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REVIEW ARTICLE

MicroRNAs in tumor stem cells

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Abstract

MicroRNAs (miRNAs) are a class of non-coding RNAs that are believed to have a significant role in tumorigenesis and cancer metastasis. Cancer stem cells play a major role in tumor recurrence, metastasis, and drug resistance. Research has shown that miRNAs can promote or inhibit the stemness of cancer stem cells and regulate the differentiation and self-renewal of cancer stem cells. In this article, the phenotype and regulatory mechanisms of miRNAs in cancer stem cells will be described, together with an explanation of their potential role in tumor diagnosis and treatment.

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MicroRNAs (miRNAs) are a class of non-coding RNAs that can control the stability and efficiency of messenger RNAs. They play significant roles in the proliferation of embryonic development, apoptotic cell differentiation, invasion and metastasis of tumors and many other biological processes [1]. Stem cells, a class of cells with self-renewal and multi-directional differentiation potential characteristics, can transform into specific tissue cells. There is evidence that a type of stem cell exists in tumor tissues, known as tumor stem cells [2]. The cycle of tumor stem cells varies and differs from normal cells, and they are capable of self-renewal and unlimited proliferation. Cell movement and migration can lead to metastasis of tumor cells. Recent research has demonstrated that miRNAs can regulate and control differentiation and self-renewal of tumor stem cells. For example, miR-302 and miR-181 can promote the formation of the cancer stem cell phenotype, while Let-7, miR-145, miR-200, miR-203, miR-128, miR-34 and miR-199b can inhibit tumor stem cell growth and promote cancer stem cell differentiation [3]. The role of miRNAs in cancer stem cells is summarized in this article.

MiRNAs

MiRNAs regulate and control a class of endogenous non-coding RNAs in eukaryotes. They consist of approximately 18–25 nucleotides and are highly conserved, temporal and tissue-specific. The first small molecule non-protein coding RNA was discovered in 1993 in worms by Rosallind *et al*, [4] which led to a better understanding of miRNAs. With the development of high throughput miRNA chip technology, additional attention has been directed toward miRNAs in the past 10 years [5]. Recent research has demonstrated that there are more than 1000 different types of human miRNAs. Additionally, each messenger RNA contains different miRNA binding sites, and every predicted target of a single miRNA contains hundreds of genes, which suggests that up to 1/3 of all human messenger RNA may be regulated by miRNAs [6]. Other studies have confirmed that miRNAs are involved in embryonic development, differentiation, apoptosis, cell proliferation, tumor development, invasion, metastasis and other biological processes [7]. Research on tumor-related miRNAs has demonstrated that abnormal expression of miRNAs exists in a variety of tumor tissues, which suggests that miRNAs may be involved in the regulation and expression of genes in a complex network of tumor formation. These findings have attracted increasing attention to understanding the specific mechanisms involved.

Tumor stem cells

Tumor stem cells are capable of self-renewal and unlimited proliferation. They can produce abnormal tumor cells, which exist in tumor tissues, and play a role in tumor survival, proliferation, metastasis and recurrence. Tumor stem cells can remain dormant for extended periods of

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time and are insensitive to chemotherapy and radiotherapy. Therefore, a relapse that occurs after complete remission of the tumor is likely due to tumor stem cells [2]. Tumor stem cells originated from the study of acute myeloid leukemia (AML) by Dick etc in 1994 [8], in which the authors identified the origin of a group of AML cells by cell transplantation into severe combined immunodeficiency (SCID) mice. The cells are capable of homing to the marrow and exhibit morphology similar to leukemia, with differentiation, proliferation and self-renewal potential. These leukemic stem cells are referred to as SCID leukemia initiating cells (SL-IC). In 2003, Clarke and his team isolated stem cells from breast cancer for the first time [9]. Since then, the use of cell surface markers ($CD34^+CD38^-$) for sorting and identification has confirmed that cancer stem cells exist in colon cancer, pancreatic cancer, lung cancer, prostate cancer, melanoma, neuroblastoma and other solid tumors [10–15].

Early studies have demonstrated that cancer stem cells represent a small portion of the total tumor cells and are capable of self-renewal and maintaining the characteristics of tumor cells in solid tumors. However, recent studies have shown that approximately 25% of the tumor cells have the same characteristics as tumor stem cells and can coexist with non-tumor stem cells. The surfaces of tumor stem cells contain specific surface markers, such as CD44, CD24 and CD133, which are highly expressed in tumor cells, and these markers may vary depending on the type of tumor in which they are found. For example: $CD34^+$/CD38 are leukemia stem cell specific markers$^+$ [8]; $CD44^+/CD24^{-/low}$/Lin-ALDH1$^+$ are breast cancer stem cell specific markers [9]; $CD133^+$ is a colon cancer stem cell specific marker [10]; $CD44^+/CD24^+/WSA^+$ are pancreatic stem cell specific markers [11]; and $CD90^+$ is a liver cancer stem cell specific marker [16]. Tumor stem cell markers can exhibit specificity in different types of tumors or tumors of different tissue. Their expression can vary with different expression of cancer genes in the same tumor tissue type. The diversity of tumor stem cell markers indicates the specificity and complexity of tumor stem cells. However, a better understanding of tumor stem cell molecular markers and associated mechanisms requires further studies.

MiRNAs and tumor stem cells

A recent study demonstrated that the expression of miRNAs is closely related to the occurrence of a wide variety of tumors, the degree of differentiation, metastasis and prognosis. Approximately 50% of miRNAs have annotations located in fragile sites in the genome, showing that miRNAs play an important role in tumorigenesis [17]. The function of these miRNAs is similar to that of tumor suppressor genes and tumor genes, and relevant miRNA mutations can activate the expression of cancer genes or lead to a lack of tumor suppressor genes and cause tumor occurrence. In addition, research has shown that miRNAs participate in different stages in the formation and progression of tumor stem cells. For example, miR-302 and miR-181 can promote the formation of the tumor stem cell phenotype [18–20]; miR-203 and miR-200 members regulate tumor stem cell "stemness" and promote differentiation of tumor stem cells [21–23]; Let-7 can regulate the self-renewal and differentiation of tumor stem cells of breast cancer [24–25]; miR-145 shows low expression in the self-renewal processing of human embryonic stem cells, but high expression during the differentiation, and is capable of controlling the number of stem cells through stem cell reassemble factor (OCT4, KLF4) [26]; some miRNAs, such as miR-128 and miR-199b, control tumor stem cell differentiation [27–28]; and miR-17 and miR-34s participate in tumor occurrence and development processing by regulating and controlling the progression of cell cycle [29–31]. Therefore, the research of miRNAs in tumors and tumor stem cells is becoming a new strategy in cancer treatment and clinical research.

Stemness of tumor stem cells controlled by miRNAs

MiR-302

The miRNA from the miR-302 gene family was first cloned from mouse embryonic stem cells and human embryonic stem cells, and it exhibited high expression in embryonic stem cells. However, the expression is lost soon after differentiation and is not expressed in adult cells. The miR-302 gene promoter is controlled by the transcription factors Oct4 and Sox2. They are essential for maintaining early stage embryonic stem cell totipotency and stemness [18]. Recent studies on the regulation of the human embryonic stem cell cycle protein cyclinD1 demonstrated that cyclinD1, which is effective in the G1 period of the cell cycle, is the target of the miR-302 gene. The expression of miR-302 can transfer the tumor cells expression of embryonic stem cell markers (for example: Oct3/4, SSEA-3, SSEA-4, Sox2 and Nanog gene) to embryonic pluripotent stem cells [18]. Therefore, the signal pathways of Oct4/Sox2-miR-302-cyclinD1 are significant in pluripotency and in the self-renewal properties of embryonic stem cells.

MiR-181

Reduced expression of the homologous protein Hox-A11 (a repressor protein that is active in the process of differentiation) results in an increased expression of miR-181 during mammalian skeletal muscle differentiation and during the process of bone reconstruction. Research has shown that, compared with undifferentiated progenitor cells, miR-181a has a high expression level in B lymphocytes, illustrating that miR-181a is a positive regula-

tory protein of B lymphocytes [19]. On the contrary, there is a high level of expression of miR-181 in hepatocellular carcinoma (HCC) cells and an especially high expression in EpCAM positive liver cells or progenitor cells, which are isolated from α-AFP positive liver tissues [20]. In addition, the study also demonstrated that miR-181 expression is significantly higher in the embryonic liver and in liver stem cells. By regulating the differentiation of liver cancer cells (inhibition of GATA6 and CDX2 genes) and activating the Wnt/beta-catenin signaling pathway (reduce NLK gene), the stemness of tumor stem cells is maintained.

MiR-203

MiR-203 is a switch for skin cell proliferation and differentiation in the process of skin formation. Research has shown that miR-203 can inhibit epidermal stem cell proliferation. By inhibiting P63 protein in the division cycle, epidermal cell differentiation can be promoted. In pancreatic tumor stem cells, miR-203 can inhibit the stemness of tumors. Furthermore, miR-203 itself is suppressed by ZEB1, which is an epithelial-interstitial transformation agonist, and is an essential factor for pancreatic cancer and colorectal cancer stem cell [21] self-renewal. Therefore, removing ZEB1 can reduce pancreatic cancer stem cells ($CD24^+/CD44^+$) and slow the formation of monoclonal undifferentiated pancreatic cancer cells, possibly producing a cure for pancreatic cancer.

MiR-200

MiR-200 can inhibit epithelial metaplasia of human breast cancer cells and can be induced by TGF-β. Research has shown that miR-200a, miR-200b and miR-200c, members of the miR-200 family, have low levels of expression in human breast cancer stem cells, breast stem/progenitor cells and embryonic tumor cells [22]. In addition, miR-200c can inhibit normal breast stem cells from differentiating into breast duct or tumor cells. miR-200c can also inhibit the "stemness" of pancreatic cancer stem cells and breast cancer stem cells [23].

Control of tumor stem cell differentiation by miRNAs

Let-7

Studies have shown that let-7 is not expressed in the embryonic L1 and L2 periods, while there is a low level of expression in the early stages of the L3 period, and higher levels of expression in the L4 and adult periods [24]. Several heterochrony genes, such as Lin-41 and daf-12, are considered to be the target of let-7. Let-7 is widely expressed in tissues of mice and human adults. However, mature let-7 was not expressed in embryonic stem cells and pluripotent stem cells of mice and humans, but was widely expressed during the process of differentiation. Let-7 can inhibit a variety of important cell cycle regulators of gene expression, such as cyclinD1, cyclinD3, cyclinA, CDK4, CCNA2, CDC25A, CDK6 and CDK8. Its abnormal expression can lead to the occurrence of lung cancer, breast cancer and many other tumors [25]. Excessive expression of let-7 can inhibit the expression of Ras-7 and HMGA of cancer genes; thus, it can inhibit the cell cycle process, leading to the formation of tumor cells. Excessive expression of let-7 in breast cancer stem cells can reduce cell differentiation and the formation of monoclonal cell mass. Increasing the expression level of breast cancer stem cells, therefore, can interfere with tumor occurrence and self-renewal. Reduced expression of let-7 can maintain an undifferentiated state in breast cancer stem cells and the proliferation potential of the monoclonal cells.

MiR-145

MiR-145 is a type of tumor suppressor gene, and the expression of miR-145 in human embryonic stem cells and the process of self-renewal is low, but increases during cell differentiation. OCT4, SOX2 and KLF4 pluripotency genes are direct targets of miR-145, which suggests that an overexpression of miR-145 can inhibit the self-renewal of human embryonic stem cells and induce directional differentiation [26]. In addition, miR-145 can also regulate the differentiation of smooth muscle cells through the target genes KLF4, troponin and ELK-1 and can become an essential factor for reprogramming fibroblast troponin. Similar to miR-145, miR-143 can also promote differentiation and inhibit the proliferation of smooth muscle cells.

MiR-199b

As a type of tumor inhibitor among miRNAs, expression of miR-199b in metastatic medulloblastoma is missing. Overexpression of miR-199b leads to reduced tumor cell proliferation and destroys the capability of tumorigenesis in brain tumor cells. Studies have shown that miR-199b is a target of the Notch transcription factor HES1 and that it regulates self-renewal of tumor stem cells [27]. Therefore, enhanced expression of miR-199b will restrain the growth of tumor stem cells, reduce the number of medulloblastoma tumor stem cells ($CD133^+$) and lead to abnormal formations of mouse cerebellar medulloblastoma in tumor transplantations.

MiR-128

Expression of miR-128 *in vitro* can reduce the proliferation of neuroglioma cells. It can also restrain the growth of neurogliomas and may also suppress the growth of neuroblastomas [28]. Compared with normal brain tissue, expression of BMI-1 in neuroblastoma rises significantly, while expression levels of miR-128 are lower, suggesting that miR-128 may inhibit proliferation of brain gliomas and self-renewal through the target gene for BMI-1.

Control of tumor stem cell cycle regulation by MiRNAs

MiR-17

MiR-17 genes encode six mature miRNAs. They can inhibit the growth of breast cancer cells and promote the growth of lung cancer and lymphoma cells, thus revealing the function of miR-17 clusters [29]. The miR-17 gene cluster can regulate the cell cycle and the occurrence of tumors using E2F, c-myc, Rb, cyclinD1 and other target transcription factors. In addition, miR-19 may be the essential part of the miR-17 tumorigenic gene cluster. miR-19 also has an important effect on gene cluster carcinogenic properties. Hence, different gene cluster parts may prompt the expression of oncogenes or tumor suppressor genes in a given tumor type [30]. A recent study demonstrated that the miR-17 gene cluster is also involved in the regulation of the stem cell phenotype and has a significant role in the embryonic development of mice and in the regulation of stem cell differentiation, which may be managed by a stem cell regulation factor known as STAT3.

MiR-34

The miR-34 family, the earliest studied miRNAs, was cloned from mouse embryonic stem cells and human embryonic stem cells, and exists in male reproductive cells. Studies have shown that miR-34 induces cell cycle blockage by the p53 pathway, inhibits cell proliferation and colony formation, and promotes cell apoptosis [31]. Overexpression of miR-34 can also reduce the formation of pancreatic cancer stem cells and inhibit the formation of monoclonal cells *in vitro* and tumor formation *in vivo*. The stem cell regulation factors Notch and bcl-2 are considered to be targets of miR-34 [32].

Future applications of MiRNA in cancer treatment

Cancer stem cells are a hot spot in oncology research, and miRNAs are ideal indicators of a curative effect and tumor prognosis [33]. Tumor inhibitory miRNAs are ideal targets for the development of new drugs, which can suppress the occurrence of tumor by enhancing biological behavior and function. Currently, synthesis of miRNAs analogues, miRNAs expression vectors and chemical modification of reverse miRNAs (such as miRNAs inhibitors) have been successfully applied in cellular and animal experiments [34–35]. Application of mice miRNA knockouts and embedded technology has confirmed that let-7 inhibits tumors in non-small cell lung cancer. Using established mRNA antisense technology, introducing anti-let-7 genes into mouse lungs to increasing tumor load, can reduce the incidence rate of K-ras-dependent lung tumors [36]. Another study demonstrated that miR-26a is highly expressed in normal tissue, but is missing in hepatocellular carcinoma. Importing it into hepatocellular carcinoma in mouse models can prevent the development of liver cancer [37]. Therefore, in the treatment of tumors, it is important to ensure the targeted delivery of the miRNAs. The current organization specific expression of miRNAs and the target delivery system of tumor miRNAs have been established, and the specificity of the tumor gene therapy ligand targeting system has been developed and patented. This approach will provide a novel method for the treatment of tumors [38].

Expectation

With additional research, miRNA may become a new target for the diagnosis and treatment of diseases. It could provide new understanding of the eukaryotic gene expression regulatory network. By changing the expression of specific miRNA levels to increase tumor chemotherapy sensitivity, especially for tumor occurrence and metastasis, tumor stem cell therapy will provide a new direction for tumor therapy.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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