Minireviews

MicroRNA-Directed Cancer Therapies: Implications in Melanoma Intervention

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ABSTRACT

Acquired tumor resistance to cancer therapies poses major challenges in the treatment of cancers including melanoma. Among several signaling pathways or factors that affect neo- carcinogenesis, cancer progression, and therapies, altered microRNAs (miRNAs) expression has been identified as a crucial player in modulating the key pathways governing these events. While studies in the miRNA field have grown exponentially in the last decade, much remains to be discovered, particularly with respect to their roles in cancer therapies. Since immune and nonimmune signaling cascades prevail in cancers, identification and evaluation of miRNAs, their molecular mechanisms and cellular targets involved in the underlying development of cancers, and acquired therapeutic resistance would help in devising new strategies for the prognosis, treatment, and an early detection of recurrence. Importantly, in-depth validation of miRNA-targeted molecular events could lead to the development of accurate progression-risk biomarkers, improved effectiveness, and improved patient responses to standard therapies. The current review focuses on the roles of miRNAs with recent updates on regulated cell cycle and proliferation, immune responses, oncogenic/epigenetic signaling pathways, invasion, metastasis, and apoptosis, with broader attention paid to melanomagenesis and melanoma therapies.

Introduction

MicroRNAs (miRNAs) are a class of evolutionally conserved single-stranded noncoding RNAs of 19–22 nucleotides (Bartel, 2004). miRNAs are encoded within the genome from intronic, exonic, or intergenic regions and are initially part of immature primary transcripts (primary miRNAs) that can be of several kilobases in length. The biogenesis of miRNA involves the cleavage of primary miRNAs by the RNAse enzyme, Drosha, followed by its transcription to 60–100 nucleotide hairpin precursor RNAs (precursor miRNA). The precursor miRNA is then transported to the cytoplasm by the nuclear export factor exportin-5 and is excised by the RNA polymerase enzyme Dicer to produce 70-nucleotide-long precursor miRNAs. Finally, putative helicase unwind these precursor miRNAs to mature ~18–24 nucleotides miRNAs (Pillai et al., 2004). Single-stranded mature miRNAs associate with argonaute proteins to form the core of a multicomponent gene regulatory complex known as the RNA-induced silencing complex (Bartel, 2004). This RNA-induced silencing complex facilitates miRNA-mediated regulation of gene expression through base pairing between miRNA and sequence(s) within the 3’ untranslated region of the target messenger RNA [mRNA, i.e., between the protein-coding region of mRNA and its poly(A) tail] (Pillai et al., 2004). The binding of miRNA to mRNA reduces translation rate and/or increases degradation of mRNA (Vasudevan et al., 2007). However, recent evidence suggests that miRNAs may also increase mRNA translation when cells are undergoing cell cycle arrest (Vasudevan et al., 2007). In general, miRNA half-life ranges from hours to days and varies depending on the organs, body fluids, and cell types (van Rooij et al., 2007). In comparison with mRNA, miRNAs are highly stable in formalin-fixed paraffin-embedded tissue blocks or biobank stored animal and human biosamples, which allow its use for localization and expression studies as biomarkers even after years of storage (Hall et al., 2012; Samir and Pessler, 2016).

miRNAs play important roles in essentially all biologic processes (Tufekci et al., 2014). The differential expression of host miRNAs during infection has supported the idea that they may constitute key players in host responses to invading pathogens (Lee et al., 1993; Ambros, 2004). It has been recognized that the regulatory roles of miRNAs are much more sophisticated than initially thought due to the cooperativity...
miRNAs and the Regulation of Cancer

In particular, miRNAs are often aberrantly expressed in several human cancers including melanoma (with numerous miRNAs being overexpressed in one type of cancer and downregulated in another) (Nelson et al., 2006; Nelson and Weiss, 2008; Cortez et al., 2011; Bonazzi et al., 2012). For example, miR-205 is upregulated in lung, bladder, and pancreatic cancers (Nelson and Weiss, 2008; Cortez et al., 2011; Bonazzi et al., 2012). In contrast, miR-205 is significantly downregulated in prostate and esophageal squamous cell carcinomas, indicating that cancer-associated miRNAs cannot be generalized (Melot and Esteller, 2011). Nonetheless, cancer-specific miRNA expression signatures may prove useful as diagnostic and therapeutic tools. Interestingly, miRNA expression signatures have been linked to several clinicopathological variables such as tumor stage and metastasis, receptor status, disease recurrence, treatment resistance, and patient survival (Andorfer et al., 2011; Jiang et al., 2012). According to the personalized medicine model, miRNA-associated molecular taxonomy could help to predict the likelihood of patients developing resistance against a particular treatment. For example, studies in breast cancer patients revealed that both miR-451 and miR-27 were involved in developing resistance to doxorubicin (Andorfer et al., 2011). Additionally, overexpression of miR-125b was shown to induce resistance of breast cancer cells to paclitaxel (Zhou et al., 2010). Therefore, the analysis of miRNAs that affect drug sensitivity represents a potentially important area of investigation in understanding mechanistic insights contributing to drug resistance and clinical management of cancers.

miRNAs and Regulation of Melanomagenesis and Progression

Melanocytes are skin cells that originate from neural crest cells and have the ability to produce melanin pigment. Melanocyte differentiation occurs via a series of steps, resulting in lineage specification of melanoblasts and transportation of mature melanosomes to keratinocytes (Ernfors, 2010). Melanomagenesis is a stepwise metamorphic process in which normal melanocytes in the epidermis gradually transform into the vertical growth phase characteristic of malignant melanomas (Bevona et al., 2003). Cutaneous malignant melanoma is a highly aggressive and metastatic malignancy accounting for the majority of skin cancer–related deaths worldwide (Villanueva and Herlyn, 2008). Among several factors, exposure to UV light, melanocyte integrity, and melanocyte homeostatic mechanisms play important roles in the transformation of melanocytes into melanomas (Gupta et al., 2005; Rigel, 2008).

The dysregulation of miRNAs has been linked to either the suppression or progression of the initiation, differentiation, development, and prognostic biomarker of melanoma (Gaur et al., 2007; Mueller et al., 2009; Chan et al., 2011; Bonazzi et al., 2012; Poliseno et al., 2012; Kozubek et al., 2013; Guo et al., 2014; Hwang et al., 2014; Knoll et al., 2014; Sun et al., 2014; Liu et al.,...
miR-15b, miR-205, miR-149*, miR-155, miR-21, miR-26a (apoptosis induction)

miR-21 (inhibition of tumor growth and augmentation of chemosensitivity)

miR-15/16, miR-41/200a, miR-96/182 family, miR-203 (inhibition of cell viability)

miR-21, miR-125b, miR-7b, miR-29c, miR-659-3p, miR-514a, miR-32, miR-579-3p, miR-7, miR-34a, miR-100 (regulation of mechanisms of action, resistance or sensitivity of combination therapy)

miR-34b/d, Let-7a, miR-182, Let-7b, miR-30b/d, miR-1908, miR-199a-5p, miR-199a-3p, miR-200c, miR-145, miR-30, miR-365, miR-203, miR-15a, miR-194, miR-21, miR-339-3p, miR-124 (induction/inhibition of migration, invasion/metastasis)

miR-200 Family (miR-200c/a) (morphological plasticity of cells)

miR-211 (various functions including regulation of melanocytic pigmentation)

miR-200c, miR-205 (tumor suppressor)

miR-196a (progression and invasiveness)

miR-149* (oncogenic regulator)

miR-506-514 cluster (melanocytic transformation & growth)

miR-30b, miR-30d, miR-34a/c, miR-494, miR-302c, miR-520c, miR-155 (cell invasion, regulation of immune cells/immune escape)

miR-375, miR-34b (cell proliferation, invasion, motility)

miR-182, miR-148a (regulation of DNA methylation)

miR-26, miR-29, miR-203 (melanocyte transformation)

Fig. 1. Schematic representation of miRNA roles in melanoma and melanoma therapy.

miR-211 has been identified as the most differentially expressed miRNA between normal melanocytes, nonpigmented melanoma cell lines, and primary melanomas in patients (Xu et al., 2012; Bell et al., 2014). Using gene expression profiling of normal and melanoma cells, Bell et al. (2014) investigated relationships between transcription factors and miRNAs that are crucial for melanoma proliferation/invasion and identified several miRNAs including miR-211, and its new target NUAK1. The ectopic expression of miR-211 in melanoma cells significantly inhibited its growth and invasion compared with parental cells, suggesting that miR-211 possesses tumor suppressor functions (Xu et al., 2012; Bell et al., 2014). This hypothesis was supported by findings that miR-211 is encoded by a region in the sixth intron of TRPM1, a candidate suppressor of melanoma metastasis (Mazar et al., 2010; Xu et al., 2012; Bell et al., 2014). Additionally, TRPM1 and miR-211 expressions were regulated by MITF, a transcription factor and master regulator of melanocyte development and function (Mazar et al., 2010). These findings indicate that the tumor suppressor activities of MITF and/or TRPM1 could be in part mediated by miR-211. Recently, several miR-211 target genes including RUNX2, IGF2R, TGFBR2, POU domain-containing transcription factor BRN2, and NFAT5 have been identified (Aftab et al., 2014). It has been proposed that miR-211 may also directly regulate melanocyte pigmentation and invasion since it is highly expressed in melanocytes and pigmented melanomas but not in nonpigmented melanomas (Aftab et al., 2014). Moreover, melanomas with greatly reduced miR-211 expression have been shown to possess highly invasive characteristics.
miRNAs in melanomagenesis and progression

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Function</th>
<th>Expression of miRNA</th>
<th>Target</th>
<th>Reference</th>
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<tbody>
<tr>
<td>miR-211</td>
<td>Tumor suppressor, regulation of melanocytic pigmentaton, increased or decreased invasiveness</td>
<td>Upregulation or downregulation</td>
<td>NUAKI, RUNX2, TGFBIR2, BRN2, NFAT5, KCNA1, TRPM1, MITF</td>
<td>Levy et al. (2010), Mazar et al. (2010), Zhou et al. (2010), Boyle et al. (2011), Xu et al. (2012), Margue et al. (2013), Bell et al. (2014)</td>
</tr>
<tr>
<td>miR-200c, miR-205</td>
<td>Tumor suppressor</td>
<td>Differential expression</td>
<td>HOX-B7, bFGF, ETS-1, BMP-4, HOX-C8, Cadherin-11, Calponin-1, Osteopontin, MTI</td>
<td>Xu et al. (2012)</td>
</tr>
<tr>
<td>miR-196a</td>
<td>Progression and invasiveness</td>
<td>Downregulation</td>
<td>HOX-B7, bFGF, ETS-1, BMP-4, HOX-C8, Cadherin-11, Calponin-1, Osteopontin, MTI</td>
<td>Braig et al. (2010), Mueller and Bosserhoff (2011)</td>
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<tr>
<td>miR-149*</td>
<td>Oncogenic regulator</td>
<td>Upregulation</td>
<td>GSK3β, Mcl-1</td>
<td>Jin et al. (2011)</td>
</tr>
<tr>
<td>miR-506-514 cluster, miR-218</td>
<td>Melanocyte transformation (melanomagenesis) and melanoma growth</td>
<td>Overexpression</td>
<td>MITF</td>
<td>Streicher et al. (2012), Guo et al. (2014)</td>
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bFGF, basic fibroblast growth factor.

(Levy et al., 2010; Mazar et al., 2010; Margue et al., 2013). In contrast, miR-211 highly expressing melanoma cells possesses reduced invasive potential independent of metastatin, an inhibitor of tumor growth (Liu et al., 2001; Aftab et al., 2014; Bell et al., 2014). In the same context, miR-200c and miR-205 have been shown to be differentially expressed between benign nevi and primary or metastatic melanoma, and they act as tumor suppressors (Xu et al., 2012). Similarly, Braig et al. (2010) investigated miR-196a downregulation, which upregulated HOX-B7 and consequently stimulated basic fibroblast growth factor signaling, resulting in upregulation of ETS-1 transcription factor and BMP-4 expression, which play crucial roles in melanoma progression. Later, using the high-throughput miRNA expression profiling approach in melanoma cells and tissue samples, Mueller and Bosserhoff, 2011 showed that miR-196a expression was significantly reduced in malignant lesions. Importantly, overexpression of miR-196a significantly reduced melanoma cell invasiveness (Mueller and Bosserhoff, 2011). In addition, HOX-C8, cadherin-11, calponin-1, and osteopontin were identified as miR-196a targets (Mueller and Bosserhoff, 2011). Moreover, miR-149*, a p53-responsive miRNA has been shown to be overexpressed in human metastatic melanoma isolates, and targets glycogen synthase kinase-3 alpha (GSK3α) to induce resistance of melanoma cells to apoptosis via increasing the expression of Mcl-1 (Jin et al., 2011). Furthermore, miR-506-514 (a cluster of 14 miRNAs on the X chromosome) and miR-218 have been demonstrated to play crucial roles in initiating melanocyte transformation (melanomagenesis) and/or promoting melanoma growth (Streicher et al., 2012; Guo et al., 2014).

miRNA and Regulation of Cell Cycle and Proliferation in Melanoma

Since cell cycle regulation is controlled by several factors including cyclin-dependent kinases (CDKs), the E2F transcription factor, and proteins such as c-myc, p27 (a tumor suppressor protein that binds to and inhibits the function of the cyclin D1-CDK4 complex), as well as PTEN (Mamillapalli et al., 2001; Walter et al., 2002; Suryadinata et al., 2010), one can postulate that miRNAs that regulate cell proliferation might directly target these cell cycle regulators (Table 2). In this regard, let-7b miRNA has been shown to target cell cycle regulators since increased let-7b expression significantly decreases melanoma cell proliferation via reducing the expressions of CDK4, cyclin D1, and cyclin D3 (Schultz et al., 2008). Using miRNA microarrays, Chen et al. (2010) analyzed

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</thead>
<tbody>
<tr>
<td>miR let-7b</td>
<td>Cell cycle regulation and proliferation</td>
<td>Upregulation</td>
<td>CDK4, cyclin D1, cyclin D3</td>
<td>Schultz et al. (2008)</td>
</tr>
<tr>
<td>miR-193b</td>
<td>Cell cycle regulation and proliferation</td>
<td>Downregulation</td>
<td>Cyclin D1</td>
<td>Chen et al. (2010)</td>
</tr>
<tr>
<td>miR-206</td>
<td>G1 cell cycle arrest and inhibition of proliferation</td>
<td>Downregulation</td>
<td>CDK4, cyclin D1, cyclin C</td>
<td>Georganagas et al. (2014)</td>
</tr>
<tr>
<td>miR-143</td>
<td>G1 cell cycle arrest and induction of apoptosis</td>
<td>Downregulation</td>
<td>Syn-1</td>
<td>Li et al. (2016)</td>
</tr>
<tr>
<td>miR-106b</td>
<td>G1 cell cycle arrest and inhibition of growth</td>
<td>Downregulation</td>
<td>P21/WAF1/Cip1</td>
<td>Prasad and Katiyar (2014)</td>
</tr>
<tr>
<td>miR-221 and miR-222</td>
<td>Cell proliferation</td>
<td>Downregulation and upregulation</td>
<td>PLZF, c-Kit, p27/Kip1/CDKN1B</td>
<td>Felicetti et al. (2008a,b), Igoucheva and Alexeev (2009), Kanemaru et al. (2011)</td>
</tr>
<tr>
<td>miR-205</td>
<td>Cell proliferation</td>
<td>Downregulation</td>
<td>E2F1 and E2F5</td>
<td>Dar et al. (2011)</td>
</tr>
<tr>
<td>miR-155</td>
<td>Cell proliferation</td>
<td>Downregulation</td>
<td>SKI</td>
<td>Levati et al. (2011)</td>
</tr>
<tr>
<td>miR-9</td>
<td>Cell proliferation and migration</td>
<td>Downregulation</td>
<td>E-cadherin, NF-kB1-Snail1</td>
<td>Liu et al. (2012a,b)</td>
</tr>
<tr>
<td>miR-145</td>
<td>Suppression of cell proliferation and migration</td>
<td>Downregulation</td>
<td>c-MYC</td>
<td>Noguchi et al. (2012)</td>
</tr>
<tr>
<td>miR-126 and miR-126*</td>
<td>Melanoma progression</td>
<td>Downregulation</td>
<td>ADAM9 MMP7</td>
<td>Felli et al. (2013)</td>
</tr>
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</table>
the expression of 470 miRNAs in benign nevi and metastatic melanoma tissues, and observed 31 differentially expressed miRNAs, of which miR-193b was significantly downregulated in melanoma tissues. Furthermore, overexpression of miR-193b in Malme-3M melanoma cells resulted in inhibition of cell proliferation via downregulating 18 genes including cyclin D1 (CCND1) (Chen et al., 2010). Similarly, downregulation of miR-206, miR-143, or miR-106b expression has been correlated with reduced growth and migration/invasion of several melanoma cell lines mediated via G1 cell cycle arrest resulting in an inhibition of CDK4, cyclin D1, cyclin C, and syndecan-1 (Syn-1) or reactivation of p21/WAF1/Cip1 or as target genes (Georgantas et al., 2014; Li et al., 2016; Prasad and Katiyar, 2014). Since cell cycle regulation controls the proliferation of cells, miR-221 and miR-222 have been shown to directly modulate the in vitro and in vivo proliferation of melanoma cells via targeting multiple signaling pathways including e-Kit or p27Kip1 and their circulating levels in malignant melanoma patients could be used as a new tumor marker (Felicetti et al., 2008a,b; Igocheva and Alexeev, 2009; Kanemaru et al., 2011). Notably, miRNAs, including miR-205, miR-149, miR-18b, miR-21, miR-203, and miR-26a have been documented to regulate cell cycle proteins in a cyclin-independent manner. In this regard, downregulation of miR-205 was reported in primary melanomas, and this regulates E2F1 and E2F5 associated with reduced growth and migration/invasion of several melanoma tissues via multiple signaling pathways including c-Kit and functions of tumor-expanded myeloid-derived suppressor cells are involved in mediating immunosuppression and/or promoting tumor growth (Sahu et al., 2014a). Liu et al. (2012b) have shown that TGF-β1-induced miR-494 expression in myeloid-derived suppressor cells favors the accumulation and functions of tumor-expanded myeloid-derived suppressor cells mediated by targeting PTEN and activation of the Akt pathway. Moreover, Arts et al. (2015) demonstrated the role of miR-155 in melanoma immune escape and functions of tumor-expanded myeloid-derived suppressor cells.

miRNA and Regulation of Melanoma Immune Responses

In addition to regulating cell cycle, miRNAs have been demonstrated to influence the host immunity against melanoma (Table 3). In this regard, the ectopic expression of miR-30b and miR-30d has been shown to target GalNac transferase GALNT7 to enhance melanoma metastasis via promoting invasion, increased synthesis of immunosuppressive cytokine IL-10, reduced immune cell activation, and recruitment, which resulted in induction of immunosuppression (Gaziol-Sovran et al., 2011). Similarly, miR-34a/c has been reported to regulate innate immune responses in melanoma cells via controlling ULBP2 expression, a stress-induced ligand of NKG2D (Heinemann et al., 2012). Since NKG2D detects early tumorigenesis, eliminates cytotoxic lymphocytes, and provides an innate barrier to tumor development, overexpression of miR-34 downregulated ULBP2 expression, and removal of ULBP2 ligand protected malignant melanoma cells from NKG2D-mediated immune surveillance (Heinemann et al., 2012). Similarly, upregulation of the NKG2D ligands MICA/B and ULBP2 has been reported to mediate natural killer cell–induced cytotoxicity of melanoma cells by 1,25(OH)2D3 treatment mediated partly via the downregulation of miR-302c and miR-520c expression (Min et al., 2013). Since suppressive immunophenotypes such as myeloid-derived suppressor cells are involved in mediating immunosuppression and/or promoting tumor growth, Liu et al. (2012b) have shown that TGF-β1-induced miR-494 expression in myeloid-derived suppressor cells favors the accumulation and functions of tumor-expanded myeloid-derived suppressor cells mediated by targeting PTEN and activation of the Akt pathway. Moreover, Arts et al. (2015) demonstrated the role of miR-155 in melanoma immune escape.

### Table 3

<table>
<thead>
<tr>
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<th>Expression of miRNA</th>
<th>Target</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-30b and miR-30d</td>
<td>Cell invasion and Immune suppression</td>
<td>Upregulation</td>
<td>UprgalNac transferases</td>
<td>Gaziol-Sovran et al. (2011)</td>
</tr>
<tr>
<td>miR-34a and miR-34c</td>
<td>Regulation of innate immunity</td>
<td></td>
<td>NKG2DL ULBP2</td>
<td>Heinemann et al. (2012)</td>
</tr>
<tr>
<td>miR-494</td>
<td></td>
<td></td>
<td>PTEN</td>
<td>Liu et al. (2012b)</td>
</tr>
<tr>
<td>miR-302c and miR-520c</td>
<td></td>
<td></td>
<td>NKG2D, MICA/B, and ULBP2</td>
<td>Min et al. (2013)</td>
</tr>
<tr>
<td>miR-155</td>
<td></td>
<td></td>
<td>IL-1β, MITF-M</td>
<td>Arts et al. (2015)</td>
</tr>
<tr>
<td>miR-375</td>
<td></td>
<td></td>
<td>Minimal CpG island methylation in melanocytes, keratinocytes, normal skin and nevus</td>
<td>Mazar et al. (2011a)</td>
</tr>
<tr>
<td>miR-34b</td>
<td>Cell invasion and motility</td>
<td>Epigenetic regulation</td>
<td>Cpg island methylation</td>
<td>Mazar et al. (2011b)</td>
</tr>
<tr>
<td>miR-162</td>
<td>Epigenetic modulation</td>
<td>UprgalNac transferases</td>
<td>Cpg island hypermethylation</td>
<td>Liu et al. (2013a)</td>
</tr>
<tr>
<td>miR-148a</td>
<td>Regulation of DNA methylation</td>
<td></td>
<td>TGFIP2</td>
<td>Tian et al. (2015)</td>
</tr>
<tr>
<td>miR-26, miR-29 and miR-203</td>
<td>Melanocyte transformation/antioncogenic</td>
<td>Epigenetic regulation</td>
<td>Dnmt3b CREB1/MITF/RAB27a</td>
<td>Gasque Schoof et al. (2015), Noguchi et al. (2015, 2016)</td>
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</table>
miR-155 in targeting the novel mechanisms of melanoma immune escape in inflammatory microenvironment mediated via the modulation of IL-1β-induced downregulation of endogenous MITF-M expression in melanoma cells.

miRNA and Epigenetic Regulation of Melanoma

Epigenetic refers to those biologic processes by which changes in phenotype or gene expression occur without changes in DNA sequences. Several miRNAs have been shown to regulate or be regulated by epigenetic modification in melanoma (de Umamuno et al., 2015) (Table 3). In this regard, Mazur et al. (2011a) have demonstrated that miR-375 epigenetically regulates the development of melanoma in patients. In this study, the authors have shown that CpG island methylation regulates miR-375 expression in WM1552C stage 3 melanoma cells following treatment with the demethylating agents 5-aza-2-deoxycytidine and 4-phenyl-butyrate (Mazar et al., 2011a). Methylation of miR-375 CpG islands was stage dependent with significant levels in stage II and III melanoma tumors compared with stage I melanomas or benign melanocytes (Mazar et al., 2011a). In another study, the expression of miR-34b was shown to be regulated by increased methylation of CpG islands, and this was apparent in stage III and IV melanoma tumors compared with stage I and II melanomas, melanocytes, and keratinocytes (Mazar et al., 2011b). Similarly, epigenetic modulation has been shown to induce overexpression of miR-182 in human melanoma cells, and CpG islands upstream of mature miR-182 were found to be hyper-methylated in melanoma cells (Liu et al., 2013a). In addition, Tian et al., 2015 reported decreased expression of miR-148a in skin cancer patients when the TGIF2 gene was targeted. In this study, the authors found that DNA methylation regulated the expression and function of miR-148a, and they concluded that this miR-148a methylation could serve as an independent potential indicator/marker in the prognosis of skin cancer. However, due to the limitation of the number of samples and experimental conditions, as well as other unfavorable factors, further studies are still necessary (Tian et al., 2015). Importantly, Gasque Schoof et al. (2015) demonstrated the roles of miR-26, miR-29, and miR-203 in the regulation of epigenetic reprogramming, and the involvement of the Dnmt3a, Dnmt3b, Mecp2, and EzH2 genes during melanocyte transformation. Similarly, DNA methylation of CpG islands upstream of the miR-203 coding region (MIR203) was detected in both human and canine melanoma cells as well as canine clinical specimens, but not in human normal melanocytes. The findings by Noguchi et al., 2015 indicated that demethylating MIR203 agents could be used as promising therapeutic targets for the treatment of human and canine melanomas. This same group later demonstrated that miR-203 functions as a common tumor suppressor miRNA in human and canine melanoma cells via its ability to directly target CREB1 and its downstream targets MITF and RAB27a (Noguchi et al., 2016).

miRNAs in Melanoma Cell Invasion and Metastasis

Metastasis of melanoma tumors to distal organs including the brain is a complex process requiring several stages from local tumor invasion to intra- and extravasation leading to the formation of macrometastases, which is the major cause of skin cancer–related mortality in the United States (Adler et al., 2017; Westphal et al., 2017) (Table 4). Several factors or signaling pathways have been shown to drive melanoma cell migration and invasion leading to metastasis including FSCN1, basigin, β3-integrin, GALANT7, MARCKS, e-MET, STAT3, PTEN, and NFκB1 (Muramatsu and Miyauchi, 2003; Boukerche et al., 2007; Estrada-Bernal et al., 2009; Elson-Schwab et al., 2010; Yang et al., 2011; Chattopadhyay et al., 2012; Liu et al., 2013b; Li et al., 2016). Importantly, a wide array of miRNAs has been identified to target the key signaling pathways including those previously mentioned (Zhang et al., 2006). In this regard, Migliore et al. (2008) have shown that miR-34a/b/c acts as a suppressor of metastasis since ectopic expression of these miRNAs directly targets the proto-oncogene MET, leading to inhibition of MET-induced signal transduction and invasive behavior of melanoma cells. Given that enhanced expression of ITGB3 increases the invasiveness of melanoma cells (Seffor et al., 1992), let-7a has been shown to reduce the invasive potential of melanoma cells via negatively regulating ITGB3 expression (Müller and Bosserhoff, 2008). Similarly, miR-182, a frequently amplified miRNA in melanoma tumors compared with benign melanocytes has been shown to promote melanoma metastasis via repressing FOXO3 and MITF-M (Sequera et al., 2009). Downregulation of miR-182 impeded the invasion via inducing apoptosis, and enhanced expression of FOXO3 or MITF-M blocked miR-182-induced proinvasive effects (Sequera et al., 2009). While several miRNAs have been shown to either promote or reduce the invasiveness of melanoma cells, Elson-Schwab et al. (2010) demonstrated that expression of miR-200 family members does not suppress invasion but regulates morphologic plasticity or leads to a switch between modes of invasion of melanoma cells. The expression of miR-200c resulted in a higher proportion of cells adopting the rounded or amoeboïd-like mode of invasion mediated via reduced expression of MARCKS, and miR-200a induced a protrusion-associated elongated mode of invasion via reduced acetylcholinesterase activity (Elson-Schwab et al., 2010). Reduced let-7b expression in melanoma cells leads to increased metastases due to enhanced expression of basigin, an invasion-associated protein, and consequently enhanced expression of extracellular matrix metalloproteinases (Fu et al., 2011), while overexpression of let-7b results in reduced basigin and MMP-9 protein expression and decreased distant metastases (Fu et al., 2011). Along similar lines, Gaziel-Sovran et al. (2011) reported that expression of miR-30b/30d in human melanoma positively correlated with the stage, metastatic potential, shorter time to recurrence, and reduced overall survival. Ectopic expression of miR-30b/30d in melanoma cells increased their metastatic behavior via direct targeting of GalNAc transferase GALT7, which resulted in reduced immune cell activation and recruitment. In addition, Yang et al. (2011) demonstrated that the overexpression of miR-21 in human melanoma requires STAT-3 activation, and regulates the metastatic behavior of B16 melanoma cells via targeting tumor suppressor (PTEN and PDCD40) and anti-proliferative (BTG2) proteins. Specific miRNAs, termed metastamirs, were reported to regulate the migration, invasion, and metastasis of melanoma cells, suggesting that they represent novel targets to inhibit melanoma progression (White et al., 2011; Segura et al., 2012). Moreover, miR-1908, miR-199a-5p, and miR-199a-3p have been shown to target ApoE, which leads to LRPI/LRP8-dependent melanoma metastasis.
and angiogenesis (Pencheva et al., 2012). Similarly, overexpression of miR-200c (in CD44 + CD133 + cancer stem cells) and miR-145 has been demonstrated to downregulate ZEB1 or FSCN1, a known regulator of cell migration to inhibit the migration, invasion, and/or tumorigenicity of melanoma cells in vitro and in vivo (Dou et al., 2013; Dynoodt et al., 2013). Using miR-30-based short hairpin RNAs against heparanase and lentiviral approaches, it was reported that miRNAs (miR-30) targeting heparanase could be used as an effective RNA interference agent to suppress melanoma metastasis (Liu et al., 2013b). Interestingly, Fu et al. (2014) demonstrated the role of miR-26a in enhancing the biogenesis of other miRNAs, especially let-7 in various cancer models including melanoma via targeting Lin28B and Zcchc11, and suppressing tumor growth and metastasis. Recent studies have demonstrated that the downregulation of miR-365, miR-203, miR-124 and miR-10b or the overexpression/upregulation of miR-15a, miR-194, and miR-21 will inhibit the growth/proliferation, invasion, and/or metastasis of malignant melanoma cells via their abilities to target distinct signaling pathways such as neuropilin 1 (NRP1), BM1, RLIP76, a stress-inducible non-ABC transporter, CDCA4, GEF-H1, STAT3, PTEN, and PDCD4 (Bai et al., 2015b; Chang et al., 2015; Alderman et al., 2016; Guo et al., 2016; Li et al., 2016; Saldanha et al., 2016a,b; Zhang et al., 2016). Importantly, the differential expression of miR-339-3p in melanoma cells and healthy melanocytes has been correlated with reduced invasion associated with decreased MCL1 expression (Weber et al., 2016).

**miRNA and Apoptotic Induction in Melanoma**

Multiple studies have highlighted the role of microRNAs, including miR-205, miR-155, miR-26a, miR-21, miR-15b, and miR-149* in apoptosis induction (Satzger et al., 2010, 2012; Dar et al., 2011; Jin et al., 2011; Levati et al., 2011; Reuland et al., 2013; Jiao et al., 2015; Mao et al., 2017) (Table 5). In this regard, Satzger et al. (2010) determined the expression levels of 16 miRNAs in normal melanocytes versus 10 melanoma cell lines and FFPE tissues of 11 melanocytic nevi versus 16 melanomas. In their study, the levels of miR-15b and miR-210 were significantly upregulated, and miR-34a was significantly downregulated. However, upon further evaluation of these three miRNAs in 128 primary melanomas from patients with detailed clinical follow-up information, only miR-15b was found to be significantly associated with poor recurrence-free survival, and overall survival. The downregulation of miR-15b in two melanoma cell lines with higher miR-15b expression resulted in reduced tumor cell proliferation and increased apoptosis, indicating the important role of miR-15b in melanoma and associated poor prognosis and tumorigenesis (Satzger et al., 2010). In another study, reduced miR-205 expression was identified in melanoma cells compared with benign nevi (Dar et al., 2011). Further analysis showed that miR-205 targets E2F1 and reduces its expression by decreasing the proliferation via inducing apoptosis mediated through the activation of p73 family members in advanced malignant melanomas (Dar et al., 2011). Importantly, miR-149* was found to be directly regulated by p53, which targets glycogen synthase kinase-3 alpha to induce resistance of melanoma cells to apoptosis mediated via increased expression of Mcl-1 (Jin et al., 2011). In addition, downregulation of miR-155 was found to be downregulated, and its ectopic expression induces apoptosis via inhibition of SKI gene expression (Levati et al., 2011). Interestingly, miR-21 expression was reported to be significantly increased in primary and malignant melanoma tissues and melanoma cells compared with benign nevi, normal skin, and melanocytic cell preparation, and that
downregulation of miR-21 in melanoma cells induces apoptosis without significantly affecting cell proliferation or via targeting PDCD4 (Satzger et al., 2012; Jiao et al., 2015). Moreover, miR-26a was found to be significantly downregulated in human melanoma cell lines compared with primary melanocytes, and overexpression of miR-26a resulted in significant and rapid cell death and repressed silencer of death domain expression that rescued melanoma cells from undergoing apoptosis, suggesting miR-26a as a potential therapeutic molecule in the treatment of melanoma (Reuland et al., 2013). Furthermore, miR-21 was reported to also regulate the therapeutic molecule in the treatment of melanoma (Reuland et al., 2013; Jiao et al., 2015).

miRNA and Melanoma Therapy

Malignant melanoma often develops resistance to most standard chemotherapeutic agents and radiation therapy (Terando et al., 2003). While new targeted therapies such as vemurafenib, which targets a BRAFV600-activating mutant kinase, have shown initial promising anti-tumor responses in melanoma patients, tumor resistance remains a significant therapeutic challenge (Wagle et al., 2011; Sosman et al., 2012; Lankenau et al., 2015; Pinto et al., 2015; Foth et al., 2016; Hackler et al., 2014). Thus, further investigation of molecular mechanisms underlying melanoma development and/or therapeutic resistance is required to design new strategies to improve the clinical outcomes in melanoma patients. Despite various reports correlating miRNA involvement with or without BRAF-mutated melanoma tumors and/or therapies in preclinical and clinical studies (Caramuta et al., 2010; Shi et al., 2014; Lankenau et al., 2015; Pinto et al., 2015; Foth et al., 2016; Mannavola et al., 2016; Saldanha et al., 2016a,b), more research is needed to develop sensitive and specific molecular tests to identify novel miRNAs that are modulated in response to resistance to standard melanoma therapies (Table 5). In a recent review, Fattore et al. (2017) highlighted the roles of miRNAs in inducing the development of resistance to BRAF and MEK inhibitors. Importantly, Kozar et al. (2017) identified the differential expression of several novel and previously reported miRNAs in BRAF inhibitor–resistant (vemurafenib and dabrafenib) melanoma cells. In addition, Jiang et al. (2012) reported that miR-21 status was an independent prognostic factor in cutaneous melanoma patients. Importantly, antisense-mediated miR-21 silencing inhibited melanoma growth via increasing apoptosis and also enhanced the chemosensitivity of human cutaneous melanoma cells, suggesting its potential in the treatment of human cutaneous malignant melanoma (Jiang et al., 2012). Using functional assays, Poell et al. (2012) highlighted the importance of miR-15/16, miR-141/200a, miR-96/182, and miR-203

<table>
<thead>
<tr>
<th>miRNA Function</th>
<th>Expression of miRNA</th>
<th>Target</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>miR-15b, miR-205, miR-149*, miR-155, miR-21, miR-26a</td>
<td>Apoptosis induction</td>
<td>Upregulation of 15b, 149* and 21 Downregulation of 155, 205, and 26a</td>
<td>E2F1 (205), SODD (26a), GSK-3α (149*), SKI (155), PDCD4 (21), SPRY1 (21) and PTEN (21)</td>
</tr>
<tr>
<td>miR-21</td>
<td>Inhibition of growth and augmentation of chemo- and radiosensitivity</td>
<td>Upregulation</td>
<td>Bax/Bcl-2 ratio</td>
</tr>
<tr>
<td>miR-15/16, miR-41/200a, miR-96/182 family of miRNAs and miR-203</td>
<td>Inhibition of cell viability</td>
<td>Downregulation</td>
<td>Survivin</td>
</tr>
<tr>
<td>miR-125b, miR-7b, miR-29c</td>
<td>Regulation of mechanisms of action of Temsirolimus and Bevacizumab combination</td>
<td>Differential expression after treatment with temsirolimus and bevacizumab combination</td>
<td>AKT, CCND1, DNMT3A/B</td>
</tr>
<tr>
<td>miR-659-3p</td>
<td>Predicts clinical outcome of carboplatin/paclitaxel-based therapy</td>
<td>Differential expression based on PFS</td>
<td>NFIX</td>
</tr>
<tr>
<td>miR-514a</td>
<td>Modulates BRAFi sensitivity</td>
<td>Overexpression</td>
<td>NF1</td>
</tr>
<tr>
<td>miR-32</td>
<td>Tumor suppressor and exhibit synergistic effects with vemurafenib</td>
<td>Poor expression</td>
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<tr>
<td>miR-579-3p</td>
<td>Resistance to targeted therapy</td>
<td>Low expression (downregulation)</td>
<td>BRAF, MDM2</td>
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<tr>
<td>miR-7</td>
<td>Reversal of resistance to targeted therapy</td>
<td>Downregulation</td>
<td>EGFR/IGF-1R/CRAF</td>
</tr>
<tr>
<td>miR-34a, miR-100 and miR-125b</td>
<td>Restoration of resistance to vemurafenib</td>
<td>High expression (upregulation)</td>
<td>CCL-2</td>
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GSK-3α, glycogen synthase kinase-3 alpha; PFS, progression free survival; SODD, silencer of death domain.
As potent inhibitors of melanoma cell proliferation since ectopic expression of these miRNAs resulted in long-term inhibition of melanoma cell expansion, both in vitro and in vivo. This study provided a comprehensive interrogation of miRNAs that interfere with melanoma cell proliferation and viability, and offered a selection of miRNAs that are promising candidates in melanoma therapy (Poell et al., 2012). Wagensen et al. (2013) studied global miRNA expression profiles using microarrays in melanoma tissues from combination-targeted therapy of temsirolimus- and bevacizumab-treated patients, and detected significant upregulation of 15 miRNAs in treated versus nontreated melanoma tissues, 12 of which possess tumor suppressor functions via their ability to target 15 different oncogenes. Of these miRNAs, miR-125b, miR-7b, and miR-29c were differentially expressed after temsirolimus and bevacizumab combination treatment. Similarly, differential expression of miR-659-3p based on progression-free survival was reported to predict the clinical outcome of carboplatin/paclitaxel-based therapy in metastatic melanoma patients (Villaruz et al., 2015). In particular, miR-514a, which plays an important role in initiating melanocyte transformation and promotion of melanoma growth, has been reported to modulate the sensitivity of melanoma resistance (Fattore et al., 2016), miR-7, miR-203, and miR-124a were associated with vemurafenib (Mishra et al., 2016). Moreover, while miR-196a is a central regulator of HOX-B7 and BMP4 expression in malignant melanoma. (Braig S, Mueller DW, Rothhammer T, and Bosserhoff AK (2010) MicroRNA miR-196a is a central regulator of HOX-B7 and BMP4 expression in malignant melanoma. J Invest Dermatol 130:2062–2070).

Conclusions

From its discovery to the present, miRNAs have represented a paradigm shift in scientific research. miRNAs may assist in the diagnosis and early detection of melanoma recurrence, and in predicting patient's outcomes/responses to therapies. Thus, the development of miRNAs as accurate progression risk biomarkers would greatly enhance the clinical management of melanoma.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Thyagarajan, Shahan, Sahu.

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