MicroRNA Regulation of Cancer Stem Cell Phenotypes

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Abstract

A small subpopulation of cancer cells, termed Cancer Stem Cells (CSCs), are primarily responsible for initiating metastasis, resistance to therapy and ultimately patient relapse. If any improvements in long-term patient survival are to be achieved, it is vital to target this highly tumorigenic population of cells. The cancer stem-like phenotype is defined by the capability to self-renew, differentiate and proliferate. A number of signaling pathways and stem cell markers have been demonstrated to regulate these vital activities, including Wnt/ β -catenin, Hedgehog, Notch, BMI-1 and HMGA2. Intense interest is now focused on developing strategies to eliminate the CSC population. In this regard, several microRNAs (miRNAs), including miR-451, miR-34a, miR-17-92 and miR-200, have been demonstrated to regulate these fundamental CSC signaling pathways and consequently modulate the CSC phenotype. Thus, modulation of CSC-related miRNAs may represent a plausible mechanism for targeting the CSC population. The pleiotropic nature of miRNA activity can be exploited in a therapeutic manner to simultaneously inhibit multiple key regulators of the CSC phenotype and potentially achieve more efficacious targeting. This article will review our current understanding of the role of miRNAs in regulating CSC signaling pathways. In particular, this review will provide a better understanding of the role of specific miRNAs in regulating the key CSC signaling pathways and highlight several miRNAs that could be therapeutically applicable to eliminate the CSC population.

Keywords: Leukemia stem cell; MicroRNA; Self-renewal

Introduction

Metastatic dissemination of disease, the emergence of resistance to therapy and ultimately relapse of the disease state are the primary cause of treatment failure among cancer patients [1-3]. In recent years, it has become apparent that a subpopulation of tumor cells is primarily responsible for driving tumor progression and resistance to therapy in some cancers [4,5]. These cells, termed Cancer Stem Cells (CSCs), possess the unique characteristics of self-renewal, ability to differentiate and high tumorigenic potential when injected into recipient mice [6]. The persistence of CSCs is believed to be the ultimate cause of treatment failure for current cancer therapy regimens [7,8]. Thus, it is imperative to develop mechanisms to specifically target and eradicate the cancer stem cell population.

MiRNAs have been receiving intense interest as promising targeted therapeutic agents. Their activity as endogenous regulators of gene expression, their stability in body fluids and the multi-target nature of their activity indicates that miRNA-mediated therapeutics could produce efficacious results with minimal antigenicity [9]. Emerging evidence suggests that miRNAs regulate key signaling pathways that control CSC self-renewal such as Wnt/ β -catenin, Notch and Hedgehog signaling [10]. Thus, therapeutic targeting of cancer stem cell associated miRNAs could represent an attractive strategy to eliminate this highly tumorigenic and therapy-resistant subpopulation of tumor cells. In order to achieve this, it is important to increase our understanding of the mechanisms of action of specific miRNAs in modulating the CSC phenotype. This review will first provide an introduction into the two scientific areas of intense interest at present, cancer stem cells and miRNAs. Subsequently, a concise overview of the role of specific miRNAs in modulating the CSC phenotype will be discussed by concentrating on the following key CSC signaling pathways: Wnt/ β -catenin, Notch, Hedgehog, BMI-1 and HMGA2 signalling.

Cancer stem cells

Tumor cells are extremely heterogeneous with regard to their tumorigenic capacity as illustrated by the failure of the vast majority of primary tumor cells to propagate tumors when injected into immunocompromised mice [11]. It is estimated that merely 0.05-1% of tumor cells will propagate secondary tumors in this manner and therefore represent the CSC population [6]. The heterogeneity that exists between individual tumor cells arises from a combination of genetic alterations, microenvironmental influences and alterations in the physical properties of the cells [12-14]. Many questions regarding the source and consequences of tumor heterogeneity remain unanswered such as to what extent is metastasis or therapy resistance a consequence of acquired genetic alterations as opposed to being dependent on the varying physical properties of the cells. For example, it has recently been suggested that varying diffusivity of cellular components could account for disparate responses to therapy [15]. CSCs represent an important subpopulation of cancer cells in many cancer types and are characterised by unlimited replicative potential and give rise to the bulk of the tumor mass by continuous self-

renewal and differentiation. CSCs have also been implicated in accelerating metastasis and facilitating resistance to chemo- and radiotherapy [5]. It has been demonstrated that human breast cancer cells displaying a distinct CSC molecular signature possess enhanced metastatic capacity [16]. Furthermore, CSC are inherently resistant to standard therapy regimes due to their reduced proliferation rate in comparison to normal cancer cells. The majority of cancer therapeutics target rapidly proliferating cancer cells and therefore quiescent CSCs evade targeting from such therapies [17]. It has also been suggested that CSC resistance to radiotherapy is mediated by preferential activation of the DNA damage response, and an increase in DNA repair capacity [18].

CSCs were initially identified in leukemia in which the transition of events from a wild-type immature cell to an immature tumorigenic cell is a well characterized event [6]. It was observed that only a small subset of slowly dividing cells was capable of inducing transplantable acute myeloid leukemia. CSCs have subsequently been identified in a range of solid tumors where the tumor initiating events are less well characterized including breast, melanoma, colon, ovarian, pancreatic and prostate cancers [19-24]. There is evidence to suggest that CSCs can originate from either normal hematopoietic stem cells or more differentiated progenitor cells, as illustrated in Figure 1. In the case of normal hematopoietic stem cells, the transition to malignant stem cell phenotype could be easily envisioned due to the fact that these

multi-potent cells already possess activation of the required self-renewal signaling pathways. Therefore, such cells merely require maintenance of this activated phenotype in contrast to *de novo* activation in order to propagate the CSC population [25]. Indeed, the leukemia stem cell population capable of propagating acute myeloid leukemia in immunocompromised mice possess a CD34⁺CD38⁻ phenotype similar to the hematopoietic stem cell phenotype, indicating a normal stem cell origin [26]. However, it has also been demonstrated that CSCs can originate from committed progenitor cells in some subtypes of AML. Indeed, Krivtsov et al. illustrated that leukemia stem cells from leukemia initiated in committed granulocyte macrophage progenitors by introduction of the MLL-AF9 fusion protein were highly tumorigenic when transferred into secondary recipient mice yet maintained an immunophenotype and gene expression pattern analogous to that of the original granulocyte-macrophage progenitor [27]. This indicates that transformational events in differentiated progenitor cells can generate CSCs by activating self-renewal capability without inducing global gene expression reprogramming.

Several molecular markers have been identified as key regulators of pluripotency, including polycomb group RING finger protein 4 (BMI-1), transcription factor SOX-2, octamer binding protein 4 (Oct 4), high mobility group AT-hook protein 2 (HMG2) [28,29]. These molecular markers play fundamental

Figure 1: Cancer stem cell development: Cancer stem cells can originate from either normal hematopoietic stem cells or more differentiated progenitor cells. The generation of cancer stem cells from normal hematopoietic stem cells requires a transformational event in conjunction with maintenance of pre-existing self-renewal capacity. Cancer stem cells generated from differentiated progenitor cells require a transformational event in addition to the activation of self-renewal signaling pathways.

roles in regulating the activity of normal stem cells and many have also been implicated in the regulation of CSC activity. In addition, three main signaling pathways have been demonstrated to regulate the unlimited proliferative capacity which defines CSCs: Wnt/ β -catenin signaling, Notch signaling and Hedgehog signaling [30]. Therapeutic targeting of these key pathways with the aim of reverting the CSC phenotype represents a plausible mechanism for eradicating the CSC population. One possible strategy is to exploit the multi-target activity of miRNAs in an effort to simultaneously inhibit the expression of multiple components of one or more of these self-renewal pathways.

MicroRNAs

Recent advances in high-throughput sequencing techniques have unearthed an extensive landscape of small non-coding RNAs in the eukaryotic genome, the most extensively studied of which are miRNAs [31]. MicroRNAs are small, single-stranded molecules of approximately 18-25 nucleotides in length that play a fundamental role in virtually all cellular processes [32-34]. MicroRNAs form an extensive complex circuitry that mediate post-transcriptional gene silencing and represent a naturally conserved mechanism of regulation that controls approximately 60% of all protein coding genes [35]. MicroRNAs bind to complementary sequences within the 3' untranslated region (UTR) of target mRNAs and mediate mRNA degradation or translational inhibition thereby reducing protein expression [36,37]. The miRNA network is known to co-ordinate normal development and control cell processes such as proliferation, differentiation and apoptosis [38]. Thus, aberrant expression of miRNAs has been linked to many pathological conditions, including the development and progression of cancer [39].

MicroRNAs are generally transcribed by RNA polymerase II (Pol II) into primary transcripts termed pri-miRNAs [40]. The primary transcript contains a region in which the sequences are imperfectly complementary forming a structure called a stem-loop or hairpin [41]. In the nucleus, the stem-loop is recognised by the ribonuclease enzyme Drosha which mediates cleavage of the pri-miRNA at the stem of the hairpin structure, releasing a small hairpin termed the pre-miRNA. The pre-miRNA is subsequently transported to the cytoplasm and further cleaved by the ribonuclease Dicer 1 releasing a ~22 nucleotide miRNA duplex [42,43].

The miRNA duplex is then unwound and one strand of the duplex, designated the guide strand, is loaded onto an Argonaute (AGO) protein and incorporated into the RNA-induced silencing complex (RISC). Within the miRISC, the miRNA functions as the guide sequence and dictates which mRNAs to interact with, it is the protein components of the miRISC that execute the silencing of target mRNAs [44]. The miRNA biogenesis pathway is depicted in Figure 2. MiRNAs can mediate mRNA cleavage and degradation at sites of extensive complementarity or mediate direct translational repression and degradation, or a combination of both mechanisms. The ability of a miRNA to target a sequence of limited complementarity allows a single miRNA to regulate the expression of a vast number of mRNA sequences.

MicroRNA regulation of CSC signaling pathways

An extensive collection of experimental data has verified the role of miRNAs in regulating all aspects of tumorigenesis in a wide range of different cancer types [45]. MicroRNAs also regulate the fate of embryonic stem cells as demonstrated by the reduced proliferation and defective differentiation associated with Dicer null and DGCR8 null embryonic stem cells [46]. Therefore, it is not surprising that CSCs have exhibited aberrant expression of several miRNAs which dysregulate CSC self-renewal capacity. An overview of miRNA-mediated regulation of the key CSC signaling pathways is illustrated in Figure 3.

Wnt/ β -catenin signaling

The Wnt/ β -catenin signaling pathway determines cell fate during embryonic development and also functions as a key regulator of homeostasis in adult self-renewing tissues [47,48]. Wnt proteins are secreted glycoproteins which interact directly with Frizzled-LRP-receptor complexes to activate downstream signaling which results in an accumulation of β -catenin in the cytoplasm. This accumulation of β -catenin is subsequently translocated to the nucleus and activates the expression of a plethora of genes associated with self-renewal [49]. Over-expression of β -catenin has been associated with an increase in the proportion of CSCs. We have previously demonstrated the requirement for Wnt/ β -catenin signaling in the development of leukemia stem cells in acute myeloid leukemia [50]. Aberrant activation of the Wnt/ β -catenin pathway in more differentiated granulocyte-macrophage progenitor cells, in which Wnt/ β -catenin signaling is normally inactive, is not sufficient to drive leukemia, but may provide a permissive environment for malignant transformation [46,50]. Wnt/ β -catenin is not an absolute requirement to enable self-renewal of adult hematopoietic stem cells, thus, inhibiting the Wnt/ β -catenin pathway represents a therapeutic opportunity for targeting leukemia stem cells.

Wnt/ β -catenin also plays a critical role in regulating the activity of CSCs in a wide range of solid cancer types. Disruption of Wnt/ β -catenin signaling, which occurs by loss of Adenomatous polyposis coli (APC), is a key event in the development of colorectal cancer stem cells [51]. MiR-451 has been identified as a regulator of Wnt/ β -catenin signaling in colon cancer. Ectopic over-expression of miR-451 significantly reduces the self-renewal capacity, tumorigenicity and chemoresistance of colonospheres. MiR-451 directly targets macrophage Migration Inhibitory Factor (MIF) gene which regulates the expression of cytochrome c oxidase subunit II (COX-2). COX-2 facilitates β -catenin accumulation which promotes CSC growth and survival [52]. In hepatocellular carcinoma stem cells, miR-181 regulates Wnt/ β -catenin activity by directly targeting the negative Wnt/ β -catenin regulator nemo-like kinase (NLK) [53].

Notch signaling

Notch signaling plays a fundamental role in many aspects of embryonic development and the control of tissue homeostasis in adult tissues. Notch is a transmembrane receptor which binds to Delta ligands and is activated by a cascade of proteolytic cleavage

Figure 2: MicroRNA biogenesis pathway: The biogenesis of miRNAs involves the production of a primary transcript (pri-miRNA) by RNA Pol II which contains a region of imperfect complementarity. The pri-miRNA is cleaved by the ribonuclease enzyme Drosha in the nucleus. The resulting pre-miRNA is exported to the cytoplasm by exportin 5 and further cleaved by Dicer to produce the mature miRNA. The mature miRNA is unwound and one strand is subsequently incorporated into the miRNA-induced silencing complex (miRISC).

events. Newly cleaved Notch is subsequently translocated to the nucleus and activates gene expression by binding to DNA-binding proteins of the CSL family [54]. Aberrant Notch signaling has been demonstrated to promote self-renewal of CSCs. In prostate CSCs, miR-141 and miR-429 directly target and inhibit the expression of the Notch signaling ligand JAGGED1, thereby reducing the rate of cell proliferation [55]. Notably, restoration of miR-34a expression in pancreatic CSCs induces a 90% reduction in the CSC population in addition to significant inhibition of tumor formation *in vivo* [56]. The miR-34a regulates pancreatic CSC self-renewal by inhibiting various components of the Notch signaling pathway including, ZEB1 and Snail [57]. The miR-34a has further been demonstrated to target Notch signaling in glioblastoma and medulloblastoma stem cells by directly regulating the Notch-1 and Notch-2 pathway components. The miR-34a expression in glioblastoma cells significantly reduced *in vivo* xenograft growth and inhibits the tumor propagating ability of medulloblastoma cells [58,59].

Hedgehog signaling

The Hedgehog signaling pathway is a critical regulator of segmental patterning during embryonic development

and controls cell proliferation, migration and differentiation [60]. Pathway activation is initiated by binding of one of the three secreted Hedgehog ligands to its receptor Patched. This binding releases Smoothened from its repressed state which modulates the expression of three glioma associated oncogene homologue (Gli) zinc-finger transcription factors. The expression of downstream target genes is modulated by the balance between activated and repressed forms of the Gli proteins [61]. Stimulation of the Hedgehog pathway in hematopoietic stem cells is known to increase self-renewal capacity. The oncogenic miR-17-92 cluster is associated with regulation of Hedgehog signaling. Indeed, enhanced expression of miR-17-92 in medulloblastoma tumors has been demonstrated to activate Hedgehog signaling and accelerate proliferation [62]. A miRNA signature of human medulloblastomas with high levels of Hedgehog signaling has identified miR-125b, miR-326 and miR-324-5p as significantly down-regulated, furthermore, the Smoothened activator and its downstream transcription factor Gli1 were identified as direct target genes [63].

BMI-1

Activation of B cell-specific Moloney murine leukemia virus

Figure 3: MicroRNA-mediated regulation of cancer stem cell signaling networks: CSC self-renewal capacity and survival is primarily driven by Wnt/ β -catenin, Notch and Hedgehog signaling. The miR-451 functions to regulate the CSC phenotype by disrupting the cytoplasmic accumulation of β -catenin. Specifically, miR-451 directly targets the macrophage Migration Inhibitory Factor (MIF) gene which regulates the expression of cytochrome c oxidase subunit II (COX-2) which, in turn, regulates β -catenin accumulation and hence CSC self-renewal and survival. MicroR-141 and miR-429 inhibit CSC proliferative capacity by targeting the Notch signaling ligand JAGGED1. In addition, miR-34a regulates CSC self-renewal by targeting the Notch signaling components ZEB1 and Snail. Hedgehog signaling is regulated by miR-125b, miR-326 and miR-324-5p which inhibit Smoothened activation following binding of Hedgehog to its receptor Patched. This subsequently inhibits the activity of the downstream glioma associated oncogene homologue (Gli) transcription factors.

integration site 1 (BMI-1), a stem cell factor and polycomb group family member, has been associated with the enhanced chemoresistance displayed by CSCs [64]. BMI-1 regulates the proliferative capacity of normal, stem and progenitor cells. Up-regulated BMI-1 expression has been identified in a variety of cancer types and positively correlates with clinical stages and poor prognosis. Loss of BMI-1 expression results in a decrease in the neural stem cell population and proliferative capacity [65]. Altering the expression of BMI-1 in breast CSCs modulates the mammosphere-initiating cell population and size [66]. The miR-200 family are known to regulate expression of BMI-1 in addition to other stem cell factors such as SOX, KLF4 and Suz-12. The miR-220b expression has been demonstrated to inhibit the formation and maintenance of mammospheres and to prolong the period of remission in mouse xenograft models when used in conjunction with chemotherapy [67]. The miR-220c has also been verified to target BMI-1 and thereby suppress CSC growth [68].

HMGA2

Another important stem cell marker and regulator of CSC self-renewal and survival is HMGA2. HMGA2 plays a role in controlling the proliferation and differentiation of cells during embryonic

development [69]. It has been found to be over expressed in several cancer types and associated with disease progression and metastasis [70,71]. HMGA2 is a direct downstream target of the let-7 family of miRNAs. Let-7 was the first miRNA identified in the human genome and extensive research has elucidated its fundamental role in controlling developmental fate and cellular differentiation. Dysregulation of let-7 expression contributes to the development of a malignant phenotype. Yu et al. discovered that let-7 expression was dramatically reduced in the breast CSC phenotype and that expression increased with differentiation [72]. Forced expression of let-7 in breast CSCs revealed its ability of modulating self-renewal, differentiation and tumorigenic potential by regulating the expression of HMGA2.

Conclusion

The realization that a CSC population embodies the true tumorigenic potential of a cancer and is primarily responsible for metastatic dissemination, emergence of therapy resistance and ultimately patient relapse, signifies the requirement for a re-evaluation of our current approach to cancer therapy. In an effort to improve long-term patient survival it is imperative to target the

CSC population. To achieve this, an increase in the understanding of the fundamental signaling pathways driving the self-renewal and survival of these cells is critically important. As illustrated here, it would appear that the CSC phenotype, regardless of its origin or anatomical predisposition, may be driven by several universal signaling pathways and markers. Thus, the development of a universal CSC therapy may be a possibility in the near future. MicroRNAs have previously been extensively demonstrated to regulate many aspects of cancer development and progression. Accordingly, attention is now focusing on the potential importance of these small non-coding RNAs in the regulation of the vital CSC population. As discussed here, miRNAs play a fundamental role in regulating the CSC phenotype facilitating the possible use of miRNA therapeutics for the eradication of the CSC population. This review provides a concise overview of currently identified CSC-modulating miRNAs including miR-451 which targets the Wnt/ β -catenin pathway, miR-141, miR-429 and miR-34a which regulate various components of the Notch signaling pathway, in addition to the Hedgehog modulating miR-17-92 polycistronic cluster. Currently, intense effort is being employed towards the development of miRNA-mediated therapeutics with technologies such as nanoparticle delivery systems demonstrating significant potential. The development of miRNA-mediated therapeutics remains in its infancy, the true clinical benefit of which remains unknown and is eagerly anticipated. In the interim, it is essential that we continue to expand our understanding of the CSC properties and continue to identify key regulators that serve as drivers of phenotype-switching in distinct cancers.

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