Advances in Experimental Medicine and Biology 889

Gaetano Santulli *Editor*

microRNA: Cancer

From Molecular Biology to Clinical Practice

Springer

Advances in Experimental Medicine and Biology

Volume 889

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

Gaetano Santulli
Editor

microRNA: Cancer

From Molecular Biology to Clinical Practice

Springer

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

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

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

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

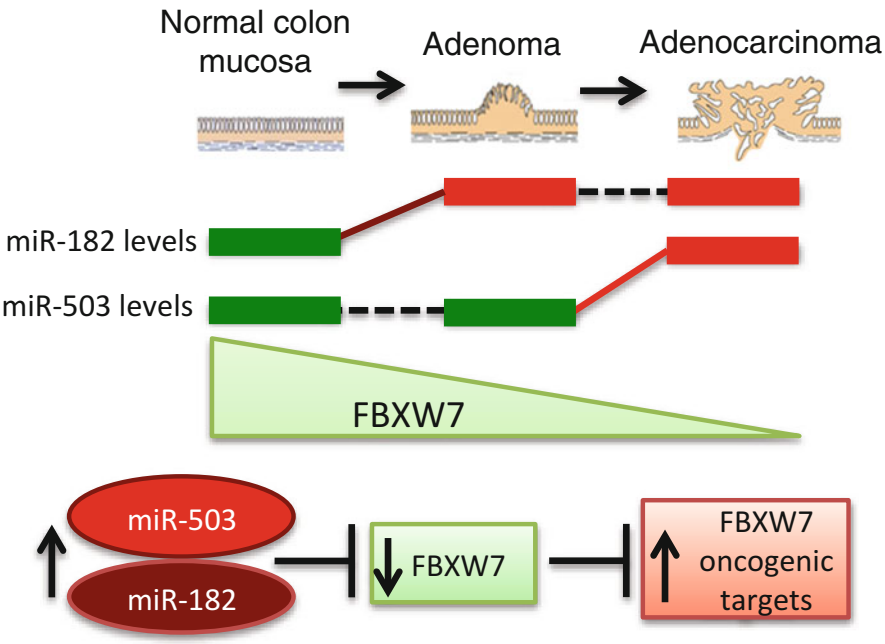

Fig. 1.1 Schematic representation of the stepwise expression of miR-182 and miR-503 levels during colon adenoma-to-adenocarcinoma progression and corresponding levels of FBWX7. Reprinted with permission from Li et al. [32]

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].

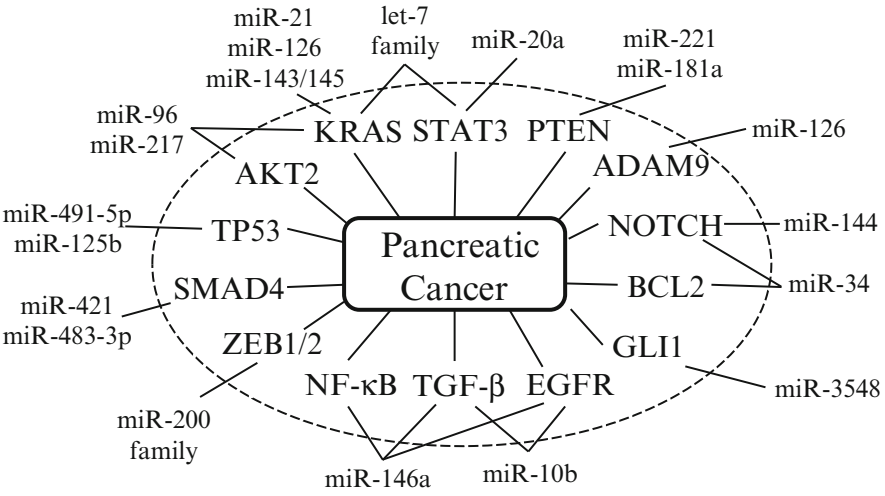

Fig. 1.2 Key dysregulated miRNA regulators of signaling pathways involved in the molecular pathogenesis of pancreatic ductal adenocarcinoma. Adapted from Chitkara et al. [82]

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 preexisting 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].

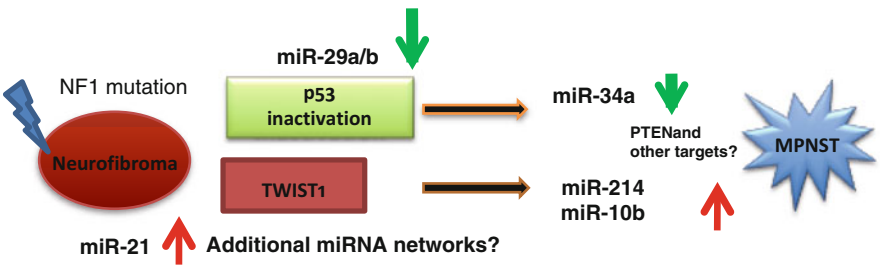

Fig. 1.3 miRNA gene networks dysregulated in the malignant transformation of neurofibroma to MPNSTs. Adapted from Subramanian and Kartha [23]

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.