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REVIEW

A Review of MicroRNA in Uveal Melanoma

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Abstract: Uveal melanoma (UM) is the most common and aggressive primary intraocular tumor in adults. UM is classified as a malignant tumor with a strong tendency of metastasis, which always leads to poor outcomes. At present, the pathogenesis of UM remains unclear and lacks effective therapies. Recent studies have shown that microRNAs (miRNAs), defined as a group of 21–23 nucleotides single-stranded noncoding RNAs, play a significant role in UM. By binding to the complementary sites within the 3′ untranslated region (3′UTR) of message RNAs (mRNAs), miRNAs regulate genes by decaying mRNAs or inhibiting their translation. Thus, miRNAs can modulate various biological behaviors of tumors, including cell proliferation, invasion and metastasis. Furthermore, miRNAs have shown clinical applications by serving as biomarkers for diagnosis and prognosis, regulating immune response, and functioning as epigenetic regulators. It is reasonable to believe that miRNAs have wide application prospects in the early diagnosis and therapy of UM.

Keywords: uveal melanoma, microRNA, biomarker, immune response, review

Uveal Melanoma

Uveal melanoma (UM) is the most common and aggressive primary intraocular tumor in adults. It has been estimated that the incidence of UM is about 5–6 per million people per year in America.[1,2] The incidence rate of UM increases with age and peaks at an age of 70.[3] UM is highly malignant with a mortality rate of 31% after 5 years following diagnosis.[4,5] The leading cause of death is tumor metastasis, which usually first occurs in liver[6] and is hard to detect at early stage.[7] Various studies have reported potential therapies against metastatic UM, including globe enucleation, local resection and radiotherapy. However, the prognosis is still very poor.[8,9] The mean survival time (MST) is only 3.6 months after the diagnosis of liver metastasis and most patients will die within 6 months.[10,11] Therefore, there is an urgent need to develop novel methods for the diagnosis and therapy to prolong the lifetime of UM patients.

MicroRNA

MicroRNAs (miRNAs) are defined as a group of 21–23 nucleotides single-stranded noncoding RNAs.[12] By binding to the complementary sites within the 3′ untranslated region (3′UTR) of message RNA (mRNA), miRNA regulates genes via decaying mRNA or inhibiting the translation.[13] Thus, miRNA plays a significant role in physiological and pathological behaviors such as cell proliferation, apoptosis, differentiation, organ formation, organism development and even diseases, which has become a research hotspot in recent years.[14,15] Increasing evidences prove that abnormal expression of miRNA is closely correlated to the onset and

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progression of multiple tumors, including osteosarcoma, hepatocellular carcinoma, prostate cancer, colorectal cancer, multiple myeloma and breast cancer.[16–18] In this review, we will discuss the dysregulations, biological functions and clinical applications of miRNAs in UM.

Biological Functions of miRNAs in UM

Roles in Cell Proliferation and Apoptosis

MiR-137: Chen et al[19] discovered a decreased level of miR-137 in UM cells. Overexpression of miR-137 might significantly inhibit cell proliferation through blocking G1/S phase transition. By using TargetScan and bioinformatics prediction, they found the potential target genes of miR-137 were c-Met, CDK6 and MITF. Another study showed that the p160 family of steroid receptor coactivators (SRCs) were pleiotropic "master regulators" of steroid hormone receptor.[20] The SRCs acted as critical oncogenic drivers and participated in tumor growth, metastasis and drug resistance. MiR-137 could downregulate the expression of SRCs and suppress cell proliferation in UM. The full list of miRNAs reported to be involved in UM to date is provided in Table 1.

MiR-145: Li et al[21] reported that in contrast to uveal melanocytes, UM cells had a lower level of miR-145. Through blocking G1 phase entering S phase, the upregulation of miR-145 could inhibit cell proliferation and promote apoptosis. Knocking down of insulin receptor substrate 1 (IRS-1), which was the target gene of miR-145, had similar outcomes to miR-145 overexpression.

MiR-144: Previous study showed the expression of miR-144 was decreased in both UM tissues and cell lines (OCM-1A, MUM-2B, MUM-2C, C918). Transfection of miR-144 could inhibit c-Met-mediated UM cell proliferation and invasion through binding with 3′ UTR of c-Met mRNA.[22]

MiR-92a-3p: Venza et al[23] found miR-92a-3p upregulated in UM cell lines and involved in apoptosis. The histone deacetylase inhibitor MS-275 could deplete the expression of miR-92a-3p and upregulate MYC Binding Protein 2 (MYCBP2), which was a target gene of miR-92a-3p. This would finally lead to the programmed cell death of UM.

MiR-181b: A recent study reported the promoting effect of miR-181b in UM.[24] Bioinformatics and functional analysis suggested that miR-181b enhanced cell cycle progression by targeting carboxy-terminal domain small phosphatase-like (CTDSPL), which led to the accumulation of downstream cell cycle effector pRB/E2F1. After transfecting miR-181b into MUM-2B cells, the percentage of G0/G1 phase cells decreased by 19%, while the ratio of S-phase cells increased about 10%, compared to the control group.

Roles in Cell Migration and Invasion

MiR-296-3p: Matrix metalloproteinase (MMP) is closely related to the formation and progression of tumors. MMP-2 and MMP-9 have been proved to participate in UM angiogenesis and metastasis. Wang et al[25] demonstrated that transfection of miR-296-3p in UM cells could repress cell proliferation, migration, invasion and stimulate apoptosis by regulating MMP-2/MMP-9.

MiR-20a/miR-155: Zhou et al[26] discovered that miR-20a functioned as an oncogenic role in UM. Transfection of miR-20a mimic into MUM-2B cells contributed to an increase in cell motility, including invasion and migration. Similarly, miR-155 also showed an oncogenic property by promoting cell proliferation and invasion in UM. And this effect could be reversed by ectopic expression of Nedd4-family interacting protein 1 (NDFIP1), which was related to the nuclear translocation and ubiquitination of Phosphatase and tensin homolog (PTEN).[27]

MiR-454/miR-367: PTEN served as a tumor suppressor gene and participated in the regulation of cancer cell cycle. By regulating PTEN, miR-454 could promote UM cell proliferation, colony formation, invasion and cell cycle progression via G1/S transition.[28] Similarly, miR-367 functioned as an oncogenic role and targeted PTEN. The anti-miR-367 transfection inhibited the proliferation and migration of MUM-2B cells and increased the proportion of cells in G0/G1 phase.[29] In addition, co-transfection with a PTEN construct lacking the 3′-UTR decreased the promoting effect of miR-367 mimic on UM cells.

MiR-224-5p: Li et al[30] found a low expression of miR-224-5p in UM cells. After transfecting miR-224-5p mimics into UM cells, they observed a decrease in cell proliferation, migration and invasion. Dual-luciferase reporter assay showed the target genes were PIK3R3 and AKT3. It is well known that the phosphatidylinositol 3 kinase (PI3K)/protein kinase B (AKT) pathway takes part in the malignant transformation of various carcinomas, and PIK3R3 is a regulatory subunit of PI3K. Therefore, miR-224-5p acts as a tumor suppressor via miR-224-5p/PIK3R3/PI3K/AKT axis.

MiR-21: Another study reported that in comparison with uveal melanocytes, the level of miR-21 was higher

Table 1 Characteristics of miRNAs in UM

miRNA	Target	Expression	Mechanism	Sample	Role	Reference
miR-137	c-Met, CDK6, MITF, p160 SRCs	Down	Inhibit cell proliferation	Cell lines (M17, M23, SP6.5, OCM-1/3, OMM1/1.3, Mel202, 92.1)	Tumor suppressor	[19,20]
miR-145	IRS-1	Down	Inhibit cell proliferation and promote apoptosis	Tissues and cell lines (OCM-1, MUM-2B)	Tumor suppressor	[21]
miR-144	c-Met	Down	Inhibit cell proliferation and invasion	Tissues and cell lines (OCM-1A, MUM-2B/2C, C918)	Tumor suppressor	[22]
miR-92a-3p	MYCBP2	Up	Inhibit apoptosis	Cell lines (OCM-1/3, 92.1)	Oncogenic	[23]
miR-181b	CTDSPL	Up	Promote cell cycle progression	Tissues and cell lines (SP6.5, VUP, OCM-1/1A, MUM-2B, 92.1)	Oncogenic	[24]
miR-296-3p	MMP-2, MMP-9	Down	Inhibit cell proliferation, migration, invasion and promote apoptosis	Tissues and cell lines (C918)	Tumor suppressor	[25]
miR-20a	Not reported	Up	Promote cell proliferation, migration and invasion	Tissues and cell lines (MUM-2B/2C)	Oncogenic	[26]
miR-155	NDFIP1	Up	Promote cell proliferation and invasion	Tissues and cell lines (OCM-1A, MUM-2B/2C, C918)	Oncogenic	[27]
miR-454	PTEN	Up	Promote cell proliferation, invasion, colony formation and cell cycle progression	Tissues and cell lines (OCM-1A, MUM-2B/2C, C918)	Oncogenic	[28]
miR-367	PTEN	Up	Promote cell proliferation and migration	Tissues and cell lines (M17/23, MUM-2B, C918)	Oncogenic	[29]
miR-224-5p	PIK3R3/AKT3	Down	Inhibit cell proliferation, migration and invasion	Tissues and cell lines (OCM-1A)	Tumor suppressor	[30]
miR-21	p53	Up	Promote cell proliferation, migration and invasion	In vivo (BALB/c) and cell lines (OCM-1A)	Oncogenic	[31]
miR-23a	Zeb1	Down	Inhibit cell migration	Cell lines (OCM-1)	Tumor suppressor	[34]

in UM cells.[31] In vitro, downregulation of miR-21 promoted apoptosis through inducing S and G2 phase arrest in MUM-2B cells, G2 phase arrest in M619 cells and G1 phase arrest in OCM-1 cells. Besides, miR-21 promoted cell migration and invasion by regulating p53 and its downstream targets glutathione S transferase pi (GST-Pi) and LIM and SH3 protein 1 (LASP1). In vivo, inhibition of miRNA-21 successfully diminished UM tumor growth in BALB/c nude mice.

MiR-23a: Epithelial-mesenchymal transition (EMT) is a process that epithelial cells evolve into mesenchymal cells via losing cell adhesion and gaining the ability of migration and invasion.[32] Therefore, EMT has been proved to occur in the initiation of tumor metastasis. Previous research demonstrated the key of EMT was the loss of epithelium-derived labelled protein E-cadherin, which was crucial for adherent junction of the epithelial cells.[33] And zinc finger protein Zeb1 was able to promote EMT through the repression of E-cadherin. Wang et al[34] reported that miR-23a could degrade Zeb1, eliminate its suppression over E-cadherin, upregulate E-cadherin, then reverse the EMT process of UM cells and finally decrease the cellular migration capacity.

Clinical Applications of miRNAs in UM

miRNAs Function as Biomarkers in UM

Increasing evidences demonstrate that the expression profile of miRNAs is able to identify cancers from normal samples and even to distinguish different subtypes and clinical stages.[35,36] Actually, miRNAs have several advantages: i) Easy accessibility. Since miRNAs exist in many kinds of body fluid such as blood, tears, vitreous humor and milk, it is

convenient to detect them in non-invasive ways.[37–39] ii) High degree of stability. MiRNAs are released from extracellular vesicles and cannot be easily decomposed by RNases.[40] Therefore, they may have a long half-life up to 24 hrs.[41] iii) High level of specificity and sensitivity. iv) Rapid and accurate detection in an economical way.[42,43] Hence, it is reasonable to believe that miRNAs may exert as promising biomarkers for the diagnosis and prognosis of UM.

Ragusa et al[44] analyzed 754 microRNAs from vitreous humor (VH), serum and their respective exosomes of six UM patients and six healthy people. An obvious overexpression of miR-146a was observed by TaqMan low-density array, indicating that miRNAs were released by the affected eye and could be referred as potential diagnostic biomarkers of UM. According to gene expression profiling (GEP), UM is divided into two groups: one is low metastatic risk group, corresponding to class 1, and the other is high metastatic risk group, corresponding to class 2.[45] Worley et al[46] found six miRNAs (let-7b, miR-199a, miR-199a*, miR-143, miR-193b and miR-652) were able to distinguish the classification of UM with 100% sensitivity and specificity. These six miRNAs were all upregulated in class 2 UM, among which let-7b and miR-199a were the most critical predictors for metastasis. Another study showed that five miRNAs (miR-214, miR-146b, miR-143, miR-199a and miR-134) expressed differently between monosomy 3 and disomy 3 UM.[47] Furthermore, miR-149* and miR-134 were strongly related to liver metastasis in UM. Triozzi et al[48] reported overexpression of three miRNAs in monosomy 3 UM, including miR-92b, miR-223 and miR-199-5p. These three molecules all targeted genes that promoted metastasis.

A recent study identified a set of twenty miRNAs associated with the prognosis of UM patients. They observed a downregulation of miR-506-514 cluster in patients with poor survival.[49] Besides, the upregulation of miR-199a-5p and miR-592 led to a worse overall survival. Xin et al[50] reported nine miRNAs closely associated with prognosis of UM through bioinformatics analysis, among which miR-4709, miR-7702, miR-365a, miR-365b, miR-195, miR-452 were identified as poor prognostic biomarkers. However, Larsen et al[51] detected no association between miRNA expression profile and prognosis based on the data from 36 UM patients. They found no evidence that miRNAs were correlated to metastasis, TNM stage or other clinicopathological prognostic features. Therefore, further research is still needed.

Therapeutic Meanings of miRNAs in UM

miRNAs Function as Epigenetic Regulators

Accumulating evidences suggest that epigenetics plays a critical role in tumor therapies. It is a novel idea to use drugs or molecules to treat cancers by targeting miRNAs, which act as epigenetic regulators.[52] Long non-coding RNAs (lncRNAs) are non-protein coding transcripts with a length of more than 200 nucleotides. Recent studies demonstrated that lncRNA could modulate miRNAs and influence tumor development.[53] Hence, they were considered as potential epigenetic drugs. Sun et al[54] reported that expression of miR-140 was inhibited in UM samples. Besides, lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) could negatively regulate miR-140 expression. Inhibition of MALAT1 could repress proliferation, migration and invasion of MUM-2C cells by targeting miR-140. Zheng et al[55] observed that miR-224-5p, which was downregulated in UM cells, had a negative correlation with lncRNA ferritin heavy chain 1 pseudogene 3 (FTH1P3). They transfected miR-224-5p mimic into UM cells that overexpressed FTH1P3. As a result, the cell proliferation, cell cycle and migration were all suppressed. The MicroRNA Target Prediction Database (miRDB) showed that FTH1P3 was a direct target gene of miR-224-5p. Lu et al[56] discovered that lncRNA HOMEOBOX A11 antisense RNA (HOXA11-AS) was upregulated, while miR-124 was downregulated in UM. The starBase and dual-luciferase reporter assays showed that HOXA11-AS might serve as a competing endogenous RNA (ceRNA) for miR-124. Western blot indicated that knockdown of HOXA11-AS downregulated enhancer of zeste homolog 2 (EZH2) expression, which was consistent with miR-124-induced decrease of EZH2 protein levels. Thus, as a tumor suppressor, miR-124 could reverse the UM cell proliferation and invasion-promoting effect of HOXA11-AS.

In addition to lncRNAs, some drugs are also able to regulate miRNAs via epigenetic mechanisms. Genistein, a kind of isoflavone, is described as an angiogenesis inhibitor as well as a phytoestrogen. Previous research demonstrated genistein might be a potential antitumor agent.[57] Sun et al[58] discovered that genistein inhibited the growth of UM cells in a dose-dependent manner. Functional assays revealed that genistein regulated the expression of miR-27a as well as its target gene zinc finger and BTB domain containing 10 (ZBTB10). Therefore, miR-27a and ZBTB10 might be involved in targeted therapeutic strategy in UM. Moreover, Chen et al[19] discovered that 5-aza-2′-deoxycytidine, which

was a DNA hypomethylating agent, could upregulate the tumor suppressor miR-137 in UM. Therefore, it is plausible that genistein, 5-aza-2′-deoxycytidine and other epigenetic drugs may have promising prospects in the treatment of UM.

miRNAs Function as Immune Regulators

The role of miRNAs in immune regulation is increasingly being recognized. For instance, miR-223 regulates myeloid-derived suppressor cells (MDSCs),[59] miR-181a regulates natural killer (NK) cells[60] and miR-199a-5p participates in regulatory T cells regulation.[61] Besides, ectopic transfection of miRNAs in mice models could boost protective immunity against malignant tumors.[62] Consequently, miRNAs can act as immune regulators and indirectly modulate biological behaviors of tumors.

Interleukin (IL)-10 is an immunoregulatory cytokine that increases in various tumors, including UM. It has been proved that IL-10 could promote tumor growth by suppressing immune response.[63] Venza et al[64] found that miR-15a, miR-211 and miR-185 were downregulated in UM cell lines. Transfection of these three miRNAs separately inhibited cell proliferation by targeting IL-10Rα gene, indicating that the IL-10/IL-10 receptor system might be regarded as a new therapeutic target for UM treatment.

Cancer stem cells (CSCs) refer to a class of special cancer cells, which are similar to normal stem cells. CSCs have an ability of self-renew and can differentiate into all cell types of a specific carcinoma.[65] There are evidences that CSC is a key factor of UM metastasis, moreover, it is susceptible to NK cell cytotoxicity.[66,67] Joshi et al[68] reported that UM CSCs could synthesize NK cell regulatory miRNAs, including miR-181a, miR-146a, miR-20a, miR-223 and miR-155. After downregulating miR-155, UM cells were more sensitive to NK cell cytolysis. Thus, anti-miR-155 therapy may inhibit UM metastasis through regulating activities of CSCs and NK cells. Likewise, Achberger et al[39] found higher levels of miR-181a, miR-146a, miR-20a, miR-223, miR-155 and miR-125b in UM patients. They also assessed the levels of these miRNAs in $CD56^+$, $CD15^+$, and $CD3^+$ cells isolated from five UM patients. The results showed miR-181a decreased in $CD3^+$ cells, miR-146a increased in all three cells, miR-20a and miR-223 increased in $CD56^+$ cells, and miR-155 decreased in $CD15^+$ and $CD56^+$ cells. These studies suggest that there is a close association between miRNAs and immune cells, further study of immune regulatory miRNAs will be necessary.

Since Tasuku Honjo and James P. Allison won the Nobel Prize for their study of programmed cell death 1 (PD-1)[69] and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4)[70] in 2018, immune checkpoint blockade has become a hotspot in antitumor field. PD-L1, the ligand of PD-1, is expressed on various types of cells, such as B cells and T cells. PD-L1 is always upregulated in tumor cells and interacts with PD-1 to deactivate T cells, which finally leads to immune escape.[71] Thus, anti-PD-1/PD-L1 antibodies (like Pembrolizumab, nivolumab) may prevent T cells from deactivation, restore immunocompetence and have a promising antitumor outcome.[72] In a retrospective study of 86 metastatic UM patients treated with pembrolizumab, the overall response rate (ORR) was 7%.[73] The median overall survival (OS) and median progression-free survival (PFS) were 10.3 and 4.8 months, respectively. Furthermore, in another multi-center study,[74] 64 patients with metastatic UM received a combined anti-CTLA-4 and anti-PD-1 treatment, the median OS and PFS were 16.1 and 3.0 months, with an ORR of 15.6%.

However, immune checkpoint blockade therapy also has disadvantages,[75] including drug resistance and reduced response rate. Given this problem, many researchers attempt to seek solutions from miRNAs. Audrito et al[76] discovered an inverse correlation between miR-17-5p and PD-L1 expression in melanoma cell lines. The luciferase reporter assay showed a direct binding between miR-17-5p and the 3′-UTR of PD-L1 mRNA. Besides, they observed a cohort of 80 metastatic melanoma (MM) patients and found a lower level of miR-17-5p in patients with $PD\text{-}L1^+$ tumors than patients with $PD\text{-}L1^-$ tumors. Li et al[77] transfected miR-28 mimic in melanoma mice models and found a decreased expression of PD-1, which meant miR-28 could silence PD-1 and revert the exhaustive status of T cells. Similarly, Xi et al[78] detected an upregulation of PD-L1 in miR-21-deficient tumor-associated macrophages (TAMs) isolated from melanoma. Besides, the combination of anti-PD-1 therapy and miR-21 depletion in TAMs had a superior anti-tumor effect than either agent alone. It was assumed that miR-21 could downregulate PD-L1 expression in TAMs via inhibiting the interferon-γ (IFN-γ)-induced signal transducers and activators of transcription 1 (STAT1) signaling pathway. In summary, it is reasonable to believe that miRNAs may play an indispensable role in immune checkpoint blockade therapy of UM.

Other Potential Therapies

Aerobic glycolysis is a common metabolic pathway in tumor cells. It provides cancer cells energy even in the presence of

abundant oxygen, thus promoting tumor growth and survival.[79] Hexokinase 2 (HK2) is an enzyme that catalyzes the rate-limiting and first obligatory step of glucose metabolism.[80] High expression of HK2 can be seen in various tumors and usually has a close relationship with poor prognosis.[81,82] Liu et al[83] discovered that miR-216a-5p could suppress glycolysis in A375 and MUM-2B cells, contributing to the suppression of cell growth both in vivo and in vitro. Functional assays suggested that HK2 was the target of miR-216a-5p. Hence, if the UM patient has a high level of HK2, miR-216a-5p supplement is a plausible therapeutic strategy. In another study, let-7b in UM cells was downregulated after exposure to irradiation, and transfection of let-7b could repress cyclin D1 expression and reinforce radio-sensitivity of UM via cell cycle arrest. It indicated let-7b might act as a radio-sensitivity enhancer and improve the curative effects of irradiation in UM.[84]

Conclusion

UM is the most common primary intraocular tumor in adults, and early metastasis is the main cause of death. Due to the lack of early diagnostic method and effective therapy, there is an urgent need to discover novel ways. By regulating target genes, miRNAs participate in various biological behaviors in tumors and consequently have many clinical applications in UM. MiRNAs can act as biomarkers in early diagnosis and prognosis of UM. MiRNAs may regulate the immunocompetence of immune cells and improve the therapeutic effect of immune checkpoint blockade in UM. MiRNAs also function as epigenetic regulators. By targeting miRNAs, epigenetic drugs can indirectly affect the biological process to acquire ideal tumor-inhibiting outcomes. Furthermore, miRNAs can even regulate the radio-sensitivity of UM. Therefore, it is reasonable to believe that miRNAs may make a breakthrough in the diagnosis and treatment of UM.

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Disclosure

All authors declare there is no conflict of interest.

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