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Review

The role of microRNAs and long non-coding RNAs in the pathology, diagnosis, and management of melanoma

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ABSTRACT

Melanoma is frequently lethal and its global incidence is steadily increasing. Despite the rapid development of different modes of targeted treatment, durable clinical responses remain elusive. A complete understanding of the molecular mechanisms that drive melanomagenesis is required, both genetic and epigenetic, in order to improve prevention, diagnosis, and treatment. There is increased appreciation of the role of microRNAs (miRNAs) in melanoma biology, including in proliferation, cell cycle, migration, invasion, and immune evasion. Data are also emerging on the role of long non-coding RNAs (lncRNAs), such as SPRY4-IT1, BANCR, and HOTAIR, in melanomagenesis. Here we review the data on the miRNAs and lncRNAs implicated in melanoma biology. An overview of these studies will be useful for providing insights into mechanisms of melanoma development and the miRNAs and lncRNAs that might be useful biomarkers or future therapeutic targets.

Introduction

Melanoma is the leading cause of skin cancer deaths in the United States [1]. Melanoma survival rates are good when the disease is detected early; precise diagnostic tests for early melanoma detection would therefore be useful, and innovative therapies to cure advanced melanomas are needed. The underlying molecular biology of melanomas is complex and involves interactions between networks of genes, signaling pathways, and gene-regulatory mechanisms, and a better understanding of these underlying molecular mechanisms is essential for translational research. In addition, the histopathologic interpretation of cutaneous melanoma remains one of the most frustrating and difficult diagnostic areas in dermatopathology, and histopathologists would benefit from sensitive and specific diagnostic biomarkers. A number of protein-coding genes [2] have been identified as potential diagnostic and prognostic biomarkers [3–9], several of which exhibit distinct expression profiles between the spectrum of malignant melanomas and their benign forms [2]. In addition, non-protein-coding RNAs (microRNAs and long non-coding RNAs (lncRNAs))[1] are emerging as early prognostic markers and therapeutic targets in a variety of diseases, and microRNAs (miRNAs) is particular have gained increasing attention due to their potential roles in tumorigenesis [10–14], not least in melanoma [15–19]. miRNAs are thought to influence cancer development by regulating transcription and translation of tumor suppressor genes and oncogenes [20–26]. Several genome-wide expression studies have implicated a number of miRNAs and lncRNAs that are potentially important regulators of melanoma development [9,19,27,28].

Melanocytes are skin cells that originate from neural crest cells and have the ability to produce the pigment melanin [1]. Melanocyte differentiation occurs via a series of steps, ultimately resulting in lineage specification of melanoblasts and transportation of mature melanosomes to keratinocytes [29,30]. Melanocytes are

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[1] *Abbreviations used:* lncRNAs, long non-coding RNAs; MITF, microphthalmia transcription factor; *TRPM1*, transient receptor potential cation channel subfamily M member 1; *RUNX2*, Runt-related transcription factor 2; *IGF2R*, insulin-like growth factor 2 receptor; *TGFBR2*, TGF-beta receptor 2; *NFAT5*, nuclear factor of activated T cells 5; NKG2D, natural killer cell immunoreceptor; bFGF, basic fibroblast growth factor; BMP-4, bone morphogenetic protein 4; CTLs, cytotoxic T lymphocytes; CDKs, cyclin-dependent kinases; kif5b, kinesin superfamily protein 5b; eIF4E, eukaryotic translation initiation factor 4; GSK3α, glycogen synthase kinase-3α; FSCN1, fascin actin-bundling protein 1; FFPE, formalin-fixed paraffin-embedded; SODD, silence of death domain; PSF, protein-associated splicing factor; SNPs, single nucleotide polymorphisms.

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characterized by the expression of melanocyte-specific proteins, including tyrosinase, tyrosinase-related protein 1 and 2, melanosomal matrix proteins (Pmel17, MART-1), and microphthalmia transcription factor (MITF) [1]. Genes such as *MITF*, [31] *PAX3*, *SOX10* [32–34], members of the Wnt and Notch signaling pathways [35–37], *KIT*, and cyclins [38] all play an important role in the development and regulation of melanocytes.

Melanogenesis is a stepwise metamorphic process in which normal melanocytes in the epidermis gradually transform into the vertical growth phase characteristic of malignant melanomas [39]. Several factors influence the transformation of melanocytes into melanomas, such as UV exposure [40], melanocyte integrity [41], melanocyte homeostatic mechanisms [42], and neural crest invasion and differentiation [43,44]. In addition to the many protein-coding genes that regulate cancer development, many non-coding genes have also been shown to play important roles in cancer prognosis, diagnosis, and therapy. These include the small RNAs, in particular miRNAs and lncRNAs. Due to wide spread increase and mortality of melanoma globally, it is important to discuss ways and means for the insight mechanism of transformation of melanocytes into melanoma. On the basis of basic information, many methods have been proposed for the prognosis and treatment of melanoma. In this review, we have discussed the possible role of miRNAs in the pathology, diagnosis, and treatment of melanoma. Although role of lncRNAs in melanoma is not fully established, still lncRNAs were given due consideration for their involvement in melanoma.

miRNAs in melanocytes and melanoma biology

miRNAs are small, non-coding RNAs that play a physiological role in the post-transcriptional fine-tuning of the expression of up to 60% of mammalian protein-coding genes [45,46]. The aberrant expression and function of miRNAs has been linked to the development and progression of many human diseases, Fig. 1 including various cancers [10–14], not least melanoma [15–19]. As a result of systematic experimental screens for miRNAs involved in the development and progression of melanoma, several groups have identified miR-211 as the miRNA most differentially expressed between normal melanocytes and non-pigmented melanoma cell lines and primary melanomas from patients [47–52]. Ectopic expression of *miR-211* in melanoma cell lines results in significant inhibition of growth and invasion compared to parental cells, suggesting that *miR-211* normally functions as a tumor suppressor in melanocytes. This hypothesis is supported by the finding that *miR-211* is encoded by a region in the sixth intron of *TRPM1* (transient receptor potential cation channel subfamily M member 1), a candidate suppressor of melanoma metastasis [47,48]. Moreover, we have also reported that the expression of *TRPM1* and *miR-211* are controlled by MITF, a master regulator of melanocyte development and function. It is therefore possible that the tumor suppressor activities of MITF and/or TRPM1 may be mediated, at least in part, by *miR-211*. Recently, several *miR-211* target genes have been identified, including Runt-related transcription factor 2 (*RUNX2)*, insulin-like growth factor 2 receptor (*IGF2R),* TGF-beta receptor 2 (*TGFBR2)*, the POU domain-containing transcription factor *BRN2,* and nuclear factor of activated T cells 5 (*NFAT5)* [48,53].

miR-211 may also directly regulate melanocyte pigmentation and invasion, since it is highly expressed in melanocytes and pigmented melanomas but not in non-pigmented melanomas (Mazar et al., personal communication). Melanomas with greatly reduced *miR-211* expression are highly invasive [47,54,55]. Conversely, melanoma cells that highly express *miR-211* have reduced invasive potential [52], independent of expression of melastatin that was able to block formation of tumor nodules [56]. Together, these findings provide strong evidence that *miR-211* plays a critical role in melanoma invasiveness and progression.

In an attempt to explain the mechanistic basis for these findings, a melanoma-specific metastasis gene network was scrutinized for overlaps between metastatic genes and miR-211 target genes [54]. Six genes overlapped: *IGF2R*, *NFAT5*, *TGFBR2*, *FBXW7*, *ANGPT1*, *IGFBPS* and *VHL*. Functional validation showed that knockdown of 'central node genes' had the same effect on melanoma cell invasion as up-regulation of *miR-211*. Of these, *TGFB* had previously been linked to melanoma progression via promotion of tissue and blood vessel invasion. More recently, Bell et al. [52] tried to establish which relationships between transcription factors and miRNAs were important for melanoma proliferation and invasion using gene expression profiling of normal and melanoma cells. Several miRNAs known to regulate proliferation and invasion were identified, including *miR-211*. A new *miR-211* target, *NUAK1*, was also identified, which was shown to play a role in melanoma cell adhesion with downregulation of *miR-211* upregulating NUAK1 and promoting adhesion, and vice versa.

miR-196a has also been shown to act as a tumor suppressor miRNA in melanoma [57]. Using a high-throughput miRNA expression profiling approach in cell lines and tissue samples, *miR-196a* expression was found to be significantly reduced in malignant lesions. Over-expression of *miR-196a* significantly reduced the invasive capacity of melanoma cells, and HOX-C8, cadherin-11, calponin-1, and osteopontin were identified asmiR-196a targets. These authors then went on to show that *miR-196a* downregulation led to upregulation of HOX-B7 and consequent stimulation of basic fibroblast growth factor (bFGF) signaling, with resulting ETS-1 transcription factor and bone morphogenetic protein 4 (BMP-4) expression, which is known play a role in melanoma progression [58]. Using similar methodology, Chen et al. showed that *miR-193b* also acts as a tumor suppressor via cyclin D1, and it was the most downregulated of 31 differentially expressed miRNAs in malignant tissue samples.

It has been shown that miRNA regulatory effect on their targets is directly correlated to mRNA decay [59,60]. mRNA decay rates in animal cells changes rapidly and with half-lives varying from minutes to days [61]. For example, for mRNAs stability in mouse embryonic stem cells the median is around 7 h, whereas some genes, including *Foxa2*, *Hes5* and *Trib1*, have half-lives under an hour [62]. In a recent study, Larsson et al., [59] reported that the short-lived transcripts are more difficult to perturb using microRNAs. Therefore, it is important to take this (mRNA stability/decay rate) in to account when designing a miRNA perturbation study.

The role of miRNAs in the immune response

Hypoxia influences the microenvironment of solid tumors, including in melanoma. One effect of hypoxia in melanoma is that it is thought to facilitate escape from immune control and promote cancer via downregulation of antigens and proteins that are necessary for an effective immune response [134,135].

Hypoxia is known to stimulate expression of several miRNAs. miR-210 is regulated during HIF1-α-dependent hypoxia in non-small cell lung cancer (IGR-Heu) and melanoma cell lines (NA-8) [124]. Reduced expression of miR-210 in melanoma cells facilitates cell lysis by antigen-specific cytotoxic T lymphocytes (CTLs). Table 1 At a gene regulatory level, PTPN1, HOXA1, and TP53I11 are target genes of miR-210 and are thought to mediate the immunosuppressive response and have been shown to be involved in immune regulation and tumor initiation [125–129]. Another microRNA, miR-34a/c, has been reported to regulate innate immune responses in melanoma cells [109]. miR-34a/c control the expression of ULBP2, which is a ligand for natural killer cell immunoreceptor (NKG2D). NKG2D usually detects early tumors, eliminates