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The role of microRNAs in the formation of cancer stem cells: Future directions for miRNAs

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The scientific investigation of microRNAs (miRNAs) and cancer stem cells is of considerable interest. Cancer stem cells are a small subpopulation of cells identified in a variety of tumors and capable of self-renewal, differentiation, chemoresistance and tumorigenesis. MiRNAs are small (18-24 nucleotide) RNAs that regulate expression of genes at the post-transcriptional level. Recently, many studies have found several miRNAs that are either upregulated or downregulated in cancer stem cells when compared to non-cancerous cells from the same tissues. Importantly, miRNAs also have a demonstrable effect on cancer stem cell features, suggesting miRNAs may play a key regulatory role in the formation of cancer stem cells. Here we discuss the potentially highly significant contribution of miRNAs to the formation of cancer stem cells.

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Introduction

OVER THE PAST FEW YEARS, microRNAs (miRNAs) have emerged as a prominent class of gene regulators (1). MiRNAs are single-stranded RNA of 18-24 nucleotides in length derived by RNase III-type enzyme cleavage of endogenous transcripts containing local hairpin structures (2, 3). MiRNA are important to cell physiology because of their ability to control messenger RNA (mRNA) translation. Functioning as a guide molecule in post-transcriptional gene silencing, miRNAs partially complement the 3'-untranslated region (UTR) of target mRNAs (4) and thereby repress gene expression (5). MiRNAs have already been shown to have key roles in diverse regulatory pathways, including embryonic development (6), apoptosis (7), cell proliferation (8), cell division (9), protein secretion (10), viral infection (11), and cell self-renewal/differentiation (12). Because of this, it is likely that dissecting the relationship of miRNA in these various pathways will provide new insight into the formation of cancer stem cells, since cancer stem cells are a pathology that can occur at any stage of development and in virtually any cell type, therefore involving all of the processes just cited.

Cancer stem cells are a small subpopulation of cells that have been identified and isolated in a plethora of tumors, including within the brain (13, 14), breast (15), bone (16), colorectum (17), head and neck (18), lung (19), skin (20), pancreas (21), prostate (22), and blood (23, 24). Cancer stem cells are capable of self-renewal, differentiation and tumorigenesis, and of critical importance, many cancer stem cells also appear resistant to traditional chemotherapeutics and are therefore likely to underlie many instances of cancer relapse (25). In support of this theory, implantation of cancer stem cells into healthy mice induces tumorigenesis (26).

It is therefore clinically relevant that aberrant miRNA expression, both as an upregulation of some miRNAs and a down regulation or absence of others, has been widely found in cancer stem cells (27). For example, breast cancer stem cells express less miR-200 than healthy breast tissue (28), and the entire miR-200 family is inhibited during breast cancer stem cells formation (29). On the other hand, hepatic cancer stem cells express high levels of mir-181 (30) and Li et al. (31) have reported a distinct miRNA expression profile of 68 miRNAs during hepatocarcino-

genesi. These examples are just a few of what appears to be a major trend (32) and it remains unclear as to which miRNA might be most important for tumorigenesis within various tissues. Expressional changes in the levels of many miRNAs could also result from cancer formation, for example, and may not contribute as a causative factor in development of the disease. Therefore, dissecting the mechanisms through which candidate miRNAs become expressed, as well as their physiological roles, promises to provide new insight into the neoplastic transformation of cancer stem cells into tumors.

MiRNAs dysregulation in cancer stem cells

Since aberrant levels of certain miRNAs are present in cancer stem cells and miRNA are able to potently affect many cellular processes, at some level we feel the observed dysregulation of miRNA contributes to the generation of cancer stem cells. As mentioned above, the key is discovering which are critical in the initial step of tumorigenesis, and more specifically, which might underlie the development of cancer stem cells.

For this reason, it is of great interest that miR-495 is upregulated early in breast cancer stem cells and promotes oncogenesis and hypoxia resistance via repression of E-cadherin and REDD1 (33). Additionally, increased expression of miR-495 is also found in breast cancer stem cells, implicating miR-495 as a potential factor that defines the tumorigenic properties in cancer stem cells. In a blind approach, Sun et al. (34) isolated breast cancer stem cells from the cancer cell line, MCF-7, using fluorescence-activated cell sorting, and performed microarray analysis to identify differentially expressed miRNAs. In their study, 19 miRNAs were revealed to have an expression level at least four times enriched in breast cancer stem cells when compared to the non-stem cell tumor cells. Using similar methods, Zhang et al. (35) isolated a population of colon cancer stem cells, analyzed the miRNA expression profile of colon cancer stem cells using miRNA array, and found 11 overexpressed and 8 underexpressed miRNAs.

MiRNAs enhance self-renewal in embryonic stem cells

Several studies have identified miRNAs that are differentially expressed between embryonic stem cells and their differentiated progeny in both mice and humans (36). Such dynamic expression profiles suggest miRNAs play important roles in maintaining stem cell self-renewal and pluripotency. For example, Ma et al. (37) found that by targeting the tumor protein 53-induced nuclear protein 1, miR-130b promotes liver tumor-initiating cell growth and self-renewal within cells containing the stem cell marker CD133. Importantly, lentiviral overexpression of miR-130b in liver cells that do not express the stem cell marker CD133, induced the potential for self-renewal. Similarly, Han et al. (38) showed that miR-29a overexpression converts non–self-renewing myeloid progenitors into self-renewing populations, and differential expression of miR-29a in mouse haematopoietic stem cells. The authors strongly suggest that miR-130b and miR-29a are potentially critical, causative factors in the establishment of cancer stem cells self-renewal by regulating the haematopoietic stem cells and promoting acute myeloid leukemia.

MiRNAs promote cell differentiation

In addition to a proven role in self-renewal, miRNAs are implicated in cell differentiation. As one example, Zhao et al. (39) revealed that miRNA let-7 is expressed in mammalian brains, exhibits increased expression during neural differentiation, and can facilitate neural differentiation when overexpressed. Their work also shows that let-7b regulates neural stem cell differentiation by targeting the stem cell regulator and transcription factor, TLX, as well as the cell cycle regulator, cyclin D1.

Other miRNAs have also been shown to play a role in differentiation, such as miR-27, which enhances differentiation of myeloblasts into granulocytes by targeting Runx1, an important effect because Runxl differentially regulates megakaryopoiesis through a functional interaction with miR-27 (40). At the same time, Chen et al. found miR-1 and miR-206 are sharply upregulated

during muscle satellite cell differentiation and downregulated following muscle injury. In addition, miR-1 and miR-206 were shown to facilitate satellite cell differentiation by restricting proliferation, though the precise targets remain unknown. Together, these experiments suggest that miRNAs participate in a regulatory circuit that allows rapid gene program transitions from proliferation to differentiation (41).

MiRNAs promote tumorigenesis

That miRNAs are involved in tissue differentiation in many cells types is important because many cancers appear to have small populations of cancer stem cells that resist anti-cancer therapies and cause tumor relapse in patients. In the transition of undifferentiated to differentiated tissue, a process that occurs in cancer stem cell cancer relapse, numerous known oncogenic miRNAs decrease in level, whereas the opposite holds true for tumor suppressive miRNAs. Any of these miRNAs might be promising targets for anti-cancer stem cell therapy. To date, there are no known miRNAs specific to cancer stem cells however, and any miRNA-based therapeutic approach would likely also affect other cell types.

Several studies have identified miRNAs that are differentially expressed between embryonic stem cells and their differentiated progeny in both mice and humans.

Still, understanding the biology of miRNA in cancer stem cells provides hope for better treatment. Lee et al. (42) reported that miR-378 enhances cell survival, tumor growth, and angiogenesis by targeting SUFU (suppressor of fused homolog) and the candidate tumor suppressor protein, FUS1. Similarly, it has been shown that miR-21 is markedly overexpressed in breast tumor tissues and functions as an oncogene by modulating tumorigenesis. MiR-21 overexpression leads to up-regulation of the protein, B-cell lymphoma 2, and as a consequence, increases tumor growth and decreases apoptosis (43). The miR-17-92 cluster comprised of seven miRNAs, is highly overexpressed in lung cancers and introduction of miR-17-92 into hematopoietic stem cells significantly accelerates the formation of lymphoid malignancies (44). Together, the evidence clearly indicates that miRNA are not only important for differentiation in non-cancerous tissues, but specific miRNA can be causative factors in the promotion of tumorigenesis.

MiRNAs enhance chemoresistance

One of the most frustrating aspects of cancer stem cells is their apparent resistance to traditional chemotherapeutics. It is possible the integrity of cancer stem cells is at least in part a function of their specific miRNA profile. For this reason, it is of great interest that three miRNAs (miR-200b, 194 and 212) are expressed at relatively low levels in non-small cell lung carcinoma cells that are resistant to the chemotherapeutic, docetaxel, while three others, (miR-192, 424 and 98) are enriched in the chemo resistant population (45). Recently, the expression of miR-140 was found to be associated with chemo sensitivity to the anti-cancer drugs, 5-fluorouracil and methotrexatein osteosarcoma. Remarkably, blocking endogenous miR-140 sensitized cancer cells to 5-FU treatment, whereas overexpression of miR-140 made tumor cells more resistant to 5-FU (46). Similarly, Hamano et al. (47) found over expression of miR-200c induces chemo resistance in esophageal cancers through activation of the AKT signaling pathway. In this study, the authors screened several miRNAs known to regulate stem cell function and found that the level of miR-200c could predict response to preoperative chemotherapy and prognosis. Collectively, these reports suggest a regulatory role of miRNAs in chemo resistance that may be of considerable importance to future therapeutic regimens.

Discussion

Cancer stem cells are a small subpopulation of cells identified in a variety of tumors and

are capable of self-renewal, differentiation, chemoresistance and tumorigenesis, processes recently proven to be controlled by miRNAs. A straightforward conclusion from this relationship therefore, is that miRNAs are involved in the birth of cancer stem cells. We have presented a subset of the data available, highlighting recent findings in a variety of cancer types. We feel that the knowledge gained by studying the role of miRNA in cancer stem cell genesis will enable development of therapeutics targeted to specific cancer stem cells. At the very least, understanding the relationship between miRNA and cancer stem cells promises to advance cancer treatments.

At present, many pharmaceutical companies have begun examining the potential of miRNAs as direct therapeutic targets. However, the basic biology of miRNA is still not fully understood, and more insight into the complex and likely interwoven role of miRNA in cancer stem cells will help. Nonetheless, while miRNAs themselves are potential targets for cancer therapy, via antagonism by anti-miRNA oligonucleotides (48), or miRNA sponges (49), studying miRNA in cancer stem cells also holds potential to reveal novel protein targets for therapeutics. Thus, the discovery that miRNA are critical in cancer stem cell genesis, chemoresistance, and relapse, offers new potential to cure the world's most pervasive disease. H

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