ABSTRACT

Cancer stem cells (CSCs), or cancer cells with stem cell properties, represent a small fraction of tumor bulk and are thought to be responsible for tumor formation and metastasis. However, the mechanisms of how CSCs are generated and regulated at the molecular level are poorly understood. Recent progress has highlighted the significance of microRNAs (miRNAs) in cancer progression and CSC function. The function and dysfunction of miRNAs in the development of cancer and CSCs have become a burgeoning area of intense research. A new finding has elucidated a mechanism of antagonistic miRNA crosstalk whereby one miRNA can inhibit another miRNA in regulating CSCs. Herein we make this short review to summarize the current understanding of the regulatory mechanisms of miRNAs in cancer and CSCs and discuss the implications of targeting CSCs for cancer therapeutics. J. Cell. Biochem. 115: 605–610, 2014.

KEY WORDS: MicroRNA; CANCER; CANCER STEM CELLS; MicroRNA-ANTAGONISM; CANCER THERAPY

Despite the continuous progress in the development of treatment strategies, cancer still remains one of the most lethal diseases plaguing our society today [Jemal et al., 2011]. The 5-year survival rate was about 68% in cancer patients, underlining that more progress is needed to understand such a disease [Baccelli and Trumpp, 2012]. The hallmarks of cancer include unlimited replication, insensitivity to anti-growth signals, avoidance of apoptosis and metastasis [Vira et al., 2012]. Of all the features, metastasis remains as the most challenging problem of cancer therapeutics. Recent studies have indicated that during cancer progression, genetic and epigenetic mechanisms may lead to the emergence of a metastatic cancer cell with stem cell properties, which is named as cancer stem cell (CSC) or cancer-initiating cell (CIC) [Visvader and Lindeman, 2008; Ito et al., 2009]. These metastatic CSCs can detach from the primary site, eventually enter the blood and seed secondary tumors in distinct organs, and are recognized as the main cause of death in cancer patients. Therefore, further understanding of the regulatory mechanisms of CSCs at molecular level is highly necessary for better cancer therapeutics.

MicroRNAs (miRNAs) are small noncoding RNAs with 21–25 nucleotides (nt) in length. They can silence their cognate target genes by inhibiting mRNA translation or degrading the mRNA molecules by binding to their 3’-untranslated region [Bartel, 2004]. MiRNAs have also been recognized as the key regulator for stemness and metastasis of cancer cells, indicating that they may play pivotal roles in cancer diseases [Calin et al., 2005; Ma et al., 2007]. Recently, several miRNAs have been elucidated to regulate CSC functions and the differential expression profiles of miRNAs from normal tissues across cancers confirm their close relation to tumorigenesis [Lu et al., 2005]. Such regulatory functions of miRNAs in CSCs have emerged as potential therapeutic candidates for cancer disease by virtue of their ability to regulate cancer progression and metastasis. Herein we review the current understanding and recent advance of miRNAs involved in the control of CSC functions, which will broaden our understanding of the regulatory mechanisms of CSCs and may contribute significantly to the cancer therapy.

CANCER STEM CELLS AND CANCER

Over the past 10 years, the concept of CSC has emerged after the identification of CSC-enriched populations in several distinct cancer entities [Reya et al., 2001]. However, the existence of such cell
populations has remained a topic of intense debate due to questions about the robustness of CSC markers [Magee et al., 2012], till new findings identified and tracked CSCs in mouse models of brain [Chen et al., 2012], skin [Driessen et al., 2012], and intestinal tumors [Schepers et al., 2012]. These findings resolved the CSC debate and provided further supports that cancer progression is mostly driven by CSCs. In relation to the origin of CSCs, it is believed that CSCs may come from two different sources. One theory suggests that CSCs originate from transformation of normal stem cells. One alternative theory points out that CSCs were generated from normal tissue cells which acquire stem cell-like properties. For instance, non-CSC cancer cells gain CSC-like properties through EMT process [Mani et al., 2008]. A great number of CSCs have been isolated using cell surface markers like CD24, CD29, CD44, CD90, CD133, aldehyde dehydrogenase1 (ALDH1) and epithelial-specific antigen (ESA) [Al-Hajj et al., 2003; Singh et al., 2003; Ginestier et al., 2007] (Table I). And it is notable that the expression of surface markers in CSCs is cancer type-specific or subtype-specific. So far, CSCs have been identified in breast, pancreatic, prostate, colon, head and neck, ovarian and liver cancers, melanoma and brain tumors [Leal and Leonart, 2012].

Classically, CSCs are defined by the following properties: (1) high tumorigenicity, (2) the ability to differentiate into non-CSC daughter cells, (3) unlimited self-renewal abilities, and (4) remarkably resistant to conventional therapies. These findings imply that CSCs may account for cancer formation, treatment resistance, metastases, and cancer progression. Hence, complete cure of cancer cannot be achieved until and unless all CSCs are totally ablated [Li et al., 2012]. Recently, miRNAs have been reported to be frequently deregulated in distinct cancer types. They function either as oncogenes or as tumor suppressors and initiate tumor formation, metastasis, epithelial–mesenchymal transition (EMT) process and the overall “stemness” of cancer cells [Ali et al., 2012, 2013]. Therefore, targeting these miRNAs appears to be very promising therapeutic strategies.

**MICRORNA REGULATION OF CANCER AND CANCER STEM CELLS**

Enormous evidence has proven that developmental genes in somatic stem cells are regulated by miRNAs and vice versa. MiRNAs can regulate mRNA at post-translational level by binding to an 8-base seed sequence at the 3′-UTR of miRNAs. Thus, miRNAs are recognized to play critical roles during development. Downregulated miRNA expression is linked to various human diseases including cancer [Mendell and Olson, 2012]. Several profiling studies have also determined potential implications of high percentage miRNAs in cancer due to their close proximity to cancer associated genomic regions and fragile sites, chromosomal breakpoints, and dysregulated expression levels in many malignancies [Garg, 2012]. Moreover, CSCs have been proposed to be originated from normal stem cells or EMT, and such dysregulation of CSCs by acquired epigenetic abnormalities may include the aberrant expression of miRNAs. Here we discuss the major findings of very recent studies highlighting the CSC-specific miRNAs in certain cancer types.

Over the past few years, research in the area of cancer biology has shown that miRNAs may have both suppressive and promoting roles in cancer progression [Hammond, 2006]. For example, studies have shown that upregulated miR-200c expression disrupts breast CSCs-mediated colony formation in vitro and inhibits tumorigenesis in vivo. In addition, miR-34 family members can be activated by p53 and mediate cell apoptosis and cell cycle arrest [He et al., 2007], and let-7 miRNAs target oncogenes like HMGa2 and RAS [Johnson et al., 2005; Mayr et al., 2007]. Both miRNAs are suppressed in various tumor types and their reintroduction can reduce tumor growth. In contrast, miR-155 and miR-181 family members (miR-181a and miR-181b) have been evidenced as oncomiRs, which could intensely promote the self-renewal, colony formation of breast cancer cells and tumor development in breast cancer [Jiang et al., 2010]. The miR-19 was identified as the major oncogenic miRNA of the miR-17–19 cluster [Mavrikas et al., 2010]. Moreover, some pathways controlled by miRNAs have been shown to play critical roles in regulating CSCs. For example, in prostate cancer cell lines, let-7b, miR-34a, miR-141, and miR-106a were reported to be inhibited in CSCs, while miR-452 and miR-301 were observed to be highly expressed [Liu et al., 2012]. In breast CSCs, miRNA profiling observed that miR-200c, miR-203, and miR-375 expressions were significantly downregulated, while the expression of miR-125b, miR-100, miR-221, and miR-222 was notably upregulated [Wang et al., 2012]. Other miRNAs that have been shown to be involved in various cancer types are roughly summarized in Table II.

Recently, a very interesting research conducted by Song et al. have observed that during the regulation of CSCs, one miRNA can function

**TABLE I. CSC Markers for Different Solid Cancer Types**

<table>
<thead>
<tr>
<th>Cancer</th>
<th>CSC markers</th>
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<tbody>
<tr>
<td>Breast</td>
<td>ALDH1, CD24, CD44, CD90, CD133, α6-integrin, Hedgehog-Gli activity</td>
</tr>
<tr>
<td>Liver</td>
<td>CD13, CD24, CD44, CD90, CD133</td>
</tr>
<tr>
<td>Lung</td>
<td>ABCG2, ALDH1, CD90, CD117, CD133</td>
</tr>
<tr>
<td>Colon</td>
<td>ABCGS, ALDH1, CD24, CD26, CD29, CD44, CD133, CD166, β-catenin activity, LGR5</td>
</tr>
<tr>
<td>Glioma</td>
<td>CD15, CD90, CD133, αc-integrin, Nestin</td>
</tr>
<tr>
<td>Melanoma</td>
<td>ABCGS, ALDH1, CD20, CD133, CD271</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>ABCG2, ALDH1, CD24, CD44, CD133, e-Met, CXCR4, Nestin, Nodal-Activin</td>
</tr>
<tr>
<td>Prostate</td>
<td>ALDH1, CD44, CD133, CD166, αβ1-integrin, αc-integrin, Trop2</td>
</tr>
<tr>
<td>Ovarian</td>
<td>CD24, CD44, CD117, CD133</td>
</tr>
</tbody>
</table>

Markers are not ordered according to their importance, and this is not an exhaustive list of all identified markers.
at another miRNA. They have demonstrated that miR-22 acts as a crucial epigenetic modifier and promoter of EMT and breast cancer stemness toward metastasis by silencing anti-metastatic miR-200 through direct targeting of the ten eleven translocation (TET) family of methylcytosine dioxygenases [Song et al., 2013a] (Fig. 1). Almost by the same mechanism of TET protein, Song et al. has also identified miR-22 as a potent proto-oncogene in hematopoietic malignancies [Song et al., 2013b]. These miRNAs might provide novel targets for efficient and specific therapies for cancer and CSCs.

In short, miRNAs have been recognized as critical regulators of cancer and CSCs. Understanding the functional role of miRNAs in a specific type of cancer, miRNA-based therapies can be targeted to these CSCs in order to correct their expression levels and restoring the tumor suppressor functions via the RNAi methods.

### THERAPEUTIC IMPLICATIONS AND FUTURE PERSPECTIVES

As discussed above, dysregulation of miRNAs has been evidenced to be implicated in cancer progression, and miRNAs may modulate tumor formation and metastasis by regulating CSC functions. For example, let-7 control cell-cycle and differentiation of breast CSCs; miR-34a can restrict the migration and invasiveness of prostate CSCs by directly targeting CD44; and miR-200c may regulate the self-renewal of breast CSCs by modulating BMI1 (Fig. 1). Moreover, recent findings also suggest that miR-22 may exert its metastatic potential by targeting miR-200, thereby inhibiting demethylation of miR-200 promoter. These new findings better our knowledge and understanding of CSC function and provide novel insight into more effective therapeutic strategies to target cancer and CSCs. Given that miRNAs exert broad regulatory functions on cancer progression and development, miRNA-based therapeutics may open up new areas in the anti-cancer arena.

However, in spite of tremendous progress in this field, we still have a long way to go before a fully understanding of such regulatory mechanism is achieved. The phenotypic consequences of manipulating miRNAs in vivo are hard to predict, and most miRNAs mentioned above were discovered and studied in C. elegans with little knowledge about their regulation in human development and physiology. Hopefully, these problems mentioned above could be solved as we get better insights into the miRNA regulation of cancer and CSCs. Moreover, different miRNAs seem to concertedly and distinctively modulate functional properties of CSCs, complete eradication of CSCs may entail targeting of multiple miRNAs. Therefore, miRNA expression profiling in CSCs or certain types of cancer at various clinical stages might have great diagnostic and prognostic values.

Overall, research into CSC will provide innovative approaches to the therapeutics of cancer diseases, and miRNA-based therapeutic strategies hold great promise in this area. Combining the potential of CSCs and the advantages provided by miRNAs, a novel therapeutic approach could fill the gap in the treatment of cancer patients.
REFERENCES


