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MicroRNA in Melanoma

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ABSTRACT

Melanoma is a highly aggressive and deadly skin cancer. Early intervention correlates with nearly 100% patient survival, but greater than 80% mortality is associated with advanced disease. Currently, few treatment options are available for patients with metastatic melanoma, and the global incidence of melanoma is increasing faster than that of other cancers. Therefore, it is vitally important to uncover and use genetic and epigenetic regulatory mechanisms at work during the development and progression of melanoma for better prevention, diagnosis, and clinical management. MicroRNA (miRNA) is a set of small, single-stranded, noncoding RNAs that target the 3′-untranslated region of an estimated 30% of all human genes to inhibit their expression. Our understanding of miRNA-mediated regulation of cancers has grown immensely over the past decade. Here we review currently available data on melanoma-associated miRNAs, highlighting those deregulated miRNAs targeting important genes and signaling pathways involved in the progression of melanocytes to primary and metastatic melanoma. Understanding the important roles of miRNAs in melanoma progression and metastasis development will contribute to the development of miRNA-targeted therapy in the future.

INTRODUCTION

Melanoma is currently the sixth most common cancer in white men and women in the United States. According to the American Cancer Society, 68,720 new cases of melanoma and 8,650 deaths were predicted for 2009.[1] Although not the most common of skin cancers, comprising only 5% of all skin cancers, it is by far the deadliest, responsible for 75% of skin cancer-related deaths. Incidence rates for white men and women in the United States are currently at 1 in 45 and 1 in 58, respectively,[1] with the incidence rate of melanoma increasing faster than any other cancer in the world. Those at high risk for developing melanoma may have any combination of fair skin and eye color, familial melanoma, presence of common or dysplastic/atypical nevi (moles), high density of freckles, intermittent sun exposure with burns, and/or other current or previous skin cancer lesions. Primary cutaneous melanoma diagnosed early in its course has an excellent outcome with early surgical intervention, but regional and distant metastatic disease has a much more dismal prognosis. Current 5-year survival rates for all races equal 91% for all disease sites (significantly higher than 1975-1977 at 82%), 99% for local disease, 65% for regional disease (including regional lymph node involvement), and only 16% for those with distant metastasis (median survival is only 7.5 months). Unfortunately, for those patients who will develop locally advanced and metastatic melanoma, current treatment options are limited. Metastatic melanoma is highly resistant to therapy; thus, the overall poor mortality rates for patients with regional and distant disease emphasize the need for better treatment modalities and recognition of early neoplasia.

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Melanocytes and Melanoma

Cutaneous melanoma arises from epidermal melanocytes of neural-crest developmental origin. Neural-crest cells, induced at the time of gastrulation, are highly invasive and undergo an epithelial-to-mesenchymal cell-type transition (EMT), as they must migrate through embryonic tissues during early development to form cartilage, bone, connective tissue, neural and glial cells, sympathoadrenal cells, and smooth muscle and pigment-producing melanocytes, among other cell types.[2] Some of these multipotent cells differentiate to melanocytes and/or the basal layer of the epidermis, as well as hair follicles, meninges, the eye, and cochlea.[2] They produce melanin pigment in response to ultraviolet (UV) radiation in melanosome organelles that may either remain in the cell or, more often, be deposited into neighboring keratinocytes via extending dendritic

processes for protecting the skin from UV radiation and reactive oxygen species. Importantly, metastatic melanoma cells have been shown to overexpress proteins associated with neural-crest cell invasion and differentiation relative to their nonmetastatic counterparts as well as to downregulate proteins characteristic of normal melanocytes, leading to the idea of tumor development and metastasis being a cell ''lineage reversion'' to a more neural-crest cell phenotype.[3,4]

The conversion of normal melanocytes to melanoma cells has thereby been attributed to a number of factors. Notably, UV exposure has been associated with the development of skin cancer, including melanoma.[5] Indeed, focused efforts on decreasing UV exposure appear to result in decreased melanoma incidence, and high sun protection factor (SPF) sunscreens afford a protective advantage against UV radiation.[6,7] Direct DNA damage by UV radiation can deregulate normal DNA repair mechanisms and result in unimpeded proliferation.

Melanocyte integrity is also highly influenced by its microenvironment. In the epidermis, melanocytes exist at roughly 1/5 the number of basal-level keratinocytes present and 1/36 of total keratinocytes. Paracrine hormone communication, direct cell-to-cell adhesion communication, and autocrine regulation serve to direct melanocyte homeostatic mechanisms, including differentiation, quiescence, and proliferative and apoptotic responses.[8] Deregulation of these processes can lead to the formation of benign and dysplastic nevi and melanoma.[9] For example, by way of the transmembrane protein, Notch, melanocyte precursors (melanoblasts) maintain their nondifferentiated status, and *Notch1* has been described as overexpressed in melanoma relative to melanocytes and benign nevi.[10-12] In a recent study, transfection of an active truncated *Notch1* transgene construct into a normal melanocyte resulted in induction of tumorigenic character, including anchorage-independent growth, increased proliferation in limited media, and increased migration and invasive capacity. The authors thus described *Notch* itself as the first melanoma-transforming oncogene.[12]

Understanding the gene expression character of early melanocytic transformation, such as Notch signaling, and further metastatic processes and their regulatory mechanisms is vital for the future of targeted melanoma therapy. Interest in epigenetics, defined as all heritable changes in gene expression not involving DNA coding, has grown significantly over the past decade because of its recognized influence on normal cellular processes and disease states; in particular, cancer. Epigenetic modes of gene expression regulation include DNA methylation (promoter methylation inhibits transcription factor binding), histone modifications (influences histone binding affinity for DNA), and RNA-associated silencing, including microRNA (miRNA).[13,14] Of these epigenetic mechanisms, messenger RNA (mRNA) silencing by miRNA may be the most complex and promising field for future cancer research and therapy.

MicroRNA

MiRNAs are a set of small [~22 nucleotides (nt)], single-stranded, non−protein-coding RNA molecules, which can recognize and bind 3′-untranslated regions (UTRs) of mRNA, blocking translation of the gene or inducing cleavage of the mRNA.[15,16] Currently, more than 700 miRNAs have been discovered in humans, ~800 are predicted as remaining, and more than 4,000 in all eukaryotic species have been examined.[17]

A primary miRNA is the result of transcription as either an independent transcript or as coexpressed from an intron of another gene by RNA polymerase II and may include multiple regions that will become mature miRNAs (multiple miRNAs that are transcribed together are termed *clusters*, ie, *miR-17-92* cluster). The primary miRNA that was coexpressed undergoes further splicing before it and the individual transcript primary miRNA are bound in the nucleus by the microprocessor complex, which consists of the RNase III-type endonuclease Drosha and its cofactor Pasha (DGCR8). This complex then crops the primary miRNA into a hairpin loop, cleaving off 3′ and 5′ regions of excess mRNA, to give a precursor miRNA of ~70 nt in length. Precursor miRNA is then actively transported to the cytoplasm by exportin-5, where it is bound by the RNAse III-type endonuclease Dicer, which removes the loop and results in a mature miRNA duplex of complementary sequences. One strand is bound by the RNA-induced silencing complex (RISC), which guides mature miRNA to target mRNA for subsequent silencing. The remaining strand is usually degraded but may be bound by the RISC and target its own mRNAs, denoted with an asterisk (eg, *miR-373* and *miR-373**).[18,19] MiRNA synthesis and function are summarized in Figure 1.

MiRNAs mediate gene expression at the posttranscriptional and translational levels in both plants and animals.[20,21] Because of their critical function in gene regulation and expression, it is important to understand their roles and significance in tumor cell development, differentiation, proliferation, and apoptosis.[22-25] Interestingly, a single miRNA potentially binds hundreds of its cognate mRNA 3′-UTR sequences. It is thus predicted that miRNAs may regulate the expression of more than 30% of all mammalian genes, acting as tumor suppressors or oncogenes, depending on which they dominantly bind and repress.[16]

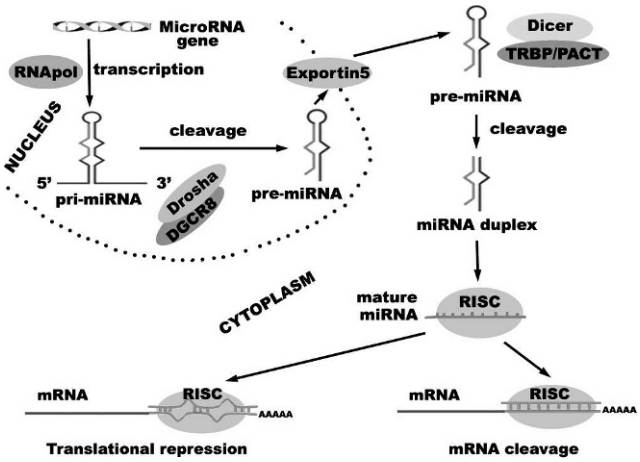

Figure 1. MicroRNA synthesis and function.

The first indication that miRNAs might function as tumor suppressor genes was derived from a study by Calin et al,[26] who found that *miR-15a* and *miR-16-1* were commonly deleted in more than 65% of patients with B-cell chronic lymphocytic leukemia. This study proved that *miR-15a* and *miR-16-1* negatively regulated Bcl2, which is an antiapoptotic protein that is often overexpressed in a variety of tumor histologies.[26] Recent reports have also demonstrated that more than 50% of miRNA genes are located in cancer-associated genomic regions or within fragile sites.[27] This suggests that miRNAs might play a more important role in the pathogenesis of human cancer than was previously realized.

MICRORNA IN MELANOMA

Overview

Compared with studies in other solid tumors, relatively few studies have focused on the role of miRNAs in the pathogenesis and development of melanoma. Zhang et al[28] published one of the first studies on miRNA in melanoma, documenting that 86% of primary melanoma cell lines had DNA copy number alterations in genomic loci containing miRNA genes. They also identified 243 miRNA genes (83 with gains vs 160 with losses) that were unique to melanoma. Using highly sensitive miRNA quantitative real-time polymerase chain reaction (qRT-PCR) techniques, Gaur et al[29] identified a uniquely clustered gene group that was able to distinguish melanoma from all other tumor histologies. Moreover, *miR-137* was identified as a trigger for micropthalmia-associated transcription factor (*MITF*), a master regulator of melanocyte development and survival of significant functional importance in melanoma.[30] *MiR-221* and *miR-222* (*miR-221/222*) possess an oncogenic role in melanoma and other cancers when their expression is deregulated.[31] Worley et al[32] identified several miRNA profiles associated with a ''good'' prognostic signature in the progression of uveal melanoma, with the overexpression of *miR-199a* and *let-7b* found primarily in metastatic lesions. It should be noted that uveal and cutaneous melanomas do have important clinical and molecular differences; for instance, uveal melanoma favors hematogenous spread rather than lymphatic, has a high propensity for metastasis to the liver and lungs, and demonstrates only rare instances of *BRAF* and *CDKN2A* mutation compared to cutaneous melanoma. *MiR-34a* appears to act as a tumor suppressor in uveal melanoma cell proliferation and migration through the downregulation of *c-Met*.[33] Furthermore, it is likely that *miR-34b*, *miR-34c*, and *miR-199a** have the capacity to impair *c-Met*–mediated invasive growth.[34] *MiR-182* ectopic expression stimulated migration of melanoma cells *in vitro* and increased their metastatic potential *in vivo*, whereas *miR-182* downregulation impeded invasion and triggered cellular apoptosis.[35] Mueller et al[36] recently identified a large cohort of miRNAs associated with the malignant transformation and progression of melanoma using *in vitro* models. Taken together, these results suggest that miRNAs play an important role in the biology and progression of melanoma. Clearly, additional studies are required to validate a distinct profile of miRNA markers with the capacity to predict the potential for developing metastatic melanoma. The Table lists notable miRNAs involved in the progression from melanocyte to metastatic melanoma, their validated or proposed targets, regulatory factors, and effects of miRNA upregulation or downregulation during the respective stages of progression.

MITF and Associated miRNAs in Melanoma

MITF is a recognized oncogene in melanoma that regulates cell proliferation and apoptosis and is overexpressed in 10% to 20% of human melanomas.[37] It is a member of the *Myc* supergene family of basic helix-loop-leucine-zipper transcription factors, necessary for functional melanocyte formation.[38] Because of the critical role of *MITF* in melanoma progression, several recent studies have explored the impact of miRNAs on melanoma through *MITF*-mediated pathways.

MicroRNAs Targeting MITF. MicroRNA.org, an online database for miRNA target prediction, provides more than 300 miRNA candidates that putatively target *MITF*, although only a few of them have been validated thus far. *MiR-137* is located at the chromosomal region 1p22, which is known to harbor an allele for melanoma susceptibility. Bioinformatic and *in vitro* analyses verified *miR-137* targeting of *MITF* in melanoma cells.[30] Segura et al[35] described *miR-182* as another negative regulator of *MITF* expression. *MiR-182* is located in 7q31-34, a chromosomal region

Table. Representative miRNAs involved in the progression of melanoma

Progression	miRNA	Target(s)	Regulatory Factor	Associations
Melanocyte	↓ **let-7a**[43]	*ITGB3*		↓ Migration, invasion
	↓ **let-7b**[42]	*CCND1*		↓ Proliferation, differentiation
	↓ **miR-155**[46]			↓ Proliferation
	↓ **miR-324-5p**[36, 76]			
	↓ **miR-34a**[33, 63, 73, 76]	*MET*	Promoter methylation	↓ Proliferation
↓				
Primary Melanoma	↑ miR-106a[36]			
	↑ **miR-126**[36, 76]			
	↑ **miR-133a**[36, 76]			
	↑ **miR-141**[36, 76]			
	↑ **miR-145**[36, 76]			
	↑ **miR-15b**[73]			↑ Proliferation, survival
	↑ **miR-200c**[36, 76]			
	↑ **miR-210**[65, 73]	*MNT*		↑ Proliferation
	↑ **miR-27b**[36, 76]			
Primary Melanoma	↓ **miR-126**[76]			
	↓ **miR-133a**[76]			
	↓ **miR-141**[76]			
	↓ **miR-199a***[34, 76]	*MET*		↓ Migration, invasion and survival
	↓ **miR-200c**[76]			
	↓ **miR-34a**[76]			
	↓ **miR-34b/c**[34]	*MET*		↓ Migration, invasion and survival
↓				
Metastasis	↑ **let-7b**[32]			
	↑ miR-106a[36]			
	↑ miR-133a[36]			
	↑ **miR-182**[35]	*MITF, FOXO3*		↑ Migration, invasion and survival
	↑ **miR-199a***[32, 36]			
	↓ **miR-133a**[76]			
	↓ **miR-146a/b**[46]			
	↓ **miR-155**[46, 76]			↓ Proliferation, survival
Melanocyte	↑ miR-133a[36]			
	↑ **miR-17-5p**[36, 46]			
↓	↑ **miR-18a**[36, 46]			
Metastasis	↑ miR-19a[36]			
	↑ miR-19b[36]			
	↑ **miR-20a**[36, 46]			
	↑ **miR-221/222**[31, 36]	*c-Kit, p27^{Kip1}*	*PLZF*	↑ Proliferation, invasion; ↓ differentiation
	↑ **miR-532-5p**[64]	*RUNX3*		↑ Invasion
	↑ **miR-92a**[36, 46]			

Abbreviations: *CCND1*: cyclin D1; *FOXO3*: forkhead box O3; *ITGB3*: integrin β3; *MET*: c-Met; *MITF*: microphthalmia-associated transcription factor; *MNT*: max-binding protein; *PLZF*: promyelocytic leukemia zinc finger; *RUNX3*: runt-related transcription factor 3.

[a] MicroRNAs (miRNAs) are organized based on their expression changes during melanoma progression, as described in the literature. Validated miRNAs (eg, as by quantitative real-time polymerase chain reaction) are in bold. Arrows indicate relative miRNA expression (upregulated or downregulated) during the respective progressive stage. Targets and regulatory factors have been described for melanoma, while the associations highlight increases or decreases (''up'' arrow and ''down'' arrow, respectively) in cell activities with expression of the miRNA.

frequently altered in melanoma. *MiR-182* was demonstrated to increase survival and invasive potential of melanoma cells by repressing *MITF* and *FOXO3*, a forkhead family transcription factor. Importantly, 7q31-34 also harbors *c-Met* (encoding hepatocyte growth factor receptor with tyrosine-kinase activity) and *BRAF* (member of the raf/mil family of serine/threonine protein kinases), two important regulators in the *MAPK/ERK* signaling pathway.[39] They found that *miR-182* was overexpressed not only in human melanoma cell lines but also in tissue specimens. These results were inversely correlated with *MITF* and *FOXO3* expression in predicting melanoma progression and development. Moreover, ectopic expression of *miR-182* in melanoma cells stimulated anchorage-independent growth and invasion, using an *in vitro*

extracellular matrix assay, and promoted melanoma lung metastasis in a mouse model, whereas *miR-182* downregulation impeded invasion and triggered apoptosis of melanoma cells.

MiRNAs Regulated by MITF at the Transcriptional Level. Ozsolak et al[40] identified a number of miRNAs to be regulated by *MITF* in melanoma cells using nucleosome mapping and linker sequence analyses. These miRNAs include some members of the *let-7* family (*let-7a-1*, *-7d*, *-7f-1*, and *-7i*), *miR-221/222*, *miR-17-92* cluster, *miR-106-363* cluster, *miR-29*, *miR-146a*, *miR-148b*, and *miR-125b*.[40]

The *let-7* family is highly conserved across species in sequence and function, the first miRNAs validated to be involved in tumorigenesis.[41] Schultz et al[42] revealed 5 members of the *let-7* family (*let-7a*, *-7b*, *-7d*, *-7e*, and *-7g*) to be significantly downregulated in primary melanoma compared to benign nevi, suggesting the *let-7* family might act as tumor suppressors in melanoma. The ectopic expression of *let-7b* diminished the anchorage-independent growth ability of melanoma cells and inhibited cell-cycle progression. Overexpression of *let-7b* eventually repressed cyclins (D1, D3, and A) and cyclin-dependent kinase 4 (*CDK4*), all of which have been described as playing a role in melanoma development.

Another study found that *let-7a* was lost in melanoma when comparing primary melanocytes and malignant melanoma cell lines, with sequence analysis suggesting that it interacts with the 3′-UTR of *integrin β3* mRNA.[43] *Integrin β3* is highly related to melanoma progression and leads to enhanced migratory and invasive potential of melanoma cells.[8] Transfection of melanoma cells with *let-7a* precursor miRNA molecules resulted in the downregulation of *integrin β3* mRNA and protein expression, which suggested that the loss of *let-7a* expression might be an essential regulatory mechanism leading to increased *integrin β3* expression in melanoma cells.[43] Müller and Bosserhoff[43] also proved that the overexpression of *let-7a* in melanoma cells reduced their invasive potential by approximately 75%, while transfection with antisense oligonucleotides that directly bind and inhibit the actions of miRNAs resulted in the induction of *integrin β3* expression and induced migration of anti-*let-7a*–transfected melanocytes. These findings revealed *let-7a* to be an important regulator of *integrin β3*, the loss of *let-7a* thus being involved in the development and progression of malignant melanoma.

The *miR-17-92* cluster locates to chromosome 13 and contains 6 members (*miR-17*, *-18a*, *-19a*, *-20a*, *-19b-1*, and *-92a-1*), while another miRNA cluster, *miR-106-363*, which shares many similarities with the *miR-17-92* cluster, locates to the X chromosome and also consists of 6 members (*miR-106a*, *-18b*, *-20b*, *-19b-2*, *-92a-2*, and *-363*). Both miRNA clusters are described as oncogenic and found highly expressed in a variety of cancers.[44,45] Mueller et al[36] compared the profiles of normal human melanocytes and well-characterized melanoma cell lines derived from primary tumors and melanoma metastases. They showed that all members of the *miR-17-92* cluster were upregulated in primary tumor cell lines compared to normal melanocytes. The expression of the *miR-17-92* cluster was even higher in metastatic cell lines, with an approximately twofold upregulation compared to primary melanoma cell lines. The expression of the *miR-106-363* cluster was similar to the expression of the *miR-17-92* cluster in melanocytes and melanoma cells. The researchers detected a strong upregulation of *miR-106a* expression in primary tumor cells and a further increase in expression level in metastatic melanoma cells.[36] In addition to finding *miR-17-5p*, *miR-18a*, *miR-20a*, and *miR-92a* overexpressed and *miR-146a*, *miR-146b*, and *miR-155* downregulated in the majority of melanoma cell lines with respect to melanocytes, Levati et al[46] found that ectopic expression of *miR-155* in melanoma cells inhibits their proliferation. These results imply involvement of the *miR-17-92* cluster in melanoma progression.

Both *miR-221* and *miR-222* are regulated by *MITF* at the transcriptional level.[31] These 2 miRNAs are clustered on the X chromosome, transcribed as a common precursor, and overexpressed in a variety of cancers with the function of repressing the *c-Kit* receptor. In normal melanocytes, stem cell factor-dependent, *c-Kit*–mediated signaling supports proliferation, migration, and differentiation of cells[47]; however, constitutive activation of the *c-Kit* receptor tyrosine kinase alone is insufficient to induce tumorigenic transformation of melanocytes *in vitro* or *in vivo*.[48] Cutaneous melanomas are often characterized with a loss of *c-Kit* expression.[49] Inhibition of the *c-Kit* receptor tyrosine kinase in *c-Kit*–positive melanoma cells showed increased apoptosis and G1-phase cell-cycle arrest,[49] while the re-expression of *c-Kit* in *c-Kit*–negative melanoma cells restored *c-Kit*–mediated apoptosis and resulted in a loss of tumorigenic potential.[50] In accordance with these observations, Felicetti et al[31] found that upregulation of *miR-221/222* repressed the expression of the *c-Kit* receptor and *p27Kip1* (cyclin-dependent kinase inhibitor 1B) tumor suppressor during melanoma progression from a weakly invasive primary tumor to a more invasive phenotype. Overexpression of *miR-221/222* in melanoma cells led to increased proliferation and invasion *in vitro* and accelerated tumor growth in a mouse melanoma model. Conversely,

treatment with anti-miRNAs against both miRNAs resulted in a reduced rate of proliferation and ability to migrate in melanoma cells with a high level of *miR-221/222*. The researchers also found that the elevated expression of *miR-221/222* in melanoma cells was caused by the loss of a transcription factor, promyelocytic leukemia zinc finger (*PLZF*). *PLZF* binds to the *miR-221/222* promoter and inhibits their transcription in normal melanocytes. Igoucheva and Alexeev[51] recently described a similar result. They confirmed that *c-Kit* was downregulated by *miR-221/222* and revealed that *c-Kit* regulation was mainly based on miRNA-dependent posttranscriptional mechanisms instead of an AP-2-dependent transcriptional mechanism.

Phosphatase and tensin homolog (*PTEN*) is an important tumor suppressor that is either mutated or deregulated in a variety of cancers, including melanoma.[52-54] It inhibits the phosphorylation/activation of Akt3 (p-Akt), a member of the serine/threonine kinase family, and the phosphoinositide 3-kinase/protein kinase B (*PI3K/AKT*) pathway, known to promote proliferation, survival, invasion, and angiogenesis in melanoma. Akt3 becomes more active (p-Akt) toward advanced, metastatic melanoma with a concomitant loss of *PTEN* expression. No miRNAs are currently described as targeting *PTEN* in melanoma, although recent reports highlighted *miR-141* in nasopharyngeal carcinoma and *miR-221/222* in aggressive non-small cell lung cancer and hepatocarcinoma as oncogenic miRNAs (oncomirs) capable of directly targeting and inhibiting the expression of the tumor suppressor *PTEN*.[55,56]

MiR-221/222 are increased from primary to metastatic melanoma samples (Table), where *PTEN* mutation is relatively rare (5%-20%).[31,52] These miRNAs act in melanoma to increase proliferation, invasion, migration, and anchorage-independent growth and to reduce differentiation and melanogenesis by downregulating the *c-Kit* receptor and *p27Kip1*. Felicetti et al[31] described *miR-221/222* overexpression in melanoma cells as being the result of a decrease in the *PLZF* transcription factor, a direct inhibitory factor of *miR-221/222*. Cyclin-dependent kinase 2 (*CDK2*) has been reported to phosphorylate *PLZF*, triggering its ubiquitination and subsequent degradation.[57] Furthermore, *p27Kip1*, which is induced to coexpress with *PTEN*, is important for the efficient induction of G1 cell-cycle arrest by *PTEN* and is necessary for *PTEN*-induced downregulation of *CDK2*.[58,59] Therefore, *PTEN* itself may be an important regulator of *miR-221/222* in melanoma as its coexpression with *p27Kip1* in turn inhibits CDK2-mediated phosphorylation of *PLZF*, thereby maintaining *PLZF* levels to bind *miR-221/222* promoters, preventing their transcription. Additionally, *PTEN* is

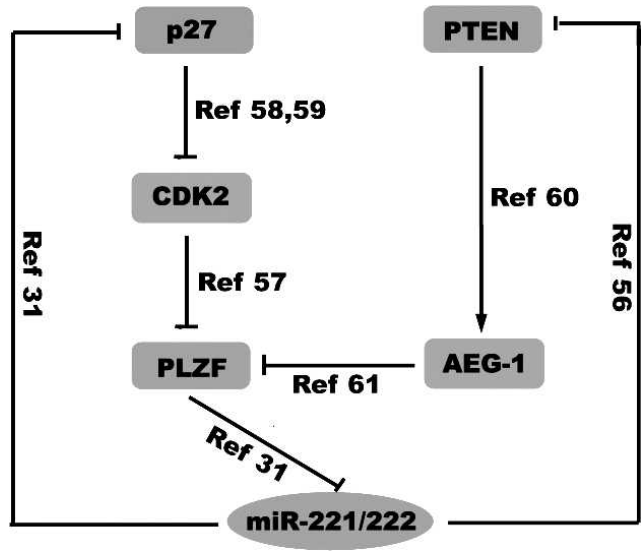

Figure 2. The regulatory network of *miR-221/222*.

important for Ha-ras–mediated astrocyte elevated gene-1 (*AEG-1*) promoter activation.[60] *AEG-1* directly binds *PLZF*, preventing it from binding its target promoters,[61] including those of *miR-221/222*. As a result, there exists a putative positive feedback loop for *miR-221/222* expression, promoting melanoma progression through the joint inhibition of *PTEN* and *p27Kip1* and blocking *PTEN/AEG-1/PLZF* and/or *p27Kip1/CDK2/PLZF*-mediated repression of *miR-221/222* (Figure 2).

MiR-34 Family and Melanoma

Recently, *miR-34* was identified as a target and a potential key effector of the tumor suppressor gene product *p53*. Ectopic expression of *miR-34a* induced a G1 cell-cycle arrest, senescence, and apoptosis, suggesting that *miR-34* may act as a tumor suppressor.[62] The altered expression of *miR-34* was also found during melanoma progression.[33,34,63] Lodygin et al[63] reported that *miR-34a* expression is silenced in several types of cancer because of aberrant CpG methylation of its promoter. Indeed, 43.2% of melanoma cell lines and 62.5% of primary melanoma samples displayed CpG methylation of the *miR-34a* promoter and loss of *miR-34a* expression, whereas the 2 samples of normal melanocytes included in the study showed little to no promoter methylation. Migliore et al[34] identified 3 miRNAs–*miR-34b*, *miR-34c*, and *miR-199a**–in melanoma cells that negatively regulate the expression of *MET*, an oncogene that encodes the tyrosine kinase receptor for hepatocyte growth factor. *MET* is frequently overexpressed in many human tumors and promotes the ''invasive growth'' that results from stimulation of cell motility and protection

from apoptosis. Exogenous expression of these miRNAs in primary melanoma cells led to a decreased MET protein expression and resulted in the impairment of *MET*-mediated motility in these cells.[34] Recently, Yan et al[33] found *miR-34a* to be actively expressed in melanocytes but not in uveal melanoma cells. The transfection of *miR-34a* into melanoma cells led to a significant repression of their growth and migration by downregulating the expression of *c-Met* directly and the expression of phosphorylated Akt (p-Akt) and other cell-cycle–related proteins indirectly.

Other miRNAs in Melanoma

Kitago et al[64] recently reported that *miR-532-5p* directly targets the runt-related transcription factor 3 (*RUNX3*) tumor suppressor during the progression from melanocyte and skin to metastatic melanoma. *MiR-532-5p* was shown to be significantly upregulated in melanoma cells compared to normal melanocytes and in metastatic melanoma tissue compared to primary melanoma tissue. Transfection of anti–*miR-532-5p* molecules into the melanoma cells rescued the expression of *RUNX3*. Methylation analysis of the *RUNX3* promoter region showed that transcriptional regulation was not a major regulatory mechanism for the downregulation of *RUNX3* expression in melanoma, suggesting that *miR-532-5p*–induced posttranscriptional regulation of *RUNX3* may play an important role in melanoma progression.

Zhang et al[65] demonstrated that the expression of *miR-210*, the most prominent miRNA upregulated by hypoxia and a direct transcriptional target of hypoxia-inducible factors, was elevated in multiple cancer types and correlated with breast cancer and melanoma metastases, respectively. *MiR-210* overexpression in cancer cells bypassed hypoxia-induced cell-cycle arrest by directly targeting the expression of *MNT*, an *Myc* antagonist. The *miR-210*–mediated abolishment of hypoxia-induced cell-cycle arrest was restored by the loss of *Myc*. This finding indicated that *miR-210* influenced the hypoxia response in tumor cells by triggering a *Myc*-like response by targeting *MNT* expression.

CLINICAL FOCUS

Several years ago, we and other groups separately demonstrated that miRNAs were relatively more stable and tolerant of RNAases than were mRNAs, both in archived tissue samples and in blood samples,[66-68] which suggests that miRNAs have the potential to be valuable, practical, and reliable biomarkers for disease states. Recently, several groups employed high-throughput microarray techniques to discover miRNA biomarkers from formalin-fixed and paraffin-embedded (FFPE) melanoma samples.[69-71] A number of miRNAs have shown the potential to become diagnostic markers for melanoma based on data from clinical samples and array analyses.[69-71] Radhakrishnan et al[72] examined the presence of oncomirs in uveal melanoma using FFPE specimens by comparing miRNA expression profiles between noninvasive tumor and melanoma metastatic to the liver. They revealed 19 miRNAs that were expressed in nonmetastatic melanoma and absent in metastatic melanoma and 11 miRNAs with the opposite expression pattern.

Satzger et al[73] determined the expression level of 16 miRNAs in 6 melanocytes versus 10 melanoma cell lines and in 11 nevi versus 16 melanoma cell lines, respectively, finding that *miR-15b* and *miR-210* were significantly upregulated in parallel with the downregulation of *miR-34a* in melanomas compared to nevi. These 3 miRNAs were then analyzed in 128 primary melanoma samples from patients with detailed clinical follow-up information. Only high expression of *miR-15b* was significantly correlated with poor recurrence-free survival and overall survival by univariate Kaplan-Meier and multivariate Cox analyses. Transfection of anti–*miR-15b* into melanoma cells led to reduced tumor cell proliferation and increased apoptosis. These results showed that *miR-15b* might be a novel melanoma biomarker contributing to poor prognosis and tumorigenesis.

Worley et al[32] were the first to use a genome-wide, microarray-based approach to investigate the value of miRNA expression patterns in predicting metastatic risk in uveal melanoma. They found that the most significant discriminator for classifying low and high metastatic risk was *let-7b* and *miR-199a* expression. A classifier system that included the top 6 miRNA discriminators accurately distinguished melanoma patient tissues of high metastatic propensity with 100% sensitivity and specificity. Their work suggested for the first time that miRNA expression might represent a highly accurate biomarker for metastatic risk in melanoma.

Because miRNAs are critical for regulating many cellular events and are highly deregulated in various cancers, including melanoma, it is likely that miRNAs could be effective targets for treatment. Sun et al[74] recently found that genistein, an isoflavone isolated from soybeans, inhibited human uveal melanoma cell growth *in vitro* and *in vivo*. Additionally, it altered the expression of *miR-27a* and its target gene *ZBTB10* (zinc finger and BTB domain containing 10; BTB represents bric-a-brac, tramtrack, Broad-Complex), hinting at the contributions of *miR-27a* to the inhibitory effect of genistein on melanoma growth. Kota et al[75] demonstrated that the expression of *miR-26a* in liver cancer cells *in vitro* induces cell-cycle arrest by targeting cyclins D2 and E2, while *miR-26a* inhibited

cancer cell proliferation and induced tumor-specific apoptosis without toxicity in a mouse model. These findings highlight the possibility that the delivery of miRNAs that are highly expressed, and therefore typically well tolerated, in normal tissues but lost in diseased cells may provide a general strategy for miRNA replacement therapies.

CONCLUSION

MiRNA regulation represents a promising, multifaceted means for targeted therapy against melanoma. Indeed, miRNAs have been known to interact with many of the most important regulatory pathways in melanoma development and progression, including *MAPK/ERK* and *PI3K/PTEN/Akt*. Their influence over normal and tumorigenic processes, particularly in regard to their small number, will almost certainly allow for the discovery of many new prognostic markers and key melanoma regulators. Furthermore, archived FFPE tissue samples will continue to be a valuable source of stable miRNAs for future comprehensive analysis. Understanding miRNA expression trends from melanocyte to metastatic melanoma and their roles in many complex regulatory networks may lead to the development of better therapeutic management.

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