

# Chapter 2

## MicroRNAs in Cancer Progression

Omozusi Andrews and James G. Patton

**Abstract** Tumor initiation and progression have been widely investigated and ongoing work implicates microRNAs (miRNAs) as central players. miRNAs can control proliferation and differentiation as well as apoptosis, consistent with miRNAs functioning as oncogenes or tumor suppressors. Specific miRNAs have been shown to play roles in the overlapping fields of cancer stem cells and chemoresistance. In this review, we summarize existing data to elucidate the role of miRNAs in tumorigenesis and potential strategies using miRNAs for cancer therapy or as biomarkers.

**Keywords** MicroRNA · Cancer · Anchorage independence · Angiogenesis · Antagomir · Biomarker · Chemotherapy · epithelial-to-mesenchymal transition (EMT) · Lymphoma · Metastasis · Oncogenes · Tumor suppressors

### 1 Cancer Overview

Cancer consists of numerous diseases characterized by misregulated and/or altered cell division. Most commonly, changes in cell cycle control arise from accumulated mutations leading to chromosomal instability, proliferation, and aggressive metastatic behavior. While some cancers harbor hereditary mutations such as colorectal cancers (Lynch syndrome with mutations in mismatch repair complex proteins) or breast cancers (mutation in BRCA1 and BRCA2 genes), the majority are not hereditary and instead arise from random somatic mutations (<http://www.cancer.gov/cancertopics/understandingcancer/genetesting/page27>). These mutations typically activate downstream pathways and share specific hallmarks as identified by Hanahan and Weinberg [1]. These hallmarks are defined as acquired functional capabilities that allow cancer cells to survive, proliferate, and disseminate. These shared characteristics include self-sufficiency in growth signals, insensitivity to antigrowth signals, the ability to evade apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion, and metastasis. Broadly, there are two defining features of cancers: upregulation of oncogenes and downregulation of tumor suppressor

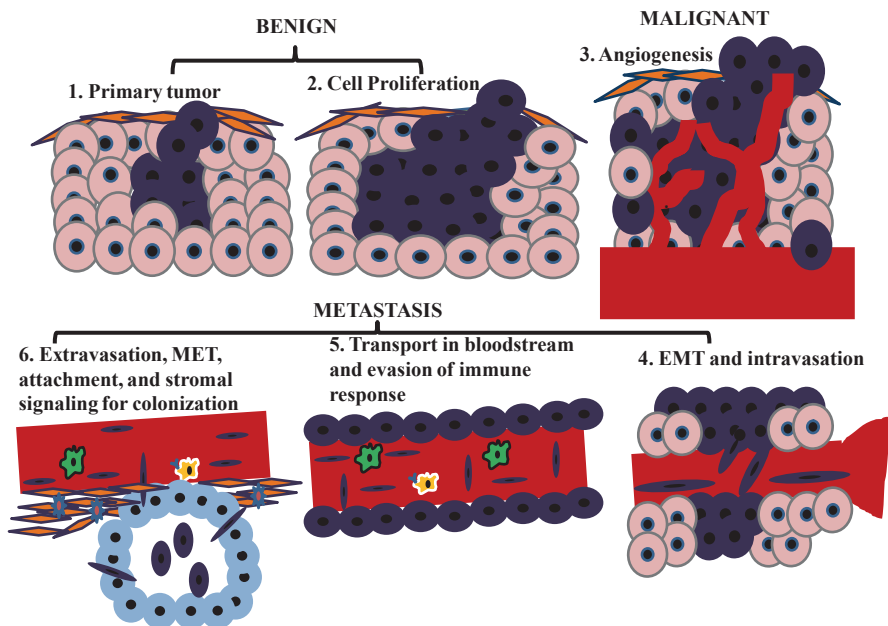
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**Fig. 2.1** Cancer progression. During the benign stage, cells acquire multiple mutations and form the primary tumor. With increasing tumor growth, angiogenesis is initiated and cells locally invade during the malignant phase. Aggressive metastasis results when tumor cells undergo epithelial-to-mesenchymal transition (EMT), enter blood vessels, and colonize secondary sites through cross talk in a tumor-permissive microenvironment

genes. However, ongoing research has revealed that induction of stromal changes and evasion of host defense mechanisms are critical for tumor progression. Tumor cells aggressively outcompete their neighbors, activate angiogenesis, detach from their primary location, intravasate into the bloodstream, survive immune clearance, extravasate out of blood vessels, and metastasize to distant sites (Fig. 2.1). Currently, additional hallmarks have emerged including reprogramming of energy metabolism and the concept of the tumor microenvironment [2].

Due to the multiple genetic events that occur in succession and accumulate over time, development of cancer typically manifests itself in older adults. According to the World Health Organization, cancer accounted for 7.6 million deaths in 2008 and 70% of all cancer deaths occurred in low-income and middle-income countries (<http://www.who.int/mediacentre/factsheets/fs297/en/index.html>). Worldwide studies have attributed 30% of cancer deaths to five behavioral and dietary risks including high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, and alcohol use (<http://www.who.int/mediacentre/factsheets/fs297/en/index.html>). According to the National Cancer Institute, it is estimated that 1,638,910 men and women will be diagnosed with cancer in 2012 in the USA (American Cancer Society: Cancer Facts and Figures 2012). The median age at death from cancers of all types was 72 years old from 2005 to 2009 (American Cancer Society: Cancer Facts and Figures 2012) with most fatalities due to secondary metastases [3]. However,

cancer is an equal opportunity disease, also affecting young children. While the majority of tumors seen in adults arise from epithelial cells, that is, solid tumors, children more commonly develop lymphomas and leukemias. Diagnosis is dependent on the tissue of origin, analysis of cell morphology, and the extent of tumor spread, broadly known as staging (<http://www.cancer.gov/cancertopics/factsheet/detection/staging>). Therapies are dependent on staging but for a more personalized treatment, tumors can be screened and targeted via detection of specific mutations associated with particular tumors. For example, lung cancers with gene amplification or tyrosine kinase mutations, the epidermal growth factor receptor (EGFR) can be treated with erlotinib, a selective inhibitor that blocks kinase activity [4]. While cancer survival has improved due to radiation administration, chemotherapy, and/or specific inhibitors, many cancers unfortunately become drug resistant, leading to recurrence.

A plausible explanation for the adverse response to chemotherapy is the heterogeneity of cancer cells and the adaptability of specific stem cell-like populations within the primary tumor [5]. Growing research on the underlying mechanism of chemotherapeutic resistance has led to a popular model hypothesizing the existence of cancer stem cells (CSCs) [6, 7]. These rare cells are hypothesized to behave similar to stem cells and are capable of self-renewal, asymmetric cell division, and multipotent differentiation. However, the origin of CSCs remains elusive and it has been postulated that CSCs arise from transforming mutations occurring in either multipotent stem cells, tissue-specific stem cells, progenitor cells, mature cells, or cancer cells [7]. Recent evidence indicates that some CSCs are distinct from stem cells and arise from progenitor cells as evidenced by induction of neurofibroma formation by differentiated glial progeny [8]. Since CSCs are rare with yet uncharacterized molecular signatures, further investigation is necessary to understand their role. If cancer initiation involves CSCs exhibiting stem cell-like behavior, then regulators of stem cells and associated pathways may be linked to cancer development including sonic hedgehog signaling in basal cell carcinoma and glioma, Notch signaling in T-cell acute lymphoblastic leukemia/lymphoma, epidermal growth factor (EGF) pathways in human squamous cell lung cancer, and the canonical Wnt pathway in colorectal cancer [9–12]. These same pathways control embryonic development, so it is conceivable that tumor cells reprogram normal cells to turn on genes required during the early proliferative phases of embryonic growth. In this way, tumor cells take advantage of genetic pathways required for normal development but perturb the balance between normal and aberrant signaling. Recently, stem cell regulators and stem cells themselves have been shown to be posttranscriptionally regulated by noncoding RNA molecules during normal animal development [13]. The focus of this chapter is on microRNAs (miRNAs) and their role in tumor progression and prevention.

## 2 miRNAs

Found in all metazoans, miRNAs are small RNA molecules that negatively regulate gene expression at the posttranscriptional level [14–16]. After processing of primary and precursor transcripts by the RNase III enzymes Droscha and Dicer,

**Table 2.1** Oncogenic miRNAs

| Oncogenic miRNAs         | Gene loci           | Validated mRNA targets                       | Cancer                                                                                   | Cellular processes                     |
|--------------------------|---------------------|----------------------------------------------|------------------------------------------------------------------------------------------|----------------------------------------|
| <i>miR-17-92 cluster</i> | Chromosome 13q31–32 | p21, Bim, Pten, CTGF, and Tsp1 <sup>42</sup> | B Cell lymphomas, lung cancer                                                            | Proliferation, apoptosis, angiogenesis |
| <i>miR-155</i>           | Chromosome 21q21    | TP53INP1                                     | Burkitt, Hodgkin’s, primary mediastinal and diffuse large-B cell lymphoma, breast cancer | Cell cycle, proliferation, apoptosis   |
| <i>miR-372/373</i>       | Chromosome 19       | LATS2                                        | Testicular cancer                                                                        | Proliferation                          |
| <i>miR-221/222</i>       | Chromosome X        | p27 Kip1                                     | Glioblastoma multiforme, prostate cancer, thyroid cancer, HCC                            | Proliferation                          |

respectively, mature miRNAs interact with Argonaute proteins and other components of the RNA-induced silencing complex (RISC) in the cytoplasm. The RISC facilitates binding of miRNAs to their messenger RNA (mRNA) targets. MiRNA–mRNA binding occurs through imperfect base pairing with the 3′ untranslated region (3′UTR) of mRNA, leading to translational repression and/or mRNA degradation. Computational predictions suggest that about one-third of all protein-coding genes are regulated by miRNAs in humans [17]. Initially discovered as regulators of animal development, miRNAs have been shown to control multiple steps in tumorigenesis, including proliferation and differentiation [18, 19]. The following sections will elucidate the lessons learned so far about miRNAs that behave as protumorigenic and antitumorigenic contributors to cancer.

3 miRNAs as Oncogenes

The first report of an oncogenic miRNA (Table 2.1) showed that the *miR-17-92* polycistron cluster is highly expressed in human B cell lymphomas [20]. The *miR-17-92* cluster is located within a genomic locus on the chromosome 13q31 that was previously established as a frequently amplified region in cases of diffuse large B cell lymphoma, follicular lymphoma, mantle cell lymphoma, primary cutaneous B cell lymphoma, and other cancer types [21]. The cluster encodes *miR-17-5p*, *17-3p*, *18*, *19a*, *20*, *19b-1*, and *92-1*. One of the defining characteristics of an oncogene is chromosomal amplification and as a proof of principle, serial transplant assays are typically used to examine the tumor-forming potential of a gene of interest. Using a mouse model of human B cell lymphoma, where the c-Myc oncogene is overexpressed by the immunoglobulin heavy chain enhancer (E $\mu$ ). He and colleagues [20] demonstrated that the *miR-17-19b* cluster accelerated tumorigenesis . Transplantation of reconstituted hematopoietic stem cells (derived from E $\mu$ –Myc mice) with *miR-17-19b* expression into irradiated recipient mice led to lymphoma development, invasion of tumor cells into other organs, and decreased survival.

Tumors continued to form after two rounds of serial transplantation, and analysis of the tumor cell population for markers of pre-B and mature B cells (CD19 and immunoglobulin M (IgM), respectively) suggested that overexpression of the cluster favored transformation of B cell progenitors [20]. Another study implicating the *miR-17-92* cluster in lung tumorigenesis used northern blotting and detected increased *miR-17-92* expression, increased copy number, and functionally enhanced lung cancer cell growth through cell proliferation assays [22]. Thus, the *miR-17-92* cluster can accurately be classified as oncogenic.

Further investigation of oncogenic miRNAs, or “oncomiRs” as they are designated, led to the detection of elevated *miR-155* expression in lymphomas derived from B cells of different developmental stages [23]. Expression of *miR-155* (found on chromosome 21q21) was derived from sequences present in the *bic* RNA, which was previously discovered as a target for insertional mutagenesis in avian B cell lymphomas [24]. *bic* cooperates with c-Myc in enhancing the growth and transformation potential of cultured chicken embryo fibroblasts. In pancreatic ductal adenocarcinoma, *miR-155* expression is upregulated and functional assays identified tumor protein 53-induced nuclear protein 1 (TP53INP1) as its target [25]. Decreased TP53INP1 occurred through translational inhibition, providing a link between *miR-155* and a regulator of cell cycle progression and apoptosis. Further evidence for the oncogenic nature of *miR-155* was observed in breast cancer [26]. Overexpression of *miR-155* triggered constitutive activation of growth pathways including the signal transducers and activators of transcription 3 (STAT3) and Janus kinase (JAK) pathways, and its effects were mediated through targeting the tumor suppressor gene, *Socs1* [26]. These experiments provided support for the contribution of miRNAs in tumorigenesis by downregulation of genes controlling cell growth and division, a mandatory prerequisite for primary tumor formation.

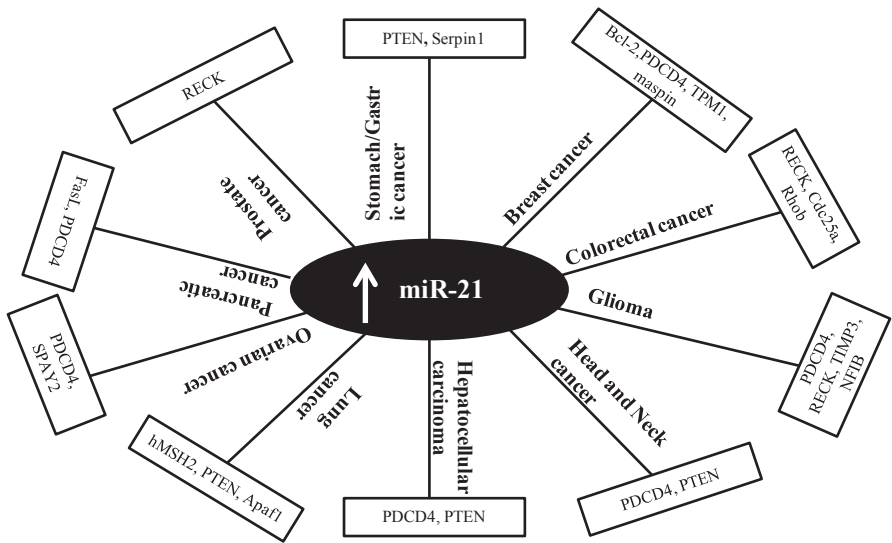
While initial findings of pro-tumorigenic miRNAs were mostly derived from DNA copy number analyses, few have been identified through targeted approaches, i.e., genetic screens. Voorhoeve and colleagues (2006) designed a library of vectors expressing the majority of cloned human miRNAs and used microarrays to examine expression [27]. With an oncogenic stress model in which human fibroblasts express a constitutively active Ras, they discovered that vectors encoding *miR-372* and *miR-373* conferred a selective growth advantage to cells that would otherwise undergo a stress response, known as oncogene-induced senescence. Their results indicated that these miRNAs act in cooperation with Ras to promote tumorigenesis and also provided evidence implicating both miRNAs in tumors that retained a wild-type (WT) copy of p53 but were nevertheless sensitive to DNA-damaging agents. Analysis of chemosensitive testicular germ cell tumors harboring WT p53 indicated high expression of *miR-372* and *miR-373* in tumors classified as embryonal carcinoma [27]. Also, germ cell lines failed to undergo growth arrest in the presence of a cell cycle inhibitor and *miR-372* and *miR-373*. Using prediction algorithms to identify targets whose 3'UTRs contained putative binding sites for both miRNAs, the serine threonine kinase large tumor suppressor homolog 2 (LATS2) was identified and validated using a luciferase reporter assay. Negative regulation of LATS2 by *miR-372* and *miR-373* occurs through a combination of RNA ablation and inhibition of

protein synthesis. Since LATS2 is a tumor suppressor, its loss in mouse embryonic fibroblasts provides a growth advantage [27]. Together, *miR-372* and *miR-373* fit the oncogenic criteria because downregulation of their mRNA targets prevents exit from the cell cycle, resulting in uncontrolled cell growth.

The classification of miRNAs as oncogenes was further substantiated when specific miRNAs were found to be overexpressed within diverse tumor types [19, 28, 29]. One such miRNA that appears to be ubiquitously required for aggressive metastatic potential is *miR-21*. Encoded on chromosome 17q, *miR-21* was originally identified as an oncogene in human glioblastoma cells [30]. Through analysis of RNA isolated from neoplastic and nonneoplastic glioma samples, *miR-21* was found to be upregulated in gliomas. Inhibition of *miR-21* expression in cultured glioblastoma cells caused a marked increase in apoptosis through activation of the caspase machinery [30]. Additional evidence for pro-tumorigenic *miR-21* was illustrated through microarray analyses that showed significant upregulation of *miR-21* in all tumors (breast, colon, lung, pancreas, prostate, and stomach) irrespective of the disease status [31]. Consistent with this, the anti-apoptotic protein Bcl-2 was found to be a direct target of *miR-21* in breast cancer cells and in a xenograft model of breast cancer [32]. Other tumor suppressor targets of *miR-21* have been identified including phosphatase and tensin homolog (PTEN), which inhibits the oncogene PI3K in AKT-mediated cell proliferation [33]. *miR-21* modulates PTEN levels in lung cancer cells resulting in increased cell growth, migration, and invasion [33].

Several reports have referred to the phenomenon of dependence on a single oncogene as “oncogenic addiction” [34–36]. A landmark in miRNA cancer research was the in vivo demonstration of oncogenic addiction in mice conditionally expressing *miR-21* [37]. Using Cre recombinase and the Tet-off system, overexpression of *miR-21* in hematopoietic tissues accelerated pre-B malignant lymphoid-like tumor formation, whereas loss of *miR-21* resulted in regression of tumors. This clearly demonstrates that *miR-21* is a bona fide oncomiR. Overexpression of *miR-21* in different cancer types (Fig. 2.2) illustrates the dysregulation and dependence on common miRNA pathways and common mRNA targets in the acceleration of tumorigenesis [38–41].

Similar to *miR-21*, the *miR-221/222* family encoded on the X chromosome has been implicated in several cancer types after initial detection in glioblastoma multiforme (GBM) [42]. Global miRNA expression profiles (miRNome) showed increased expression of *miR-221/222* in patient tissue samples and GBM cell lines. Similar microarray analyses uncovered an increase in *miR-221/222* expression in papillary thyroid carcinoma, consistent with decreased Kit receptor expression, a gene with *miR-221/222* binding sites [43]. Also, prostate cancer cells showed increased *miR-221/222* expression, particularly in highly aggressive PC3 cells (cells derived from a distal metastasis) compared to LNCaP cells (slow-growing cells derived from local lymph node metastasis) [44]. Overexpression of both miRNAs in the slowly growing cell line increased the number of cells entering S-phase. For these experiments, targeted inhibition of both miRNAs caused increased expression of p27<sup>Kip1</sup>. Similarly, treatment of tumor-bearing mice with intravenous injection of



**Fig. 2.2** miR-21 expression in cancer. Diverse and redundant mRNA targets of *miR-21* have been identified in multiple tissues. Some targets like Bcl-2 and Apatf regulate apoptosis, while others control cell growth and invasion (Cdc25a, PDCD4, PTEN, RECK, RhoB, and TIMP3)

antisense oligonucleotides against *miR-221* resulted in increased survival in mice injected with human hepatocellular carcinoma (HCC) [45].

4 miRNAs as Tumor Suppressors

Located in a cluster on chromosome 13q14.3, the first-described tumor suppressor miRNA was the *miR-15/16* family [46] (Table 2.2). Deletions and translocations in this region were found in ~65 % of B cell chronic lymphocytic leukemia (CLL). An initial inverse correlation was recognized between *miR-15/16* and the anti-apoptotic regulator protein Bcl2, which is overexpressed and a hallmark of CLL [47]. Other *miR-15/16* family members harbor the same 9-base-pair Bcl2-complementary sequence, providing evidence for a putative interaction consistent with experiments that showed that *miR-15/16* posttranscriptionally regulates Bcl2. Re-introduction of *miR-15/16* in a leukemia-derived cell line lacking both miRNAs resulted in strong reduction in Bcl2 levels. Also, cells transfected with plasmids expressing *miR-15/16* demonstrated increased apoptosis as measured by DNA fragmentation, activation of caspases, and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining of individual cells [47].

Tumor growth relies on genetic alterations coupled with external communication to and from the environment. A critical process for tumor expansion is the establishment of stromal progression and the metastatic niche [48]. Tumor cells

**Table 2.2** *Tumor suppressor miRNAs*

| Tumor suppressor miRNAs | Gene loci          | Validated mRNA targets  | Cancer                                                                | Cellular processes                  |
|-------------------------|--------------------|-------------------------|-----------------------------------------------------------------------|-------------------------------------|
| <i>miR-15/16</i>        | Chromosome 13q14.3 | Bcl2, FGF-2, FGFR1      | Chronic lymphocytic leukemia                                          | Apoptosis, proliferation            |
| <i>let-7</i>            | Chromosome 21q21   | Ras, Hmga2              | Lung, breast, ovarian, prostate cancers, HNSCC, sarcoma <sup>61</sup> | Proliferation                       |
| <i>miR-34</i>           | Chromosome 1p36    | SIRT1                   | Colon cancer                                                          | Epigenetic modification of histones |
| <i>miR-29</i>           | Chromosome X       | DNMT3A/3B, CDK6, IGF-1R | Mantle cell lymphoma, leukemia                                        | DNA methylation, proliferation      |

interact with the extracellular matrix (stroma) and upon metastasis, distant stromal–tumor interactions form a niche that facilitates tumor growth. Distinct cell types configure the stromal architecture including fibroblasts that often receive signals (cytokine and chemokine) from tumor cells that prime the stroma for tumor growth. Investigation of prostate cancer–stromal interaction showed downregulation of *miR-15/16* in comparison to stroma surrounding noncancerous tissues [49]. Candidate mRNA targets were screened using bioinformatic prediction algorithms and luciferase reporter assays and found that the 3'UTR of fibroblast growth factor 2 (FGF-2) and fibroblast growth factor receptor 1 (FGFR1) can be silenced by *miR-15/16*, consistent with increased FGF-2 and FGFR1 in prostate cancer [49]. Reconstitution of carcinoma-associated fibroblasts with *miR-15/16* also showed a reduction of FGF-2 and FGFR1. Last, subcutaneous co-injection of prostate cancer cells with fibroblasts transduced with *miR-15/16* expression vectors dramatically reduced tumor growth in immunocompromised mice [49]. Histological analyses showed decreased parenchymal invasion, impaired angiogenic behavior, and reduction of FGF-2 expression in the stroma. These experiments show how miRNAs can regulate multiple aspects of cancer development including primary tumor growth and metastatic spreading.

*let-7*, the first conserved miRNA discovered in *Caenorhabditis elegans*, plays a pivotal role in regulating animal development and has been implicated in tumorigenesis [50, 51]. In humans, there are 13 *let-7* family members encoded on nine different chromosomes [52]. Microarray analysis of *let-7* levels in lung cancer patients with squamous cell carcinoma showed a significant decrease in *let-7* expression and follow-up experiments support the idea that *let-7* negatively regulates Ras, consistent with increased levels of oncogenic Ras in lung cancer [51]. Indeed, *let-7 g* reduced luciferase activity when the K-Ras 3'UTR was fused to luciferase in mouse lung adenocarcinoma cells. *let-7* also repressed the levels of c-Myc using the same reporter system.

Typically, *let-7* loss is due to chromosomal deletions but mutations in the 3'UTR of a *let-7* target can also block *let-7* function [53]. Chromosomal translocations associated with human tumors disrupted repression of the oncogenic chromatin-associated *let-7* target high-mobility group protein A2 (Hmga2) by deletion of 3'UTR

regions targeted by *let-7* [53]. *let-7* regulates Hmga2 by interacting with seven conserved 3'UTR elements. Expression of truncated Hmga2 lacking the *let-7* sites induced higher colony formation on soft agar and anchorage-independent behavior. Consistent with this, subcutaneous injection of mouse embryonic fibroblast cells with full length, WT Hmga2 produced no tumors, whereas fibroblasts harboring mutated *let-7* sites or 3'UTR truncations in Hmga2 generated tumors at the sites of injections [53]. These data provide in vivo support for a tumor suppressor function for *let-7* through Hmga2 regulation.

One of the most widely studied anti-tumorigenic proteins is p53, the most commonly mutated gene in numerous cancers [54]. Originally identified as a transcription factor, p53 also acts as a DNA damage response protein and apoptotic regulator. He and colleagues (2007) examined global changes in miRNA levels in p53-deficient mouse embryonic fibroblasts and found that *miR-34* expression correlates with p53 status [55]. Conditional activation of p53 or induction of p53 increased *miR-34* levels. Analysis of p53 binding showed that p53 occupies specific *miR-34* promoter regions. Ectopic expression of *miR-34* inhibited growth of human primary fibroblast cells, in addition to inducing cell cycle arrest in G1 after addition of *miR-34* in immortalized mouse cells and human tumor cells. Upregulation of *miR-34* also produced changes in mRNA expression patterns among a large number of genes implicated in cell proliferation including cyclin-E and CDK-4 [55]. These experiments suggest a novel mechanism of p53-mediated regulation of cell proliferation through activation of *miR-34*. Therefore, *miR-34* can be considered a tumor suppressor and, indeed, the chromosomal region 1p36 encoding *miR-34* is frequently deleted in various cancers including colon cancer [56]. For targets of *miR-34*, increased silent mating type information regulator 1 (SIRT1) expression was observed after *miR-34a* inhibition [57]. SIRT1 is known to regulate apoptosis in response to oxidative and genotoxic stress through nicotinamide adenine dinucleotide (NAD)-dependent deacetylation (reviewed in [58]). Cell survival decreased when *miR-34a* was introduced into colon cancer cells but SIRT1 re-expression partially blocked *miR-34a*-mediated cell death. Interestingly, SIRT1 can deacetylate histones associated with the p53 gene, suggesting a positive feedback loop between p53, *miR-34a*, and SIRT1 [55].

Besides regulating histone acetylation, miRNAs can also regulate DNA methylation. DNA methylation patterns are frequently altered in cancer [59]. The human DNA methyltransferases DNMT3A and DNMT3B are targets of *miR-29* [60]. The *miR-29* family includes *miR-29a*, *miR-19b-1*, *miR-29b-II*, and *miR-29*, located on chromosome 7q32, a region frequently deleted in various leukemias. Lung cancer cells transfected with *miR-29* showed a global reduction of DNA methylation and reduced promoter methylation and therefore expression of the tumor suppressor genes FHIT and WWOX (normally hypermethylated and silenced in lung cancer) [60]. In a mantle cell lymphoma model, *miR-29* levels were found to be controlled by c-Myc [61]. Promoter analysis showed that Myc, HDAC3, and EZH2 form a repressive complex to epigenetically repress *miR-29* transcription in Myc-expressing lymphoma cells. Loss of *miR-29* results in upregulation of CDK6 and insulin-like growth factor 1 receptor (IGF-1R), pro-

growth genes that promote Myc-associated lymphomagenesis [61]. *miR-29* loss therefore confers a growth advantage, supporting its designation as a tumor suppressor miRNA.

## 5 miRNA Function in Hallmarks of Cancer

As mentioned above, cancer cells have shared characteristics known as the hallmarks of cancer. Although the majority of the pro-tumorigenic and anti-tumorigenic miRNAs mentioned so far regulate early tumor events including cell growth, apoptosis, and invasion, several findings have also implicated miRNAs in later stages of tumor progression. These include angiogenesis, epithelial-to-mesenchymal transition (EMT), intravasation into the bloodstream, evading the immune response in transit, extravasation, formation of micrometastases, and, eventually, colonization at a secondary site. Angiogenesis is a key step of the malignant tumor phase and *miR-296* is associated with this process via targeting hepatocyte growth factor-regulated tyrosine kinase substrate (HGS) mRNA, resulting in HGS-mediated degradation of the growth factor receptors vascular endothelial growth factor receptor 2 (VEGFR2) and platelet-derived growth factor receptor-beta (PDGFR $\beta$ ) [62]. Administration of antagomirs targeting *miR-296* resulted in reduced tumor volume. *miR-9* is elevated in human breast cancer cell lines and is also associated with angiogenesis, possibly through the targeting of E-cadherin [63]. Inhibition of E-cadherin by *miR-9* resulted in increased  $\beta$ -catenin and VEGF levels in breast cancer cells. Transplantation of breast cancer cells expressing *miR-9* showed increased MECA-32 (endothelial cell antigen) staining and a greater than tenfold increase in the density of intratumoral microvessels [63]. Mammary fat pad injections of the highly metastatic 4T1 breast cancer cells transfected with an *miR-9* sponge showed a ~50% decrease in lung metastases. These results support a pro-metastatic role for *miR-9* in later stages of tumor progression.

Tumor cells undergo an EMT resulting in the loss of cell adhesion and detachment [64]. As a result of EMT, cancer cells at the invasive front gain enhanced migratory capacity that facilitates intravasation into the bloodstream. Importantly, these cells lose E-cadherin expression while activating mesenchymal gene products like vimentin and N-cadherin. *miR-200* and *miR-205* inhibit the expression of two zinc-finger containing transcription factors, ZEB1 AND ZEB2 that target and repress E-cadherin [65]. Human epithelial kidney cells transfected with inhibitors against *miR-200* displayed minimal E-cadherin expression, increased ZEB1 and ZEB2 expression, elongated cell morphology, and increased invasion. Transfection of *miR-200a/b* and *miR-205* into MDCK-Pez-transformed cells resulted in re-expression of E-cadherin and a rounded epithelial-like morphology, in addition to loss of ZEB1 mRNA and protein levels. In breast cancer cells, a reciprocal pattern was observed between *miR-200* and ZEB1 and ZEB2 with a loss of *miR-200* in mesenchymal-like cell lines compared to epithelial-like cells [65]. Last, patient samples of less invasive ductal tumors were found to express *miR-200* with coincidently increased

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