miRNA Deregulation in Cancer Cells and the Tumor Microenvironment

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ABSTRACT
miRNAs are a key component of the noncoding RNA family. The underlying mechanisms involved in the interplay between the tumor microenvironment and cancer cells involve highly dynamic factors such as hypoxia and cell types such as cancer-associated fibroblasts and macrophages. Although miRNA levels are known to be altered in cancer cells, recent evidence suggests a critical role for the tumor microenvironment in regulating miRNA biogenesis, methylation, and transcriptional changes. Here, we discuss the complex protumorigenic symbiotic role between tumor cells, the tumor microenvironment, and miRNA deregulation.

Significance: miRNAs play a central role in cell signaling and homeostasis. In this article, we provide insights into the regulatory mechanisms involved in the deregulation of miRNAs in cancer cells and the tumor microenvironment and discuss therapeutic intervention strategies to overcome this deregulation.

INTRODUCTION
miRNAs, a key component of the noncoding RNA family, are involved in multiple cellular functions (1). Since the discovery of these short RNA molecules in Caenorhabditis elegans, miRNAs have been recognized to play multifaceted roles in controlling cellular functions by repressing target genes (1–6). miRNA genes in humans and many other organisms are located in varying genomic contexts, which include intergenic and intragenic noncoding RNA regions in introns or sometimes within an exon of the gene. Mature miRNA biogenesis starts with RNA polymerase II processing of long non–protein coding RNA primary transcripts, called precursor miRNAs (7). These transcripts are further processed by DROSHA and its binding partners, such as DGCR8, leading to primary miRNAs (pri-miRNA). After these pri-miRNAs are translocated into the cytoplasm via exportin 5, they bind to DICER and RNA-induced silencing complex (RISC), which includes argonaute proteins. In conjunction with RISC, a guide strand helps to navigate the mature miRNAs to the target mRNA, consequently resulting in downregulation of target genes (ref. 7; Fig. 1).

Although miRNA biogenesis is a tightly regulated process, deregulation of miRNAs caused by alterations in the biogenesis pathway proteins, including DROSHA, DICER, and AGO2, has been recognized to occur in cancer cells (8–10). In addition to autonomous cancer cell gene changes, the tumor microenvironment can directly influence miRNA levels. These alterations can occur as a result of either biogenesis defects under the influence of hypoxia (11–15) or miRNA transcriptional changes (16–18). Despite biogenesis defects and global downregulation in miRNAs (8, 9, 14, 15, 19–21), many oncogenic miRNAs are significantly increased in cancer (16, 22–27). Mechanisms by which expression of oncogenic miRNAs is increased in cancer are diverse and individual miRNA–dependent (e.g., increased transcription of specific miRNAs). Here, we summarize recent advances in understanding the complex interplay between miRNA deregulation and the tumor microenvironment.

Part I: Cancer Cells and Deregulation of miRNAs
Key Downregulated Enzymes in the miRNA Biogenesis Pathway in Cancer
More than 6 years ago, downregulation of DROSHA and DICER, two key enzymes involved in miRNA biogenesis, was reported in many cancers, including ovarian, lung, and breast cancers (8, 9). Such changes are functionally relevant because cells with deficient biogenesis exhibit defects in miRNA processing (9). Since then, several other studies have demonstrated the importance of downregulated DROSHA and DICER expression in an array of cancer types (19–21,
Figure 1. Summary of canonical miRNA biogenesis pathway. MiRNA genes are transcribed from intergenic or intragenic regions of noncoding RNA transcripts mediated by RNA polymerase II, called pri-miRNA. These long pri-miRNAs are processed by the DROSHA–DGCR8 complex to form precursor miRNAs (pre-miRNAs) of approximately 60 nucleotides in length. EXPO5 mediates the export of these pre-miRNAs to the cytoplasm for further processing by DICER. DICER is a ribonuclease, which cleaves pre-miRNAs to form mature miRNAs of approximately 20 nucleotides in length. One of the strands of mature miRNA (guide strand) gets incorporated into RISC involving DICER and AGO2 enzymes to target mRNAs to cause degradation or translational suppression of gene. The canonical miRNA biogenesis pathway is significantly perturbed in cancer by several proteins at various stages, as highlighted in red. At the gene level, transcripts are altered in cancer by transcription factors, such as MYC, or by epigenetic modifications. DROSHA-mediated miRNA processing is suppressed in cancer by hypoxia, involving ETS1/ELK1 transcriptional repression of the DROSHA gene. Several studies have highlighted DICER downregulation in cancer mediated by several factors, such as TAP63, hypoxia-mediated epigenetic changes, and miR-103/107. EGFR has been reported to bind to AGO2, resulting in phosphorylated AGO2 with decreased association to RISC. TRBP, TAR RNA-binding protein.
Recent studies have shown that miR-103/107 and Let-7 target the regulation in cancer contains multiple layers of complexity. This role for mutant nucleus. Also, genomic studies showed a tumor-promoting XPO5 resulted in entrapment of precursor miRNAs in the also been reported. In this study, a genetic mutation in (39) that prevents precursor miRNAs from being processed of a select set of miRNAs (37). This observation is important because cancer cells contain several upregulated miRNAs despite the decrease in DROSHA or DICER downregulation owing to loss of TAP63 in cancer has been observed (31). In that study, loss of DICER led to decreased miR-130b and increased cancer cell–invasive potential. Mutant p53 has also been shown to result in DICER downregulation in a p63-independent manner (32), suggesting that DICER downregulation in cancer contains multiple layers of complexity. This is further illustrated by the observation that some miRNAs target the DICER 3′ untranslated region. Two independent studies have shown that miR-103/107 (33) and Let-7 (34) can target DICER, and loss of these miRNAs is related to increased tumor growth. MiR-200 was one of the main miRNAs downregulated by low DICER as a result of miR-103/107 direct targeting, and this led to increased cancer metastasis (33).

In addition to DROSHA and DICER, other enzymes in the miRNA biogenesis pathway, such as TARB2 and AGO2, have also been reported to be downregulated in cancer. In sporadic and hereditary carcinomas, mutations leading to a truncated form of TARB2 protein can impair DICER function (35). Downregulation of TARB2 protein expression in cancer stem cells was shown to be important for metastasis signaling (36). EGFR-dependent AGO2 phosphorylation impairs AGO2 binding to DICER, resulting in increased miRNA biogenesis (12). Although downregulation of these key enzymes involved in biogenesis is important for cancer progression, additional alterations in miRNAs (unrelated to biogenesis enzymes) have also been reported. For example, DNA damage induces ATM kinase-mediated phosphorylation of KH-type splicing regulatory protein, which leads to increased processing of a select set of miRNAs (37). This observation is important because cancer cells contain several upregulated miRNAs despite the decrease in DROSHA or DICER enzymes, suggesting that alternative mechanisms process some of the miRNAs involved in oncogenic signaling. Likewise, Hippo protein sequesters DDX17 and leads to decreased miRNA production (38). A genetic defect in XPO5 (39) that prevents precursor miRNAs from being exported to the cytoplasm for processing by DICER has also been reported. In this study, a genetic mutation in XPO5 resulted in entrapment of precursor miRNAs in the nucleus. Also, genomic studies showed a tumor-promoting role for mutant XPO5 via increased expression of oncogenes such as EZH2, MYC, and KRAS, due to loss of the corresponding targeting miRNAs.

Key miRNAs Downregulated in Cancer and Implications

Some of the main miRNAs downregulated in cancer are those in the miR-200 family. These miRNAs are involved in many diverse functions, such as induction of epithelial-to-mesenchymal transition (EMT) via downregulation of E-cadherin and consequent increases in ZEB proteins (40–42). MiR-200 targets ETS1, and loss of miR-200-mediated repression of ETS1 under hypoxic conditions leads to angiogenic responses in cancer cells (43). We have demonstrated that miR-200 influences angiogenesis indirectly via downregulation of CXCL1 and IL8, which are major cytokines in the tumor microenvironment (44). Overall, miR-200 acts as a master regulator of several cancer cell signaling pathways, and targeting this miRNA could be an important strategy for cancer treatment.

Members of the Let-7 family can also regulate cancer stem cells by targeting Hras and HMGA2 (27, 45). Additional roles of Let-7 relate to cell proliferation and regulation of several cell-cycle regulators (46). In silico and tumor sample analyses have shown that a master regulatory network of miRNAs is involved in the mesenchymal phenotype of cancer cells (47). Some of the key miRNAs identified were miR-506, miR-200, and miR-25 (47). These networks can lead to increased tumor metastasis. Specifically, miR-506 was shown to target SNAI2, an EMT-promoting protein, and overexpression of miR-506 in cancer cells resulted in decreased tumor metastasis. MiR-10b is another important miRNA induced by TWIST1, which targets HOXD10, leading to increased RHOC protein levels and, subsequently, increased metastasis (18).

Oncogenic miRNAs in Tumor Progression

Oncogenic miRNAs targeting key tumor-suppressor genes have been reported. Considering the significant downregulation in miRNAs due to defective miRNA biogenesis, it is of great interest to understand how these oncogenic miRNAs are increased in cancer. Two of the major oncogenic miRNAs reported are the miR-17–92 cluster (48, 49) and miR-21 (50–52). Noncoding RNA C13orf25 encodes the miR-17–92 cluster and is known to be upregulated in several cancers (48). Amplification of the 13q31-32 locus is attributed to increased expression of this noncoding RNA region, resulting in increased expression of miRNAs in the cluster. Two of the miRNAs in this cluster, miR-17 and miR-20a, target E2F1, a cell-cycle regulator involved in cell division and apoptosis (49). In breast cancer, miR-21 showed a significant increase in expression and was correlated with poor patient survival. PDCD4, a protein with a role in promotion of cellular apoptosis, was the prime target of miR-21 in breast and colon cancers, leading to increased tumor growth (50, 51). One of the main questions concerning oncogenic miRNAs is how does the expression of a select set of oncogenic miRNAs remain at an elevated level despite the decreases in miRNA biogenesis in cancer cells. One answer could be selective processing of miRNAs by binding to RNA-binding proteins such as KSRP (53). Some miRNAs (e.g., miR-21) bind to KSRP, and the entire pre-miRNA–KSRP complex gets loaded into RISC at higher affinity, leading to increased processing (53). Recently, hypoxia was found to result in downregulation of miRNAs in cancer cells via decreased DROSHA and DICER (11, 14, 15). Hypoxia in the tumor is a dynamic process, and it is
Figure 2. Illustration of cancer cells and tumor microenvironment–deregulated miRNA target networks leading to tumor growth and progression. 

A, MiRNAs play a very important role in the transformation of normal fibroblasts (NF) to CAFs. For example, miR-320 targets ETS2 and controls oncogenic secretome secretion. This oncogenic secretome converts NFs to CAFs in the tumor microenvironment, leading to increased tumor growth via inflammation.

B, Inflammation in the tumor microenvironment results in alterations in several key miRNAs, such as Let-7 and miR-155, which target a multitude of mRNAs that are involved in proinflammatory signaling. Macrophages (MAC), T cells, and dendritic cells, all of which are important immune cells found in the tumor microenvironment, deregulate miRNAs that promote tumor growth.

C, Pro-tumoral gene changes (cell survival, immune escape) 

D, Key challenges in developing miRNA therapeutics include developing novel tumor-targeting nanoparticle delivery systems and better stable miRNA mimics or anti-miRNAs.
possible that miRNAs, which are oncogenic in nature, are processed in the normoxia phase of the tumor and diffused into other areas to regulate gene expression and promote tumor growth. Alternatively, another potential explanation for increased oncogenic miRNAs in cancer is via transcriptional increase at precursor levels. Considering that miRNA biogenesis is decreased in cancer, but not completely lost, it is possible that the high input of precursor miRNAs to the remaining miRNA processing enzymes would result in expression of these miRNAs. However, these two theories will require further investigation.

Collectively, studies suggest that a complex interplay of miRNAs and their corresponding targets in cancer lead to augmented cancer growth or metastasis. The ability of miRNAs to target multiple genes provides an opportunity to interrupt this oncogenic network; this concept is discussed further in Part III.

Part II: Tumor Microenvironmental Factors Influence Tumor Progression through miRNA Deregulation

Tumor growth and metastasis are highly dependent on interactions between the tumor and the associated microenvironment. For every step in tumor growth and metastasis, intricate molecular interactions occur among tumor microenvironmental cells, such as fibroblasts and immune-related cells. Additional factors associated with cancer cells, such as hypoxia, as well as tumor-derived factors, such as cytokines, also influence the tumor microenvironment. Conversely, proteins secreted from cells in the tumor microenvironment can influence cancer cells. Several miRNAs have been shown to play critical roles in the interactions between the tumor and the tumor microenvironment (Fig. 2). In the following sections, we summarize and discuss the potential of these findings to inform a better understanding of processes involved in cancer growth and metastasis and opportunities for innovative clinical interventions.

MiRNAs and Cancer-Associated Fibroblasts

Cancer-associated fibroblasts (CAF) are known to influence tumor growth by modulating inflammation or direct cell-to-cell communication. In addition, fibroblasts provide a stromal framework to cancer cells during early growth and development, leading to malignant transformation. These extensive roles have been reviewed elsewhere (54, 55). One of the early studies involving the role of miRNAs in the transformation of normal fibroblasts to CAFs focused on PTEN-regulated miR-320 (ref. 56; Fig. 2A). Using a Pten knockout mouse model, the authors found that loss of Pten in stromal fibroblasts resulted in activation of an oncogenic secretome (56). Downregulation of miR-320 and upregulation of one of its direct targets, Ets2, with loss of Pten is a key event in oncogenic secretome signaling, leading to increased angiogenesis and tumor formation (56). miR-320 was found to regulate CAF-secreted proteins, including MMP9, MMP2, LOXL2, and EMILIN2, which are known to enhance tumor metastasis by programming the tumor microenvironment via degradation of extracellular matrices.

An miRNA array-based analysis comparing normal fibroblasts with CAFs identified an miRNA signature of CAFs: 3 miRNAs were upregulated (miR-221-5p, miR-31-3p, and miR-221-3p) and 8 miRNAs were downregulated (miR-205, miR-200b, miR-200c, miR-141, miR-101, miR-342-3p, Let-7g, and miR-26b) in CAFs compared with normal fibroblasts (ref. 57; Fig. 2A). Many of these miRNAs are either functionally oncogenic (upregulated miRNAs) or tumor suppressors (downregulated miRNAs). However, the role played by these miRNAs in fibroblasts is not well understood. One could speculate that these miRNAs alter the chemokines secreted by fibroblasts (e.g., miR-320) or could alter fibroblast phenotypes to change tumor stromal compartments to facilitate migration and invasion. In CAFs isolated from ovarian cancer samples, 2 other miRNAs, miR-31 and miR-214, were downregulated and miR-155 was upregulated (58). Expression of miR-155 in normal fibroblasts resulted in the conversion of the fibroblasts to a CAF-like phenotype (58). In addition, the authors identified CCL5, an important chemokine in the tumor microenvironment, as a target of miR-214, which is downregulated in CAFs (58). These data support the idea that miRNAs in fibroblasts could alter the tumor microenvironment by changing proteins, such as chemokines, to have a progrowth phenotype.

Tumor-Related Inflammation, Immune Cells, and miRNAs

Inflammation plays a pivotal role in the development and progression of cancer through modulation of immune cells, cytokines, and angiogenesis (59). Considering the role of miRNAs in regulating genes related to inflammation, such as those regulating cytokines (60), it is not surprising that miRNAs can influence tumor inflammation, leading to progrowth features. For example, Let-7 is reported to be involved in an epigenetic switch, leading to tumor transformation (ref. 61; Fig. 2B). Increased transcriptional activity of LIN28 leads to reduced Let-7 and de-repression of the IL6 cascade involving STAT3, leading to transformation of normal cells into cancer cells owing to significantly increased inflammation (61). Interestingly, this functions as a positive loop because IL6 can activate NFkB. IL6 signaling–mediated STAT3 activation is not limited to Let-7 miRNA regulation alone. In a breast cancer model, increased STAT3 signaling was observed, and loss of miR-146b owing to methylation in the promoter region was reported (26). Members of the miR-146 family are reported to be elevated in a NFkB-dependent manner, regulating innate immune responses (62). These complex signaling networks are highlighted in Fig. 2B. miR-146b targets the tumor necrosis factor receptor–associated factor 6 and IL1 receptor–associated kinase 1 genes, which are involved in Toll-like receptor cytokine signaling (62). Inhibition of this signaling could be an important step for cancer cells to interfere with the immune response in severe inflammatory settings during cancer initiation and development.

Another important miRNA involved in the modulation of immune responses and inflammation is miR-155. Multiple studies have reported that miR-155 promotes growth in several types of cancer, including breast and lung cancers. Oncogenic miR-155 downregulates SHIP1, an important modulator of immune responses, which is involved in activation of AKT signaling during the cellular response to lipopolysaccharide (ref. 63; Fig. 2B). The role of miR-155 in targeting WEE1, an important cell-cycle regulator involved
in DNA damage response during inflammation and during cancer development, has also been reported (64). A tight link between miR-155 levels and DNA damage leading to increased mutation rates under inflammatory conditions has been suggested (64). miR-155 deficiency leads to accumulation of SOCS1, causing defective cytokine signaling through STATS (65). Using mouse models, Dudda and colleagues demonstrated that enforced SOCS1 silencing induced tumor destruction (65).

In addition to miRNAs deregulating key cytokines and inflammatory responses, leading to modulation of immune responses, a direct role of miRNAs in immune cells, such as T cells and B cells, has been reported. miR-181a expression in mature T cells increases the sensitivity of T cells to antigens, and inhibition of miR-181a results in impaired selection of antigens (ref. 66; Fig. 2C). Downregulation of multiple phosphatases by miR-181a leads to a reduction of T-cell receptor signaling (66). This is highly relevant to cancer development, considering that high CD8+ T-cell influx is observed during inflammation and cancer development.

In adult T-cell leukemia, constitutively active NFκB signaling is reported to have a causative role in cancer development (67). miR-31 is lost in adult T-cell leukemia and negatively regulates the NFκB pathway by directly targeting NFκB-inducing kinase, leading to apoptosis resistance (ref. 67; Fig. 2C). In addition, hypoxia-upregulated miR-210 (68) acts in a feedback loop to regulate HIF1α, a key regulator of the transcription of genes related to Th17 polarization (69). Thus, miR-210 may act as an important regulator under disease conditions involving hypoxia to modulate immune responses to cancer antigens, although further investigation is needed to clearly define the multiple roles of miR-210.

Another detailed study of the role of miRNAs in B-cell–related lymphoma development focused on the role of miR-21 as an oncogenic miRNA (23). Overexpression of miR-21 led to a pre–B-cell malignant lymphoid-like phenotype, and when miR-21 was inactivated, the tumors regressed owing to a pre–B-cell malignant lymphoid-like phenotype, and inhibited lymphoma development focused on the role of miR-210. miR-210 may act as an important regulator under disease conditions involving hypoxia to modulate immune responses to cancer antigens, although further investigation is needed to clearly define the multiple roles of miR-210.

Role of Hypoxia in miRNA Biogenesis

Hypoxia is common in the tumor microenvironment and can influence tumor progression by altering cancer and host cell interactions and molecular signaling. During tumor growth and metastasis, cancer cells encounter significant amounts of hypoxia owing to improperly developed and tortuous blood vessels. Key contributions of hypoxia to cancer progression, with an emphasis on protein signaling and clinical implications, are highlighted elsewhere (75, 76). In human endothelial cells, DICER-dependent miR-185 was found to be decreased under chronic hypoxic conditions, and this resulted in increased HIF2α (Fig. 3A); however, biologic endpoints have yet to be defined in this setting (11). Interestingly, suppression of angiogenesis after complete loss of Dicer has been reported (77). In tumors from Dicer−/− mice, a significant increase in hypoxia was found to be caused by reduced angiogenesis resulting from derepression of a HIF1-inhibiting factor, FIH1 (77).

In patients with breast cancer, tumor hypoxia is associated with reduced DICER expression (14). The underlying mechanism was found to be related to inhibition of the oxygen-dependent H3K27me3 demethylase KDM6A/B, which resulted in increased DROSHA and Dicer expression in cancer cells, leading to derepression of DICER under hypoxic conditions (ref. 15; Fig. 3A). Functionally, this leads to decreased processing of the miR-200 family, resulting in EMT and associated stem cell phenotypes (14). In a parallel study, a significant reduction in RNA biogenesis was found to occur as a result of decreased DROSHA and DICER in ovarian and breast cancers. ETS1 and ELK1 mediate DROSHA promoter methylation, leading to downregulation of DICER under hypoxic conditions (ref. 15; Fig. 3A). Functionally, this leads to decreased processing of the miR-200 family, resulting in EMT and associated stem cell phenotypes (14). In a parallel study, a significant reduction in RNA biogenesis was found to occur as a result of decreased DROSHA and DICER in ovarian and breast cancers. ETS1 and ELK1 mediate DROSHA promoter methylation, leading to downregulation of DICER under hypoxic conditions (ref. 15; Fig. 3A). Functionally, this leads to decreased processing of the miR-200 family, resulting in EMT and associated stem cell phenotypes (14). In a parallel study, a significant reduction in RNA biogenesis was found to occur as a result of decreased DROSHA and DICER in ovarian and breast cancers. ETS1 and ELK1 mediate DROSHA promoter methylation, leading to downregulation of DICER under hypoxic conditions (ref. 15; Fig. 3A). Functionally, this leads to decreased processing of the miR-200 family, resulting in EMT and associated stem cell phenotypes (14).
Role of microRNAs in Tumor Progression

**Figure 3.** Tumoral hypoxia functions as a master regulator of microRNAs. A, hypoxia leads to decreased DICER expression in an HIF-dependent manner in endothelial cells and via methylation of DICER in cancer cells. B, DROSHA is downregulated under hypoxic conditions by two transcription factors, ETS1 and ELK1, which bind to the DROSHA promoter region. This binding results in downregulation of DROSHA expression through promoter methylation. C, AGO2, an important enzyme component of the RNA-induced silencing complex, is functionally downregulated via phosphorylation by EGFR under hypoxic conditions in cancer cells. The downregulation of these three key biogenesis components under hypoxic conditions results in various gene changes important for cancer cell survival and tumor metastasis. D, several microRNAs are regulated by hypoxia through mechanisms unrelated to biogenesis. For example, miR-210 is upregulated by the HIF1α transcription factor and is involved in several hypoxia cancer cell signaling pathways. Also, miR-34 and miR-199a are significantly downregulated under hypoxic conditions, leading to altered prometastatic signaling.
Another important protein in the miRNA biogenesis pathway is AGO2, which is part of the RISC. In cancer cells, under hypoxic conditions, EGFR phosphorylates AGO2 at Tyr393, resulting in decreased AGO2 function (ref. 12; Fig. 3C). AGO2 phosphorylation was found to result in decreased DICER–AGO2 interaction, leading to decreased miRNA maturation and function (12). However, another study reported that AGO2 protein levels were increased owing to posttranslational changes in hydroxylation under hypoxic conditions (78). Collectively, hypoxia plays a multifaceted role in deregulating miRNAs, leading to tumor progression (Fig. 3).

Functional Implications of Hypoxia-Deregulated miRNAs

By comparing breast cancer cell lines cultured under normoxic and hypoxic conditions, Kulshreshtha and colleagues identified an miRNA signature of hypoxia (68). One of the miRNAs in this group was miR-210, a transcriptional target of HIF1α (ref. 16; Fig. 3D). AGO2 immunoprecipitation and RNA-sequencing analyses have identified more than 50 potential gene targets of miR-210, and these targets are involved in the response to hypoxia, which improves cell survival. In orthotopic mouse models of head and neck and pancreatic cancers, loss of miR-210 resulted in decreased tumor initiation or growth (16). The role of miR-210 in mitochondrial dysfunction under hypoxic conditions has also been reported. miR-210 was identified as one of the highly upregulated miRNAs in samples from patients with advanced-stage lung cancer (79).

Microarray-based mRNA pathway analyses have suggested that cell lines with increased miR-210 have increased apoptosis. However, target analysis showed that miR-210 targets succinate dehydrogenase complex subunit B (SDHB), leading to stabilization of HIF1α and cell survival under hypoxic conditions (79). Another study showed that miR-210 plays a cytoprotective role by targeting apoptosis-inducing factor mitochondria-associated 3 (AIFM3), known to cause cell death (ref. 24; Fig. 3D). Negative regulation of NFκB in murine macrophages by miR-210, resulting in decreased cytokines, suggests that the role of miR-210 is not limited to cancer cell signaling (80). Increased miR-210 levels in the plasma result in decreased IL6/STAT signaling (81). miR-210 is also involved in Th17 differentiation. HIEF1α is reported to be a target of miR-210 in T cells, and under hypoxic conditions, deletion of miR-210 promoted Th17 differentiation (69). Th17 differentiation could lead to either protumor or antitumor effects; thus, the role of miR-210 in Th17 differentiation under hypoxic conditions is an important question to be answered.

miR-34 is downregulated under hypoxic conditions and influences cancer cells and the tumor microenvironment (57). NOTCH1 and JAG1 are targets of miR-34a, and transfection of cells with miR-34a was found to result in reversal of EMT (57). In prostate cancer, miR-34 was found to be involved in cancer stem cell signaling by directly targeting CD44 (82). In colorectal cancer, downregulation of miR-34 resulted in increased IL6 signaling, leading to EMT and cancer metastasis (83). Altogether, these data suggest that miR-34 is part of the mechanism that leads to a hypoxia-induced increase in cancer metastasis (Fig. 3D).

Another miRNA proven to play an important role in cell response to hypoxia is miR-199a. Targeting of MTOR and MET by miR-199a resulted in increased sensitivity to doxorubicin (ref. 84; Fig. 3D). Targeting of PPARD by miR-199a in the setting of cardiac hypoxia resulted in a metabolic shift toward glycolysis. Mice treated with antagomir-199a displayed improved cardiac function and restored mitochondrial fatty acid oxidation (85). Although that study was not conducted with a cancer mouse model, its findings demonstrate the importance of miR-199a in modulating hypoxia metabolism. Recently, the role of miR-199 in the regulation of HIF1α and HIF2α signaling in ovarian cancer has been reported (ref. 86; Fig. 3D). Decreased miR-199a expression under hypoxic conditions resulted in increased HIF levels. Exogenous expression of miR-199a decreased HIF levels, cell migration, and ovarian cancer metastasis (86).

Part III: Therapeutic Targeting of miRNA Deregulation

miRNAs have a unique advantage for targeted therapy because single miRNAs can target multiple genes. As highlighted in earlier reviews, miRNA or siRNA delivery to tumors is an attractive, yet challenging opportunity for improving therapy for cancer (87–90). Some of the major challenges and current advances are highlighted below.

Finding the Right Target

One of the early miRNA therapeutic strategies that showed significant impact on tumor growth was the delivery of miR-34a and Let-7 in lung cancer models (91). In this study, effective delivery of miRNA mimics miR-34a and Let-7 was demonstrated in orthotopic models of non-small cell lung cancer. Encouraging results from preclinical studies involving miR-34a in several types of cancer have increased efforts to move miR-34a delivery as a therapeutic strategy into clinical trials (92). Delivery of miR-200 in ovarian, lung, breast, and renal cancer preclinical models significantly reduced tumor metastasis and angiogenesis and induced vascular normalization by targeting IL8 and CXCL1 (44). Combining miRNA with siRNA is another attractive approach that may allow a “boosting” effect for targeting oncogenic pathways. Combined systemic delivery of miR-520d-3p with EPHA2 siRNA resulted in robust antitumor effects (93). In this study, miR-520d was shown to target EPHA2 and EPHB2, and combining the miR-520d replacement with siRNA-mediated depletion of EPHA2 resulted in synergistic effects on reducing tumor growth. These and other studies demonstrate the use of miRNA mimics to replenish the lost miRNAs as a viable option for cancer therapy.

Designing a Delivery System for miRNAs

One of the major challenges in developing miRNA therapeutics is the high vulnerability of RNA molecules to nucleases. Hence, design of novel nanoparticle platforms is needed to allow intracellular delivery with minimal toxicity while providing protection to RNA molecules from nucleases. Several lipid-based carriers (91, 94) have been developed and tested in preclinical models, and some are in clinical trials. For example,
MRX34, a lipid-based nanoparticle–miR-34 system, is currently in phase I clinical testing and has shown great promise (92). Another approach is use of neutral liposomal particle 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC; ref. 94). This delivery platform has already been used for miR-200 (44), miR-520d (93), and miR-506 (47) in several cancer types in preclinical models. Use of DOPC for delivery of siRNA against EPHA2 is currently in phase I clinical trials. Completely unrelated to cancer, use of miRNAs as therapy has been employed against hepatitis C virus infections (95). Inhibition of miR-122 by locked nucleic acid–based inhibitor resulted in a significant reduction in hepatitis C virus RNA in patients, as reported in a phase Ia clinical trial (NCT01200420). Apart from these clinical delivery platforms, several novel siRNA or miRNA delivery systems are under development. One such approach includes spherical nucleic acid nanoparticle conjugates with a gold nanoparticle core. Using this system, siRNA against EGFR was successfully delivered to skin and showed significant reduction in EGFR levels (96). In glioblastoma models, delivery of siRNAs against Bcl2L12 resulted in a significant reduction in tumor growth, and these nanoparticles were able to cross the blood–brain barrier, providing a potentially significant advantage in treating brain cancers (97). In a colon cancer xenograft mouse model, delivery of miR-145 and miR-33a using polyethylenimine particles resulted in a significant reduction in tumor growth (98). Use of adenovirus in delivering miRNAs is another approach. Using this approach, investigators delivered miR-26a to liver tumors; the study showed a significant reduction in tumor growth with restored miR-26a expression (99). If the results from the above approaches continue to show success and enter into the clinic, use of noncoding RNAs as therapeutics could emerge as a key technology for the treatment of cancer and other diseases (Fig. 2D).

CONCLUSION

In summary, we have highlighted recent advances in the understanding of tumor microenvironmental interactions mediated by miRNAs. As highlighted in Figs. 2 and 3, several miRNAs target important cancer cell–regulatory molecules and are involved in a complex network of signaling between cancer cells and the tumor microenvironment. In addition to their involvement in direct cell-to-cell signaling, several miRNAs are secreted through microvesicles or exosomes and affect cancer cell growth and metastasis. All of these microenvironmental changes are suggestive of a complex signaling network between tumor cells and stromal components and conditions such as hypoxia, CAFs, and endothelial cells (Figs. 2 and 3). Some of the current challenges in RNAi and miRNA therapeutics involve selecting the right target and optimizing the delivery systems. Advances in RNAi and miRNA therapeutics have enabled us to target miRNA alterations in a highly specific and robust manner in preclinical models. Nevertheless, studies of miRNA-mediated interactions, specifically those focused on understanding the origin of miRNA alterations, are needed to improve targeted therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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