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Medical Hypothesis

No small matter: microRNAs -- key regulators of cancer stem cells

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Abstract: Emerging evidence demonstrates that both tumor suppressor and oncogenic miRNAs play an essential role in stem cell self-renewal and differentiation by negatively regulating the expression of certain key genes in stem cells. It seems logical that they may also be critical players in cancer stem cells. Though small in size, miRNAs play a key role in the epigenetic regulation of cancer stem cells. Specifically, the imbalance of oncogenic vs. tumor suppressor miRNAs may lead to dysregulation of cancer stem cells, thus causing excessive self-renewal and survival of cancer stem cells, and resistance to chemo/radiotherapy. We postulate that restoring the balance of miRNAs will correct this dysregulation via the direct and simultaneous modulation of downstream stem cell pathways involved in cancer stem cell self-renewal and/or differentiation. The resultant restoration of key regulatory pathways could improve therapeutic response. Restoring tumor suppressor miRNAs and/or inhibiting oncogenic miRNAs may provide a novel molecular therapy for human cancers, potentially via modulating cancer stem cells.

Keywords: microRNAs, stem cells, cancer, regulators, oncogene

Introduction

Tumor recurrence accompanied by resistance to treatment, i.e., the tumors respond to initial therapies but eventually re-emerge and become resistant to chemo/radiotherapy, remains a daunting clinical challenge. Recent studies suggest that the drug-resistance of recurring tumors may be due to a special small subpopulation of the malignancy named tumor-initiating cells or cancer stem cells [1]. Cancer stem cells are thought to comprise only a fraction of the tumor and maintain self-renewal, unlimited growth and differentiation capabilities. These attributes make them responsible for tumor outgrowth, progression, drug-resistance and metastasis [2].

Existing knowledge about the hierarchical role of non-malignant stem cells in tissue maintenance has driven much of the research into cancer stem cell biology. Adult stem cells are found in numerous tissues of the body and play a role in tissue development, replacement and repair [3]. Breast stem cells exhibit the two hallmarks of stem cells, multipotency and self-renewal. It has been shown that a single cell enriched with certain cell surface markers has the ability to grow a fully functional mammary gland *in vivo* [1, 4]. Similarly, a single cancer stem cell will establish a new tumor that displays the same heterogeneity of surface markers found in the tumor from whence it came. The cancer stem cell hypothesis arose from observations in haematopoietic malignancies but has since expanded to solid tumors. By using *in vitro* culture techniques and *in vivo* transplant models, investigators have established evidence of cancer stem cells in colon, pancreas, prostate, brain and breast cancers [4-12]. The prevailing view is that cancer stem cells are a small sub-population of cells capable of self-renewal and differentiation, although in some types of human cancers such as melanoma,

such tumorigenic cells may not be rare [13]. To be maximally effective, cancer therapy must be directed against both the largely quiescent pool of cancer stem cells as well as the more actively proliferating bulk tumor cells [14]. This may be possible if specific stem cell signals are inhibited using molecularly targeted therapy, while at the same time attacking proliferating cells by conventional means [15, 16]. To achieve this goal, developments in microRNA regulation present exciting prospects.

MicroRNAs (miRNAs) are a conserved class of naturally occurring 20-22nt non-coding RNAs that regulate gene expression by binding to mRNA, leading to mRNA degradation or translational inhibition [17]. Our group recently reviewed miRNA biogenesis and outlined how the majority of miRNAs achieve specificity and activate the RNA interference pathway by 6-8 complementary base pair interactions with the 3' UTRs of their targets [18]. By this mechanism, they can regulate a variety of biological processes, including developmental timing, signal transduction, tissue differentiation and maintenance, disease (for an interesting analysis of miRNA and disease processes see [19]), and carcinogenesis [17]. Indeed, multiple miRNAs have been reported as either oncogenes or tumor suppressors. Emerging evidence demonstrates that miRNAs play an essential role in stem cell self-renewal and differentiation by negatively regulating the expression of certain key genes in stem cells [17]. One study has shown that microRNA-21 knockdown disrupts glioma growth *in vivo* and displays synergistic cytotoxicity with neural precursor cell delivered S-TRAIL in human gliomas [20]. mi-R21 itself is an oncogene that is overexpressed in certain breast cancers and can affect tumorigenesis through modulating key players in apoptotic control including the proto-oncogene Bcl-2 [21-23]. Other miRNAs, such as miR-15 and miR-16, have been reported to downregulate Bcl-2. Their deletion in cancer allows for apoptosis inhibition when Bcl-2 is subsequently expressed at high levels [24, 25]. The obstacle to apoptosis due to overexpression of Bcl-2 results in an increased number of stem cells *in vivo* [26]. This suggests that apoptosis plays a role in regulating the microenvironments of stem cells [27].

We recently demonstrated that miR-34 plays an important role in the p53 tumor suppressor network. Restoration of miR-34 reestablishes the tumor-suppressing signaling pathway in human gastric and pancreatic cancer cells lacking functional p53 [28, 29]. As a result, miR-34 potently inhibits tumorsphere formation and growth in p53-mutant human gastric cancer cells, providing the first proof-of-concept that there is a potential link between the tumor suppressor miR-34 and cancer cell self-renewal [28]. In a different model system, we recently reported that miR-34 inhibits CD44+/CD133+ pancreatic cancer stem cells self-renewal *in vitro* and tumor-initiation *in vivo* [29]. The mechanism of miR-34-mediated suppression of gastric and pancreatic cancer stem cell self-renewal might be related to the direct modulation of downstream targets Bcl-2 and Notch, implying that miR-34 may be involved in cancer stem cell self-renewal / differentiation decision-making [18, 29]. Our data suggest that miR-34 may hold significant promise as a new class of molecular therapy for human gastric and pancreatic cancer, potentially by modulating cancer stem cells.

Discussion and Future Directions

miRNAs regulate a variety of biological processes, including developmental timing, signal transduction, tissue differentiation and maintenance, disease, and carcinogenesis [17, 30]. Altered expression of specific miRNA genes contributes to the initiation and progression of cancer [30]. Disruption of miRNA expression levels in tumor cells may result from distorted epigenetic regulation of miRNA expression, abnormalities in miRNA processing genes or proteins, and the location of miRNAs at cancer-associated genomic regions [31]. Clearly, miRNAs play a critical role in carcinogenesis as well as tumor progression [18, 32]. Certain abnormal miRNA expression levels cause cancer stem cell dysregulation, resulting in unlimited self-renewal and cancer progression [30, 33]. Therefore, microRNA expression is a vital key to cancer stem cell dysregulation [18]. In addition, a number of miRNAs have been identified within cancers to function as either oncogenes or tumor suppressors [34, 35]. Silber et al. reported that miR-124 and miR-137 induce differentiation of neural stem cells and glioblastoma stem cells and induce glioblastoma cell cycle arrest [36]. These results suggest that the targeted delivery of miR-124 and miR-137 to glioblastoma cells may be therapeutically efficacious for the treatment of this disease [36]. Another re-

cent study shows that miRNA Let-7 regulates self-renewal of breast cancer stem cells [33]. These miRNAs offer great promise for cancer therapy because they might have potential to regulate aberrant miRNA expression in cancer stem cells [18]. Thus miRNA therapy could be a powerful tool to correct the cancer stem cell dysregulation and its resulting excessive self-renewal and cancer progression in patients.

We have demonstrated the importance of miR-34 and its relation to the p53 tumor suppressor network and cancer stem cells [18, 28, 29]. miR-34 targets Notch, cMET, HMGA2 and Bcl-2, genes involved in the self-renewal and survival of cancer stem cells, suggesting a potential role of miR-34 in cancer stem cells [18]. Restoration of miR-34 was able to re-establish the tumor suppressing signaling pathway in cancer cells lacking functional p53. More significantly, miR-34 can inhibit cancer cell growth and tumor initiation via inhibiting the self-renewal of cancer stem cells. This indicates that miR-34 can function as a tumor suppressor gene in p53-deficient cancer cells and that its loss-of-function plays a role in the dysregulated self-renewal in cancer stem cells [29]. More importantly, miR-34 restoration led to an 87% reduction of the CD44+/CD133+ tumor initiating cells (cancer stem cells), accompanied by significant inhibition of tumorsphere growth and tumor initiation *in vivo* [29].

If our hypothesis holds true, miRNAs like miR-34 may play an important role in cancer stem cell self-renewal / differentiation processes. Delineating the role of miR-34 in regulation of cell growth and tumor progression, and its relationship with cancer stem cells, will help us better understand the p53 tumor suppressor signaling network, facilitate our research in carcinogenesis and cancer therapy, and build a solid foundation for our exploration of novel strategies in diagnosis, treatment and prevention of cancer. Modulating cancer stem cell signaling pathways to improve chemotherapeutic response is an exciting new avenue for molecularly targeted therapy. The restoration of tumor suppressor miRNAs and/or inhibition of oncogenic miRNAs provide novel prospects for clinical innovation.

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