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Regulation of miRNAs by agents targeting the tumor stem cell markers DCLK1, MSI1, LGR5, and BMI1

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Abstract

Gastrointestinal cancers such as colorectal, pancreatic, liver, gastric, and esophageal, are the most common forms of malignant cancers. MicroRNAs (miRNA) play important role in regulating gastrointestinal cancer progress either as potent oncogenes or tumor suppressors. In this report, we will discuss the importance of several tumor suppressors involved in colon or pancreatic cancer. Some recent studies on tumor stem cells and regulation of these miRNAs via agents targeting the tumor stem cell markers doublecortin-like kinase 1 (DCLK1), Musashi-1 (MSI1), polycomb protein BMI1, and WNT genes (LGR5 and ASCL2) will also be discussed. Agents such as siRNA/shRNA, small molecule kinase inhibitors, and general herbal drugs (curcumin) targeting these tumor stem cell markers and tumor suppressor miRNAs could be the perfect therapeutic agents for the treatment of these cancers.

Keywords

Tumor stem cell markers; DCLK1; MSI1; LGR5; BMI1; Tumor suppressor miRNAs

Introduction

Cancer is perceived as a system-level, network phenomenon [1]. Gastrointestinal cancers are one of the leading causes of cancer related deaths worldwide. Among them, colorectal

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Conflict of Interest

Sripathi M. Sureban and Dongfeng Qu declare that they have no conflict of interest. Dr. Courtney W. Houchen is a co-founder of COARE Biotechnology Inc.

Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

cancer (CRC) is the third leading cause of cancer deaths in the U.S., with a 6% 5-yr survival rate for stage IV disease [2]. Although the mortality rate continues to decline, nearly 136,000 new cases of CRC were expected to be diagnosed, resulting in nearly 51,000 deaths annually [3]. Pancreatic ductal adenocarcinoma (PDAC) is another devastating human cancer. It is the fourth leading cause of cancer-related deaths in the US and despite more than 10 years of FDA-approved therapeutic regimens and marked improvements in medical and surgical care, no significant impact on PDAC patient survival has been observed [4]. The 5-year survival rate continues to remain below 5% [5]. In 2014, an estimated 46,420 Americans were expected to be diagnosed, and ~39,590 were expected to die from the disease [6]. It has the highest mortality rate of all major cancers; 74% of patients die within the first year of diagnosis and 94% of patients die within five years [7].

A cancer stem cell (CSC) or tumor stem cell (TSC) is defined as a cell within a tumor that is able to self-renew and to produce the heterogeneous lineages of cancer cells that comprise the tumor [8]. Epithelial-mesenchymal transition (EMT) is a key feature in cancer invasion and metastasis that is linked to a TSC phenotype in CRC and is responsible for increased metastatic spread and high mortality [9, 10]. Overexpression of EMT transcription factors in CRC cells induces EMT and a CSC-like phenotype [9, 10]. TSCs are often resistant to chemotherapy and radiation therapy, which explains the modest progress in advancing therapies against cancers [11–15]. Li *et al* identified a putative CSC population in PDAC, which has a 100-fold increased tumorigenic potential compared to the rest of the population [16]. It has been demonstrated that EMT plays a key role in cancer invasion and metastasis [17, 18]. EMT-type cells in PDAC have increased expression of the stem cell markers CD24, CD44, and ESA, and increased sphere-forming capacity, thus suggesting a link between EMT and CSCs in PDAC [15, 19]. EMT in CSCs may play a critical role in tumorigenesis in general and PDAC in particular [20].

MicroRNAs (miRNAs) are a class of small non-coding single-stranded RNA molecules (18 – 25 nucleotides) that bind to target mRNA at coding or untranslated regions. Upon binding, miRNAs post-transcriptionally regulate/target mRNAs by either degrading or translationally repressing them. miRNAs can act as oncogenes or tumor suppressors and can regulate the expression of hundreds of target mRNAs simultaneously. With these properties, miRNAs can control a variety of important cellular functions including cell proliferation, self-renewal, stem cell maintenance and differentiation. Several miRNAs are shown to regulate CSCs in various cancers. Some of the examples of miRNAs are *let-7*, *miR-200*, and *miR-143/145*. This review will explore the regulatory role of these and other tumor suppressor miRNAs and agents that regulate them.

Main text

Cancer is a multistep process where a cell undergoes various genetic changes to convert from normal to metastatic cancer cell. These stages are pre-malignant, invasive, and metastatic. Cancer initiation and progression is due to dysregulation of genes involved in uncontrolled cellular proliferation, differentiation, and apoptosis. A growing body of evidence suggests that CSCs may play a decisive role in the development and progression of cancer [8, 21]. Furthermore, in this aspect miRNAs also play a crucial role.

1. miRNA biogenesis

Several investigators over the past years have demonstrated that miRNA biogenesis is a process that involves two steps cleavage events that takes place in nucleus and cytoplasm, one by ribonuclease III and the other by Drosha and Dicer [22–24]. Following this, miRNA is transcribed to primary miRNA which is further processed into precursor miRNA (pre-miRNA) and finally into a mature miRNA. These mature miRNAs finally bind to the mRNAs and regulate them.

2. The importance of miRNAs in cancer biology

miRNAs are dysregulated in majority of the cancers, either by overexpression of oncogenic miRNAs (e.g., *miR-155*, *miR-17-5p* and *miR-21*) [25, 26] or by downregulation of tumor suppressor miRNAs (e.g., *miR-34*, *miR-15a*, *miR-16-1*, *let-7*, *miR-200*, and *miR-143/145*) [27–30]. Among the tumor suppressor miRNAs, *let-7*, *miR-200*, and *miR-143/145* are involved in gastrointestinal CSCs.

The *let-7* and *miR-200* families are downregulated in various cancers and are well known to be the regulators of key differentiation programs during development. Loss of *let-7* in cancer results in progression and dedifferentiation, and the *miR-200* family has been shown to be a key regulator of EMT. Furthermore, recent studies have linked *let-7* and *miR-200* with stem cell maintenance and EMT. Moreover, EMT is tightly regulated by miRNA-dependent mechanisms [31–34]. *Let-7* family was among the first to be identified in *Caenorhabditis elegans* and demonstrated a role in cancer. *Let-7* family one of the largest families of miRNAs with twelve members (*let-7-a1*, *a2*, *a3*, *b*, *c*, *d*, *e*, *f1*, *f2*, *g*, *I* and *miR-98*) is located in different chromosomes. Further studies have demonstrated its role in regulating various genes including cMYC, CDC25A, CDK6, HRAS, NRAS, HMGA2, and IMP-1 in various cancers. *miR-200* family consists of five members (*miR-200a*, *b*, *c*, *miR-141*, and *miR-429*) within two clusters on two different chromosomes. These miRNAs target two E-box binding transcription factors ZEB1 and ZEB2 that are the key repressors of E-cadherin. These transcription factors play an important role in EMT and cancer metastasis.

miR-143/145 cluster is located on human chromosome 5q and has been reported to be downregulated in cancers. Collective data suggest that they possess tumor suppressor activity [35, 36]. Reduced *miR-143/145* expression is a common feature of many tumor types including colorectal carcinoma and PDAC [35–37]. Moreover, overexpression of these miRNAs inhibits proliferation and activates apoptosis of cancer cells [37]. The *miR-143/145* cluster has been demonstrated to inhibit *KRAS* and its downstream effector RREB1 [37]. It has been recently demonstrated that treatment with *miR-143/145* blocked the growth of PDAC xenografts [37, 38]. Studies have demonstrated increased expression of epidermal growth factor receptor (EGFR) in various cancer including colorectal and pancreatic [39, 40]. Also, EGF signaling inhibition leads to inhibition of cancer initiation and progression [41]. Studies have reported that *miR-145* inhibits cell proliferation of human lung adenocarcinoma by targeting EGFR, indicating that *miR-145* is a tumor suppressor miRNA [42]. It was also demonstrated that EGFR suppresses *miR-143/145* in colon cancer tumor xenografts [43]. These data taken together indicate that there is a negative feedback loop mechanism between EGFR and *miR-143/145* similar to *KRAS/RREB1* and *miR-143/145*.

3. Colon and pancreatic tumor stem cell markers

CD133, CD44+CD24⁻, and ALDH1 are some of the several putative colorectal and pancreatic tumor stem cell markers that have been identified. Other putative tumor stem cell markers which when targeted have shown significant anti-cancer activities. These include polycomb complex protein BMI1, Doublecortin-like kinase 1 (DCLK1), Musashi-1 (MSI1), Achaete scute-like 2 (ASCL2), and leucine-rich repeat containing G-protein coupled receptor 5 (LGR5). In this article, we will focus on the agents targeting these tumor stem cell markers.

3a. miRNAs regulated by agents targeting DCLK1 and MSI1—DCLK1 is a microtubule-associated protein with a C-terminal serine/threonine kinase domain. It is a putative stem cell marker for small intestine and pancreas [44, 45]. The Dclk1⁺ cell population demonstrates enriched expression of putative CSC markers such as CD133, CD24/CD44/ESA, and ALDH [46–48]. Introducing the DCLK1 specific siRNA (siDCLK1) into both human colon cancer and pancreatic cancer cell lines results in: 1) up-regulating *miR-200a, b, c*, and down-regulating several genes of mesenchymal profile, including ZEB1, ZEB2, SNAIL, SLUG, TWIST, angiogenic factors VEGFR1 and VEGFR2, thus suggesting that DCLK1 may regulate EMT and angiogenesis; 2) up-regulating *miR-144* and *let-7a* microRNAs, while down-regulating NOTCH1, c-MYC, and KRAS, suggesting that DCLK1 may promote tumorigenesis; 3) up-regulating *miR-143/145* microRNA, and down-regulating OCT4, SOX2, NANOG, KLF4, RREB1, suggesting that DCLK1 may also promote pluripotency [31, 49]. Introducing siDCLK1 into solid tumor xenografts originated from either human colon or pancreatic cancer cell line results in the inhibition of tumor growth [31, 49, 50]. A recent study reported that down-regulating DCLK1 using a small molecule kinase inhibitor, XMD8-92, also resulted in the up-regulation of *miR-200a, b, c*, *miR-143/145*, *miR-144*, and *let-7a* [51]. XMD8-92 treated tumors demonstrated significant downregulation of DCLK1 and its downstream targets (c-MYC, KRAS, NOTCH1, ZEB1, ZEB2, SNAIL, SLUG, OCT4, SOX2, NANOG, KLF4, LIN28, VEGFR1, and VEGFR2) [51]. Furthermore, LRRK2-IN-1 (DCLK1 inhibitor) has been demonstrated to possess significant activity against colorectal and pancreatic cancer [52]. Collective considerations of these data indicate that targeting DCLK1 regulates important miRNAs that are known to play important role in CSCs. These miRNAs inhibit various oncogenic pathways including EMT, pluripotency, angiogenesis and cell survival that is an important target for treatment of cancer.

MSI1, a RNA-binding protein and a neural stem cell marker is known to regulate and target mNumb mRNA, and other genes involved in cell cycle regulation, proliferation, and apoptosis. Recent studies indicated that MSI1 is an ISC marker and is overexpressed in colorectal and esophageal adenocarcinoma. We have demonstrated that MSI1 regulates tumor suppressor miRNAs *let-7a* and *miR-200a* in colorectal cancer cells. siRNA-mediated knockdown of MSI-1 thus resulting in increased expression of *let-7a* and *miR-200a* [53]. These data demonstrate that siRNA targeting MSI1 results in induced expression of tumor suppressor miRNAs and leading to an effective anti-cancer activity. Additional studies have demonstrated that various tumor suppressor miRNAs including *miR-34a*, *miR-101*, *miR-128*, *miR-137*, and *miR-138* have putative binding sites in the 3' untranslated region of MSI1

mRNA. These miRNAs have been shown to downregulate MSI1 resulting in decreased cancer cell proliferation [54].

3b. miRNAs regulated by agents targeting WNT signaling genes—A recent study by Tsuji et al has demonstrated the role of LGR5 and GATA6 transcription factor in the regulation of miR-363 in colorectal cancer. They demonstrated that GATA6 directly enhances the expression of LGR5 (upregulated in colorectal cancers). Furthermore, overexpression of miR-363 directly suppresses GATA6 thereby resulting in decreased expression of LGR5 and thus indicating the importance of *miR-363* in colorectal cancer suppression. Additionally, knockdown of ASCL2 a basic helix-loop-helix (bHLH) transcription factor (putative intestinal stem cell marker) by RNA interference resulted in tumor growth arrest by a *miRNA-302b*-related mechanism [55]. Following the knockdown of ASCL2, increased expression of miRNA *let-7b* was also observed. Recently, Kantara et al. reported that curcumin (active ingredient in roots of turmeric plant) downregulates both DCLK1 and LGR5 expressions in colon cancer cells resulting in colorectal cancer cells three-dimensional spheroid culture and tumor xenograft growth arrest [56]. These data indicate that the tumor suppressor miRNAs including *let-7*, *miR-200*, and *miR-143/145* may be affected following the treatment with curcumin.

3c. miRNAs regulated by agents targeting BMI1—Lineage tracing studies indicate that Polycomb protein BMI1 is an intestinal stem cell marker that labels the quiescent stem cell population [57]. It is overexpressed in different cancer types [58]. Recently it has been demonstrated that BMI1 is overexpressed and *miR-16* is repressed in tumor stem-like cells. Following the inhibition of BMI1 an increased expression of *miR-16* was observed that ultimately reduced the tumor size indicating the role of BMI1 in regulating tumor suppressor miRNAs [59]. Studies have demonstrated that *miR-200c* is down-regulated in various cancers and is known to suppress BMI1 mRNA. Recently, a study by Yin et al has shown that knockdown of BMI1 increased the sensitivity of breast cancer cells to 5-Fu; this also resulted in reduced CSC-like subpopulation. They also demonstrated a feedback loop and inverse expression pattern between BMI1 and *miR-200c*. Overexpression of *miR-200c* and *miR-203* downregulated BMI expression, and on the contrary overexpression of BMI1 inhibited the expression of *miR-200c*. This study suggests a cross talk between BMI1 and tumor suppressor miRNA. *miR-200c* induction and BMI1 inhibition results in cancer stem cell enrichment inhibition and susceptible to apoptosis [60].

Conclusion

In this review, we provide evidences to demonstrate the regulation of miRNAs by the agents targeting cancer stem cell markers: DCLK1, MSI1, LGR5, ASCL2 and BMI1. These agents include siRNAs, small molecule kinase inhibitors, and natural chemopreventive agent such as curcumin. We propose that some of the anti-cancer effects of these agents are through these tumor suppressor miRNAs.

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