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REVIEW

Challenge and promise: the role of miRNA for pathogenesis and progression of malignant melanoma

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Abstract microRNAs are endogenous noncoding RNAs that are implicated in gene regulation. More recently, miRNAs have been shown to play a pivotal role in multiple cellular processes that interfere with tumorigenesis. Here we summarize the essential role of microRNAs for human cancer with special focus on malignant melanoma and the promising perspectives for cancer therapies.

Keywords microRNA · Cancer · Malignant melanoma · Human cancer metastasis

Abbreviations

miRNA	microRNA
RISC	RNA-induced silencing complex
UTRs	untranslated regions
AMOs	anti-microRNA oligonucleotides
dsRNA	double-stranded RNA
snRNA	small nuclear RNA

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Introduction

Malignant melanoma is characterized as a malignant neoplasm that is initially composed of proliferation of aberrant melanocytes. These atypical melanocytes have distinct defects in cellular processes that include cell cycle regulation, cell signalling, cell adhesion, cell differentiation, and cell death (Bartel 2004). Furthermore, malignant melanoma is one of the most aggressive cancer types in humans with propensity for distant metastasis behavior, and metastasized melanoma is resistant to many of the available therapies. At present, dacarbazine has the best efficacy with a response rate ranging from 5% to 29% and a short 4-month median response duration (Blower et al. 2007). Notably, the incidence of melanoma is progressively increasing in the past decades (1 in 74 Americans will develop melanoma during his lifetime). Moreover, death that is caused by malignant melanoma is also increasing, as its mortality rate has risen by 2% annually since 1960 (Bartel 2004; Eigentler et al. 2003).

The limited success of available treatments underlines the needs to develop new therapeutic and preventive approaches for malignant metastasized melanoma (Grabacka et al. 2006). Therefore, in recent years, new molecular therapy analyzes the possibility of miRNA involvement in malignant melanoma treatment. Molecular biology studies have long reported the existence of noncoding RNAs in different organisms, among which miRNAs represent a new highly impressive class to be studied in tumor biology field. microRNA, which is a short noncoding negative regulator of gene expression, interacts with messenger RNA (mRNA) and could be intimately involved in the development of cancer (Halaban et al. 2009).

Previous studies have demonstrated an association between tumor incidence and gene mutations, while recent

studies have reported that the expression of these genes might be modified by deregulated miRNAs without any concomitant alteration or mutation in gene sequences. Changes in the sequence of miRNA or in the miRNA target region of a transcript might also have a major impact on posttranscriptional regulation (Hammond 2006). Therefore, it remains to be investigated to what extent miRNAs expression in malignant melanoma cell lines could affect tumor development, metastasis, and response to treatment.

microRNA biogenesis

miRNAs are generally 18 to 25 nt long. They were first described in the early 1990s in the worm *Caenorhabditis elegans* as regulators of development and differentiation. miRNAs are produced from either their own genes or from introns by RNA polymerase II, which catalyzes the transcription of DNA to synthesize precursors of mRNA and most snRNA and microRNA (Hammond 2006; Horwich and Zamore 2008) (Fig. 1).

MiRNAs are initially expressed as primary miRNA (pri-miRNA), which are apparently transcribed by RNA polymerase II, in the cell nucleus, and include a cap and poly(A) tail. The miRNA portion of the pri-miRNA transcript likely forms a hairpin with signals for dsRNA-specific nuclease cleavage (Horwich and Zamore 2008). This processing is performed in animals by a protein complex known as the microprocessor complex, consisting of the dsRNA-specific ribonuclease Drosha that digests the pri-miRNA in the nucleus to release hairpin and the dsRNA binding protein Pasha. Then these pre-miRNAs of approximately 70 nt are processed to mature miRNAs in the cytoplasm by interaction with the endonuclease Dicer, a member of the RNaseIII, which also initiates the formation of the RNA-induced silencing complex (RISC). To control the translation of target mRNAs, the double-strand RNA produced by Dicer proceed to strand separate, and the single-strand mature miRNA must associate with the RISC. This complex is responsible for the gene silencing observed due to miRNA expression (Igoucheva and Alexeev 2009).

microRNA function and mechanism of action

microRNAs are short noncoding RNAs that act as endogenous regulators of hundreds of target genes. In fact, these miRNAs may influence cellular processes such as development, cell proliferation, apoptosis, survival, and differentiation by controlling the expression levels of hundreds of mRNA species (Horwich and Zamore 2008; Kurihara and Watanabe 2004). The fact that remarkably small numbers of nucleotides are required for miRNA-directed target repression suggest that each miRNA can regulate numerous mRNAs, and up to one third of human mRNA are predicted to serve as a potential miRNA target (Lehmann et al. 2008).

Recently, it has been shown that miRNAs are well recognized as gene regulators via binding to the 3'-untranslated regions of their respective targets protein coding transcript. Additionally, miRNA can use a mechanism of gene regulation termed *translational repression*, which does not result in the degradation of their mRNA

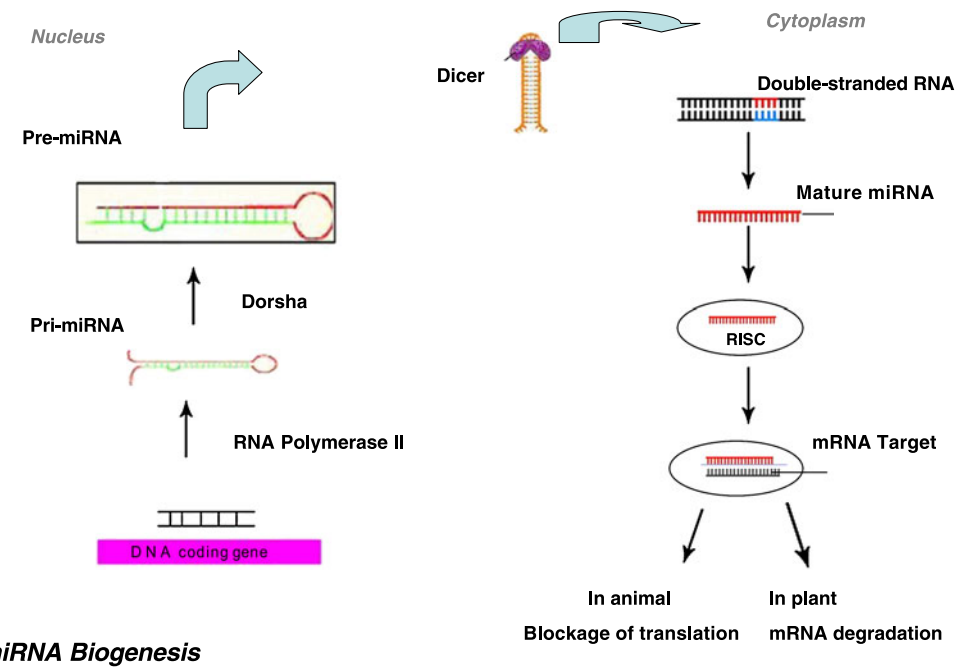

Fig. 1 miRNA biogenesis: miRNAs originate in the nucleus as hairpin precursors and then are processed by the RNaseIII enzymes, Drosha and Dicer, to yield double-stranded RNA. One strand is selected to function as mature miRNA and loaded into the RISC, while another strand is degraded. Then miRNA binds imperfectly with its target mRNA in animal, whereas it binds with perfect complementarity in plants, resulting in mRNA translational repression

targets (Halaban et al. 2009; Kurihara and Watanabe 2004). These miRNAs act by binding imperfectly within the 3' (untranslated regions [UTRs]) of their mRNA targets, inducing concomitant repression of their translation. miRNAs that use this mechanism are able to reduce the protein levels of their target genes, while the mRNA levels are not affected. However, in plants, miRNAs have perfect sequence complementarity with mRNA targets, which lead to irreversible endonucleolytic cleavage and degradation of the mRNA. The degradation pathway and the translational repression pathway both result in posttranscriptional gene silencing (Kurihara and Watanabe 2004).

The tissue-restricted expression of many miRNAs suggests that they might be involved in the differentiation and development in a cell-type and tissue-specific manner. For example, miR-1 and miR-133 have a role in the development of the heart and skeletal muscle, while miR-181 is playing an essential role in B-cell-progenitor determination and hematopoietic differentiation (Liu et al. 2008; Lewis et al. 2005).

Expression profile of microRNA in cancer

Despite a poor characterization of the biological functions and the target genes of miRNAs, it has been confirmed that their expression is changing in tumorigenesis. They are strongly involved in differentiation and development, two main features that are deregulated in cancer (Halaban et al. 2009; Lujambio et al. 2008). Additionally, the human genome contains up to 1000 miRNA, of which more than 300 miRNA have been identified (Liu et al. 2008; Ma et al. 2007). Recent studies have shown that the expression of miRNAs affects the activities of their targeted mRNA encoding proteins. These target mRNAs may have oncogenic or tumor suppressor functions. As a consequence, the cancer-related functions of miRNAs frequently depend on the biological properties of their target genes (Kurihara and Watanabe 2004).

Recently, it has been demonstrated that microRNAs that are up-regulated in tumors and whose targets are tumor suppressor genes might be considered as oncogenes, as the overexpression of these miRNAs down-regulates the level of tumor suppress proteins. Conversely, the miRNAs that are down-regulated in cancer and whose target are oncogenes are classified as tumor suppressors as summarized in Table 1 (Halaban et al. 2009; Lujambio et al. 2008). For example, miRNAs such as miR-124a has been found to be down-regulated in colon, breast, and lung carcinoma cell lines, suggesting that it can act as tumor suppressor gene. These data convincingly show that normal miRNA expression is essential for proper development and differentiation, and abnormal cell proliferation and differentiation, which are the hallmark of human cancers, are associated with aberrant miRNA expression profile (Lujambio et al. 2008).

Accumulating evidence is showing that miRNA expression might be affected by epigenetic mechanisms such as DNA methylation and histone modifications. Moreover, it has been shown that alterations in the expression of miRNAs may be achieved by treatment with epigenetic modulator drugs. For example, let-7a-3 locus, which is involved in lung adenocarcinoma and controls the activity of the human oncogene RAS, is generally hypomethylated, and its expression can be epigenetically modulated (Kurihara and Watanabe 2004; Lujambio et al. 2008). In contrast, mi124a3, mir-9-1, and mir-152 are hypermethylated in 34% to 86% of all primary human breast cancers (Ma et al. 2009). Similarly, miR-127, whose target gene is BCL6, is found to be remarkably up-regulated in cancer cell lines after treatment with 5-aza-2'-deoxycytidine, a potent DNA methylation inhibitor, and 4-phenylbutyric acid, a histone deacetylase inhibitor (Seike 2009).

Signature of microRNA in human cancer metastasis

The metastatic process associates with the capacity of tumor cells to invade neighboring tissues, enter the

Table 1 Tumor suppressor and oncogenic miRNAs and their target genes: The biological role of individual miRNA is correlated to the function of its target gene. *IRS* insulin receptor substrate-1 (oncogene), *AT1R* angiotensin II type 1 receptor, which is responsible for the activity of the main effector hormone, *LATS2* serine/threonine-protein kinase (which is involved in P53 function), *HES1* hairy and enhancer of split (tumor suppressor), *E2F1* transcription factor (plays a crucial role in the control of cell cycle and action of tumor suppressor proteins)

Tumour suppressor miRNA	Target gene	Reference	Oncogenic miRNA	Target gene	Reference
Let-7	oncogeneRAS	Lujambio et al. 2008	miR-372	P53	Ma et al. 2007
miR-17-5p	c-Myc	Ma et al. 2007	miR-373	LATS2	Lujambio et al. 2008
miR-20a	c-Myc	Ma et al. 2007	miR-155	AT_1R	Lujambio et al. 2008
miR-126	IRS-1	Saito et al. 2009	miR-9-1	HES1	Ma et al. 2007
miR-15a	BCL-2	Lujambio et al. 2008	miR-17-92	E2F1	Liu et al. 2008
miR-16	BCL-2	Lujambio et al. 2008	miR-221	P27	Rigel and Carucci 2000

systemic circulation, reside in distant capillaries, and translocate into surrounding tissues. Most importantly, metastasis is responsible for 90% of mortality in solid tumors. It has been reported that aberrant expression of miRNAs that regulate tumor suppressor or oncogenes not only plays an essential role in the development of human cancer, but also associates with the metastatic behavior of tumors (Meng et al. 2006).

Three metastasis-promoting miRNAs have been described: miR10b, miR-373, and miR-529-c. In contrast, other miRNAs such as miR-126 and miR-335 inhibit metastatic process (Molnára et al. 2008).

miR-10b has been reported to indirectly activate the prometastatic gene RHOC by suppressing homeobox D10 (HOXD10), thus, leading to tumor invasion and metastasis. This miRNA is up-regulated exclusively in metastatic cells (Si et al. 2006)

Additionally, miR-373 can also promote tumor invasion and metastasis, at least in part by regulating the gene CD44, which is involved in cell–cell interactions, cell adhesion, and migration. For example, analysis of normal/tumor breast samples has shown an up-regulation of miR-373 in cancer, in particular, in samples exhibiting lymph node metastasis (Molnára et al. 2008). However, miR-335 works as metastasis suppressor by inhibiting certain genes that are associated with human metastasis, particularly the oncogene SOX4 and tenascin C, an extracellular matrix component. Interestingly, these remarkable studies suggest the potent role of miRNAs as stimulators and inhibitors of metastasis and identify several target genes involved in cell's metastatic capacity; these data support the molecular link between miRNA deregulation and a specific tumor behavior and exert the possibility of using miRNAs within therapeutic approaches for the prevention of tumor metastasis (Meng et al. 2006; Molnára et al. 2008).

microRNA expression in melanoma

There is a lack of studies about the role of microRNA in the development and progression of melanoma. Most importantly, the comparison of normal human epidermal melanocytes with established melanoma cell lines reports that a set of miRNAs can play a role in malignant melanoma transformation and in the progression and metastasis of the tumor (Mueller et al. 2009). One of these studies analyzed 283 known human miRNA gene in 227 human ovarian cancers, breast cancers, and melanoma samples and reported that 86% of melanoma cell lines have alteration in genomic loci containing miRNA genes (Ma et al. 2007). Further studies, which analyzed miRNAs that were differentially expressed among six tissue groups (CNS, colon, leukemia, ovarian, renal and melanoma), showed that expression profile of mir-141, mir-147, mir-192, and mir-335 might provide a useful tool to predict malignant melanoma development, because of their differential expression in melanoma tissue when compared to tissue group (Negrini and Adrian Calin 2008).

The comparison of the miRNAs expression profiles in melanocytes with that in melanoma cells identified 49 miRNAs, which are up-regulated tenfold in primary melanoma cell lines, and 14 miRNAs, which are down-regulated only by threefold. On the other hand, to investigate miRNAs that might be associated with metastatic melanoma, it has been reported that 11 miRNAs are up-regulated and 2 miRNAs are down-regulated in metastasized melanoma cell lines compared with primary melanoma cell lines (Mueller et al. 2009; Negrini and Adrian Calin 2008).

Another study demonstrated down-regulation of let-7 family members in primary cutaneous melanoma compared with benign nevi (Rigel and Carucci 2000).

This miRNA was previously reported to be involved in tumor invasion and metastasis by suppressing the p53 pathway and cooperating with oncogenic RAS to induce cellular transformation (Saito et al. 2006; Rothhammer and Bosserhoff 2007). Therefore, the differential expression of some miRNAs in primary malignant melanoma and metastasized melanoma cells in comparison to benign melanocytes opens new horizons in the understanding of the pathogenesis and prognosis of malignant melanoma on basis of miRNA expression profile (Lujambio et al. 2008; Satzger et al. 2009).

Normalizing miRNAs expression in cancer therapy

Since deregulated miRNAs expression has been reported in many types of human cancer and as miRNA expression has been observed to affect the activities of targeted mRNA encoding proteins that have oncogenic or tumor suppressor functions, an exciting new area of research involves correcting miRNA expression (Ma et al. 2007; Suárez and Sessa 2009).

Normalizing the expression of down-regulated miRNAs or inhibition of overexpressed miRNAs may contribute to rebalanced expression of large genes implicated in oncogenesis and tumor progression. For this reason, targeting of miRNAs might provide an important therapeutic strategy for human cancer (Lujambio et al. 2008; Ma et al. 2007)

Inhibition of overexpressed miRNAs has been achieved by the intervention of anti-miRNA oligonucleotides (AMOs), which are complementary to the miRNAs, while the induction of down-regulated miRNAs might be performed by using

expression systems that use viral or liposomal delivery systems for the vectors (Suárez and Sessa 2009; Zhang et al. 2007).

miRNAs have been speculated to play a potential role in sensitivity or resistance to specific drugs. In this filed, one study found that the inhibition of miRNA-21 using antisense oligonucleotides enhanced growth inhibition of MCF7 cells by topotecan by approximately 40% (Voorhoeve et al. 2006). Similar results have been found in lung cancer cells and cholangiocytes cell lines when treated with AG1478 and gemcitabine, respectively (Wurdinger and Costa 2006; Zhang et al. 2006).

In summary, understanding the miRNA signature in human biology including their expression profile and their target genes may eventually provide critical information for an understanding of tumorigenesis and metastasis and support the possibility of developing new technologies in the treatment of human malignancies.

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