chen G, Zhai Y, Bai R, Hu H, Lu L, Xu Z. miR-409-3p inhibits HT1080 cell proliferation, vascularization and metastasis by targeting angiogenin. Cancer Lett. 2012;323(2):171–9.
62. Chase DR, Enzinger FM. Epithelioid sarcoma: diagnosis, prognostic indicators, and treatment*'+. Am J Surg Pathol. 1985;9(4):241–63.
63. Enzinger F. Epithelioid sarcoma. A sarcoma simulating a granuloma or a carcinoma. Cancer. 1970;26(5):1029–41.
64. Baratti D, Pennacchioli E, Casali PG, Bertulli R, Lozza L, Olmi P, Collini P, Radaelli S, Fiore M, Gronchi A. Epithelioid sarcoma: prognostic factors and survival in a series of patients treated at a single institution. Ann Surg Oncol. 2007;14(12):3542–51.
65. Callister MD, Ballo MT, Pisters PW, Patel SR, Feig BW, Pollock RE, Benjamin RS, Zagars GK. Epithelioid sarcoma: results of conservative surgery and radiotherapy. Int J Radiat Oncol Biol Phys. 2001;51(2):384–91.
66. Gasparini P, Facchinetti F, Boeri M, Lorenzetto E, Livio A, Gronchi A, Ferrari A, Massimino M, Spreafico F, Giangaspero F. Prognostic determinants in epithelioid sarcoma. Eur J Cancer. 2011;47(2):287–95.

67. Casanova M, Ferrari A, Collini P, Bisogno G, Alaggio R, Cecchetto G, Gronchi A, Meazza C, Garaventa A, Di Cataldo A. Epithelioid sarcoma in children and adolescents. Cancer. 2006;106(3):708–17.
68. Izumi T, Oda Y, Hasegawa T, Nakanishi Y, Iwasaki H, Sonobe H, Goto H, Kusakabe H, Takahira T, Kobayashi C. Prognostic significance of dysadherin expression in epithelioid sarcoma and its diagnostic utility in distinguishing epithelioid sarcoma from malignant rhabdoid tumor. Mod Pathol. 2006;19(6):820–31.
69. Papp G, Krausz T, Stricker TP, Szendrői M, Sápi Z. SMARCB1 expression in epithelioid sarcoma is regulated by miR-206, miR-381, and miR-671-5p on Both mRNA and protein levels. Genes Chromosomes Cancer. 2014;53(2):168–76.
70. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol. 2008;110(1):13–21.
71. Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, Banham AH, Pezzella F, Boultwood J, Wainscoat JS, Hatton CS, Harris AL. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol. 2008;141(5):672–5.
72. Agaoglu FY, Kovancilar M, Dizdar Y, Darendeliler E, Holdenrieder S, Dalay N, Gezer U. Investigation of miR-21, miR-141, and miR-221 in blood circulation of patients with prostate cancer. Tumor Biol. 2011;32(3):583–8.
73. Cheng H, Zhang L, Cogdell DE, Zheng H, Schetter AJ, Nykter M, Harris CC, Chen K, Hamilton SR, Zhang W. Circulating plasma MiR-141 is a novel biomarker for metastatic colon cancer and predicts poor prognosis. PLoS One. 2011;6(3), e17745.
74. Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Newell J, Kerin MJ. Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg. 2010;251(3): 499–505.
75. Fujiwara T, Kawai A, Nezu Y, Fujita Y, Kosaka N, Ozaki T, Ochiya T. Circulating microRNAs in sarcoma: potential biomarkers for diagnosis and targets for therapy. Chemotherapy. 2014;3(123):2.
76. Miyachi M, Tsuchiya K, Yoshida H, Yagyu S, Kikuchi K, Misawa A, Iehara T, Hosoi H. Circulating muscle-specific microRNA, miR-206, as a potential diagnostic marker for rhabdomyosarcoma. Biochem Biophys Res Commun. 2010;400(1):89–93.
77. Weng Y, Chen Y, Chen J, Liu Y, Bao T. Identification of serum microRNAs in genome-wide serum microRNA expression profiles as novel noninvasive biomarkers for malignant peripheral nerve sheath tumor diagnosis. Med Oncol. 2013;30(2):531.
78. Fujiwara T, Katsuda T, Hagiwara K, Kosaka N, Yoshioka Y, Takahashi R, Takeshita F, Kubota D, Kondo T, Ichikawa H. Clinical relevance and therapeutic significance of microRNA-133a expression profiles and functions in malignant osteosarcoma-initiating cells. Stem Cells. 2014;32(4):959–73.
79. Osaki M, Takeshita F, Sugimoto Y, Kosaka N, Yamamoto Y, Yoshioka Y, Kobayashi E, Yamada T, Kawai A, Inoue T. MicroRNA-143 regulates human osteosarcoma metastasis by regulating matrix metalloprotease-13 expression. Mol Ther. 2011;19(6):1123–30.

Chapter 11
microRNA and Bone Cancer

Mary Nugent

Abstract MicroRNA molecules have a variety of roles in cellular development and proliferation processes, including normal osteogenesis. These effects are exerted through post-translational inhibition of target genes. Altered miRNA expression has been demonstrated in several cancers, both in the tumor tissue and in the peripheral circulation. This may influence carcinogenesis if the specific miRNA targets are encoded by tumor suppressor genes or oncogenes. To date, most research investigating the role of microRNAs and primary bone tumors has focused on osteosarcoma and Ewing sarcoma. Several microRNAs including the *miR-34* family have been implicated in osteosarcoma tumorigenesis via effects on the Notch signaling pathway. Progression, invasion, and metastasis of osteosarcoma tumor cells is also influenced by microRNA expression. In addition, microRNA expression may affect the response to chemotherapy in osteosarcoma and thus hold potential for future use as either a prognostic indicator or a therapeutic target. The EWS-FLI1 fusion protein produced in Ewing sarcoma has been shown to induce changes in miRNA expression. MicroRNA expression profiling may have some potential for prediction of disease progression and survival in Ewing sarcoma. There is limited evidence to support a role for microRNAs in other primary bone tumors, either malignant or benign; however, early work is suggestive of involvement in chondrosarcoma, multiple osteochondromatosis, and giant cell tumors of bone.

Keywords miRNA • Osteosarcoma • Ewing sarcoma • Chondrosarcoma

Introduction

Malignant lesions in bone encompass primary bone tumors, lesions resulting from hematologic malignancies, and secondary metastatic deposits from primary tumors at other sites. Although the most frequently encountered malignant tumors with bone involvement are metastases from carcinomas [1], the relationship between microRNA

M. Nugent (✉)
Department of Trauma and Orthopaedics, St. James Hospital, Dublin 8, Ireland
e-mail: nugentmary@gmail.com

© Springer International Publishing Switzerland 2015

G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5_11

(miRNA) and these tumors is covered extensively in other chapters, as are the main hematological malignancies. Therefore this chapter focuses mainly on miRNA and primary bone tumors with only a brief section considering metastasis to bone.

The field of microRNA research is relatively young, with identification of the first of these small single-stranded non-coding ribonucleic acids (RNAs) as recently as 1993 (in *C. elegans*) [2]. Since then more than 35,000 mature miRNA sequences in 223 species have been identified with over 2600 in humans at the time of writing (although this number continues to grow exponentially) [3–8].

Initially the biological roles of these molecules were unknown; however, those functions are now known to include roles in regulation of biochemical pathways in cell differentiation, cell-cycle progression, and apoptosis [9]. MiRNAs downregulate the expression of target genes either by induction of messenger RNA (mRNA) degradation or by translational inhibition at the post-transcription level [9–13].

MiRNA expression was first linked with cancer in 2002. This association was initially with chronic lymphocytic leukemia (CLL), but subsequently with many other types of malignancies [14]. Since then expression of many individual miRNAs has been found to be altered in malignancy (either overexpressed or reduced, depending on the specific miRNA). It is thought that some of these miRNAs behave as tumor-suppressor genes while others effectively function as oncogenes with roles in tumorigenesis, typically through influencing cell proliferation, migration, and invasion or effects on apoptosis [15].

Thus, dysregulation of miRNA expression may contribute to development of cancer through loss of the normal regulatory controls in cell turnover and proliferation. In addition to contributing to oncogenesis, knowledge of the mechanisms of action and functions of miRNAs may also represent opportunities for development of diagnostic and therapeutic interventions for management of malignancy and other diseases.

Primary bone tumors include a wide range of tumor types with variable clinical outcomes and prognoses; however, many of these are relatively rare with a correspondingly low volume of relevant research. Therefore the vast majority of the currently available evidence for miRNA involvement in primary bone tumors relates mainly to osteosarcoma and Ewing sarcoma and there is a relatively incomplete knowledge of the relationship with other less common tumors. Nevertheless this is a rapidly expanding field of research, not just in relation to osteosarcoma, but also chondrosarcoma and giant cell tumor (GCT) of bone, both of which have been the subject of some recently published studies.

MicroRNAs in Normal Osteogenesis

MiRNAs are involved in the control of many cellular processes including development, differentiation, apoptosis, and metabolism (see Chap. 1 of the volume "microRNA: Basic Science" for an introduction to miRNA biology). They are known to have a number of regulatory roles in normal bone metabolism and

osteogenesis. Although these functions are becoming more clearly understood, many of the mechanisms involved have not been clearly elucidated to date.

Normal bone turnover and homeostasis is dependent on the interplay between osteoblasts and osteoclasts among other factors. Both osteoclastogenesis and osteoblastogenesis are tightly controlled by several biochemical pathways, many of which are in turn regulated by specific miRNAs. One of the most important pathways in control of normal osteoclastogenesis is the Notch signaling pathway which encompasses a number of factors such as Notch-1, Notch-2, JAG1, and Hay1 [16]. Osteoblastogenesis is also regulated by several gene factors including special AT-rich sequence-binding protein 2 (Satb2), runt-related transcription factor 2 (Runx2), Osterix (Osx), collagen type I alpha 1 (CollIa1), and osteocalcin (Ocn) [16]. Several individual miRNAs have been found to influence these pathways, most notably the *miR-34* family. *mir-34a* functions as a suppressor of excessive osteoclastogenesis through inhibition of transforming growth factor β-induced factor 2 (Tgif2) while *miR-34c* has been shown to regulate both osteoblastogenesis and osteoclastogenesis through targeting of Runx2, Satb2, and multiple targets in the Notch signaling pathway [16, 17].

Other miRNAs have also been identified as important regulators of osteogenesis—these include *miR-26a, miR-133a*, and *miR-542-3p*. Expression of *miR-26a* is increased during receptor activator of nuclear kappa B ligand (RANKL)-induced osteoclastogenesis. *mir-26a* inhibits RANKL-induced osteoclast formation and may also have a role in the regulation of osteoclast function through blocking of resorption pit formation [18]. *miR-133a* also serves as an important regulator in osteogenesis. Bone morphogenetic protein (BMP)-induced osteogenesis is associated with marked downregulation of *miR-133a* expression which can target and suppress Runx2 expression to inhibit osteoblast differentiation [19]. *MiR-542-3p* regulates osteoblast differentiation by directly affecting the activity of BMP-7, a member of the transforming growth factor β (TGF-β) family that has a major role in osteoblast differentiation and function [20].

It is likely that many other individual miRNAs are also involved in the regulation of these processes, although their specific roles have not yet been elucidated.

MicroRNAs in Oncogenesis

Recent work has detected significant changes in miRNA expression patterns in a range of human diseases including many malignancies and several other disease processes [21]. Each miRNA has specific target mRNAs and, when these mRNAs are encoded by tumor suppressor genes or oncogenes, changes in the miRNA expression level can directly influence oncogenesis. These effects are complex as each individual miRNA has a potentially large number of targets (up to hundreds in some cases). Thus even small variations in expression level may have significant implications for the cell processes [22, 23]. Many of the human miRNA genes identified to date are thought to be located in cancer-associated regions or at fragile sites

of chromosomes which are prone to deletion, amplification, and mutations in cancer cells [21, 22, 24]. Any changes in these chromosome regions will therefore have direct effects on the expression level of the miRNAs encoded there.

As alluded to earlier, miRNAs function as negative regulators of gene expression, with an inverse relationship between changes in miRNA expression and that of the relevant target gene(s). Thus overexpression of oncogenic miRNAs can contribute to tumorigenesis by promoting cellular proliferation and evasion of apoptosis. Conversely, reduced expression of tumor-suppressive miRNAs may have similar effects as they no longer exert the same inhibitory influence on their oncogenic targets [14]. Specific miRNAs may be either increased or decreased in pathological states such as cancer and several different miRNAs may show aberrant expression within a single disease process. While some of these changes in miRNA expression levels seem to be common to a range of malignancies (and, in some cases, other disease processes), many seem to be tissue-specific and characteristic of cancer type [22, 24].

Osteosarcoma

Osteosarcoma is the most common malignant primary tumor of bone. It occurs mainly in adolescents and young adults and has an incidence of approximately 4–5 cases per million [25]. Osteosarcoma cells arise from mesenchymal stem cells (MSC) which undergo disruption to normal osteoblast differentiation.

The tumors generally affect the metaphyses of long bones although they can occur in other locations [26, 27]. Since the advent of chemotherapy the 5-year survival rate has markedly improved to approximately 60–70 % overall [28]. Response of the tumor to chemotherapy is one of the most important prognostic indicators in osteosarcoma and 10-year survival rates of up to 73 % are possible in those with a good response to chemotherapy.

Unfortunately, despite maximal chemotherapy, a significant proportion of patients still respond poorly and have a risk of relapse or metastasis even after curative resection and chemotherapy [25, 28, 29]. There are currently no established biomarkers which can be used in clinical practice to identify those patients with particularly aggressive tumors or those who are likely to respond well to conventional chemotherapy regimes.

Tumorigenesis in Osteosarcoma

Development of malignant osteosarcoma cells is thought to be influenced by several different processes, many of which are at least partially regulated by miRNAs. Thus disruption of the normal miRNA expression patterns may have a profound influence on osteosarcoma tumorigenesis.

One of these processes is the Notch signaling pathway which is involved in maintaining the balance between cell proliferation and differentiation. Altered Notch signaling has been associated with various disorders, including cancer [30, 31]. As described above, the *miR-34* family (*miR-34a*, *miR-34b*, and *miR-34c*) affects expression of several target genes involved in this pathway. *MiR-34c* has been shown to inhibit osteoblast differentiation and increase osteoclastogenesis through suppression of Notch signaling (resulting in inhibition of osteoprotegrin expression) in mouse models [16]. Reduced expression levels for the entire *miR-34* family are seen in osteosarcoma tissue samples compared to adjacent normal tissues [32]. These changes in *miR-34* expression levels are also thought to influence the activity of the P53 tumor-suppressor pathway as *miR-34a* has been shown to be a direct transcriptional target of the P53 and to inhibit osteosarcoma cell proliferation and metastasis, possibly via downregulation of the c-Met gene [33].

Diallyl trisulphide (DATS) is a naturally occurring organosulfur compound derived from *Allium* vegetables. It can inhibit cell-cycle progression and induce apoptosis [34]. One study found that DATS suppressed cell survival, invasion, and angiogenesis in osteosarcoma cells. These effects were associated with decreased expression of Notch-1 and its downstream genes as well as increased expression of a panel of tumor-suppressive miRNAs including *miR-34a*, *miR-143*, *miR-145,* and *miR-200b/c* that are typically lost in osteosarcoma [35]. Reexpression of *miR-34a* and *miR-200b* by transfection led to reduced expression of Notch-1 [35].

Lysophosphatidic acid acyltransferase β (LPAATβ) is thought to regulate osteosarcoma cell proliferation, at least partially via the mammalian target of rapamycin (mTOR) gene and Raf-1 signaling pathways; however, the mechanisms responsible for regulation of LPAATβ expression remain unclear. Enhanced LPAATβ expression correlates with osteosarcoma cell proliferation and this is inhibited by inducing overexpression of *miR-24,* which is reduced in osteosarcoma cells [36].

Insulin-like growth factor-1 receptor (IGF1R) is a receptor tyrosine kinase that mediates IGF1-induced signaling events and has pivotal roles in cellular processes such as cell proliferation, migration, and differentiation [37]. *MiR-16* overexpression inhibits the Raf1-MEK1/2-ERK1/2 pathway and silences IGF1R, inducing cell-growth inhibition and a G0/G1 phase arrest; however, *miR-16* is downregulated in osteosarcoma cell lines and tissues [38].

The chromosome 14q32 locus encodes a microRNA cluster which includes *miR-127-3p*, *miR-154*, *miR-299-5p*, *miR-329*, *miR-337-3p*, *miR-376a*, *miR-376c*, *miR-377*, *miR-382*, *miR-409-3p*, *miR-409-5p*, *miR-410*, *miR-432*, *miR-453*, *miR-493*, *miR-495*, *miR-654-5p*, and *miR-758*. This cluster of miRNAs is significantly downregulated in osteosarcoma compared to normal bone tissues [39]. The c-Myc oncogene which is known to be dysregulated in many malignant tumors is inhibited by this miRNA cluster. As c-Myc inhibits apoptosis, miRNA downregulation at this locus results in inhibition of apoptosis through increased c-Myc activity [39, 40].

Other miRNA clusters have also been implicated in osteosarcoma tumorigenesis. The *miR-17-92* cluster, also known as oncomir-I, and its two paraloga *miR-106a-363* and *miR-106b-25* were among the earliest families of miRNAs found to be upregulated in several malignant tumors. Several of the miRNAs related to this cluster have

been shown to accelerate tumor development, induce angiogenesis, prevent apoptosis, and influence osteoblastic proliferation and differentiation [41, 42]. Many of these cluster-related miRNAs were consistently upregulated in tumor biopsy samples compared to both human osteoblasts and MSC [43].

Numerous other individual miRNAs have been shown to have consistently altered expression levels in osteosarcoma. Tables 11.1 and 11.2 list those with increased and decreased expression levels respectively, along with their roles and target genes, where known.

Progression and Invasion of Osteosarcoma

Osteosarcoma may metastasize to other sites and in approximately 20–25 % patients there is clinically or radiographically detectable metastatic disease at presentation. In those who have confirmed metastatic disease at the time of presentation, a 5-year survival of only around 30 % is likely [81]. The most common site for initial

Table 11.1 MiRNAs shown to have increased expression levels in osteosarcoma tissues or cell lines (with their role and target gene where known)

miRNA	Role and target gene (if known)
miR-9	Higher levels associated with increased tumor size and metastases [44]
miR-19a	Induce angiogenesis and prevent apoptosis [41–43]
miR-19b	Induce angiogenesis and prevent apoptosis [41–43]
miR-20a	Induce angiogenesis and prevent apoptosis [41–43]
miR-21	Cell invasion and migration via regulation of RECK [45, 46]
miR-92a	Induce angiogenesis and prevent apoptosis [41–43]
miR-93	Increases cell proliferation and invasion [47]
miR-106b	Induce angiogenesis and prevent apoptosis [41–43]
miR-128	Associated with metastases, target PTEN [48]
miR-135b	[49, 50]
miR-146a-5p	[49]
miR-150	[50]
miR-181a	[51]
miR-181b	[51]
miR-181c	[51]
miR-199b-5p	Involved in Notch signaling [52]
miR-210	[53]
miR-214	Promotes cell proliferation, invasion and tumor growth in nude mice, reversible by overexpression of leucine zipper (LZTS1) [54]
miR-221	Induces cell survival via inhibition of PTEN [55]
miR-542-5p	[50]
miR-652	[50]

Table 11.2 MiRNAs shown to have decreased expression levels in osteosarcoma tissues or cell lines (with their role and target gene where known).

miRNA	Role and target gene (if known)
miR-16	Inhibition of cell proliferation via IGF1R [38, 51]
miR-24	Inhibition of osteosarcoma cell proliferation via LPAATβ downregulation [36]
miR-29a	Induces apoptosis [56]
miR-29b	Inhibits cell proliferation, migration and invasion, induces apoptosis via inhibition of vascular endothelial growth factor (VEGF) [51, 56, 57]
miR-31	Osteogenic differentiation of mesenchymal stem cells via regulation of bone transcription factor Osterix [58]
miR-34a	Inhibition of cell proliferation via Notch-1 inhibition [35, 59, 60]
miR-34b	[61]
miR-34c	Suppresses proliferation of osteoblasts by downregulation of Runx2 [62]
miR-100-3p	[49]
miR-101	Decreases expression of mTOR gene, inhibiting proliferation and promoting apoptosis [63]
miR-125b	Suppresses proliferation via downregulation of STAT3 [64]
miR-126	Inhibits cell proliferation, migration and invasion via Sox2 [65]
miR-127-3p	Loss results in inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-132	Facilitates angiogenesis [66, 67]
miR-133a	Promotes apoptosis by targeting Bcl-xL and Mcl-1 [68]
miR-133b	Inhibits osteosarcoma cell proliferation, migration and invasion, and promotes apoptosis via target genes BCL2L2, MCL-1, IGF1, MET, phospho-Akt and FAK [69]
miR-135b	Inhibits cell proliferation, migration and invasion, reduces c-Myc protein product [70]
miR-142-5p	[51]
miR-143	[35, 46]
miR-145	Inhibition of cell proliferation via Notch-1 inhibition [35, 71]
miR-154	Loss results in inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-183	Suppresses Ezrin-linked migration and invasion [72]
miR-199a-3p	Regulates cell proliferation [46, 73]
miR-199b-5p	[46, 49]
miR-200b	Inhibition of cell proliferation via Notch-1 inhibition [35]
miR-200c	Inhibition of cell proliferation via Notch-1 inhibition [35]
miR-206	Involved in apoptosis and inhibition of cell invasion and migration [74]
miR-223	Regulates Ect2 [75]
miR-299-5p	Loss results in inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-320	Inhibition of cell proliferation through negative regulation of fatty acid synthase [76]

(continued)

Table 11.2 (continued)

miRNA	Role and target gene (if known)
miR-329	Loss results in inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-335	Suppresses migration and invasion by targeting ROCK1 [77]
miR-337-3p	Loss results in inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-340	Suppresses proliferation, migration and invasion by targeting ROCK1 [78]
miR-376a	Loss results in inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-376c	Loss results in inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-377	Loss results in inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-382	Loss results in inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-409-3p	Loss results in inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-409-5p	Loss results in inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-410	Loss results in inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-424	Inhibits migration and invasion via fatty acid synthase [79]
miR-432	Inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-451	Inhibits cell proliferation and migration, increases apoptosis [80]
miR-453	Inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-493	Inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-495	Inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-654-5p	Inhibition of apoptosis through increased c-Myc activity [39, 40]
miR-758	Inhibition of apoptosis through increased c-Myc activity [39, 40]

metastatic disease in osteosarcoma is the lung and the second most common is another bone distant from the primary tumor [82]. In general, patients with metastases limited to the lungs have a better outcome than do those with metastases to other sites or to the lungs combined with other sites [81, 83].

The ability for osteosarcoma cells to migrate and invade is influenced by miRNAs, although not all of the mechanisms involved have been fully elucidated yet. One such mechanism is thought to be related to expression of Fas receptors (also known as apoptosis antigen 1 or tumor necrosis factor receptor superfamily member 6 (TNFRSF6)) on the osteosarcoma cell surface. The Fas ligand (FasL) is constitutively expressed in lungs and can mediate apoptosis in Fas^{+} cells, thus the ability of osteosarcoma cells to metastasize to lungs is inversely correlated with cell surface Fas expression [84, 85]. *miR-20a* is encoded by the *miR-17-92* cluster and overexpression in cell lines results in downregulation of Fas expression [86]. As described earlier, many of the miRNAs from the *miR-17-92* cluster are upregulated in osteosarcoma.

This is not the only miRNA mediated means of metastases in osteosarcoma. One study showed that *miR-143* was the most downregulated miRNA in human osteosarcoma cell lines in a mouse model; however, in vitro transfection of *miR-143* decreased cell invasiveness and intravenous injection of *miR-143* sup-

pressed lung metastasis of 143B sarcoma cells in the mouse model thus supporting a possible role for low *miR-143* expression in the development of lung metastasis [87].

Rho-associated, coiled-coil-containing protein kinase 1 (ROCK1)is a GTP-dependent serine/threonine protein kinase interacting with the Rho G-protein through its Rho-binding domain, and thereby mediating Rho signaling [88]. It has a role in regulation of the actin cytoskeleton reorganization and has been identified as a target gene for a number of miRNAs including *miR-145*, *miR-335*, and *miR-340*, all of which have been found to be underexpressed in osteosarcoma [77, 78, 89]. *miR-335* expression is lower in osteosarcoma tissues and cell lines than in normal controls, and the extent of this downregulation is significantly associated with lymph node metastases [77]. ROCK1 expression has been found to be inversely correlated with *miR-335* in osteosarcoma tissues [77]. Knockdown of ROCK1 inhibits osteosarcoma cell migration and invasion in vitro, however, as ROCK1 is also a target of *miR-340* which is frequently downregulated in osteosarcoma tumors, changes in its expression level may be due to underexpression of either one or both of these miRNAs [77, 78]. The extent of downregulation of *miR-340* in osteosarcoma appears to be associated with larger tumor size, positive metastasis and a poor response to pre-operative chemotherapy in a pediatric osteosarcoma population [90].

Upregulation of ROCK1 is also significantly associated with positive metastasis and a poor response to pre-operative chemotherapy. In this study, a combination of *miR-340* downregulation and ROCK1 upregulation occurred more frequently in osteosarcoma tissues from patients with positive metastases than those with no metastases [90]. Ectopic expression of *miR-145* has also been shown to suppress protein expression of ROCK1 in cell lines without affecting the ROCK1 mRNA level. This resulted in reduced cell proliferation and invasion, which was reversed by restoration of ROCK1, however at the time of writing there are no published studies confirming a role for *miR-145* in promotion of metastases via ROCK1 in vivo [89].

Several other miRNAs have been linked to osteosarcoma progression and invasion or specifically associated with development of metastases, mainly through promotion of cell proliferation and migration. These include *miR-26a*, *miR-27a, miR-34a, miR-93, miR-132, miR-133a, miR-135b, miR-144*, *miR-181c, miR-183, 199a-3p, miR-206, miR-214, miR-217,* and *miR-451*.

MiR-26a expression is markedly reduced in both osteosarcoma tissues and cell lines. This is found more frequently in tissue specimens taken from patients with disease at an advanced clinical stage and with presence of distant metastases compared to patients with early stage, non-metastatic disease. Although the precise mechanism of action is yet to be fully evaluated, *miR-26a* can directly inhibit enhancer of zeste homolog2 (EHZ2) [91]. EHZ2 is the catalytic subunit of polycomb repressive complex 2, which mediates epigenetic gene silencing by trimethylating histone H3 lysine 27. EHZ2 is known to enhance tumorigenesis through aberrant silencing of tumor-suppressor genes and is commonly overexpressed in several types of cancer [92].

Tissue samples from patients with non-metastatic osteosarcomas have been found to display higher expression levels of *miR-27a, miR-93,* and *miR-181c* compared to tissues from patients with metastatic osteosarcomas [47, 51, 93]. *miR-27a* expression is also increased in some other cancers. It is known to target mitogen-activated protein kinase kinase 4 (MAP2K4), thus reducing MAP2K4 levels. MAP2K4 normally functions to inhibit cell proliferation and migration through the JNK/p38 signaling pathway; however, this inhibition is reduced when MAP2K4 expression is lower [93]. The precise mechanism of action for *miR-93* and *miR-181c* to influence metastasis is not yet fully established.

MiR-34a expression is reduced in several cancers. The c-Met oncogene has been identified as one of its targets and the normal suppression effect of *miR-34a* on c-Met is reduced in the presence of lowered expression. In osteosarcoma, a single nucleotide polymorphism (SNP) in the pre-*miR-34a* coding region has been identified that reduces *miR-34a* expression and promotes osteosarcoma cell proliferation and migration. These effects on osteosarcoma cell proliferation were shown in vitro; however, reduced *miR-34a* expression levels have also been shown in tissue and serum samples from patients [94]. *MiR-34a* and *miR-199a-3p* together have been shown to inhibit in vitro proliferation of human osteosarcoma cells. This may be mediated through inhibitory effects on targets including mTOR and the MET proto-oncogene which regulate P53-mediated apoptosis [59].

MiR-132 expression is lower in osteosarcoma tissues compared to normal non-cancerous tissue. Although the precise details of its mechanism of action remain unclear, *miR-132* is known to target cyclin E1 (CCNE1) which is involved in cell proliferation [95].

MiR-133a is downregulated in both human osteosarcoma cell lines and osteosarcoma tissues when compared to normal tissues and cells. This decrease in tissue levels of *miR-133a* is directly correlated with tumor progression and prognosis [68]. In vitro restoration of *miR-133a* reduced cell proliferation, promoted cell apoptosis, and suppressed tumorigenicity in tumor cell lines [68]. Known targets of *miR-133a* include B-cell lymphoma-extra large (Bcl-xL) and myeloid cell leukemia 1 (Mcl-1) expression, and the anti-tumor effect is probably due to targeting and repression of these genes [68].

MiR-135b expression is significantly decreased in both osteosarcoma tissues and osteosarcoma cell lines when compared to paired samples of adjacent non-tumor bone tissue. In addition, lower levels of *miR-135b* tissue specimens were associated with the presence of metastases. In vitro inhibition of *miR-135b* resulted in accelerated rates of osteosarcoma cell proliferation, migration, and invasion. In the same study, ectopic expression of *miR-135b* leads to a significant reduction in detectable levels of the c-Myc protein product, thus suggesting this pathway as a mechanism for the effects of *miR-135b* suppression [70].

MiR-144 is also downregulated in both osteosarcoma cell lines and primary tissue samples. Its downstream target is known to be transgelin (TAGLN), expression levels of which are inversely correlated with those of *miR-144* in vitro. Ectopic expression of *miR-144* prevents cell proliferation and invasion [96].

Lower expression of *miR-183* in tumor tissues correlates with the presence of lung metastases and local recurrence [72]. *MiR-183* is thought to inhibit the metastasis of osteosarcoma through inhibiting motility and invasion, mainly by downregulation of the Ezrin gene [97]. Tissue expression of *MiR-206,* which is thought to act as a promoter of apoptosis, is inversely associated with clinical stage in osteosarcoma [74].

MiR-217 targets the Wiskott Aldrich syndrome protein family member 3 (WASF3) gene [98]. The protein products of this gene are involved in transmission of signals from tyrosine kinase receptors and small GTPases to the actin cytoskeleton, thus playing an important role in regulating cytoskeletal dynamics [99]. *MiR-217* is significantly downregulated in both osteosarcoma tissues and osteosarcoma cell lines, and lower tissue levels are associated with the presence of metastases. Inhibition of *miR-217* in vitro markedly suppressed cell proliferation, invasion, and migration [98].

Osteosarcoma tissues also express lower levels of *miR-451* compared to noncancerous bone tissue. This downregulation occurred more frequently in specimens from patients with a more advanced clinical stage of disease, presence of metastases and those who had a poor response to chemotherapy. After *miR-451* transfection in vitro, cell proliferation, migration, and tumorigenesis were all inhibited in osteosarcoma cell lines and with an associated increase in apoptosis [80].

Finally, *miR-214* is significantly upregulated in osteosarcoma tissues compared to normal tissues, and also in osteosarcoma cell lines. Both the phosphatase and tensin homolog (PTEN) gene, which regulates epidermal growth factor receptor (EGFR), and the leucine zipper (LZTS1) gene have been identified as downstream targets of *miR-214* [54, 100, 101]. Higher tissue *miR-214* levels demonstrate a significant association with shorter overall and progression-free survival in patients with osteosarcoma [100]. In addition, in functional assays, *miR-214* promoted cell proliferation, invasion, and tumor growth in nude mice. These effects were reversible by overexpression of LZTS1 [54].

Prognosis and Prognostic Indicators in Osteosarcoma

As referred to above, the most important prognostic indicator in osteosarcoma is the response to neoadjuvant chemotherapy. A tumor necrosis rate of >90 % post-chemotherapy is deemed a good response, while those patients with necrosis of <90 % are considered chemoresistant or poor responders. To date, there is no satisfactory means of selecting those who are likely to respond well; however, miRNAs appear to play a role in modulating the response to chemotherapy.

A number of studies have looked at the effects of miRNAs on commonly used chemotherapy agents such as methotrexate, ifosfamide, 5-fluorouracil, and raltitrexed. In one study, a panel of five miRNAs were studied in 27 fresh frozen paraffin-embedded (FFPE) tissue samples, cell lines, and samples from a rat model. Significant differences in miRNA expression between good responders to ifosfamide

and poor responders were observed (*miR-92a, miR-99b, miR-193a-5p,* and *miR-422a* were increased and *miR-132* was reduced in good responders) [102]. *MiR-221* is significantly increased in osteosarcoma cell lines compared to osteoblasts and has been shown to induce cell survival and cisplatin resistance and reduce apoptosis while *miR-221* knockdown reverses these effects [55]. *MiR-210* expression is also increased in osteosarcoma tissues compared to non-cancerous bone tissues from the same patients and upregulation is associated with larger tumor size, poor response to pre-operative chemotherapy and lower overall survival and progression-free survival [53]. Overexpression of *miR-140* has also been shown to cause chemoresistance to methotrexate and 5-fluorouracil in xenograft models [103]. In one small study, tissue samples from patients with chemoresistant osteosarcomas underexpressed *miR-15b* and *miR-451* compared to samples from those with chemosensitive tumors [51].

Other miRNAs have also been implicated in the development of chemoresistance in osteosarcoma. The high-mobility group box 1 (HMGB1) gene plays an important role in facilitating autophagy and promoting drug resistance in osteosarcoma cells. One study demonstrated that *miR-22* downregulates HMGB1 expression and blocked the HMGB1-mediated autophagy process during chemotherapy (in vitro). Osteosarcoma cell proliferation, migration, and invasion were inhibited by this blockage of autophagy [104].

Expression of *miR-128* is increased and expression of its downstream target, PTEN, frequently decreased in osteosarcoma. One study demonstrated that elevated *miR-128* levels and reduced PTEN expression either alone or in combination were associated with a poor response to neoadjuvant chemotherapy and were also independent prognostic factors for both disease-free and overall survival [48].

Similarly, *miR-223* is reduced in osteosarcoma tissues compared to non-tumor bone tissue with a concomitant increase in expression of its target, epithelial cell transforming sequence 2 (Ect2). Ect2 is involved in cell-cycle progression and cell proliferation. Combined low expression of *miR-223* and increased Ect2 expression is associated with higher tumor grades, a poor response to chemotherapy, presence of metastases, and disease recurrence. This combination is also an independent prognostic factor for the shortest periods of overall and disease-free survival [75].

A study analyzing cancerous and non-cancerous bone tissues in 92 pediatric patients with osteosarcoma showed significant upregulation of *miR-214* in the osteosarcoma tissues [100]. This study also found significant correlations between *miR-214* expression and prognostic factors such as tumor size, presence of metastases, and poor response to neoadjuvant chemotherapy as well as shorter overall and progression-free survival [100].

The TWIST gene product belongs to the family of basic helix-loop-helix (bHLH) transcription factors and plays an essential role in differentiation of all mesoderm derived tissues. This gene is frequently deleted in osteosarcoma tumors and the presence of TWIST haploinsufficiency has been associated with poorer overall outcomes [105]. TWIST has been reported to decrease osteosarcoma cell survival against cisplatin by inhibiting β-catenin signaling and endothelin-1/endothelin A receptor signaling pathways. This suggests that TWIST may be an important

negative regulator in the development of chemoresistance in osteosarcoma [106, 107]. Recently *miR-33a* has been shown to inhibit expression of TWIST. *miR-33a* is overexpressed in chemoresistant osteosarcoma tumors compared to those with a good response to chemotherapy and thus may influence chemosensitivity via its effect on TWIST expression [108].

A number of additional miRNAs have shown altered expression associated with poor disease-free and/or overall survival in osteosarcoma, including *miR-9, miR-132, miR-145, miR-183, miR-206,* and *miR-214*.

MiR-9 expression is significantly elevated in osteosarcoma tissues when compared to non-cancerous bone tissue. The level of expression is significantly associated with tumor size, stage of disease, and the presence of distant metastatic lesions. Patients whose tumors expressed lower levels of *miR-9* also had a significantly increased survival in comparison to those with the highest *miR-9* Levels [44].

MiR-132 expression levels are also frequently altered in osteosarcoma. Low expression is associated with a poorer overall and disease-free survival in patients with primary osteosarcoma [67]. Low *miR-145* expression is also seen in osteosarcoma (and many other malignancies). It has also been shown to be an independent prognostic factor for both overall and disease-free survival in a cohort of 166 patients with osteosarcoma. Those who had lower osteosarcoma tissue levels of *miR-145* had significantly poorer 5-year overall and disease-free survival [71].

Also referred to in the previous section, *miR-183* acts as a tumor suppressor by inhibition of migration and invasion through its targeting of an oncogene, Ezrin. Downregulation of *miR-183* negatively correlates with upregulation of Ezrin in tissue specimens from patients with osteosarcoma. Furthermore, a combination of low *miR-183* levels and elevated Ezrin expression was associated with high tumor grades, a poor response to chemotherapy, presence of metastases, and recurrence of disease. This combination was also an independent prognostic factor for overall and disease-free survival [109].

As referred to earlier, underexpression of the miRNA cluster at the 14q32 chromosomal locus is seen in osteosarcoma and thought to have an inhibitory effect on apoptosis. An inverse correlation has been demonstrated between aggressive tumor behavior (such as increased metastatic potential and accelerated time to death) and the residual expression of representative 14q32 miRNAs (*miR-134, miR-382,* and *miR-544*) in samples from human osteosarcoma patients [110]. In addition, a study of 65 human FFPE osteosarcoma tissues (from biopsies) and 26 paired post-chemotherapy resection specimens demonstrated an association between expression of several 14q32-associated miRNAs (*miR-139-5p, miR-299, miR-299-3p, miR-323-3p, miR-323-5p, miR-379, miR-382, miR-411, miR-493, miR-539,* and *miR-758*) and recurrence-free survival [110]. Thus the level of expression of this miRNA cluster appears to be predictive of response to chemotherapy, recurrence, and overall survival [110, 111].

Table 11.3 summarizes the miRNAs that have been found to have some prognostic value in osteosarcoma to date.

Table 11.3 MicroRNAs as prognostic indicators in osteosarcoma

miRNA	Association with prognosis
miR-9	Higher expression associated with shorter survival [44]
miR-19a	Levels inversely associated with survival (part of miR-17-92 cluster) [43]
miR-19b	Levels inversely associated with survival (part of miR-17-92 cluster) [43]
miR-20a	Levels inversely associated with survival (part of miR-17-92 cluster) [43]
miR-34a	Associated with greater event-free and overall survival [112]
miR-92a	Levels inversely associated with survival (part of miR-17-92 cluster) [43]
	Increased in good responders to ifosfamide for osteosarcoma [102]
miR-99b	Increased in good responders to ifosfamide for osteosarcoma [102]
miR-106b	Levels inversely associated with survival (part of miR-17-92 cluster) [43]
miR-128	Inversely associated with disease-free and overall survival [48]
miR-132	Associated with disease-free and overall survival in osteosarcoma [67]
	Reduced in good responders to ifosfamide for osteosarcoma [102]
miR-139-5p	Part of 14q32 cluster, inverse association with recurrence-free survival [110]
miR-140	Associated with chemoresistance to methotrexate and 5-fluorouracil [103]
miR-145	Inversely associated with disease-free and overall survival in osteosarcoma [71]
miR-183	Inversely associated with lung metastases and local recurrence in osteosarcoma, inhibition of motility and invasion via downregulation of the Ezrin gene [72, 97]
miR-193a-5p	Increased in good responders to ifosfamide for osteosarcoma [102]
miR-206	Inversely associated with clinical stage in osteosarcoma—functions as a promoter of apoptosis [74]
miR-210	Associated with larger tumor size, poor response to pre-operative chemotherapy and lower overall survival and progression-free survival in osteosarcoma [53]
miR-223	Underexpression combined with elevated Ect2 associated with high tumor grade, poor response to chemotherapy, presence of metastases, recurrence and shorter overall and disease-free survival [75]
miR-299	Part of 14q32 cluster, inverse association with recurrence-free survival [110]
miR-299-3p	Part of 14q32 cluster, inverse association with recurrence-free survival [110]
miR-323-3p	Part of 14q32 cluster, inverse association with recurrence-free survival [110]
miR-323-5p	Part of 14q32 cluster, inverse association with recurrence-free survival [110]
miR-335	Inversely associated with presence of lymph node metastases in osteosarcoma through effects on cell migration and invasion via ROCK1 target gene [77]
miR-379	Part of 14q32 cluster, inverse association with recurrence-free survival [110]
miR-382	Part of 14q32 cluster, inverse association with recurrence-free survival [110]
miR-411	Part of 14q32 cluster, inverse association with recurrence-free survival [110]
miR-422a	Increased in good responders to ifosfamide for osteosarcoma [102]
miR-493	Part of 14q32 cluster, inverse association with recurrence-free survival [110]
miR-539	Part of 14q32 cluster, inverse association with recurrence-free survival [110]
miR-758	Part of 14q32 cluster, inverse association with recurrence-free survival [110]

Tumor Markers in Osteosarcoma

Tumor markers are increasingly used as tools to aid early detection, monitoring of therapeutic response or detection of recurrence in a number of malignancies. Unfortunately there are no well-established minimally invasive tumor markers in osteosarcoma; however, the identification of a reliable indicator of disease activity could potentially have significant benefits, either in diagnosis or in evaluation of an individual's response to treatment. Any potential tumor marker needs to be obtainable through relatively noninvasive and low-risk techniques in order to have a widespread useful clinical application.

The majority of research to date relating to altered miRNA profiles in osteosarcoma (and other primary bone tumors) is based on quantification of miRNA expression in tumor tissue or in vitro osteosarcoma cell lines. However, recent studies relating to other malignancies have demonstrated that miRNAs are detectable in blood, serum and plasma. Furthermore, these circulating miRNAs are sufficiently stable for extraction and quantification. Circulating levels of miRNAs appear to be reflective of altered tissue expression in disease states [113–116].At the time of writing, a Pubmed search revealed only five published studies investigating the relationship between circulating miRNAs and primary bone tumors. All of these are specific to osteosarcoma, with no published work to date in relation to other primary bone tumors.

The earliest study in this area quantified serum levels of *miR-21* in 65 osteosarcoma patients and 30 healthy controls. Aberrant *miR-21* expression is not unique to osteosarcoma as it has previously been shown to be overexpressed in several different malignancies; however, this was the first study to investigate circulating levels in osteosarcoma patients. In this cohort, serum *miR-21* levels were significantly higher in the osteosarcoma patients than the healthy controls. Levels were also significantly correlated with advanced clinical stage and poor response to chemotherapy [117].

A subsequent study quantified plasma levels of *miR-21, miR-143,* and *miR-119a-3p*. All of these miRNAs have previously been shown to have altered expression levels in osteosarcoma tissues. Plasma levels of *miR-21* were significantly higher while plasma *miR-199a-3p* and *miR-143* were decreased in samples from 40 osteosarcoma patients compared with 40 healthy controls. Circulating *miR-21* and *miR-143* expression correlated with both metastasis status and histological subtype; however, *miR-199a-3p* levels only correlated with histological subtype [46].

More recently, both *miR-196a* and *miR-196b* levels were found to be significantly higher in tumor tissues compared to non-cancerous bone tissues and also in corresponding serum samples (from the same patients) compared to healthy controls. Elevation of both sera and tissue levels both occurred more frequently in patients with high-grade tumors, known metastases or recurrent disease. High serum *miR-196a*, high serum *miR-196b,* and combined high serum levels of both *miR-196a* and *miR-196b* were all independent prognostic factors for overall survival and disease-free survival [118].

Plasma levels of *miR-34b* have also been shown to be significantly lower in patients with osteosarcoma than healthy controls. These plasma *miR-34b* levels were also significantly decreased in patients with metastatic disease compared to those with no known metastases [61].

Finally, expression levels of both *miR-133b* and *miR-206* were significantly decreased in osteosarcoma tissues compared to matched non-cancerous tissues and also in serum samples from patients with osteosarcoma compared to healthy controls. This downregulation occurred more frequently in patients with high-grade tumors, known metastases, and recurrent tumors. Low *miR-133b* expression, low *miR-206* expression, and combined underexpression of both *miR133b* and *miR-206* were all independent prognostic factors for overall and disease-free survival [119].

Although none of these miRNAs are yet clinically useful as minimally invasive tumor markers, this is an area which holds huge potential for the future. It may be the case that the ultimate circulating tumor markers will be based on a panel of aberrant miRNAs, rather than overexpression or underexpression of a single miRNA.

Ewing Sarcoma

Ewing sarcoma is the second most common primary bone tumor in children and young adults (after osteosarcoma) with an overall incidence of approximately 1.43 cases per million people. Despite significant progress in treating Ewing sarcoma, the prognosis remains poor for those with primary disseminated disease at diagnosis (up to 20 %), with an event-free survival of less than 20 % overall [120].

This malignancy is a member of the Ewing family of tumors (EFT) and is distinguished from most other primary tumors of bone by a characteristic translocation which most commonly (in approximately 85 % of cases) involves chromosomes 11 and 22 [121]. Other translocations have been identified in addition to that at t(11;22)—a minority of cases have variant translocations at t(21;22) or t(7;22) [122, 123]. All three of these translocations give rise to fusion genes comprising EWS and one of the several of its family members of transcription factors [124]. The most common fusion gene product formed is EWS-FLI1 which is expressed in 85–90 % of EFT [124, 125]. EWS/FLI1 forms a transcriptional complex with RNA helicase A (RHA) and this complex has been implicated in the pathogenesis of Ewing sarcoma [126].

Several miRNAs have altered expression levels in Ewing sarcoma, apparently due to changes induced by EWS/FLI1. These include *let-7a, miR-22, miR-23a, miR-31, miR-34a, miR-92a, miR-125b, miR-126, miR-130b, miR-145, miR-490-3p,* and *miR-708. Let-7a* is a direct EWS-FLI-1 target implicated in EFT cell tumorigenicity through its effect on expression of the target oncogene HMGA2 [127]. Systemic delivery of *let-7a* into EFT bearing mice restored its expression in tumor cells, decreased HMGA2 expression levels, and resulted in EFT growth inhibition in vivo [127].

MiR-22 is also repressed by the EWS/FLI1 gene fusion product. Its targets include lysine-specific demethylase 3A (KDM3A), a chromatin modifier whose demethylase activity in the promoter regions tends to enhance gene expression. Induced *miR-22* overexpression in Ewing sarcoma cell lines resulted in inhibition of colony formation and lower expression levels of KDM3A [128]. *MiR-31* was shown to reduce proliferation, decrease invasiveness, and induce apoptosis in several Ewing sarcoma cell lines, although the precise mechanisms of action are yet to be fully elucidated [129].

Microarray analysis of samples from 49 patients with primary Ewing sarcoma identified a signature of 5 miRNAs (*miR-23a, miR-34a, miR-92a, miR-130b,* and *miR-490-3p*) as an independent predictor of risk for disease progression and survival [112]. Furthermore, *miR-34a* expression was significantly associated with both event-free and overall survival in this study. Those patients who had the highest expression levels of *miR-34a* experienced no adverse events in 5 years, in contrast to those with the lowest expression levels who had disease recurrence within 2 years [112]. Further functional analysis of *miR-34a* in EWS cell lines indicated that when *miR-34a* expression was enforced, cells were less proliferative and showed greater sensitivity to doxorubicin and vincristine [112]. This is consistent with a subsequent study which also found that higher expression of *miR-34a* in localized Ewing sarcoma tumors was significantly related to better event-free and overall survival. Expression of *miR34a* was also notably lower in metastatic lesions than in primary tumors [130]. *miR-34a* expression was inversely correlated with expression of cyclin D1 and Ki-67. Ki-67 has previously been shown to be a poor prognostic indicator in localized Ewing sarcoma [130]. Cyclin D1 has a role in regulation of the cell cycle but is also involved in induction of cellular migration and invasion, enhancement of angiogenesis, inhibition of mitochondrial metabolism, and enhancement of DNA damage sensing and DNA damage repair [131].

MiR-125b expression is reduced in Ewing sarcoma; however, in vitro restoration of *miR-125b* expression in the A673 cell line significantly inhibited cell proliferation, migration, and invasion. It also arrested cell-cycle progression and induced cell apoptosis. These effects appear to be mediated via suppression of phosphoinositide-3-kinase catalytic subunit delta (PIK3CD) which downregulates expression of phospho-AKT and phospho-mTOR and inhibit cell-cycle progression [132].

Unlike most of the other miRNAs investigated so far in relation to Ewing sarcoma, *miR-126* has significantly increased expression levels in Ewing sarcoma tissue samples when compared to MSC [129]. Its precise role in Ewing sarcoma pathology is not yet fully explored.

MiR-145 is significantly reduced in Ewing sarcoma cell lines; however, its expression is increased on knockdown of EWS-FLI1. In the same study, ectopic expression of *miR-145* in Ewing sarcoma cell lines strongly reduced EWS-FLI1 protein levels and transfection of an anti-miR to *miR-145* increased the EWS-FLI1 levels. This modulation of EWS-FLI1 protein was mediated by *miR-145* targeting the FLI1 3′ UTR. Forced *miR-145* expression halted the Ewing sarcoma cell line growth [133].

Table 11.4 Some of the miRNAs shown to be underexpressed in Ewing sarcoma—the role of each miRNA has not yet been fully elucidated

miRNA	Role and target gene (if known)
let-7a family	Increased expression of HMGA2 oncogene promotes cell growth in EFT [127]
miR-22	Repression by EWS/FLI1 enhances gene expression through KDM3A demethylase activity [128]
miR-30a-5p	Binds to CD99 3′ UTR decreasing cell proliferation and invasion [135]
miR-31	Reduces proliferation and increases apoptosis [129]
miR-34a	Reduces cell proliferation and increases sensitivity to doxorubicin (targets cyclin D1 and Ki-67) [112, 130, 131]
miR-125b	Reduces proliferation, migration and invasion, induces apoptosis [132]
miR-145	Negative feedback control of EWS-FL1 [133, 136]
miR-708	Regulation of DNA repair and transcription via *EYA3* gene [134]

The DNA repair protein and transcriptional cofactor, eyes absent homolog 3 (EYA3), is highly expressed in Ewing sarcoma cell lines compared with MSC and it is regulated by the EWS/FLI1 fusion protein transcription factor via repression of *miR-708,* a microRNA that targets the 3′ UTR of EYA3 [134]. EYA3 expression levels show an inverse relationship with *miR-708* expression in Ewing sarcoma tissue samples and are associated with chemoresistance [134].

Table 11.4 lists several miRNAs which are significantly underexpressed in Ewing sarcoma.

Other Primary Bone Tumors

As stated previously, published research to date in the area of miRNAs and primary bone tumors focuses mainly on osteosarcoma and Ewing sarcoma. In relation to other types of primary bone tumors, there is a paucity of information regarding the role of miRNAs, with a relatively small number of studies relating to chondrosarcoma and GCT of bone and no significant body of knowledge regarding other more rare tumors. However, given the exponential rate of growth in miRNA-based research in recent years, this deficit is likely to be addressed in the future.

Chondrosarcoma

Chondrosarcoma is the second most common primary bone malignancy overall and represents approximately 20 % of malignant primary tumors of bone. Unlike osteosarcoma and Ewing sarcoma, it mainly affects adults and is relatively rare in children

and adolescents [137]. The best indicator of prognosis in chondrosarcoma is the histological grade, with high-grade tumors predictably having worse outcomes [121]. Surgical resection forms the mainstay of current chondrosarcoma treatment as these tumors are resistant to both radiation and standard chemotherapy regimes [138].

At the time of writing, there are only a very small number of published studies investigating microRNA expression in chondrosarcoma. These did identify alterations in miRNA expression, thus it is possible that the role of miRNAs in chondrosarcoma is as extensive as for other malignancies; however, the dearth of evidence in this regard makes this somewhat speculative. The following miRNAs have been shown to be significantly dysregulated in chondrosarcoma: *let-7a, miR-20a, miR-96, miR-100, miR-125b, miR-136, miR-183, miR-192, miR-199a, miR-222, miR-335, miR-376a, miR-490-3p, miR-509-3p, miR-518b, miR-550,* and *miR-589*. Potential targets and mechanisms of action in chondrosarcoma have not yet been established for all of these miRNAs. Some, such as the *let-7a* family, are not unique to chondrosarcoma and are dysregulated in many malignancies.

One study found that several miRNAs (*let-7a, miR-100, miR-136, miR-222, miR-335,* and *miR-376a*) had significantly reduced expression levels in chondrosarcoma tissue samples from 20 patients and chondrosarcoma cell lines when compared to expression in normal chondrocytes. In addition, *miR-96* and *miR-183* showed increased expression in the chondrosarcoma tissue samples [139]. A separate study also identified *miR-100* as a tumor suppressor that is downregulated in chondrosarcoma cells and human chondrosarcoma tissues [140]. In addition, mTOR (involved in control of protein synthesis) was identified as a direct target for inhibition by *miR-100* and cisplatin-resistant chondrosarcoma cells were shown to have lower expression levels of *miR-100*. Conversely, forced overexpression of *miR-100* inhibited mTOR and sensitized chondrosarcoma cells to cisplatin [140]. This finding may point to a future means of expanding the treatment options for patients with chondrosarcoma, given the limited sensitivity to current chemotherapy and radiation regimes.

MiR-199a has also been found to be reduced in human chondrosarcoma cells. It is thought to be suppressed by the chemokine CCL5 and this suppression promotes vascular endothelial growth factor (VEGF) expression and induces angiogenesis in human chondrosarcoma cells [141]. *miR-518b* expression is increased in human chondrosarcoma cell lines, with associated downregulation of the anti-apoptotic protein Bcl-2 and upregulation of the pro-apoptotic protein Bax [142].

Finally, human chondrosarcoma cell lines treated with antiproliferative proline-rich polypeptide (PRP-1), an inhibitor of mTORC1, showed a significant upregulation in the expression of three tumor suppressor miRNAs (*miR-20a, miR-125b,* and *miR-192*) and downregulation of four oncomiRs (*miR-490-3p, miR-509-3p, miR-550,* and *miR-589*) [143].

When considered together, these studies are suggestive of a role for miRNAs in chondrosarcoma; however, at this stage there is insufficient information for a direct clinical application.

Multiple Osteochondromatosis

Multiple osteochondromatosis (MO) is an autosomal dominant disease characterized by the growth of multiple osteochondromas. Although benign, they have the potential to undergo malignant transformation into peripheral chondrosarcomas, estimated to occur in 1–5 % of patients [144]. There is currently insufficient evidence to determine the role of miRNAs in this pathological process; however, one study did investigate miRNA expression in cartilage samples from 19 unrelated MO patients and 4 controls (undergoing surgery for reasons unrelated to MO). This showed a range of differentially expressed miRNAs on microarray, with a signature of eight miRNAs (*miR-21, miR-140, miR-145, miR-214, miR-195, miR-199a, miR-451,* and *miR-483*) whose combined expression could distinguish between MO samples and control growth plate samples [145]. However, this is a small study and the only one in the area, therefore the role of miRNA expression in MO is largely unknown.

Giant Cell Tumor of Bone

Giant cell tumor of bone (GCTB) is a benign lesion; however, it may be locally aggressive and can occasionally metastasize to the lungs [146]. GCTB constitutes approximately 5 % of all primary bone tumors and is most commonly located in the metaphyseal region of long bones. These tumors induce expansive osteolytic defects associated with bone destruction and consist histologically of multinucleated giant cells, histiocytes, and fibroblast-like stromal cells. These giant cell tumor stromal cells (GCTSC) are thought to develop from MSC (MSC) and are the neoplastic components of GCTB. They play a role in localized osteolysis by secreting cytokines and matrix metalloproteinases (MMPs), particularly high levels of MMP-13 which acts as an interstitial collagenase and influences osteoclast differentiation and bone resorption [147–149].

To date there are very few studies investigating a possible relationship between miRNAs and GCTB. One small study examined tissue samples from 10 GCTB patients, half of whom developed lung metastases and demonstrated a reduced level of *miR-136* in tissues taken from patients with metastases compared to those with non-metastatic tumors. In addition, *miR-136* expression was shown to be inversely related to the expression of nuclear factor 1B (NF1B) in the primary tumor tissues that developed metastases. NF1B is a predicted target gene of *miR-136* [150].

A second study found a significant reduction in the expression of *miR-126-5p* in GCTSCs compared to MSCs. *miR-126-5p* was also shown to repress MMP-13 at the posttranscriptional level thus loss of this control is likely to facilitate GCTB expansion [151].

In addition, *miR-127-3p, miR-136, miR-376a, miR-376c,* and *miR-377* are all underexpressed in cultured GCTSCs compared to MSCs. Interestingly, all five of these miRNAs are encoded within the Dlk1-Dio3 locus on chromosome 14 [152].

Clearly further studies are required before these findings can be extrapolated into clinically useful information.

Metastatic Bone Tumors

Malignant tumors involving bone are most commonly metastases from a variety of carcinomas. The most frequent malignancies involved are prostate, breast, and lung cancer, however renal cell carcinoma, thyroid carcinoma, and melanoma are also common [1].

MiRNAs were first linked to metastatic disease in 2007 when *miR-10b* was shown to initiate breast cancer invasion and metastasis [153]. Following this, a miRNA hypermethylation profile characteristic of human metastasis was identified. This was highly suggestive of DNA methylation-associated silencing of tumor-suppressor miRNAs as a plausible mechanism for the development of human cancer metastasis [154]. This study used colorectal cancer (SW620), melanoma (IGR37) and head and neck cancer (SIHN-011B) cell lines derived from lymph node metastases. The authors showed that reintroduction of *miR-148a, miR-34b,* and *miR-34c* into cancer cells with epigenetic inactivation inhibited motility, tumor growth, and metastasis formation in xenograft models, with associated downregulation of miRNA oncogenic target genes such as c-Myc, E2F3, cyclin-dependent kinase 6 (CDK6), and TGIF2 [154]. Additionally, *miR-224* overexpression is thought to promote metastasis of human breast cancer cells to bone via inhibition of the Raf kinase inhibitor protein (RKIP), which normally functions as a tumor suppressor [155].

A Chinese study using human small cell lung cancer cells in mouse xenograft models found that levels of *miR-335* and *mir-29a* were reduced in SBC-5 cells (which metastasize to bone) compared to SBC-3 cells (which do not), as were RANKL and IGF-1R (key mediators in bone metastases) [156]. This study also demonstrated that overexpression of *miR-335* in SBC-5 cells significantly reduced cell migration, invasion, proliferation, colony formation, and osteoclast induction in vitro [156]. Overexpression of *miR-335* reduced RANKL and IGF-1R expression [156]. Loss of *miR-335* was associated with metastatic osteolytic skeletal lesions [156]. Expression of *miR-33a* is significantly reduced in lung cancer and underexpression may be associated with bone metastasis as a result of increased activity of parathyroid hormone-related protein (PTHrP) and the resultant osteoclastogenesis [157].

A crucial step in the development of tumor cell metastases is the epithelial–mesenchymal transition (EMT). The EMT process is regulated by several factors, including human enhancer of filamentin 1 (HEF1). HEF1 promotes EMT, thus encouraging migration, invasion, and metastases. It is negatively regulated by binding of *miR-145* to its 3′ UTR which serves to reduce HEF1 expression levels [158]. HEF1 levels show a negative correlation with *miR-145* in prostate cancer and are higher in those with bone metastases, higher prostate-specific antigen (PSA) levels or higher Gleason grades [158]. *MiR-143* has also been associated with bone

metastasis of prostate cancer and may also be involved in the regulation of EMT [159]. *MiR-203* acts as an “anti-metastatic” miRNA in prostate cancer through regulation of pro-metastatic genes including zinc finger E-box binding homeobox 2 (ZEB2), Bmi, survivin, and bone-specific effectors including Runx2. Expression is attenuated in metastatic prostate cancer and reintroduction or overexpression in cell lines has been shown to suppress metastases [160, 161].

Potential Clinical Applications of miRNAs in Bone Cancer

There are no currently available miRNA-based diagnostic tests or therapeutic interventions for management of bone cancers. However, this is a rapidly expanding area of research with immense potential for useful clinical application in the future.

MiRNAs are readily detectable in cell lines and in fresh frozen or FFPE tissues [162]. Although the majority of published work focuses on tissues and cell lines, recent evidence indicates that they are also resistant to degradation by endogenous circulating ribonucleases and hence stable in circulation, thus readily quantifiable in plasma, serum, and whole blood [116]. As outlined in the osteosarcoma section of this chapter, circulating miRNAs do appear to reflect the pathological changes in miRNA profiles within tissues. Thus, utilizing miRNA-based blood testing for diagnosis or monitoring the response to treatment may be a potentially significant advance. miRNA profiling may also have a future role in identifying those individuals who are most likely to respond to chemotherapy, thus improving patient selection for treatments which frequently have considerable adverse effects.

Future work on miRNA pathways as therapeutic targets may also allow development of chemotherapy regimes that are more specifically tailored to the disease process under treatment. Potential therapeutic options include means of reducing the expression of pro-oncogenic miRNAs, such as miRNA silencing, antisense blocking, and miRNA modifications. Alternatively, inducing higher levels of those miRNAs known to have a tumor suppressor function may be effective in limiting tumor growth or metastasis.

Use of miRNA signatures to ensure targeted delivery of treatment is an area that shows promise. A recently published study investigated the use of the miRNA response elements (MREs) of *miR-34* and *miR-122* to enable selective expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by adenoviral vectors in osteosarcoma cells. The main issue currently limiting the application of TRAIL gene therapy is its lack of selectivity for tumor cells. However, adenovirus (Ad)-TRAIL-34-122, which expressed TRAIL in a *miR-34* and *miR-122*-regulated manner, was shown to selectively express TRAIL in the osteosarcoma cells. Higher levels of apoptosis and cytotoxicity were also detected in the osteosarcoma cells, compared with the normal cells and subsequent animal experiments suggested that Ad-TRAIL-34-122 reduced the growth of osteosarcoma xenografts without significant liver toxicity [163]. This may lead to future clinical benefits from miRNA-based therapies for patients with osteosarcoma and other tumors.

This is an exciting new area of cancer research and management with immense potential for diagnostic and therapeutic advances. However, despite this enormous potential, thorough validation and evaluation of unwanted or unforeseen side effects is essential for any novel therapy based on miRNA biology.

References

1. Ibrahim T, Mercatali L, Amadori D. Bone and cancer: the osteoncology. Clin Cases Miner Bone Metab. 2013;10(2):121–3.
2. 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):843–54.
3. miRBase 21. Manchester: University of Manchester; 2014. http://www.mirbase.org/index.shtml. Accessed June 2014 to 22nd Dec 2014
4. Griffiths-Jones S. The microRNA registry. Nucleic Acids Res. 2004;32(Database issue): D109–11.
5. Griffiths-Jones S. miRBase: the microRNA sequence database. Methods Mol Biol. 2006;342:129–38.
6. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res. 2006;34(Database issue):D140–4.
7. Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res. 2011;39(Database issue):D152–7.
8. Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res. 2014;42(Database issue):D68–73.
9. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet. 2004;5(7):522–31.
10. Valencia-Sanchez MA, Liu J, Hannon GJ, Parker R. Control of translation and mRNA degradation by miRNAs and siRNAs. Genes Dev. 2006;20(5):515–24.
11. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281–97.
12. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. Nat Cell Biol. 2009;11(3):228–34.
13. Hammond SM, Caudy AA, Hannon GJ. Post-transcriptional gene silencing by double-stranded RNA. Nat Rev Genet. 2001;2(2):110–9.
14. Mirnezami AH, Pickard K, Zhang L, Primrose JN, Packham G. MicroRNAs: key players in carcinogenesis and novel therapeutic targets. Eur J Surg Oncol. 2009;35(4):339–47.
15. Nugent M. MicroRNA function and dysregulation in bone tumors: the evidence to date. Cancer Manag Res. 2014;6:15–25.
16. Bae Y, Yang T, Zeng HC, Campeau PM, Chen Y, Bertin T, et al. miRNA-34c regulates Notch signaling during bone development. Hum Mol Genet. 2012;21(13):2991–3000.
17. Krzeszinski JY, Wei W, Huynh H, Jin Z, Wang X, Chang TC, et al. miR-34a blocks osteoporosis and bone metastasis by inhibiting osteoclastogenesis and Tgif2. Nature. 2014;512(7515): 431–5.
18. Kim K, Kim JH, Kim I, Lee J, Seong S, Park YW, et al. MicroRNA-26a Regulates RANKL-Induced Osteoclast Formation. Mol Cells. 2015;38(1):75–80.
19. Li Z, Hassan MQ, Volinia S, van Wijnen AJ, Stein JL, Croce CM, et al. A microRNA signature for a BMP2-induced osteoblast lineage commitment program. Proc Natl Acad Sci U S A. 2008;105(37):13906–11.
20. Kureel J, Dixit M, Tyagi AM, Mansoori MN, Srivastava K, Raghuvanshi A, et al. miR-542-3p suppresses osteoblast cell proliferation and differentiation, targets BMP-7 signaling and inhibits bone formation. Cell Death Dis. 2014;5:e1050.

21. Li S, Fu H, Wang Y, Tie Y, Xing R, Zhu J, et al. MicroRNA-101 regulates expression of the v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS) oncogene in human hepatocellular carcinoma. Hepatology. 2009;49(4):1194–202.
22. Slaby O, Svoboda M, Michalek J, Vyzula R. MicroRNAs in colorectal cancer: translation of molecular biology into clinical application. Mol Cancer. 2009;8:102.
23. Rossi S, Kopetz S, Davuluri R, Hamilton SR, Calin GA. MicroRNAs, ultraconserved genes and colorectal cancers. Int J Biochem Cell Biol. 2010;42(8):1291–7.
24. Wijnhoven BP, Michael MZ, Watson DI. MicroRNAs and cancer. Br J Surg. 2007;94(1):23–30.
25. Kobayashi E, Hornicek FJ, Duan Z. MicroRNA involvement in osteosarcoma. Sarcoma. 2012;2012:359739.
26. Ottaviani G, Jaffe N. The epidemiology of osteosarcoma. Cancer Treat Res. 2009;152:3–13.
27. Tang N, Song WX, Luo J, Haydon RC, He TC. Osteosarcoma development and stem cell differentiation. Clin Orthop Relat Res. 2008;466(9):2114–30.
28. Bielack SS, Kempf-Bielack B, Delling G, Exner GU, Flege S, Helmke K, et al. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. J Clin Oncol. 2002;20(3):776–90.
29. Geller DS, Gorlick R. Osteosarcoma: a review of diagnosis, management, and treatment strategies. Clin Adv Hematol Oncol. 2010;8(10):705–18.
30. Wang Z, Li Y, Banerjee S, Sarkar FH. Emerging role of Notch in stem cells and cancer. Cancer Lett. 2009;279(1):8–12.
31. Wang Z, Li Y, Kong D, Ahmad A, Banerjee S, Sarkar FH. Cross-talk between miRNA and Notch signaling pathways in tumor development and progression. Cancer Lett. 2010;292(2):141–8.
32. He C, Xiong J, Xu X, Lu W, Liu L, Xiao D, et al. Functional elucidation of MiR-34 in osteosarcoma cells and primary tumor samples. Biochem Biophys Res Commun. 2009;388(1):35–40.
33. He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, et al. A microRNA component of the p53 tumour suppressor network. Nature. 2007;447(7148):1130–4.
34. Li J, Liu W, Zhao K, Zhang Y, Li X, Yang Q, et al. Diallyl trisulfide reverses drug resistance and lowers the ratio of CD133+ cells in conjunction with methotrexate in a human osteosarcoma drug-resistant cell subline. Mol Med Rep. 2009;2(2):245–52.
35. Li Y, Zhang J, Zhang L, Si M, Yin H, Li J. Diallyl trisulfide inhibits proliferation, invasion and angiogenesis of osteosarcoma cells by switching on suppressor microRNAs and inactivating of Notch-1 signaling. Carcinogenesis. 2013;34(7):1601–10.
36. Song L, Yang J, Duan P, Xu J, Luo X, Luo F, et al. MicroRNA-24 inhibits osteosarcoma cell proliferation both in vitro and in vivo by targeting LPAATbeta. Arch Biochem Biophys. 2013;535(2):128–35.
37. Annunziata M, Granata R, Ghigo E. The IGF system. Acta Diabetol. 2011;48(1):1–9.
38. Chen L, Wang Q, Wang GD, Wang HS, Huang Y, Liu XM, et al. miR-16 inhibits cell proliferation by targeting IGF1R and the Raf1-MEK1/2-ERK1/2 pathway in osteosarcoma. FEBS Lett. 2013;587(9):1366–72.
39. Maire G, Martin JW, Yoshimoto M, Chilton-MacNeill S, Zielenska M, Squire JA. Analysis of miRNA-gene expression-genomic profiles reveals complex mechanisms of microRNA deregulation in osteosarcoma. Cancer Genet. 2011;204(3):138–46.
40. Thayanithy V, Sarver AL, Kartha RV, Li L, Angstadt AY, Breen M, et al. Perturbation of 14q32 miRNAs-cMYC gene network in osteosarcoma. Bone. 2012;50(1):171–81.
41. Xiang J, Wu J. Feud or friend? The role of the miR-17-92 cluster in tumorigenesis. Curr Genomics. 2010;11(2):129–35.
42. Zhou M, Ma J, Chen S, Chen X, Yu X. MicroRNA-17-92 cluster regulates osteoblast proliferation and differentiation. Endocrine. 2014;45(2):302–10.

43. Arabi L, Gsponer JR, Smida J, Nathrath M, Perrina V, Jundt G, et al. Upregulation of the miR-17-92 cluster and its two paraloga in osteosarcoma—reasons and consequences. Genes Cancer. 2014;5(1–2):56–63.
44. Xu SH, Yang YL, Han SM, Wu ZH. MicroRNA-9 expression is a prognostic biomarker in patients with osteosarcoma. World J Surg Oncol. 2014;12:195.
45. Ziyan W, Shuhua Y, Xiufang W, Xiaoyun L. MicroRNA-21 is involved in osteosarcoma cell invasion and migration. Med Oncol. 2011;28(4):1469–74.
46. Ouyang L, Liu P, Yang S, Ye S, Xu W, Liu X. A three-plasma miRNA signature serves as novel biomarkers for osteosarcoma. Med Oncol. 2013;30(1):340.
47. Montanini L, Lasagna L, Barili V, Jonstrup SP, Murgia A, Pazzaglia L, et al. MicroRNA cloning and sequencing in osteosarcoma cell lines: differential role of miR-93. Cell Oncol (Dordr). 2012;35(1):29–41.
48. Tian Z, Guo B, Yu M, Wang C, Zhang H, Liang Q, et al. Upregulation of micro-ribonucleic acid-128 cooperating with downregulation of PTEN confers metastatic potential and unfavorable prognosis in patients with primary osteosarcoma. Onco Targets Ther. 2014;7:1601–8.
49. Lauvrak SU, Munthe E, Kresse SH, Stratford EW, Namlos HM, Meza-Zepeda LA, et al. Functional characterisation of osteosarcoma cell lines and identification of mRNAs and miRNAs associated with aggressive cancer phenotypes. Br J Cancer. 2013;109(8):2228–36.
50. Lulla RR, Costa FF, Bischof JM, Chou PM, de F Bonaldo M, Vanin EF, et al. Identification of differentially expressed microRNAs in osteosarcoma. Sarcoma. 2011;2011:732690.
51. Jones KB, Salah Z, Del Mare S, Galasso M, Gaudio E, Nuovo GJ, et al. miRNA signatures associate with pathogenesis and progression of osteosarcoma. Cancer Res. 2012;72(7):1865–77.
52. Won KY, Kim YW, Kim HS, Lee SK, Jung WW, Park YK. MicroRNA-199b-5p is involved in the Notch signaling pathway in osteosarcoma. Hum Pathol. 2013;44(8):1648–55.
53. Cai H, Lin L, Tang M, Wang Z. Prognostic evaluation of microRNA-210 expression in pediatric osteosarcoma. Med Oncol. 2013;30(2):499.
54. Xu Z, Wang T. miR-214 promotes the proliferation and invasion of osteosarcoma cells through direct suppression of LZTS1. Biochem Biophys Res Commun. 2014;449(2):190–5.
55. Zhao G, Cai C, Yang T, Qiu X, Liao B, Li W, et al. MicroRNA-221 induces cell survival and cisplatin resistance through PI3K/Akt pathway in human osteosarcoma. PLoS One. 2013;8(1):e53906.
56. Zhang W, Qian JX, Yi HL, Yang ZD, Wang CF, Chen JY, et al. The microRNA-29 plays a central role in osteosarcoma pathogenesis and progression. Mol Biol (Mosk). 2012;46(4):622–7.
57. Zhang K, Zhang C, Liu L, Zhou J. A key role of microRNA-29b in suppression of osteosarcoma cell proliferation and migration via modulation of VEGF. Int J Clin Exp Pathol. 2014;7(9):5701–8.
58. Baglio SR, Devescovi V, Granchi D, Baldini N. MicroRNA expression profiling of human bone marrow mesenchymal stem cells during osteogenic differentiation reveals Osterix regulation by miR-31. Gene. 2013;527(1):321–31.
59. Tian Y, Zhang YZ, Chen W. MicroRNA-199a-3p and microRNA-34a regulate apoptosis in human osteosarcoma cells. Biosci Rep. 2014;34(4).
60. Yan K, Gao J, Yang T, Ma Q, Qiu X, Fan Q, et al. MicroRNA-34a inhibits the proliferation and metastasis of osteosarcoma cells both in vitro and in vivo. PLoS One. 2012;7(3):e33778.
61. Tian Q, Jia J, Ling S, Liu Y, Yang S, Shao Z. A causal role for circulating miR-34b in osteosarcoma. Eur J Surg Oncol. 2014;40(1):67–72.
62. van der Deen M, Taipaleenmaki H, Zhang Y, Teplyuk NM, Gupta A, Cinghu S, et al. MicroRNA-34c inversely couples the biological functions of the runt-related transcription factor RUNX2 and the tumor suppressor p53 in osteosarcoma. J Biol Chem. 2013;288(29):21307–19.
63. Lin S, Shao NN, Fan L, Ma XC, Pu FF, Shao ZW. Effect of microRNA-101 on proliferation and apoptosis of human osteosarcoma cells by targeting mTOR. J Huazhong Univ Sci Technolog Med Sci. 2014;34(6):889–95.

64. Liu LH, Li H, Li JP, Zhong H, Zhang HC, Chen J, et al. miR-125b suppresses the proliferation and migration of osteosarcoma cells through down-regulation of STAT3. Biochem Biophys Res Commun. 2011;416(1–2):31–8.
65. Yang C, Hou C, Zhang H, Wang D, Ma Y, Zhang Y, et al. miR-126 functions as a tumor suppressor in osteosarcoma by targeting Sox2. Int J Mol Sci. 2014;15(1):423–37.
66. Yang J, Zhang W. New molecular insights into osteosarcoma targeted therapy. Curr Opin Oncol. 2013;25(4):398–406.
67. Yang J, Gao T, Tang J, Cai H, Lin L, Fu S. Loss of microRNA-132 predicts poor prognosis in patients with primary osteosarcoma. Mol Cell Biochem. 2013;381(1–2):9–15.
68. Ji F, Zhang H, Wang Y, Li M, Xu W, Kang Y, et al. MicroRNA-133a, downregulated in osteosarcoma, suppresses proliferation and promotes apoptosis by targeting Bcl-xL and Mcl-1. Bone. 2013;56(1):220–6.
69. Zhao H, Li M, Li L, Yang X, Lan G, Zhang Y. MiR-133b is down-regulated in human osteosarcoma and inhibits osteosarcoma cells proliferation, migration and invasion, and promotes apoptosis. PLoS One. 2013;8(12):e83571.
70. Liu Z, Zhang G, Li J, Liu J, Lv P. The tumor-suppressive microRNA-135b targets c-myc in osteosarcoma. PLoS One. 2014;9(7):e102621.
71. Tang M, Lin L, Cai H, Tang J, Zhou Z. MicroRNA-145 downregulation associates with advanced tumor progression and poor prognosis in patients suffering osteosarcoma. Onco Targets Ther. 2013;6:833–8.
72. Zhu J, Feng Y, Ke Z, Yang Z, Zhou J, Huang X, et al. Down-regulation of miR-183 promotes migration and invasion of osteosarcoma by targeting Ezrin. Am J Pathol. 2012;180(6):2440–51.
73. Duan Z, Choy E, Harmon D, Liu X, Susa M, Mankin H, et al. MicroRNA-199a-3p is downregulated in human osteosarcoma and regulates cell proliferation and migration. Mol Cancer Ther. 2011;10(8):1337–45.
74. Bao YP, Yi Y, Peng LL, Fang J, Liu KB, Li WZ, et al. Roles of microRNA-206 in osteosarcoma pathogenesis and progression. Asian Pac J Cancer Prev. 2013;14(6):3751–5.
75. Zhang H, Yin Z, Ning K, Wang L, Guo R, Ji Z. Prognostic value of microRNA-223/epithelial cell transforming sequence 2 signaling in patients with osteosarcoma. Hum Pathol. 2014;45(7):1430–6.
76. Cheng C, Chen ZQ, Shi XT. MicroRNA-320 inhibits osteosarcoma cells proliferation by directly targeting fatty acid synthase. Tumour Biol. 2014;35(5):4177–83.
77. Wang Y, Zhao W, Fu Q. miR-335 suppresses migration and invasion by targeting ROCK1 in osteosarcoma cells. Mol Cell Biochem. 2013;384(1–2):105–11.
78. Zhou X, Wei M, Wang W. MicroRNA-340 suppresses osteosarcoma tumor growth and metastasis by directly targeting ROCK1. Biochem Biophys Res Commun. 2013;437(4):653–8.
79. Long XH, Mao JH, Peng AF, Zhou Y, Huang SH, Liu ZL. Tumor suppressive microRNA-424 inhibits osteosarcoma cell migration and invasion via targeting fatty acid synthase. Exp Ther Med. 2013;5(4):1048–52.
80. Yuan J, Lang J, Liu C, Zhou K, Chen L, Liu Y. The expression and function of miRNA-451 in osteosarcoma. Med Oncol. 2015;32(1):324.
81. Kager L, Zoubek A, Potschger U, Kastner U, Flege S, Kempf-Bielack B, et al. Primary metastatic osteosarcoma: presentation and outcome of patients treated on neoadjuvant Cooperative Osteosarcoma Study Group protocols. J Clin Oncol. 2003;21(10):2011–8.
82. Kempf-Bielack B, Bielack SS, Jurgens H, Branscheid D, Berdel WE, Exner GU, et al. Osteosarcoma relapse after combined modality therapy: an analysis of unselected patients in the Cooperative Osteosarcoma Study Group (COSS). J Clin Oncol. 2005;23(3):559–68.
83. Bacci G, Rocca M, Salone M, Balladelli A, Ferrari S, Palmerini E, et al. High grade osteosarcoma of the extremities with lung metastases at presentation: treatment with neoadjuvant chemotherapy and simultaneous resection of primary and metastatic lesions. J Surg Oncol. 2008;98(6):415–20.

84. Lafleur EA, Koshkina NV, Stewart J, Jia SF, Worth LL, Duan X, et al. Increased Fas expression reduces the metastatic potential of human osteosarcoma cells. Clin Cancer Res. 2004;10(23):8114–9.
85. Worth LL, Lafleur EA, Jia SF, Kleinerman ES. Fas expression inversely correlates with metastatic potential in osteosarcoma cells. Oncol Rep. 2002;9(4):823–7.
86. Huang G, Nishimoto K, Zhou Z, Hughes D, Kleinerman ES. miR-20a encoded by the miR-17-92 cluster increases the metastatic potential of osteosarcoma cells by regulating Fas expression. Cancer Res. 2012;72(4):908–16.
87. 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. Mol Ther. 2011;19(6):1123–30.
88. Schofield AV, Bernard O. Rho-associated coiled-coil kinase (ROCK) signaling and disease. Crit Rev Biochem Mol Biol. 2013;48(4):301–16.
89. Lei P, Xie J, Wang L, Yang X, Dai Z, Hu Y. microRNA-145 inhibits osteosarcoma cell proliferation and invasion by targeting ROCK1. Mol Med Rep. 2014;10(1):155–60.
90. Cai H, Lin L, Tang M, Wang Z. Combined microRNA-340 and ROCK1 mRNA profiling predicts tumor progression and prognosis in pediatric osteosarcoma. Int J Mol Sci. 2014;15(1):560–73.
91. Song QC, Shi ZB, Zhang YT, Ji L, Wang KZ, Duan DP, et al. Downregulation of microRNA-26a is associated with metastatic potential and the poor prognosis of osteosarcoma patients. Oncol Rep. 2014;31(3):1263–70.
92. Simon JA, Lange CA. Roles of the EZH2 histone methyltransferase in cancer epigenetics. Mutat Res. 2008;647(1–2):21–9.
93. Pan W, Wang H, Jianwei R, Ye Z. MicroRNA-27a promotes proliferation, migration and invasion by targeting MAP2K4 in human osteosarcoma cells. Cell Physiol Biochem. 2014;33(2):402–12.
94. Lv H, Pei J, Liu H, Wang H, Liu J. A polymorphism site in the premiR34a coding region reduces miR34a expression and promotes osteosarcoma cell proliferation and migration. Mol Med Rep. 2014;10(6):2912–6.
95. Wang J, Xu G, Shen F, Kang Y. miR-132 targeting cyclin E1 suppresses cell proliferation in osteosarcoma cells. Tumour Biol. 2014;35(5):4859–65.
96. Zhao M, Huang J, Gui K, Xiong M, Cai G, Xu J, et al. The downregulation of miR-144 is associated with the growth and invasion of osteosarcoma cells through the regulation of TAGLN expression. Int J Mol Med. 2014;34(6):1565–72.
97. Zhao H, Guo M, Zhao G, Ma Q, Ma B, Qiu X, et al. miR-183 inhibits the metastasis of osteosarcoma via downregulation of the expression of Ezrin in F5M2 cells. Int J Mol Med. 2012;30(5):1013–20.
98. Shen L, Wang P, Yang J, Li X. MicroRNA-217 regulates WASF3 expression and suppresses tumor growth and metastasis in osteosarcoma. PLoS One. 2014;9(10):e109138.
99. Suetsugu S, Takenawa T. Regulation of cortical actin networks in cell migration. Int Rev Cytol. 2003;229:245–86.
100. Wang Z, Cai H, Lin L, Tang M. Upregulated expression of microRNA-214 is linked to tumor progression and adverse prognosis in pediatric osteosarcoma. Pediatr Blood Cancer. 2014;61(2):206–10.
101. Wang X, Sun J, Fu C, Wang D, Bi Z. MicroRNA-214 regulates osteosarcoma survival and growth by directly targeting phosphatase and tensin homolog. Mol Med Rep. 2014;10(6):3073–9.
102. Gougelet A, Pissaloux D, Besse A, Perez J, Duc A, Dutour A, et al. Micro-RNA profiles in osteosarcoma as a predictive tool for ifosfamide response. Int J Cancer. 2011;129(3):680–90.
103. Song B, Wang Y, Xi Y, Kudo K, Bruheim S, Botchkina GI, et al. Mechanism of chemoresistance mediated by miR-140 in human osteosarcoma and colon cancer cells. Oncogene. 2009;28(46):4065–74.

104. Guo S, Bai R, Liu W, Zhao A, Zhao Z, Wang Y, et al. miR-22 inhibits osteosarcoma cell proliferation and migration by targeting HMGB1 and inhibiting HMGB1-mediated autophagy. Tumour Biol. 2014;35(7):7025–34.
105. Entz-Werle N, Lavaux T, Metzger N, Stoetzel C, Lasthaus C, Marec P, et al. Involvement of MET/TWIST/APC combination or the potential role of ossification factors in pediatric high-grade osteosarcoma oncogenesis. Neoplasia. 2007;9(8):678–88.
106. Zhou Y, Zang X, Huang Z, Zhang C. TWIST interacts with endothelin-1/endothelin A receptor signaling in osteosarcoma cell survival against cisplatin. Oncol Lett. 2013;5(3):857–61.
107. Wu J, Liao Q, He H, Zhong D, Yin K. TWIST interacts with beta-catenin signaling on osteosarcoma cell survival against cisplatin. Mol Carcinog. 2014;53(6):440–6.
108. Zhou Y, Huang Z, Wu S, Zang X, Liu M, Shi J. miR-33a is up-regulated in chemoresistant osteosarcoma and promotes osteosarcoma cell resistance to cisplatin by down-regulating TWIST. J Exp Clin Cancer Res. 2014;33:12.
109. Mu Y, Zhang H, Che L, Li K. Clinical significance of microRNA-183/Ezrin axis in judging the prognosis of patients with osteosarcoma. Med Oncol. 2014;31(2):821.
110. Sarver AL, Thayanithy V, Scott MC, Cleton-Jansen AM, Hogendoorn PC, Modiano JF, et al. MicroRNAs at the human 14q32 locus have prognostic significance in osteosarcoma. Orphanet J Rare Dis. 2013;8:7.
111. Kelly AD, Haibe-Kains B, Janeway KA, Hill KE, Howe E, Goldsmith J, et al. MicroRNA paraffin-based studies in osteosarcoma reveal reproducible independent prognostic profiles at 14q32. Genome Med. 2013;5(1):2.
112. Nakatani F, Ferracin M, Manara MC, Ventura S, Del Monaco V, Ferrari S, et al. miR-34a predicts survival of Ewing's sarcoma patients and directly influences cell chemo-sensitivity and malignancy. J Pathol. 2012;226(5):796–805.
113. Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Kerin MJ. Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg. 2010;251(3):499–505.
114. Nugent M, Miller N, Kerin MJ. Circulating miR-34a levels are reduced in colorectal cancer. J Surg Oncol. 2012;106(8):947–52.
115. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 2008;18(10):997–1006.
116. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008;105(30):10513–8.
117. 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. J Int Med Res. 2012;40(6):2090–7.
118. Zhang C, Yao C, Li H, Wang G, He X. Combined elevation of microRNA-196a and microRNA-196b in sera predicts unfavorable prognosis in patients with osteosarcomas. Int J Mol Sci. 2014;15(4):6544–55.
119. Zhang C, Yao C, Li H, Wang G, He X. Serum levels of microRNA-133b and microRNA-206 expression predict prognosis in patients with osteosarcoma. Int J Clin Exp Pathol. 2014;7(7):4194–203.
120. Stahl M, Ranft A, Paulussen M, Bolling T, Vieth V, Bielack S, et al. Risk of recurrence and survival after relapse in patients with Ewing sarcoma. Pediatr Blood Cancer. 2011;57(4):549–53.
121. Schwab JH, Springfield DS, Raskin KA, Mankin HJ, Hornicek FJ. What's new in primary bone tumors. J Bone Joint Surg Am. 2012;94(20):1913–9.
122. Sorensen PH, Lessnick SL, Lopez-Terrada D, Liu XF, Triche TJ, Denny CT. A second Ewing's sarcoma translocation, t(21;22), fuses the EWS gene to another ETS-family transcription factor. ERG Nat Genet. 1994;6(2):146–51.
123. Jeon IS, Davis JN, Braun BS, Sublett JE, Roussel MF, Denny CT, et al. A variant Ewing's sarcoma translocation (7;22) fuses the EWS gene to the ETS gene ETV1. Oncogene. 1995;10(6):1229–34.

124. Riggi N, Stamenkovic I. The biology of Ewing sarcoma. Cancer Lett. 2007;254(1):1–10.
125. Kovar H. Downstream EWS/FLI1—upstream Ewing's sarcoma. Genome Med. 2010;2(1):8.
126. Kinsey M, Smith R, Iyer AK, McCabe ER, Lessnick SL. EWS/FLI and its downstream target NR0B1 interact directly to modulate transcription and oncogenesis in Ewing's sarcoma. Cancer Res. 2009;69(23):9047–55.
127. De Vito C, Riggi N, Suva ML, Janiszewska M, Horlbeck J, Baumer K, et al. Let-7a is a direct EWS-FLI-1 target implicated in Ewing's sarcoma development. PLoS One. 2011;6(8):e23592.
128. Parrish JK, Sechler M, Winn RA, Jedlicka P. The histone demethylase KDM3A is a microRNA-22-regulated tumor promoter in Ewing Sarcoma. Oncogene. 2013;34(2):257–62.
129. Karnuth B, Dedy N, Spieker T, Lawlor ER, Gattenlohner S, Ranft A, et al. Differentially expressed miRNAs in Ewing sarcoma compared to mesenchymal stem cells: low miR-31 expression with effects on proliferation and invasion. PLoS One. 2014;9(3):e93067.
130. Marino MT, Grilli A, Baricordi C, Manara MC, Ventura S, Pinca RS, et al. Prognostic significance of miR-34a in Ewing sarcoma is associated with cyclin D1 and ki-67 expression. Ann Oncol. 2014;25(10):2080–6.
131. Pestell RG. New roles of cyclin D1. Am J Pathol. 2013;183(1):3–9.
132. Li J, You T, Jing J. MiR-125b inhibits cell biological progression of Ewing's sarcoma by suppressing the PI3K/Akt signalling pathway. Cell Prolif. 2014;47(2):152–60.
133. Ban J, Jug G, Mestdagh P, Schwentner R, Kauer M, Aryee DN, et al. Hsa-mir-145 is the top EWS-FLI1-repressed microRNA involved in a positive feedback loop in Ewing's sarcoma. Oncogene. 2011;30(18):2173–80.
134. Robin TP, Smith A, McKinsey E, Reaves L, Jedlicka P, Ford HL. EWS/FLI1 regulates EYA3 in Ewing sarcoma via modulation of miRNA-708, resulting in increased cell survival and chemoresistance. Mol Cancer Res. 2012;10(8):1098–108.
135. Franzetti GA, Laud-Duval K, Bellanger D, Stern MH, Sastre-Garau X, Delattre O. MiR-30a-5p connects EWS-FLI1 and CD99, two major therapeutic targets in Ewing tumor. Oncogene. 2013;32(33):3915–21.
136. Riggi N, Suva ML, De Vito C, Provero P, Stehle JC, Baumer K, et al. EWS-FLI-1 modulates miRNA145 and SOX2 expression to initiate mesenchymal stem cell reprogramming toward Ewing sarcoma cancer stem cells. Genes Dev. 2010;24(9):916–32.
137. David E, Blanchard F, Heymann MF, De Pinieux G, Gouin F, Redini F, et al. The bone niche of chondrosarcoma: a sanctuary for drug resistance, tumour growth and also a source of new therapeutic targets. Sarcoma. 2011;2011:932451.
138. Onishi AC, Hincker AM, Lee FY. Surmounting chemotherapy and radioresistance in chondrosarcoma: molecular mechanisms and therapeutic targets. Sarcoma. 2011;2011:381564.
139. Yoshitaka T, Kawai A, Miyaki S, Numoto K, Kikuta K, Ozaki T, et al. Analysis of microRNAs expressions in chondrosarcoma. J Orthop Res. 2013;31(12):1992–8.
140. Zhu Z, Wang CP, Zhang YF, Nie L. MicroRNA-100 resensitizes resistant chondrosarcoma cells to cisplatin through direct targeting of mTOR. Asian Pac J Cancer Prev. 2014;15(2):917–23.
141. Liu G, Huang Y, Tzeng H, Tsai C, Wang S, Tang C. CCL5 promotes vascular endothelial growth factor expression and induces angiogenesis by down-regulating miR-199a in human chondrosarcoma cells. Cancer Lett. 2014;357(2):476–87.
142. Liang W, Li X, Li Y, Li C, Gao B, Gan H, et al. Gallic acid induces apoptosis and inhibits cell migration by upregulating miR-518b in SW1353 human chondrosarcoma cells. Int J Oncol. 2014;44(1):91–8.
143. Galoian KA, Guettouche T, Issac B, Qureshi A, Temple HT. Regulation of onco and tumor suppressor MiRNAs by mTORC1 inhibitor PRP-1 in human chondrosarcoma. Tumour Biol. 2013;35(3):2335–41.
144. Schmale GA, Conrad 3rd EU, Raskind WH. The natural history of hereditary multiple exostoses. J Bone Joint Surg Am. 1994;76(7):986–92.
145. Zuntini M, Salvatore M, Pedrini E, Parra A, Sgariglia F, Magrelli A, et al. MicroRNA profiling of multiple osteochondromas: identification of disease-specific and normal cartilage signatures. Clin Genet. 2010;78(6):507–16.

146. Raskin KA, Schwab JH, Mankin HJ, Springfield DS, Hornicek FJ. Giant cell tumor of bone. J Am Acad Orthop Surg. 2013;21(2):118–26.
147. Ghert M, Simunovic N, Cowan RW, Colterjohn N, Singh G. Properties of the stromal cell in giant cell tumor of bone. Clin Orthop Relat Res. 2007;459:8–13.
148. Cowan RW, Mak IW, Colterjohn N, Singh G, Ghert M. Collagenase expression and activity in the stromal cells from giant cell tumour of bone. Bone. 2009;44(5):865–71.
149. Mak IW, Cowan RW, Popovic S, Colterjohn N, Singh G, Ghert M. Upregulation of MMP-13 via Runx2 in the stromal cell of giant cell tumor of bone. Bone. 2009;45(2):377–86.
150. Mosakhani N, Pazzaglia L, Benassi MS, Borze I, Quattrini I, Picci P, et al. MicroRNA expression profiles in metastatic and non-metastatic giant cell tumor of bone. Histol Histopathol. 2013;28(5):671–8.
151. Wu Z, Yin H, Liu T, Yan W, Li Z, Chen J, et al. MiR-126-5p regulates osteoclast differentiation and bone resorption in giant cell tumor through inhibition of MMP-13. Biochem Biophys Res Commun. 2014;443(3):944–9.
152. Lehner B, Kunz P, Saehr H, Fellenberg J. Epigenetic silencing of genes and microRNAs within the imprinted Dlk1-Dio3 region at human chromosome 14.32 in giant cell tumor of bone. BMC Cancer. 2014;14:495.
153. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature. 2007;449(7163):682–8.
154. Lujambio A, Calin GA, Villanueva A, Ropero S, Sanchez-Cespedes M, Blanco D, et al. A microRNA DNA methylation signature for human cancer metastasis. Proc Natl Acad Sci U S A. 2008;105(36):13556–61.
155. Huang L, Dai T, Lin X, Zhao X, Chen X, Wang C, et al. MicroRNA-224 targets RKIP to control cell invasion and expression of metastasis genes in human breast cancer cells. Biochem Biophys Res Commun. 2012;425(2):127–33.
156. Gong M, Ma J, Guillemette R, Zhou M, Yang Y, Hock JM, et al. miR-335 inhibits small cell lung cancer bone metastases via IGF-1R and RANKL pathways. Mol Cancer Res. 2014;12(1):101–10.
157. Kuo PL, Liao SH, Hung JY, Huang MS, Hsu YL. MicroRNA-33a functions as a bone metastasis suppressor in lung cancer by targeting parathyroid hormone related protein. Biochim Biophys Acta. 2013;1830(6):3756–66.
158. Guo W, Ren D, Chen X, Tu X, Huang S, Wang M, et al. HEF1 promotes epithelial mesenchymal transition and bone invasion in prostate cancer under the regulation of microRNA-145. J Cell Biochem. 2013;114(7):1606–15.
159. Peng X, Guo W, Liu T, Wang X, Tu X, Xiong D, et al. Identification of miRs-143 and -145 that is associated with bone metastasis of prostate cancer and involved in the regulation of EMT. PLoS One. 2011;6(5):e20341.
160. Saini S, Majid S, Yamamura S, Tabatabai L, Suh SO, Shahryari V, et al. Regulatory role of mir-203 in prostate cancer progression and metastasis. Clin Cancer Res. 2011;17(16): 5287–98.
161. Viticchie G, Lena AM, Latina A, Formosa A, Gregersen LH, Lund AH, et al. MiR-203 controls proliferation, migration and invasive potential of prostate cancer cell lines. Cell Cycle. 2011;10(7):1121–31.
162. Meng W, McElroy JP, Volinia S, Palatini J, Warner S, Ayers LW, et al. Comparison of microRNA deep sequencing of matched formalin-fixed paraffin-embedded and fresh frozen cancer tissues. PLoS One. 2013;8(5):e64393.
163. Xiao F, Chen J, Lian C, Han P, Zhang C. Tumor necrosis factor related apoptosis inducing ligand induces cytotoxicity specific to osteosarcoma by microRNA response elements. Mol Med Rep. 2015;11(1):739–45.

ERRATUM

microRNA: Cancer

Gaetano Santulli

© Springer International Publishing Switzerland 2015
G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental
Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5

DOI 10.1007/978-3-319-23730-5_12

One of Dr. Santulli's affiliations was incorrect and the other was missing on the copyright page.

The incorrect affiliation reads:

Gaetano Santulli
Columbia University Medical Center
New York, NY, USA

The corrected and missing affiliations are given below:

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

"Federico II" University Hospital, Naples, Italy

The online version of the updated book can be found at
http://dx.doi.org/10.1007/978-3-319-23730-5

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

© Springer International Publishing Switzerland 2016
G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental
Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5_12

Index

A
Aberrant expression, 3, 7, 10, 204
Angiogenesis
  energy metabolism, 94
  genomic instability, 93
  hypoxia, 92
  immune system and inflammation, 94–95
  migration, invasion, and metastasis, 93
  stimulators and suppressors, 92

B
Bone cancer
  chondrosarcoma, 218–219
  clinical applications, 222, 223
  dysregulation, 202
  ewing sarcoma
    characteristic translocation, 216
    DNA repair protein, 218
    event-free survival, 216
    EWS-FLI-1 target, 216
    microarray analysis, 217
    miR-125b expression, 217
    miR-145, 217
    transcriptional cofactor, 218
  GCTB, 220, 221
  metastasis, 221–222
  multiple osteochondromatosis, 220
  osteogenesis, 202–203
  osteosarcoma
    Fas receptors, 208
    initial metastatic disease, 206–208
    miR-26a expression, 209
    miR-34a expression, 210
    miR-217, 211
    miR-132 expression, 210
    miR-133a expression, 210
    miR-135b expression, 210
    miR-144 expression, 210
    MSC, 204
    prognostic indicator, 211–215
    relapse/metastasis risk, 204
    ROCK1, 209
    survival rate, 204
    tissue samples, 210
    tumor markers, 215–216
    tumorigenesis, 204–206
Burkitt's lymphoma, 45

C
Cancer antigen 125 (CA125), 125, 126
Chondrosarcoma, 218–219
Chronic lymphocytic leukemia (CLL)
  clinical heterogeneity, 24
  genomic alterations, 24
  incidence, 23
  lymphatic tissues, 24
  malignant cells genome, 25
  miRNAs
    comprehensive profiling, 27
    deregulation, 26
    diagnostic and prognostic factors, 34–35
    disease onset/progression, 27
    experimental approaches, 27
    functions, 26
    hypoxia and angiogenesis, 32
    miR-155 and miR-150, 30
    miR-181, 28

© Springer International Publishing Switzerland 2015
G. Santulli (ed.), *microRNA: Cancer*, Advances in Experimental Medicine and Biology 889, DOI 10.1007/978-3-319-23730-5

Chronic lymphocytic leukemia (CLL) (*cont.*)
miR-29, 28
mouse models, 32–34
pathogenesis, 27, 30
proliferation and apoptosis, 31
RNA-inhibition therapy, 35–36
RNA polymerase II, 29
self-sufficiency, 32
signature profile, 27
3' UTR, 26
TLC1 functions, 28
translation repression and activation, 26
pathological pattern, 24
signaling pathways, 24
tumor-suppressor gene, 25
Colorectal cancer (CRC, 10
angiogenesis
energy metabolism, 94
genomic instability, 93
hypoxia, 92
immune system and inflammation, 94–95
migration, invasion, and metastasis, 93
stimulators and suppressors, 92
anti-miR-122 drug, 97
apoptosis, 91–92
cell proliferation, 90–91
clinical implications
argonaute2 protein, 95
biomarker profile, 96
blood-based diagnostic markers, 96
feces, 97
MiR-29a levels, 96
prognostic factors, 95
wash fluid, 96
drug resistance, 97
growth suppressors, 90–91
high mortality rates, 90
immortalization, 92
malignant transformation (*see* Malignant transformation)
Cutaneous T-cell lymphoma (CTCL), 46

E
Epithelial ovarian cancer (EOC). *See* Ovarian cancer
Epithelial to mesenchymal transition (EMT), 128. *See also* Prostate cancer
Ewing sarcoma
characteristic translocation, 216
DNA repair protein, 218
event-free survival, 216
EWS-FLI-1 target, 216
microarray analysis, 217
miR-125b expression, 217
miR-145, 217
transcriptional cofactor, 218

F
Fecal occult blood test (FOBT), 96
Fibroblast growth factor receptor 3 (FGFR3) expression, 134
Follicular lymphoma (FL), 46

G
Gastrointestinal stromal tumor (GIST)
chromosomal changes, 53
deregulation
chromosomal alterations, 60–61
clinical and pathological features, 58–60
clinical implications, 62–63
genetic alterations, 60–61
imatinib resistance, 61–62
KIT and ETV1, 58
ICC function, 52
oncogenic mutations, 52–53
treatment, 53–55
Giant cell tumor of bone (GCTB), 220, 221

H
High-grade serous ovarian cancer (HGSOC), 128
High-mobility group box 1 (HMGB1) gene, 212

I
Interstitial cells of Cajal (ICC) function, 52

L
Leiomyosarcoma (LMS), 181, 185
Lung cancer
biomarkers
ADC *vs*. noncancerous tissues, 159
Ambion microarrays, 159
Croce lab, 159
diagnostic efficacy, 162
evaluation, 162
noncancerous lung tissue, 159
primary lung tumors, 162
SCC *vs*. noncancerous tissues, 159
types, 162

cancer-associated genomic regions, 154
chemotherapy resistance, 156–157
miRNAs
brain metastases, 165
classifiers, 166
clinicopathologic characteristics, 163
early detection, 165
frozen resected specimens, 163
healthy smokers, 166
miR-21 and miR-10b expression, 167
miR-34a expression, 163
miR-Test, 168
NGS, 166, 167
PDK1, 167
postoperative survival, 163
prognostic factor, 163
serum and tissue samples, 166
sputum, 165
tumor-specific exosomes, 166
oncogenic miR-17-92 cluster, 155
oncosuppressor agents, 155
radiotherapy resistance, 157, 158
SNP, 154
transcriptional silencing, 155
tumor suppressor protein p53, 156
Lysophosphatidic acid acyltransferase β (LPAATβ), 205

M
Malignant lymphoma
ALCL, 44
C13orf25, 42
classification, 41
DLBCL, 43
DLEU2, 42
dysregulation
B-cell lymphomas, 45
Burkitt's lymphoma, 45
CTCL, 46
discovery, 42–43
follicular lymphoma, 46
MCL, 45
MZLs, 46–47
NK/T-cell lymphoma/leukemia, 44
Malignant peripheral nerve sheath tumors (MPNSTs)
development, 9
downregulation, 10
gene expression analysis, 10
human dermal and plexiform neurofibromas, 9
neurofibromatosis type 1, 9
prostate cancer, 11–12
soft tissue sarcomas, 189, 190, 194
upregulation, 10
Malignant transformation
benign neoplasms, 1
colorectal cancer
adenoma formation, 5
benign polyp, 4
chromosomal instability, 5
clinical stages, 3
dysregulation, 3, 6
identification and validation targets, 4
mechanism, 5
microsatellite instability, 5
mutations, 3
oncogenic pathways, 6
MPNSTs, 9–10
pancreatic cancer
cause of, 6
early diagnosis, 8–9
early mutations, 6
histological features, 6
miR-155, 8
miR-21, 7
miR-221, 7
PanIN, 7
PanINs, 6
Mammalian target of rapamycin (mTOR), 138
Mantle cell lymphoma (MCL), 30, 45
Marginal zone lymphomas (MZLs), 46–47
Mesenchymal stem cells (MSC), 204
Multiple osteochondromatosis (MO), 220

N
Next-Generation Sequencing (NGS), 166

O
Ovarian cancer
biomarkers, 125, 126
causes, 121
diagnosis, 124
factors, 121
histological classification, 122–124
incidence, 120
microRNAs
ALK7 levels, 139
benign ovarian neoplasm, 134
chemosensitivity, 127
chemotherapy, 128
cisplatin, 137, 138, 140
E-cadherin, 128
EMT, 128
exosomes, 136, 137

Ovarian cancer (*cont.*)
- FGFR3 expression, 134
- healthy controls, 135
- HGSOC, 128
- miR-7, 129
- miR-9, 129
- miR-15a and miR-16, 129
- miR-21 expression, 132, 137
- miR-22, 129
- miR-25, 133
- miR-26a inhibition, 136
- miR-29b, 130
- miR-31 expressions, 140
- miR-34, 130
- miR-100, 131
- miR-124, 130
- miR-125b, 138
- miR 133a, 131
- miR-145, 131
- miR-148b, 132
- miR-152, 130
- miR-199a, 138
- miR-200 family, 127, 128
- miR-203, 132
- miR-378, 133
- miR-497, 130
- miR-508-3p, 132
- miR-509-5p, 132
- miR-510, 132
- mTOR, 138
- overexpressed microRNAs, 134
- paclitaxel-resistant cell lines, 138, 139
- platinum resistance, 138
- potent suppressor, 134
- presurgical plasma samples, 135
- primary surgical cytoreduction, 137
- prognosis, 126, 135
- relapse-free survival, 133
- RT-PCR, 134
- SEOC, 134
- taxol-resistant cells, 140
- taxol-resistant patients, 139
- tumor *vs*. normal tissues, 126

risk reduction, 121, 122
screening, 125
symptoms, 124
treatment, 124

P

Pancreatic cancer, 10
- chemoresistance, 77–79
- deregulation, 73–74
- diagnostic markers, 79–80
- initiation and promotion, 75–76
- malignant transformation (*see* Malignant transformation)
- metastasis, 76–77
- prognostic assessment, 80–81
- survival rate, 72
- therapeutic strategies, 81

Pancreatic intraepithelial neoplasias (PanINs), 6
Platinum-based chemotherapy, 124
Polycomb repressive complexes (PRC), 110
Prostate cancer
- aberrant tumor-stromal interactions
  - biochemical recurrence, 113
  - breast cancer-associated exosomes, 112, 113
  - cancer growth and metastasis, 111
  - lncRNAs, 113
  - miR-409 expression, 111, 112
  - potential biomarker and therapeutic targets, 113
  - secretory microvesicles, 112
- $ARCaP_M$ cells, 109
- DLK1-DIO3 cluster, 106, 107
- epigenetic silencing
  - miR-101, 111
  - miR-181a and miR-181b, 110
  - miR-200 cluster, 110
  - miR-203 expression, 110
  - PRC, 110
- malignant transformation, 11–12
- miR-145 and miR-143 expression, 109
- miR-154* expression, 109
- miR-379 expression, 109
- miR-409-3p expression, 107, 108
- VHL protein, 108

R

Real-time reverse polymerase reaction (RT-PCR), 134
Rho-associated, coiled-coil-containing protein kinase 1 (ROCK1), 209

S

Single nucleotide polymorphism (SNP), 154
Soft tissue sarcomas
- miRNA
  - angiosarcomas, 190
  - epithelioid sarcomas, 191, 193
  - fibrosarcomas, 191
  - liposarcoma, 186, 187
  - LMSs, 181, 185

MPNST, 189, 190, 194
mutations, 181
rhabdomyosarcoma, 187, 188, 193, 194
synovial sarcomas, 188, 189
undifferentiated/unclassified sarcomas, 185
treatment outcomes, 180

T
T-cell activation, 24, 55, 95
T-cell leukemia lymphoma 1 (TLC1) functions, 28

V
Von–Hippel–Lindau protein (VHL), 107

W
Wiskott Aldrich syndrome protein family member 3 (WASF3) gene, 211