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MicroRNA deregulation in cancer cells and the tumor microenvironment

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Abstract

MicroRNAs (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 pro-tumorigenic symbiotic role between tumor cells, the tumor microenvironment, and miRNA deregulation.

Keywords

MicroRNAs; Hypoxia; *DICER*; Drosha; miRNA biogenesis; tumor progression

Introduction

MicroRNAs (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

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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-miRNAs). 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 (7) (Figure 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, 2 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, 28, 29); this finding is often associated with poor patient survival.

Possible mechanisms for DROSHA regulation include transcriptional activation *via* MYC (28) or downregulation *via* ADARB1 (19) proteins. DROSHA was found to be transcriptionally increased by MYC (28), leading to increased miRNA processing in A549 lung cancer cells. However, other independent groups using patient sample analysis of DROSHA expression have shown that DROSHA downregulation in lung cancer was correlated with poor survival (8, 30). These observations suggest intratumoral heterogeneity in cancer. Downregulation of DROSHA expression by ADARB1 in chronic lymphocytic leukemia can lead to decreased miR-15/16 expression and increased oncogenic signaling (19). For DICER, direct binding of Tap63 transcription factor to the *DICER* promoter has been demonstrated, and 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 TARBP2 and AGO2, have also been reported to be downregulated in cancer. In sporadic and hereditary carcinomas, mutations leading to a truncated form of TARBP2 protein can impair DICER function (35). Downregulation of TARBP2 protein expression in cancer stem cells was shown to be important for pro-metastasis signaling (36). EGFR-dependent *AGO2* phosphorylation impairs AGO2 binding to DICER, resulting in decreased 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 IL-8, 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 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 (Figure 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 (CAFs)—CAFs 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 (56) (Figure 2, Panel A). 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.

A miRNA array-based analysis comparing normal fibroblasts with CAFs identified a 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 (57) (Figure 2, Panel A). 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 pro-growth 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 modulating

genes related to inflammation, such as those regulating cytokines (60), it is not surprising that miRNAs can influence tumor inflammation, leading to pro-growth features. For example, Let-7 is reported to be involved in an epigenetic switch, leading to tumor transformation (61) (Figure 2, Panel B). Increased transcriptional activity of *LIN28* leads to reduced Let-7 and de-repression of the IL-6 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 IL-6 can activate NF-κB. IL-6 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 NF-κB–dependent manner, regulating innate immune responses (62). These complex signaling networks are highlighted in Figure 2, Panel B. MiR-146b targets the tumor necrosis factor receptor–associated factor 6 and IL-1 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 (63) (Figure 2, Panel B). 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 *Socs-1*, causing defective cytokine signaling through *Stat5* (65). Using mouse models, Dudda et al. demonstrated that enforced *Socs-1* silencing augmented 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 (66) (Figure 2, Panel C). 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 NF-κB 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 NF-κB pathway by directly targeting NF-κB–inducing kinase, leading to apoptosis resistance (67) (Figure 2, Panel C). In addition, hypoxia-upregulated miR-210 (68) acts in a feedback loop to regulate HIF1-α, 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 apoptosis (23). Increased expression of miR-21 in CAFs was found to be induced by SMAD7/TGF-beta signaling (70), which resulted in increased inflammation. MiRNAs can activate Toll-like receptor signaling by acting as ligands. Consistent with this function, miR-21 and miR-29a have been found to be secreted from cancer cells and bind to murine *Tlr7* and human *TLR8*, suggesting a role for these miRNAs as ligands for protein molecules (71). This leads to an activated inflammatory response in the tumor microenvironment, contributing to aggressive tumor behavior.

Tumor-associated macrophages are a key component of the tumor microenvironment and are known to promote an inflammatory network to modulate immune responses. MiR-155 and miR-342-5p have emerged as important regulators of inflammatory responses (25). MiR-342-5p directly targets *AKT1* and increases levels of pro-inflammatory mediators such as NOS2 and IL-6 in macrophages *via* upregulation of miR-155. Although these findings were related to atherosclerosis, they are highly relevant to cancer and inflammation in the tumor microenvironment because inflammation can drive malignant transformation. In a study of primary murine macrophages, O'Connell et al. found that after the macrophages' exposure to inflammation stimulants, miR-155 levels were significantly increased *via* Toll-like receptor ligands through myeloid differentiation factor 88 or TRIF-dependent pathways (72). Later, the same group identified inositol phosphatase SHIP1 as a primary target of miR-155. Comparing *Ship1* levels between LPS-treated wild-type and *miR-155*$^{-/-}$ primary macrophages, the authors demonstrated that *Ship1* is repressed by physiologically regulated miR-155 (63) (Figure 2, Panel C). MiR-511 also modulates genetic programming of tumor-associated macrophages. Restoration of miR-511 led to a decreased pro-tumoral gene signature in tumor-associated macrophages, as well as reduced tumor growth (73).

In addition to macrophages, dendritic cells can influence tumor growth. For example, dendritic cell signaling *via SP1* transcription factor–mediated increased expression of miR-27a can lead to altered NF-κ B and MAPK activity (74) (Figure 2, Panel C). As a result of the hampered cytokine signaling, increased levels of *miR-27a* led to decreased dendritic cell–mediated differentiation of Th1 and Th17 cells and increased tumor growth *in vitro* and *in vivo* (74).

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 HIF2-α (Figure 3, Panel A); however, biological 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 de-repression of a *HIF1*-inhibiting factor, Fih1 (77).

In patients with breast cancer, tumor hypoxia is associated with reduced *DICER* expression (14). The underlying mechanism of hypoxia was found to be related to inhibition of oxygen-dependent H3K27me3 demethylase *KDM6A/B*, which resulted in increased *DICER* promoter methylation, leading to downregulation of DICER under hypoxic conditions (15) (Figure 3, Panel A). 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 miRNA 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 under hypoxic conditions, resulting in decreased expression of *DROSHA* (14) (Figure 3, Panel B). This decrease in *DROSHA* (*via ETS1/ELK1*) and *DICER* (*via KDM6A/B*) results in a global decrease in mature miRNAs. Cells under hypoxic conditions showed consistent upregulation of the pro-metastatic genes *RHOB1, TAGLN, SRTAD1, TXNIP, JAG1, CTGF*, and *JUN*, owing to downregulation of corresponding miRNAs Let-7a, miR-135a, miR-146a, and miR-30c (14) (Figure 3, Panel B).

Another important protein in the miRNA biogenesis pathway is AGO2, which is part of the RNA-induced silencing complex. In cancer cells, under hypoxic conditions, EGFR phosphorylates *AGO2* at Tyr 393, resulting in decreased *AGO2* function (12) (Figure 3, Panel C). *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 post-translational changes in hydroxylation under hypoxic conditions (78). Collectively, hypoxia plays a multi-faceted role in deregulating miRNAs, leading to tumor progression (Figure 3).

Functional implications of hypoxia-deregulated miRNAs—By comparing breast cancer cell lines cultured under normoxic and hypoxic conditions, Kulshreshtha et al. identified a miRNA signature of hypoxia (68). One of the miRNAs in this group was miR-210, a transcriptional target of HIF1-α (16) (Figure 3, Panel D). AGO2 immunoprecipitation and RNA sequencing analysis has 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 *SDHD*, leading to stabilization of HIF1-α and cell survival under hypoxic conditions (79). Another study showed that miR-210 plays a cytoprotective role by targeting apoptosis-

inducing factor mitochondrion-associated 3 (*AIFM3*), known to induce cell death (24) (Figure 3, Panel D). Negative regulation of NF-κ B 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 placenta result in decreased *IL6/STAT* signaling (81). MiR-210 is also involved in TH17 differentiation. *HIF1A* 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 pro- 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 (Figure 3, Panel D).

Another miRNA proven to play an important role in cell response to hypoxia is miR-199a. Targeting of *MTOR* and *c-MET* by miR-199a resulted in increased sensitivity to doxorubicin (84) (Figure 3, Panel D). Targeting of *PPAR*δ 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 *HIF-1*α and *HIF-2*α signaling in ovarian cancer has been reported (86) (Figure 3, Panel D). 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 cancers 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 IL-8 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) (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 2a clinical trial (NCT01200420). Apart from these clinical delivery platforms, several novel siRNA or miRNA delivery systems are under development. One such effort 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 advance in treating brain cancers (97). In colon cancer xenograft mouse model, delivery of miR-145 and miR-33a using Polyethylenimine (PEI) particles resulted in 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 treatment of cancer and other diseases (Figure 2, Panel D).

Conclusion

In summary, we have highlighted recent advances in the understanding of tumor microenvironmental interactions mediated by miRNAs. As highlighted in Figures 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 (Figures 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.

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Statement of significance

MicroRNAs 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.

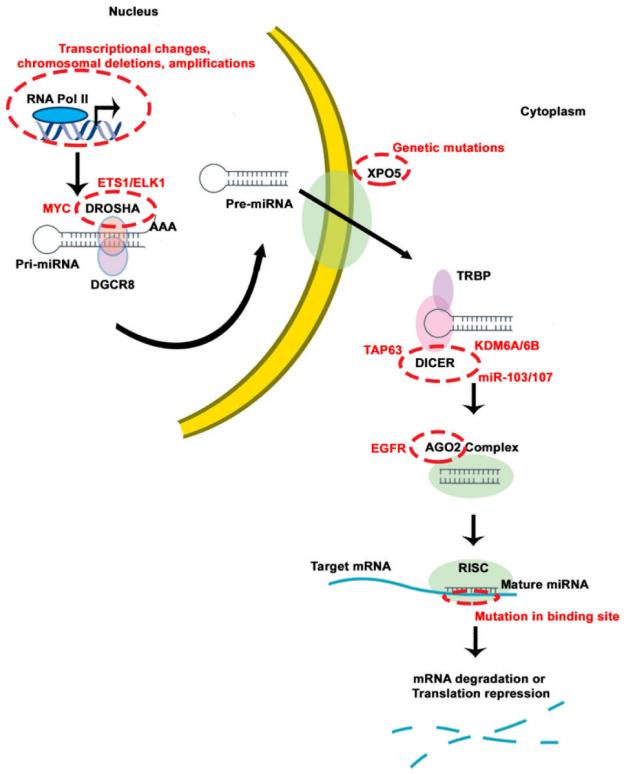

Figure 1. Summary of canonical miRNA biogenesis pathway

MicroRNA genes are transcribed from intergenic or intragenic regions of noncoding RNA transcripts mediated by RNA polymerase II, called primary miRNAs (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 (RNAi induced silencing complex) 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. 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 *DROHSA* 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 is TAR RNA binding protein.

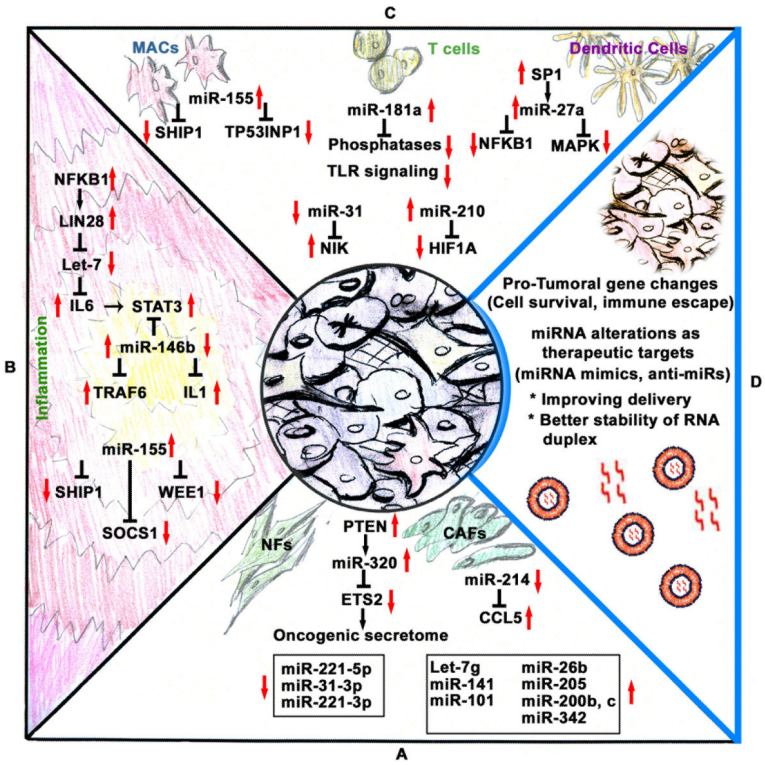

Figure 2. Illustration of cancer cells and tumor microenvironment-deregulated microRNA target networks leading to tumor growth and progression

Panel A, MiRNAs play a very important role in the transformation of normal fibroblasts (NFs) to cancer-associated fibroblasts (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. **Panel 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 pro-inflammatory signaling. **Panel C**, Macrophages (MACs), T cells, and dendritic cells, all of which are important immune cells found in the tumor microenvironment, deregulate miRNAs that promote tumor growth. **Panel D**, Key challenges in developing miRNA therapeutics include developing novel tumor targeting nanoparticle delivery systems and better stable miRNA mimics or anti-miRs.

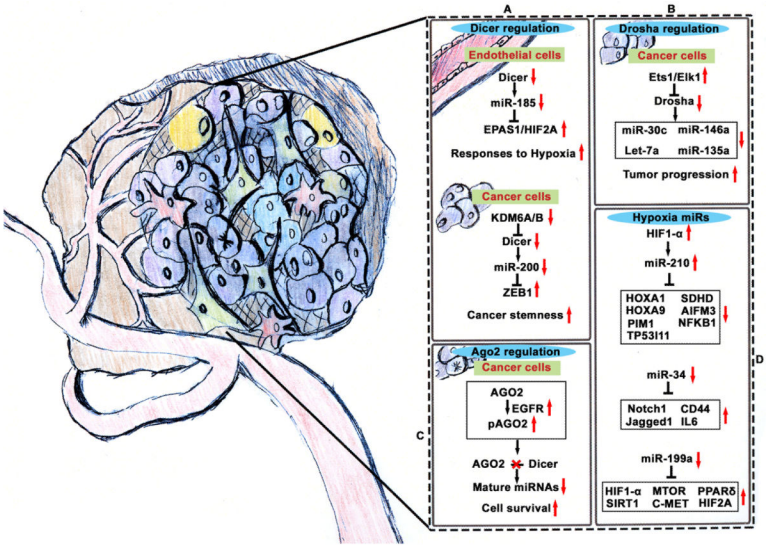

Figure 3. Tumoral hypoxia functions as a master regulator of microRNAs

Panel A, Hypoxia leads to decreased *DICER* expression in a HIF-dependent manner in endothelial cells and *via* methylation of *DICER* in cancer cells. **Panel B**, *DROSHA* is downregulated under hypoxic conditions by 2 transcription factors, ETS1 and ELK1, which bind to the *DROSHA* promoter region. This binding results in downregulation of *DROSHA* expression through promoter methylation. **Panel 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 3 key biogenesis components under hypoxic conditions results in various gene changes important for cancer cell survival and tumor metastasis. **Panel D**, Several miRNAs are regulated by hypoxia through mechanisms unrelated to biogenesis. For example, miR-210 is upregulated by the HIF1-α 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.