Current View of miRNA with Tumor Suppressor Function, Exploring MDS and AML as Models

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ABSTRACT: Since the discovery of microRNAs (miRNAs) in 1993, their role in controlling a wide variety of complex and seminal cellular functions through control of gene expression continues to be elucidated. Studies of the past decade have shown that miRNAs are able to activate or suppress target genes that are key players in the molecular pathways found to be deregulated in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). The ability of miRNAs to act as tumor suppressor genes has been demonstrated in a number of studies in both human samples and cell lines as well as in murine models of AML and MDS. The focus of this review will be to examine the complex interaction of specific miRNAs with genes that have been implicated in MDS/AML and which may eventually become therapeutically relevant.

KEYWORDS: myelodysplastic syndromes, acute myeloid leukemia, cancer, let-7a, miR-29b, miR-145

Introduction

In the last 30 years, the understanding of molecular mechanisms of neoplasia and malignancy has increased exponentially, with mutations in oncogenes and tumor suppressors as some of the earliest culprits described. In 1986, Friend et al used restriction fragment length polymorphisms to discover the first of such mutations that causes a familial form of cancer known as retinoblastoma, fittingly naming the encoded protein the retinoblastoma protein (pRb).[1] Proof that mutations resulting in aberrant gene product from a single gene could cause cancer was revolutionary, and sparked the rush for further discovery of genomic mutations. It took years to recognize that there may be mechanisms outside of traditional genomics underlying cancer development. Eventually, the advent of epigenetics and the discovery of gene regulation via microRNA (miRNA) revealed a multifaceted and complex understanding of cancer development.

miRNAs were discovered in *Caenorhabditis elegans* in 1993 as regulators of a mechanism by which small RNA transcripts could regulate expression of larger genes with vital cellular functions.[2] Shortly after, many miRNAs were both identified and mapped within the human genome. The potential for miRNA to act in a manner similar to these tumor suppressor proteins was established when miRNA targets were found to be related to fundamental cellular processes such as proliferation, apoptosis, and differentiation,[3] while downregulation of these miRNAs was seen in many types of cancerous tissues.[4–7] Our understanding of the complex mechanisms by which miRNA regulates larger cellular processes is incomplete, and the way they act as tumor suppressors is even less clear. Nevertheless, many recent developments have highlighted their critical importance in neoplasia, and investigation into miRNAs' roles as tumor suppressors is an exploding field of research in modern oncology. Our research and clinical practice focus on myelodysplastic syndromes (MDS). Here, we will analyze recent developments in miRNA research as tumor suppressors as well as discuss future directions for this research using MDS and acute myeloid leukemia (AML), a clinically distinct yet related entity, as models.

MDS

MDS comprise a heterogeneous group of hematologic disorders that arise from a clonal expansion of hematopoietic stem and progenitor cells. As in all cancers, this clonal expansion leads to a competitive advantage over neighboring cells. The clinical effects include refractory anemia, leukopenia, and thrombocytopenia leading to chronic fatigue and transfusion dependence, as well as increased risk of infection and bleeding. There may also be a limited increase in bone marrow blast cells, insufficient to be considered leukemia, with one-third of MDS cases eventually transforming to AML. The cytopenias found in this disease arise from early apoptosis in the myeloid maturation process,[8,9] but unlike in leukemia, cells retain their capacity to mature fully.[10] There are many subtypes of this disease, stratified by pathologic findings in peripheral blood samples and bone marrow biopsies, karyotype abnormalities, and defining mutations. The combination of these factors determines the patient's prognosis, risk for transformation to AML, and response to treatment. However, many factors driving disease phenotype and treatment susceptibility or resistance have yet to be determined. Because of this, approved therapies such as lenalidomide, azacitidine, and decitabine work only in some MDS patients who have the subtypes they are approved for. The complex nature of these syndromes' underlying causes makes targeted therapy difficult, because even within defined subtypes there is great heterogeneity. The activity of miRNA suggests that they may play an important role in the development of MDS and many other cancers by potentiating the effects of abnormal cellular processes and even driving disease progression as the primary underlying cause, making them additional targets for therapy.
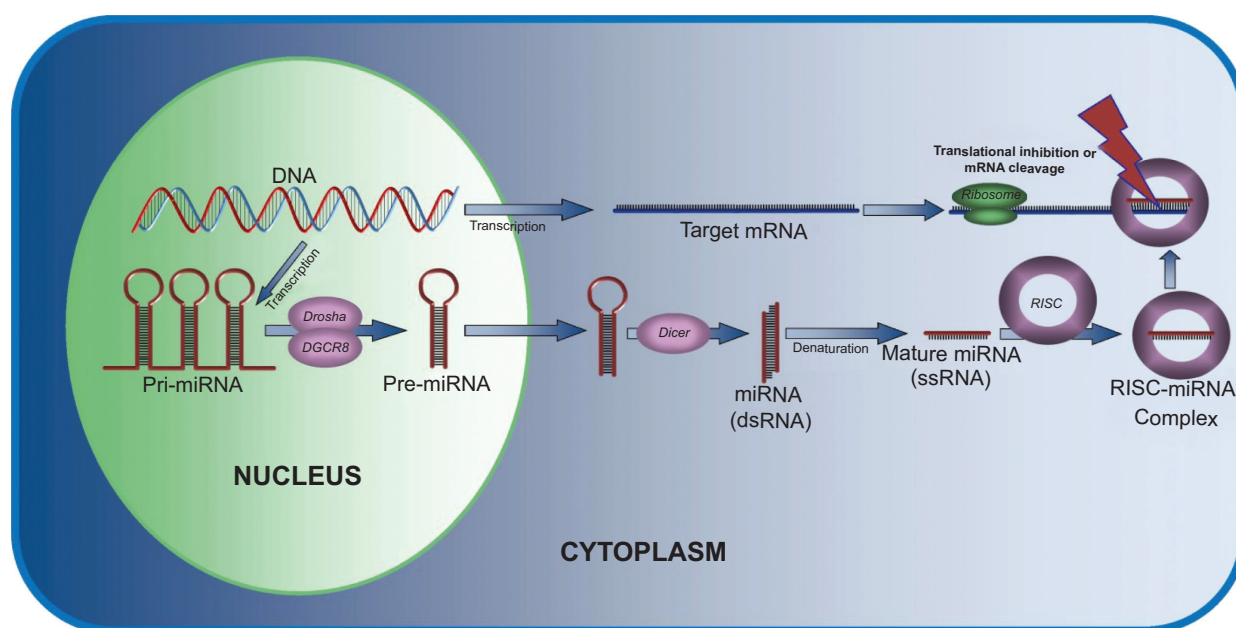
Biogenesis of miRNAs

miRNAs are transcribed as primary RNA (pri-miRNA), ranging from hundreds to thousands of base pairs long with a hairpin-loop secondary structure (Fig. 1).[11] Processing with the nuclear microprocessor complex by the proteins Drosha (an RNase III) and DiGeorge critical region 8 (DGCR8) leaves a hairpin-loop precursor miRNA (pre-miRNA) of approximately 70 base pairs.[12–15] These molecules leave the nucleus and are further processed by Dicer, another RNase III, leaving short 18–25 base pair dsRNAs in the cytoplasm.[16]

Denaturation to ssRNA allows the active miRNA strand to complex with RNA-induced silencing complex (RISC) to bind complementary sequences on target mRNAs. This complex then uses enzymatic endonuclease activity to silence the target mRNA.[17] miRNA may also act to block or enhance translation by binding to their target mRNA's complementary sequence in the 5′ untranslated region (UTR) and either activating or inhibiting the ribosomal complex.[18,19] Mechanisms by which miRNA may act in a cell are still being discovered, though most commonly they act as inhibitors of their target mRNA.

Molecular Mechanisms of miRNAs as Tumor Suppressors

To act as a tumor suppressor, a miRNA must downregulate target mRNA that could otherwise cause a neoplastic or dysplastic phenotype by leading to aberrant proliferation, reversion of differentiation, or increased metastasis. Well-characterized examples of these tumor suppressor miRNAs are becoming increasingly abundant in the literature (Table 1).

Owing to their short length of approximately 21 base pairs, miRNAs have complementary sequences throughout

Figure 1. A schematic showing the miRNA biogenesis and mechanism of action.

Table 1. Common tumor suppressor miRNAs.

miRNA	SELECT TARGET GENES	TUMOR TYPES STUDIED (MDS, AML, AND OTHERS COMMONLY ASSOCIATED)
let-7 family	RAS, PRDMI, HMGA2, E2F2, c-Myc, cyclin D2, BCL-XL	MDS, AML, Colorectal, Lung, Ovarian, Breast, Prostate
miR-15/16	Bcl-2, WT-1, MCL1, JUN, ETS1, COX-2, VEGF, VEGFR2, FGFR1, FGF2, CEBPβ, CDC25a, CCNE1,MDM4, PI3KCa, BMI-1	AML, CLL, Multiple Myeloma, Lung, Gastric, Ovarian, Colon
miR-17-5p	P21, BIM (BCL2L11), E2F1, AIB1	MDS, AML, Breast, CLL
miR-29 family	MCL-1, CDK6, TCL1, DNMT1, DNMT3A, DNMT3B	AML, CLL, Lymphoma, Liver, Lung, Breast
miR-34	E2F3, Notch1, CDK 4, CDK 5, Bcl-2, HMGA2, MET, Snail1	Breast, Colorectal, Pancreatic
miR-124a	CDK6	MDS, ALL, Medulloblastoma
miR-127	Bcl-6	Breast, Lymphoma
miR-143	Ras, ERK5, Bcl-2	MDS, Gastric, Colorectal, Prostate
miR-145/146a	TIRAP, TRAF6, Fli-1, ERG, IRAK1	MDS, AML, Breast, Gastric
miR-181	TCL1, E2F5, eIF5A	AML, CLL, Colorectal
miR-200 family	VEGFR1, ZEB1, ZEB2	Breast, Bladder, Gastric, Ovarian, Renal cell carcinoma, Prostate, Lung, Pancreatic

Abbreviations: CLL, chronic lymphocytic leukemia; ALL, acute lymphocytic leukemia.

the genome (target multiplicity), leading to an exponentially larger effect on the cell than the suggested small change in miRNA levels. This is important when considering what type of effect miRNAs have on cells when downregulated in cancer or when increasing expression for therapeutic purposes (Tables 2 and 3).

The essential role of miRNA dysregulation in development of myelodysplasia and leukemia was well demonstrated by one group who showed that in mice osteoprogenitor cells (not hematopoietic progenitor cells), knockout of DICER1 led to MDS and AML because of abnormal niche regulation.[20] This finding reinforces the fact that not only are miRNAs important to cancer development and progression of the cells in which they are deregulated, but they can also transform other cell types located regionally or distantly by altering their signaling (Fig. 2). This review will explore this complex relationship with a number of examples showing the different mechanisms by which miRNA may act as tumor suppressors.

miR-145 and miR-146a in MDS, and miRNA as a Primary Driver of Disease

Located near the commonly deleted region (CDR) of chromosome 5 in the del(5q) subtype of MDS, miR-145 and miR-146a are more commonly downregulated in patients who have 5q-syndrome than MDS patients without this deletion.[21,22] These two miRNAs have been shown to be mediators of the toll-like receptor (TLR) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways,[22,23] which are essential inflammatory signals in MDS. Abnormal niche maintenance has been implicated in development of hematopoietic dysplasia,[20] which may be because of abnormal cytokine

release or cell signaling abnormalities, which can be modulated by these two miRNA.

Both of these miRNAs act as tumor suppressors in CD34+ bone marrow (BM) stem cells by targeting TIRAP and TRAF6, respectively, which allows for regulation of NF-κB signaling. When TRAF6 is upregulated or these miRNAs are downregulated, there is constitutive activation of this pathway and development of a del(5q) phenotype. miR-145 is also a regulator of FLI1, which has an important role in the balance between erythroid and megakaryocyte lineage differentiation.[24] The combined hemizygous knockdown of miR-145 and RPS14 (another gene in the CDR) led to an increase in the megakaryocyte/erythroid ratio, a del(5q)-like phenotype, and thus suggests the important role of miR-145 in this process.

miR-146a has also been shown to be important to the regulation of inflammation, myeloproliferation, and oncogenesis.[25] Knockout of this gene led to hypersensitivity to lipopolysaccharide (LPS), leading to over-production of many cytokines, increase in TRAF6 and IRAK1 protein levels in macrophages, and autoimmunity. Aging null mice also develop MDS, BM failure, and myeloproliferative neoplasms (MPNs). These findings have direct implications for the evolution of MDS and highlight the role of abnormal niche maintenance as an underlying cause of MDS.

Because of these essential roles in regulation of pathways associated with the pathophysiology of MDS, miR-145 and miR-146a, as well as their targets and downstream proteins in those pathways, are choice candidates for targeted therapy. For example, IRAK1, a protein kinase over-expressed in MDS and validated target of miR-146a, when inhibited using a small molecule inhibitor in MDS clones drove the cells to increased apoptosis and impaired their expansion.[26] Lenalidomide,

Table 2. miRNA as tumor suppressors in MDS.

STUDIES USING MICROARRAY TECHNOLOGY DOWNREGULATED miRNA EMPHASIZED	STUDIES FROM MURINE MODELS	STUDIES FROM PRIMARY PATIENT SAMPLES AND CLINICAL STUDIES
Downregulated miRNA in MDS compared to normal BM samples included miR-10a, miR-16, miR-25, miR-125b, miR-143, let-7a, and more. miR-451 was downregulated compared to controls in all MDS subtypes.[21]	EVI-1 was known to cause an MDS like phenotype when over-expressed, and led to early mortality. EVI-1 causes methylation of the miR-124 promoter. The resultant miR-124 decrease is associated with cell cycle and cell regeneration abnormalities in hematopoietic stem cells, resulting in marrow failure and death.[28]	Low levels of let-7a and miR-16 in the serum of patients with MDS was found to be a positive prognostic marker for increased overall and progression-free survival versus high levels of these miRNA, although lower levels of these miRNA were found in MDS patients compared to healthy controls. In addition, the level of these miRNA was found to be an independent risk factor for overall survival when stratified by IPSS group, without significant correlation to cytopenia or karyotype.[31]
miR-16 is significantly downregulated in high-risk versus low-risk MDS patients.[22]		
In CD34+ progenitor cell samples from bone marrow of patients with 5q-syndrome compared to normal controls, the former displayed lower expression levels of miR-128b, miR-146a, miR-342, miR-378, and miR-520c.[23]	Patients with del(5q) were found to have under-expression of miR-145, as well as increased levels of Fli-1. Inhibition of miR-145 or increased expression of Fli-1 leads to decreased production of erythroid lineage cells and increased production of megakaryocyte lineage cells. Reversal of the expression of these molecules has the opposite effect on myeloid cell differentiation. These findings were confirmed in a mouse model when studying myeloid differentiation. Additionally, miR-145 and RPS14 co-suppression leads to a del(5q)-like phenotype in CD34+ progenitor cells, and miR-145 forced expression in CD34+ cells showed a rescue of the MDS phenotype only in the del(5q) samples, not in MDS samples without del(5q).[29]	Two miRNA (miR-17-5p and miR-20a) were found to be under-expressed in high risk MDS compared to low risk. In addition, let-7a was decreased in high and intermediate risk MDS compared to low risk. There was a corresponding increase in their target proteins E2F1, MYC, BCL2, CCND1, and KRAS. All with known involvement in neoplasia and E2F1 known to have elevated concentration in two-thirds of MDS patients.[32]
miR-103, miR-140, miR-150, miR-342, miR-378, and miR-632 were all significantly downregulated in MDS compared to normal controls.[24]		Decreased miR-124 levels in bone marrow mononuclear cells of MDS patients was observed compared to that of normal controls and AML patients, with elevated levels of methylation of the miR-124 promoter in 7 of 11 MDS patients and a significant correlation between methylation status and miR-124 levels.[33]
let-7e, miR-124, miR-146a, miR-150, miR-197, miR-206, miR-326, and miR-875-5p were all found to be downregulated in MDS compared to normal controls. miR-486-5p was also shown to be significantly decreased in high risk MDS versus low risk. miR-150 has a known critical role in megakaryocyte lineage commitment, indicating a potential role in dysplastic myeloid hematopoiesis when dysregulated.[25]		Ezatiostat was shown to have some success in treatment of patients with low risk MDS, and the miRNA signature of under-expressed miR-129 and over-expressed miR-155 prior to beginning therapy was predictive of a positive response to therapy.[34]
miR-103-1, miR-140, miR-150, miR-342, miR-378, miR-483, miR-632, and miR-636 were all downregulated in MDS samples compared to normal controls. Hypermethylation in the promoters of miR-140, miR-378, and miR-632 was found, and host genes were identified for these miRNA and the others that were found to be downregulated. Some of these genes, including IGF2, RPS11, and SRSF2, have known roles in hematopoiesis and involvement in MDS. In addition, All-Trans Retinoic Acid (ATRA) therapy of cell lines led to a relative upregulation of the following miRNAs and their paired host genes during myeloid maturation: miR-103 & PANK3, miR-140 & WWP2, miR-342 & EVL, miR-632 & ZNF207, and miR-636 & SRSF2.[26]	Downregulation of miRNA located near the commonly deleted region (CDR) of 5q-syndrome (miR-143, miR-145, and miR-146a) was found in patient samples with del(5q) versus normal karyotype MDS or healthy controls. Two targets of miR-145 and miR-146a were confirmed, TIRAP and TRAF6 respectively. Simultaneous knockdown of miR-145 and miR-146a or forced expression of TRAF6 in HSPCs transplanted in a murine model led to a del(5q)-like phenotype, with some mice progressing to marrow failure or AML.[30]	Significant increases in miR-143 and miR-145 after therapy were associated with a positive response to lenalidomide therapy in patients with 5q-syndrome. Lenalidomide activity was specific to progenitor cells depleted of these two miRNA.[35]
		32 patients with low or int-1 risk anemic primary del(5q)-type MDS were treated with lenalidomide, and were found to have downregulation of RPS-14, miR-145, miR-146a, and miR-155 at baseline, with increased expression over the course of treatment.[36]
miR-196a, miR-423-5p, miR-507, miR-525-5p, miR-583, miR-940, miR-1284, miR-1305, and HS_122.1 were found to be downregulated in MDS. Patients with del(5q) had a downregulation of miR-143 and 378, which were localized to the CDR at 5q32, while other miRNA in this region (miR-145 and miR-146a) were not found to have significantly decreased expression. Compared to healthy controls, miR-10a was increased in 5q-syndrome and RA/RARS patients, but decreased in RCMD, advanced MDS, and secondary AML.[27]		miR-221, normally involved in erythrocyte differentiation via c-Kit regulation, was found to be downregulated in 11 patients with secondary AML compared to normal controls, and downregulated in MDS patients with RA/RCMD subtype but not in other MDS subtypes.[37]

Table 3. miRNA as tumor suppressors in AML, with focus on those relevant to MDS pathogenesis.

STUDIES FROM CELL LINES
miR-15a/16-1 forced expression leads to inhibition of cell proliferation in AML cell lines. WT1, a predicted target of these miRNA, showed a significant downregulation of protein level, but no reduction in mRNA level or luciferase activity when fused with the WT1 3'UTR. This indicates an indirect regulatory mechanism of WT1 by miR-15a/16-1 that causes abnormal hematopoiesis in leukemia.[79]
Alternative isoforms of BARD1 are oncogenic in some cancer types, and are downregulated by Vorinostat therapy (an HDAC inhibitor) in AML cells via increased expression of the tumor suppressors miR-19a and miR-19b. Alternate BARD1 isoforms were also recognized as potential biomarkers for diagnosis and prognosis.[80]
miR-27a and its miRNA cluster are downregulated in leukemia cells and patient samples. miR-27a expression leads to increased cellular apoptosis by targeting 14-3-3θ, a known anti-apoptotic gene acting via negative regulation of Bax and Bad proteins.[81]
miR-29b, a known tumor suppressor in AML, was shown to decrease expression of DNMT3A and 3B, as well as DNMT1, leading to re-expression of downstream genes via promoter demethylation.[51]
The miR-29 family was found to inhibit DNA methyltransferases as well as TET1 and TDG, two factors involved in DNA demethylation. Over-expression of miR-29 showed upregulation of DNA methylation in some cancer cells and downregulation in others, indicating that miR-29 may be acting as a stabilizer from changes in DNA methylation.[55]
CEBPA, essential for normal granulopoiesis and commonly deregulated in AML, leads to upregulation of the miR-29a/b cluster, often lost in AML.[82]
miR-150 has absent or low expression in the blocked differentiation state of AML, and higher levels associated with both normal hematopoiesis and differentiation of AML blast cells corresponding to MYB downregulation and activation of myeloid differentiation signals including CEPBA and CEBPE.[83]
miR-193a was found to be suppressed in AML blasts but not in normal BM cells, and was predicted to target c-Kit. The levels of the two were inversely correlated in leukemia samples. miR-193a was then shown to reduce c-Kit levels with forced expression, as well as inhibit cell growth and lead to apoptosis and differentiation of hematopoietic progenitors. When an inhibitor of miR-193a was added to 5-azacytidine therapy, the anti-leukemic effects of the latter drug were reduced.[84]
The most common AML-fusion protein, AML1/ETO, was shown to be a negative regulator of miR-223. The fusion protein recruits chromatin remodeling enzymes to the promoter site for this miRNA, leading to epigenetic silencing and a leukemic phenotype. Forced miR-223 expression, demethylating agents, or knockdown of the fusion protein all led to increased miR-223 expression and increased cellular differentiation, and thus a reduction of the leukemic phenotype.[85]
miR-370 was found to be downregulated in AML cell expression analysis, with upregulation of the oncogene FoxM1. RNAi of miR-370 showed increased proliferation of AML cell lines, and over-expression of this miRNA showed growth arrest and senescence. When these cell lines were treated with 5-aza-2'deoxycytidine (a DNA demethylating agent), upregulation of miR-370 was observed, indicating epigenetic regulation in leukemic cells.[86]
miR-495 was shown to be significantly downregulated in MLL-rearranged samples compared to both non-MLL-rearranged samples and normal controls. Over-expression of miR-495 showed reduced MLL-fusion-mediated cell transformation, and in BM transplantation studies showed decreased leukemogenesis in a murine model. In human leukemic cell lines with MLL-rearrangements, over-expression of miR-495 causes apoptosis. Concomitant over-expression of PBX3 or MEIS1, known targets of miR-495, leads to reversal of the effects of miR-495 over-expression and induction of the leukemic phenotype.[87]
STUDIES FROM MURINE MODELS
A novel HDAC inhibitor, AR-42, increases miR-29b levels in AML, leading to downregulation of miR-29b targets. In combination with decitabine, AR-42 led to increased anti-leukemic activity in AML compared to decitabine alone.[56]
miR-29b used as a targeted therapy was found to have higher delivery efficiency as a transferrin-conjugated nanoparticle than as a free miRNA, with the former showing significant downregulation of the miR-29b targets DNMTs, SP1, FLT3, KIT, and CDK6 and a distinct reduction in the leukemic phenotype in the murine model. In addition, treatment with the nanoparticles before decitabine therapy in the mice showed significant improvement in therapeutic results compared to decitabine alone.[57]
Ectopic miR-181a, previously shown to correlate to positive outcomes when increased in cytogenetically normal AML, was shown to inhibit tumor growth when expressed. In addition, lenalidomide therapy in these leukemic mice led to an increase in miR-181a via a truncated CEBPA isoform, acting as an enhancer of transcription of this miRNA, sensitizing these cells to chemotherapy.[64]
STUDIES FROM PATIENT SAMPLES AND CLINICAL STUDIES
Downregulation of miRNA let-7a was observed in AML cells during CXCR4 activation, and upregulation of let-7a during CXCR4 inhibition. Using primary patient samples, it was found that this mechanism was via activation of the YY1 transcription factor with CXCR4 expression levels, leading to MYC and BCL-XL transcription in AML cells. Additionally, let-7a over-expression in primary human AML xenografts led to increased sensitivity to cytarabine, leading to increased survival in these mice.[29]
miR-26a is downregulated in blasts from AML patients and is upregulated during myeloid differentiation of these cells. This increase in expression corresponded to a decline in the oncogenes c-Myc and EZH2, as well as cyclin E2 downregulation to inhibit cell cycle progression. Upregulation of miR-26a also increases the differentiation and anti-proliferative properties of 1, 25-Vitamin D_3.[30]
Low dose decitabine was given to older patients with AML who were previously untreated, started on a 10-day schedule. A 52% CR rate was seen in patients with a normal karyotype and 50% with a complex karyotype, with median disease-free and overall survival of 46 and 55 weeks, respectively. Higher levels of miR-29b were significantly associated with a positive clinical response.[31]
Bortezomib (a proteasome inhibitor) and decitabine (a DNA demethylating agent) both upregulate miR-29b in AML cells. Additionally, patients taking decitabine with high miR-29b expression have a better response to treatment than those with lower levels. Combination therapy showed upregulated levels of miR-29b, leading to SP1/NF-κB signal reduction and subsequent FLT3 downregulation. This corresponded to a 7/19 complete remission or incomplete CR rate.[32]
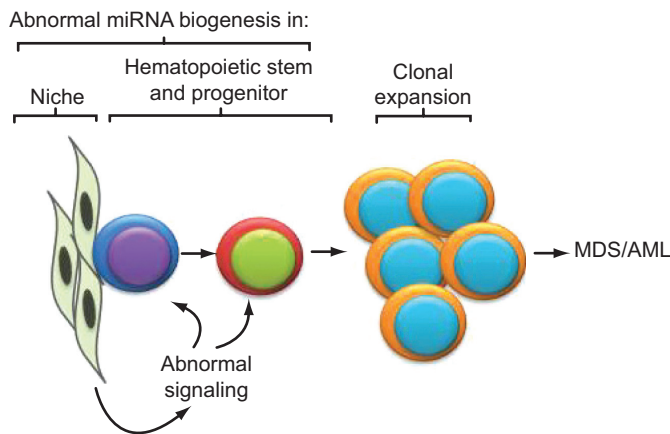
Figure 2. A schematic showing how abnormal miRNA biogenesis or regulation results in MDS/AML. Deregulation of one or more miRNAs in the hematopoietic microenvironment (niche) results in abnormal signaling to hematopoietic cells. Also, deregulation of miRNA within the hematopoietic stem and progenitor cells results in a clonal expansion leading to MDS/AML.

approved as the first-line agent for patients with 5q-syndrome, results in an increase in both of these miRNAs in BM of treated patients emphasizing the importance of these miRNAs in disease progression.[27] Targeting these miRNAs for future drug development, either via increasing endogenous expression or by exogenous delivery, would possibly lead to significantly improved therapy for MDS patients with del(5q) and under-expression of miR-145 and miR-146a.

Let-7a in MDS and AML, and the Many Roles of a miRNA

MiRNA array analysis has consistently revealed downregulation of miR-let-7a in MDS,[28] but shows a more variable expression pattern in AML with low levels in some studies and elevated levels in others.[23,25,26,29–33] Let-7a is traditionally considered a tumor suppressor,[34] downregulating various oncogenes including all three members of the Ras family,[35] EZH2,[36] and Myc.[37] In a study utilizing in silico modeling of miRNA function, analyzing most of the known miRNAs and their target pathways to relate expression and function, let-7a was near the top of the list of miRNAs having the largest number of targets with 45 types of pathways predicted to be targeted.[38] This indicates a large magnitude of effect within the cell with altered regulation and likely an extreme importance to cellular function.

Further studies have revealed the important role for this miRNA in abnormal hematopoiesis. Downregulation of let-7a leads to upregulation of important genes in carcinogenesis, including c-Myc and BCL-XL in AML cell lines, leading to a worsening leukemic phenotype.[29] Interestingly, the variability of this miRNA in AML indicates a more complex role in AML pathogenesis. Elevated levels were found to be associated with poor outcome,[39] while downregulation

increased chemoresistance of tumor cells in vitro.[29] In addition, a recent study in MDS patients showed that lower levels of let-7a in circulation were predictive of a better prognosis in MDS patients, though lower levels were found in MDS patients overall compared to healthy controls with normal hematopoiesis.[40]

Although circulating levels do not correlate necessarily to the activity level within a cell, this discrepancy between serum levels and function indicates a poor understanding of how this miRNA acts in hematopoietic cells versus other types of cancer to lead to either MDS or AML development, or in the transition to secondary AML as disease progresses. Virtually all miRNAs have been shown to exhibit target multiplicity within a cell, including the tumor suppressor miRNAs miR-202, miR-101, miR-302b, and miR-29b with 90, 65, 49, and 40 predicted target pathways, respectively.[38] This indicates that tumor suppressor miRNAs have extremely complex regulatory mechanisms in many cancers, and that their many roles will lead them to be difficult yet highly important targets for therapy.

miR-124 in MDS and Epigenetic Suppression

miR-124 is an established tumor suppressor and has been shown to be downregulated in MDS.[41,42] This miRNA is involved in a number of cancers, targeting oncogenes such as Slug in metastatic breast cancer,[43] cyclin-dependent kinase 6 (CDK6) in acute lymphoblastic leukemia,[44] and CDK4, cyclin D2, and EZH2 in uveal melanoma.[45] The most well-recognized mechanism for its downregulation in cancer is via epigenetic silencing because of methylation of its promoter.

EVI-1 is an oncogene that acts via epigenetic regulation of the phosphatase and tensin homolog/Akt/mammalian target of rapamycin (PTEN/Akt/mTOR) axis, an important pathway in cancer development.[46] EVI-1 has been implicated in the pathogenesis of MDS,[42,47,48] as well as many other types of cancers, and has been shown to be involved in miR-124-3 regulation via promoter methylation.

EVI-1 has been shown to directly interact with DNMT3A and 3B at the miR-124 promoter site,[49] indicating its role in de novo sequence-targeted DNA methylation leading to leukemogenesis. The driver of disease is abnormal regulation of EVI-1; however, it seems that suppression of miR-124 in this pathway ultimately causes the disease phenotype. Promoter methylation as the driver of this suppression was confirmed, and it was shown that this downregulation of miR-124 causes a significant oncogenic phenotype of cell cycle abnormalities, BM failure, and death in mice.[42,47] Additionally, it was found that while epigenetic regulation was usually the primary mechanism for miR-124 suppression, it was not the mechanism driving suppression in all diseased individuals,[50] indicating that miR-124 dysregulation may be as important in pathogenesis as EVI-1, and thus just as important as a target for therapy.

Many other miRNAs are involved in epigenetic regulation as well, including miR-29b,[51] let-7a,[36] and miR-26a in

MDS and AML,[30] and more in other cancer types. From the study of miR-124, it appears that epigenetic dysregulation of miRNA can alone lead to a cancerous phenotype, and that miRNA changes seen in MDS and AML are often not unique to these diseases, and may be applicable to many cancer subtypes.

miR-29b in AML, and miRNA as Targets of Therapy and as Therapy Themselves

Independently, methylation status and NF-κB signaling are potential mechanisms driving cancer; however, these pathways have been found to be modulated by the tumor suppressor miR-29b in AML pathology. This miRNA downregulates a number of oncogenes including Mcl-1 (a protein in the Bcl-2 family) which regulates the rate of apoptosis,[52] Tcl-1 in T-cell leukemias,[53] and DNMT3A and 3B in both AML and lung cancer,[51,54] with a total of 40 predicted target pathways.[37] miR-29b is downregulated in AML,[26] and was shown to act as a driver of the disease phenotype when suppressed because of increased methylation via DNMT1/3A/3B.[51] It was also found to inhibit Ten-Eleven Translocation 1 (TET1) and Thymine-DNA Glycosylase (TDG), both involved in DNA methylation regulation.[55]

Most miRNAs that are dysregulated in cancer are potential targets for therapy, including all discussed here, though some miRNAs will work better than others as monotherapy or single drug targets. For example, miR-29b has been found to be specifically targeted by bortezomib and decitabine, commonly available therapies. In AML, decitabine was shown to have a good response as the first-line therapy in a subset of AML patients, and higher levels of miR-29b significantly correlated with a positive clinical response.[31] Combining the two drugs for treatment of elderly AML patients led to increased expression of miR-29b, with corresponding NF-κB signal reduction, FLT3 downregulation, and clinical response in 44% of the patients in the phase I trial.[32] In addition, in experimental therapies on leukemic and patient cell lines and in mouse models, upregulation of miR-29b in combination with decitabine actually improves therapeutic efficacy compared to decitabine alone. This was achieved with the experimental drug AR-42 and with miR-29b conjugated to nanoparticles,[56,57] both leading to a reduction in leukemogenesis in their respective models. This indicates that not only are tumor suppressor miRNAs good targets for therapy and markers of response via endogenous upregulation, but they may also be used eventually in a clinical setting by exogenous delivery.

The Future of Therapy Related to miRNA

Available chemotherapeutic drugs often already target miRNA expression as part of their mechanism of action. In MDS, this includes lenalidomide,[27] HDAC inhibitors,[58] DNA demethylating agents like decitabine,[59] and other chemotherapeutic agents across all cancer types. However, more specific targeting of the miRNA identified as tumor suppressors and identifying the signaling pathways they affect for further drug development are the ultimate goals for personalized therapy to improve patient care.

To achieve this goal, we must consider which miRNAs are drivers of the malignant phenotype and how we want to upregulate that tumor suppressor activity. There are a number of mechanisms being implemented now which use exogenous miRNA in the lab to directly treat cancer cells. Two well-studied models are (1) attaching the target miRNA to a molecule that stabilizes it in the blood and leads to improved tissue delivery, commonly studied as conjugation to liposomes or other more advanced nanoparticles,[60,61] or (2) delivery using a virus (commonly adenovirus, though other models exist) that contains the target miRNA of interest in its genome.[62] Both methods have advantages and disadvantages, and need much more testing before reaching clinical practice.

Resistance to chemotherapy has also been found to be mediated by miRNA and their target oncogenes. One group showed that downregulation of miR-181a and subsequent upregulation of Bcl-2 in leukemia cells drove chemoresistance to Ara-C,[63] one of the most common drugs used in AML. The introduction of this miRNA into the cells increased chemosensitivity via apoptosis. Additionally, miR-181a upregulation after lenalidomide therapy led to chemotherapy sensitization to Ara-C in a subset of AML patients.[64] This type of miRNA treatment, as adjunct therapy for other chemotherapeutic agents, is a mechanism that has therapeutic implications in clinical practice.

Levels of miRNA in patients with cancer can also be helpful, especially to determine responsiveness to treatment or relapse of disease, as discussed previously related to let-7a.[40] Ezatiostat treatment in low-risk MDS was found to be more effective in patients with low miR-129 and high miR-155 expressions before therapy.[65] Both of these studies indicate the variability and utility that tracking miRNA levels can offer.

Although the use of natural agents for therapeutic purposes has existed for centuries, the molecular mechanisms of their efficacy have only recently been investigated. The potential use of such substances in combination with other drugs for cancer therapy is an active area of research. Green tea contains a compound known as epigallocatechin gallate (EGCG) that was recently shown to increase expression of the tumor suppressor miR-16. This subsequently suppresses Bcl-2 in hepatocellular carcinoma cells, leading to apoptosis.[66] Similar results were found for curcumin, a compound found naturally in turmeric (a spice that has been praised for its anti-cancer properties). This compound blocks the NF-κB pathway to lead to increased apoptosis in prostate cancer cells, by lowering Bcl-2 expression and increasing procaspase-3 and procaspase-8 levels,[67] as well as downregulating a number of oncogenic factors and growth factors in breast cancer cells.[68] It has since been shown to have a large role in altering expression of miRNA in cancer cells.[69] Curcumin was also shown to

decrease WT1 levels in leukemic cell lines and primary AML cells via upregulation of miR-15a/16-1.[70] Researchers are even synthesizing analogs to study their effects on miRNA to strengthen this therapeutic effect.[71] EGCG and curcumin are only two of many natural compounds being studied for their anti-cancer properties, making this an exciting young field in the search for drugs that can increase treatment efficiency.

Conclusion

While numerous miRNAs act as tumor suppressors, and more are likely to be identified, we have limited our review to those that have been shown to have a modulating role on pathways known to be deregulated in MDS and AML. The initial realization that miRNAs were active in hematopoiesis opened an exciting new avenue for drug development. AML and MDS are both diseases that desperately need new approaches for therapy, as a majority of patients are either resistant to current therapies or eventually relapse. The studies outlined here provide insight into how knowledge of miRNA expression and function in cancer evolution can be translated into developing targeted therapies. First, miRNA dysregulation may act as part of the larger mechanism underlying what is driving the cancerous phenotype, and may also be the primary driver of the disease. Second, miRNA can cause an oncogenic phenotype, and can also act as mediators of chemoresistance and chemosensitivity in cells, both of which are important therapeutic targets. Third, altered epigenetics such as DNA methylation status and chromatin alteration often play a role in miRNA dysregulation in cancer. Fourth, miRNA signatures or circulating miRNA levels may act as markers for personalization of therapy or prognostic determination. Fifth, miRNA can be targeted to improve cancer therapy by agents that are already in the market or are in development, and also may eventually be used as therapeutic agents themselves. Finally, natural agents are currently being explored for their effects on oncogenesis related to miRNA expression, and are showing promising results for future use as isolated natural compounds or as analog therapies.

These discoveries lead to the conclusion that tumor suppressor miRNAs are essential mediators of cancer development and will undoubtedly be crucial targets for cancer therapy and clinical practice in the future. The complexities of miRNA regulation of target oncogenes, and their effects on subsequent signaling pathways and disease phenotype, are under intense investigation to develop a more comprehensive model. This is especially important in light of the multiple target genes that can be modulated by a single miRNA. However, tissue or cell-type specificity may minimize some of the unwanted side effects. miRNAs are the new weapons in the arsenal of the fight against this extremely complex disease called cancer.

Author Contributions

NI researched and wrote the first draft of the manuscript. NI, NG, AA, and AR contributed to the writing of the manuscript, agreed with manuscript conclusions, jointly developed the structure and arguments for the paper, and made critical revisions and approved the final version. All authors reviewed and approved the final manuscript.

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