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The Role of MicroRNAs in Regulating Cancer Stem Cells

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1. Introduction

Stem cells are a rare population of cells that have the ability to self-renew (to replenish the stem cell pool) and to differentiate (to produce daughter cells that will perform the physiological functions of tissues and organs). Although stem cells exist in different tissues, organs, and developmental stages. However, stem cells differ to some degree with regard to their developmental potency; life span, and notably their potential for self-renewal and proliferation capacity.

Stem cell self-renewal and differentiation is regulated by signaling pathways, transcription factors, and micro RNAs (miRNAs). Some key transcription factors directly regulate the expression of miRNAs in stem cells. Meanwhile, miRNAs target key transcription factors and either repress or induce their expression in stem cells to regulate self-renewal and differentiation. Thereby, the miRNA regulatory network and the signaling pathways cross-talk to each other to orchestrate stem cell maintenance and cell fate decision. Dysregulation of core signaling pathways, transcription factors and miRNAs associated with normal stem cells can lead to carcinogenesis. Thus, understanding the regulation of normal stem cell is crucial for understanding the molecular mechanisms underline carcinogenesis.

In this chapter, we review the characteristics and functions of miRNAs and cancer stem cells (CSCs), focusing on the roles of miRNAs in regulating CSCs. First, we provide an introduction to stem cells and CSCs. Then, we describe the signaling pathways that regulate stem cell self-renewal and differentiation. In particular, we review the Wnt/β-catenin, Hedgehog (HH), and Notch pathways. Next, we discuss the epithelial–mesenchymal transition (EMT), CSCs, and miRNAs that play roles in regulating stem cells. Finally, we summarize the current status and discuss future perspectives.

2. Stem cells

Depending on their differentiation potentials, human stem cells can be classified into totipotent, pluripotent, and multipotent (http://stemcells.nih.gov/info/scireport). **Totipotent** cells have the potential to form any of the differentiated cells in a living organism from a single cell. Thus, these cells have ability to form extraembryonic membranes and tissues; the embryo itself, and all postembryonic tissues and organs. At the

very early stage of embryo development, each cell in the blastomere is totipotent. **Pluripotent** cells can differentiate to form tissues of any of the three germ layers: ectoderm, endoderm, or mesoderm. However, a pluripotent cell cannot form an entire living organism. Embryonic stem cells (ESCs) are pluripotent stem cells derived from the inner cell mass of the human blastocyst. ESCs can differentiate into specialized cells, and have an unlimited capacity for self-renewal. **Multipotent** cells—adult stem cells—have a differentiation ability that is limited to a specific tissue- or organ. Tissue-specific adult stem cells are responsible for organogenesis; tissue maturation, repair and regeneration, and maintenance; and balancing the cellular turnover. To fulfill these responsibilities; first, an adult stem cell is an undifferentiated cell that is found in a differentiated tissue and has the capacity to become specialized to yield all of the cell types of the tissue from which it originated; second, a stem cell has capacity to self-renewal (Spradling et al., 2001). They can undergo two kinds of cell division: symmetric and asymmetric. In symmetric division, a stem cell divides into two identical daughter cells, which are both identical to the originating stem cell. This type of division is crucial for expanding the stem cell pool, most likely in very early embryonic development. In contrast, in asymmetric division, a stem cell divides into one daughter progenitor cell (also known as a precursor cell), which eventually differentiates into a mature cell, and one new stem cell, which is identical to the originating stem cell. This process maintains stem cell number, and this feature also distinguishes the stem cell self-renewal from other proliferative processes. Normal adult stem cell can divide asymmetrically to maintain the population of stem cells and differentiated cells. The processes that regulate the balance between asymmetric and symmetric division of stem cells are unclear.

A progenitor cell is a partially specialized cell that can divide and yield two specialized cells. Progenitor cells can be distinguished from adult stem cells as follows. When a stem cell divides, at least one of the two new cells is always identical to the originating stem cell and can replicate itself. In contrast, when a progenitor cell divides, it gives rise to two progenitor cells or two specialized cells, neither of which can replicate itself. Progenitor cells can replace cells that are damaged or dead, thereby maintaining the integrity and functions of a tissue or an organ such as the liver or the brain. Examples of stem and progenitor cells:

- Hematopoietic stem cells (adult stem cells) from the bone marrow that give rise to erythrocytes, lymphocytes, plateles, monocytes, and granulocytes.
- Mesenchymal stem cells (MSCs) are a subset of nonhematopoietic multipotent stem cells (adult stem cells) that are found primarily within the bone marrow and give rise to stromal cells; within the adipose tissue that give rise to adipocytes (Bieback et al., 2008; Digirolamo et al., 1999). MSCs have also been isolated from the umblical cord (fetal stem cells). Mesenchymal stem cells can self-renew and are defined as cells that differentiate into a variety of mesenchyme-derived cell types: fibroblasts, chondrocytes, osteoblasts, myoblasts, and neural stem cells; the latter cells have the potential to differentiate into neurons, astrocytes, and oligodendrocytes (Barry and Murphy, 2004; Halleux et al., 2001).
- Epithelial stem cells (progenitor cells) that give rise to the various types of skin cells.
- Muscle stem cells that give rise to differentiated muscle tissue.
- Intestinal stem cells.

On the other hand, accumulating data show that different stem cells have distinct potential to proliferate, and some adult stem cells from one tissue are capable of differentiating into the specialized cell types of another tissue (Herzog et al., 2003; Krause, 2002a). This phenomenon

is referred to as stem cell plasticity. For example, under specific experimental conditions, adult stem cells from bone marrow can differentiate into cells that resemble neurons (Herzog et al., 2003; Krause, 2002a). Growing evidence indicates that, given the right environment (environmental niche), some adult stem cells are capable of being genetically reprogrammed to differentiate into tissues other than the ones from which they originated.

Regardless of division type, stem cell self-renewal is especially important in tissues with high self-renewal capacity, such as the intestinal cells and bone marrow, and also in tissue repair after injury. Adult tissues that undergo turnover throughout life are maintained via a very small portion of cells—adult stem cells that live through the entire life span of an organism. These stem cells can maintain homeostasis even in mitotically inactive adult tissues, such as the brain (Bartlett, 1982; Ricci-Vitiani et al., 2008). Even though stem cells have an extensive capacity for self-renewal, in fact they remain quiescent most of the time and may undergo a limited number of self-renewing divisions in adult life (Cheshier et al., 1999). This may be because, despite their proliferative capacity, stem cells often arrest at a G_0-like cell cycle phase or checkpoint (Cheshier et al., 1999). In addition, the differentiation and self-renewal rates differ depending on the stem cell type (Ahn and Joyner, 2005; Hu et al., 2004).

Adult stem cells are not easy to characterize. To date, adult stem cells have been characterize *in vitro* by using their differentiations patterns and cell surface markers. Stem cells have been identified in bone marrow, blood, the cornea and retina, the brain, skeletal muscle, dental pulp, liver, skin, the intestinal tract, pancreas, ovary, breast, lung, prostate and head and neck (http://stemcells.nih.gov/info/scireport). Thus, stem cells have been found in tissues that develop from all three embryonic germ layers.

3. Signaling pathways in stem cells

In both pluripotent and multipotent cells, self-renewal and cell fate decision are regulated by a complex set of factors and pathways. Each process: self-renewal and differentiation requires unique molecular programs specific to each pluripotent or multipotent cell. For example, in ESCs, self-renewal requires that the unique molecular program of the pluripotent state be maintained, whereas to differentiate into various lineages, ESCs must shift to alternative molecular programs that inhibit self-renewal and promote differentiation (Marson et al., 2008a). Understanding how cells switch between self-renewal and differentiation, and discovering which factors or signaling pathways control which daughter cell of an adult stem cell remains a stem cell and which undergoes differentiation, is crucial to understand the mechanism of tumorigenesis.

Several "stemness" factors are required to ensure appropriate ESC behavior (pluripotency). A core network of factors, including transcription factors and RNA binding proteins (Oct4, Sox2, Nanog, Klf4, c-Myc, Tcf3, and Lin28), is involved in the circuits that regulate ESC pluripotency. (Marson et al., 2008a). Some of these regulatory factors are tissue or cancer specific; for example, Oct4 is expressed only in the inner cell mass of the embryo and not in the trophectoderm. Some of these key regulators of ESC identity, such as Oct4, Sox2, and Nanog are expressed only in specific human cancer types (Gidekel et al., 2003; Rodriguez-Pinilla et al., 2007; Santagata et al., 2007). Thus, regulatory networks can determine classes of stem cells, such as ESCs, neural stem cells, or breast stem cells or other tissue specific stem cells (Muller et al., 2008). Sox2 and Nanog can also reprogram differentiated human cells into ESC-like induced pluripotent stem cells (Park et al., 2008; Wernig et al., 2007).

Pluripotency and the unlimited potential for self-renewal are the characteristics that distinguish ESCs from adult (tissue-specific) stem cells, which have more limited self-renewal and tissue-specific differentiation potential. The common feature of stem cells (ESCs and adult stem cells) is self-renewal. Not surprisingly, the stem cell niche and signaling pathways such as Wnt/β-catenin, Notch, Hedgehog TGF-β, and Bmi-1 are involved in the regulation of normal self-renewal programs, the balance between self-renewal and differentiation (Dontu et al., 2004; Reya and Clevers, 2005; Schofield, 1978; Song et al., 2007; Taipale et al., 2002). Accumulating evidence indicates that networks that balance proto-oncogenes (promoting self-renewal) and tumor suppressors, which act as gatekeepers (limiting self-renewal) and caretakers (maintaining self-renewal) is also involved in tissue stem cell self-renewal programs (He et al., 2009). For example, the p53, PTEN, and INK4A pathways are involved in stem cell self-renewal (Armesilla-Diaz et al., 2009b; Cicalese et al., 2009; Lowe and Sherr, 2003; Nagao et al., 2008; Zheng et al., 2008a) (Table 1). Therefore, it is not surprising that these transcription factors (*PTEN*, *TP53* and *INK4A*) are deleted or mutated in multiple CSCs.

Signaling pathway	Type of stem or progenitor cell	References
Wnt/ β-catenin	Hematopoietic stem cells Epidermal stem cells Gastrointestinal stem cells Neural stem cells Embryonic stem cells Dental pulp stem cells	(Luis et al., 2009; Reya et al., 2003) (Zhu and Watt, 1999) (Brittan and Wright, 2002; He et al., 2004) (Kalani et al., 2008) (Melchior et al., 2008; Tam et al., 2008) (Scheller et al., 2008)
Notch	Gastrointestinal progenitor cells Mammary stem/progenitor cells Liver stem cells Muscle progenitor cells Hematopoietic stem cells	(Fre et al., 2005) (Bouras et al., 2008; Dontu et al., 2004) (Zong et al., 2009) (Buas and Kadesch, 2010; Conboy et al., 2003; Conboy and Rando, 2002) (Varnum-Finney et al., 2000)
Hedgehog	Hematopoietic stem cells Neural stem cells Mammary stem cells	(Bhardwaj et al., 2001) (Palma et al., 2005; Wechsler-Reya and Scott, 1999) (Liu et al., 2006)
Bmi-1	Mammary stem cells Hematopoietic stem cells	(Liu et al., 2006) (Park et al., 2003)
PTEN	Neural stem cells	(Groszer et al., 2006; Groszer et al., 2001; Nagao et al., 2008; Zheng et al., 2008a)
p53	Mammary stem cells Neural stem cells	(Cicalese et al., 2009) (Armesilla-Diaz et al., 2009a; Zheng et al., 2008a)

Table 1. Signaling pathways involved in stem cell self-renewal.

Moreover, signaling pathways cross-talk or interact with each other to regulate stem cell behavior. For example, hypoxia-inducible factor-1α and Notch signaling interact to regulate medulloblastoma precursor cell proliferation and differentiation (Pistollato et al., 2010). The Notch and EGFR pathways interact with each other to regulate the number of neural stem cells (NSCs) (Aguirre et al., 2010). Key pathways including Wnt, HH, Notch, and Bmi-1 and transcription factors including *TP53* and *PTEN* are involved in the development of various organs during embryogenesis and in the regulation of self-renewal and differentiation in both normal adult stem cells (Molofsky et al., 2004), and CSCs such in normal adult SCs and CSCs in glioblastoma (Zheng et al., 2008b). Dysregulation of these core pathways (e.g. Wnt, HH, Notch) and transcription factors (*TP53* and *PTEN*), which associated with normal stem cells is also plays a role in the cancer development (Zheng et al., 2008b). Here, we focus on the Wnt/β-catenin, HH, and Notch pathways.

3.1 Wnt/β-catenin pathway

Two kinds of Wnt signaling pathways exist: the canonical Wnt pathway, in which Wnt ligands signal through the stabilization of β-catenin, and the noncanonical Wnt pathway, which is β-catenin-independent. The canonical Wnt pathway is activated when Wnt ligands bind to cell surface receptors composed of a member of the Frizzled protein family and one of the co-receptors LRP5 and LRP6 and hyperphosphorylate the Dishevelled (Dsh) protein, thereby activating it. Activation of Dsh prevents the phosphorylation of β-catenin and inhibits the formation of β-catenin destruction complex (glycogen synthase kinase 3β [GSK-3β], adenomatous polyposis coli [APC], casein kinase 1α [CK1α] and Axin) which leads to the stabilization of hypophosphorylated β-catenin and, thereby, to its translocation to the nucleus where it interacts with transcription factors (T cell factor/lymphoid enhancer factor [TCF/LEF]). Thus the β-catenin/TCF/LEF complex activates the transcription of target genes. In the absence of Wnt ligands, β-catenin destruction complex hyperphosporylates β-catenin, thereby this complex (hyperphosphorylated β-catenin, APC, Axin, GSK-3β, and CK1α) is thus a target for ubiquitination and degradation by the proteasome (Schweizer and Varmus, 2003).

The noncanonical Wnt signaling pathway requires Frizzled receptors and the proteoglycan co-receptor Knypek. In this pathway, Dsh localizes in the cell membrane and activates Rho through Daam1. Dsh induce cellular response by stimulating calcium flux and activating the calcium-sensitive kinases protein kinase C and calmodulin-dependent protein kinase II (Veeman et al., 2003).

Wnt signaling has been studied intensively in embryonic development. The response of cells to the Wnt pathway is tissue-dependent. Wnt signaling is involved in many key developmental processes, such as cell survival, proliferation, inhibition of apoptosis, stem cell maintenance, differentiation, and cell fate decision, and in the development of a variety of organ systems, including the cardiovascular system, central nervous system, kidney, and lung (Ille and Sommer, 2005; Peifer, 2000; Vainio et al., 1999a; Vainio et al., 1999b; Wodarz and Nusse, 1998). For example, the canonical Wnt pathway plays a crucial role in the development of intestinal tissue by regulating the self-renewal, migration and proliferation of intestinal stem and progenitor cells, and tissue self-renewal in hair follicles and bone growth plates (Clevers, 2006). The Wnt pathway also interacts with other pathways to regulate stem cell processes. For example, bone morphogenetic protein (BMP) inhibits Wnt signaling to negatively regulate stem cell proliferation (He et al., 2004), BMP signaling thereby represses de novo crypt formation and polyp growth, and mutations in BMP

pathway genes lead to formation of crypts and generation of benign polyps (Reya and Clevers, 2005).

In addition to biologic and developmental process, Wnt signaling is also involved in genetic processes. For example, APC has been shown to be involved in regulating mitotic spindle assembly, orientation of chromosomes during mitotic division, and chromosome segregation (Kaplan et al., 2001). Abnormalities in the orientation of chromosomes during mitotic division may contribute to numeric chromosomal aberrations in cancer cells (Peifer, 2000).

3.2 Hedgehog pathway

Three HH ligands have been identified—Sonic HH, Desert HH, and Indian HH (Cohen, 2003). In the presence of ligands, these ligands bind to the transmembrane receptor Patched 1 (Ptch 1), which inhibits Smothened (Smo). The binding of HH ligands relieves Smo inhibition, leading to activation of the Gli transcription factors Gli1 and Gli2 (activator) and Gli3 (suppressor). Activated Gli accumulates in the nucleus and controls the transcription of HH target genes. In the absence of HH ligands, Ptch1 inhibits Smo, and cannot activate Gli (Pasca di Magliano and Hebrok, 2003). The HH signaling pathway regulates cell proliferation through Cyclin D1 and FoxM1, apoptosis through Bcl-2, EMT through Snail and E-cadherin, and self-renewal through Bmi-1 (Kasper et al., 2009). Bmi-1 has been shown to be a key regulator of the self-renewal of NSCs and both normal and leukemic stem cells (Lessard and Sauvageau, 2003; Molofsky et al., 2003; Park et al., 2003). HH signaling has been shown to play a critical role in the development of many systems, including the limb, brain, spinal cord, thalamus, and teeth. The HH pathway is also important in cell proliferation, differentiation, and stem cell maintenance during embryogenesis (Ma et al., 2002), and in the self-renewal and maintenance of NSCs (Ahn and Joyner, 2005; Lai et al., 2003; Palma et al., 2005), mammary stem cells (Liu et al., 2006).

Either aberration of genes in the HH signaling pathway or aberrant activation of HH signaling results in tumorigenesis. For example, germline mutations in Patch, which functions as a tumor suppressor has been found in basal cell carcinomas and Gorlin syndrome (Cohen, 2003). Activation of HH signaling is implicated in small cell lung cancer, digestive tract tumor, pancreatic carcinoma, breast cancer and prostate cancer (Karhadkar et al., 2004; Olsen et al., 2004).

3.3 Notch pathway

Members of the Notch gene family encode transmembrane receptors that are crucial for cell fate decision. Four Notch receptors (Notch1, Notch2, Notch3 and Notch4) and five ligands (Jagged-1 [JAG1] and JAG2, three Delta-like [DLL1, DLL2, and DLL4]) have been found. These receptors and ligands are expressed in different combinations in most cell types (Mumm and Kopan, 2000). After ligand binding, Notch receptors are activated via cleavages of ADAM metallopeptidase domain 17 (ADAM17) and presenilin-1, which result in the release and translocation of the Notch intracellular domain (NICD) to the nucleus and the activation of HES (Hes/E(spl) family) and HEY (Hesr/Hey family) families through interaction of NICD with sequence-binding protein (Mumm and Kopan, 2000). Notch signaling is crucial for arterial-venous differentiation, for self-renewal and differentiation in hematopoietic stem cells (Krause, 2002b), maintenance of the mammary stem cell population (Bouras et al., 2008), for adult neurogenesis (Androutsellis-Theotokis et al., 2006), and for the activity of myogenic muscle stem and progenitor cells (Buas and Kadesch, 2010; Conboy et

al., 2003; Conboy and Rando, 2002). Notch signaling is involved in the self-renewal process mostly in rapidly renewing tissues, such as the hematopoietic system (Mercher et al., 2008; Wu et al., 2007), the intestine, skin, highly proliferative ESCs, and the intestine, in which the epithelium is renewed every 4-5 days (Dontu et al., 2004). Notch and Wnt signaling cooperate to regulate self- renewal and cell fate in the adult intestine (Chiba, 2006; Fre et al., 2005; Wang and Hou, 2010), and inhibition of Notch/γ–secretase induces proliferation in intestinal crypt cells and the formation of polyps (van Es et al., 2005). Adult epidermal stem cells reside in the epidermal basal layer and in the bulge region of the hair follicle (Ambler and Maatta, 2009). In addition to differentiation and self-renewal, the Notch pathway is also involved in other developmental processes, including EMT, proliferation, apoptosis, and cell adhesion during embryogenesis (Zong et al., 2009).

EMT was originally defined as a cellular reorganization process that is essential for embryonic development. EMT results in a loss of cell to cell adhesive properties, a loss of cell polarity, and a gain of the invasive and migratory features of mesenchymal cells (Thiery et al., 2009). During embryogenesis, EMT leads progenitor/precursor cells to migrate to distant sites within the embryo to form new tissues (Shook and Keller, 2003). The EMT process is reversible. EMT also occurs during tumorigenesis; the process is similar to EMT during the embryogenesis, but instead of forming new tissue, it allows some CSCs to become metastatic while keeping the features of the original tissue. It is not surprising that, the same, or similar, core signaling pathways (Wnt, HH and Notch) that regulate stem cell self-renewal are also involve regulation of EMT together as are niche factors (Mani et al., 2008; Vincan and Barker, 2008; Yang and Weinberg, 2008).

The dysregulation of signaling pathways by mutations and/or by genomic and epigenetic aberrations, which are involved in the regulation of stem cell function as well as in EMTs during embryonic development may play a crucial role in the development of cancer. CSCs and normal stem cells use many of the same signaling pathways, such as Wnt, HH, and Notch, but the difference is CSC use dysregulated way of these signaling pathways (Takebe et al., 2010). Upto now, aberrant Notch signaling has been shown in multiple human cancers including hepatocellular carcinoma, hepatoblastoma, colorectal cancer, acute myeloid leukemia, chronic myeloid leukemia, multiple myeloma, gastric cancer, and Wilms' tumor which also shows dysregulation of Wnt signaling (de La Coste et al., 1998; Kim et al., 2009; Koesters et al., 1999; Martin et al., 2010 ; Reya and Clevers, 2005) (Table 2).

4. Cancer stem cells

Normal stem cells and CSCs share several important properties, including the ability to self-renew. The signaling pathways and transcription factors that are involved in the self-renewal of normal stem cells have all been implicated in the development of cancers, but in CSCs the pathways are dysregulated and the factors are aberrantly expressed. CSCs can be distinguished from normal stem cells by the following.

1. CSCs have the capacity for self-renewal like normal stem cells, but CSCs have a different self-renewal rate from normal stem cells.
2. CSCs have the capacity to differentiate into cells of the specific tissue, but aberrantly (Singh et al., 2003).
3. CSCs have the ability to develop tumor when transplanted into the proper environment.
4. CSCs have the capacity for tumor metastasis.

5. CSCs have the abilty to repopulate the tumor, causing relapse, and can become resistant to different therapeutic agents.
6. CSCs are identified by characteristic cell surface markers.

Aberrant activation of an individual signaling pathway or cross-talk between pathways may result in tissue-specific carcinogenesis (Sun et al., 2010). Thus, an understanding of the pathways that govern the self-renewal and cell fate decisions of normal stem cells, and how these pathways are dysregulated and which of them are dysregulated during carcinogenesis, is of utmost importance. In many cases, self-renewal regulators have surprisingly similar functions in CSCs and normal stem cells (Tables 1 and 2). For example,

Signaling pathway	**Type of cancer**	**References**
Wnt/ β-catenin	Liver	(Ma et al., 2007)
	Breast	(Korkaya et al., 2009)
	Chronic myeloid leukemia	(Zhao et al., 2007)
	Acute myeloid leukemia	(Wang et al., 2010)
	Colon	(Polakis, 2000; Vermeulen et al.)
	Prostate	(Bisson and Prowse, 2009; Shahi et al., 2011)
	Intestine	(Fre et al., 2009)
	Skin	(Chan et al., 1999)
Notch	Liver	(Ma et al., 2007)
	Colon	(Sikandar et al., 2010)
	Breast	(Bouras et al., 2008; Dontu et al., 2004)
	Intestine	(Fre et al., 2009)
	Prostate	(Shahi et al., 2011)
	T-cell leukemia	(Aster et al., 2010)
Hedgehog	Liver	(Ma et al., 2007)
	Breast	(Liu et al., 2006)
	Pancreatic	(Li et al., 2007)
	Glioblastoma	(Ingham, 2008)
	Chronic myeloid leukemia	(Dierks et al., 2008; Zhao et al., 2009)
	Colon	(Varnat et al., 2009)
	Multiple myeloma	(Peacock et al., 2007)
	Medulloblastoma	(Berman et al., 2002)
	Basal cell carcinoma	(Gailani and Bale, 1999)
Bmi-1	Breast	(Liu et al., 2006)
	Head and neck squamous cell cancer	(Prince et al., 2007)
	Acute myeloid leukemia	(Lessard and Sauvageau, 2003)
PTEN	Breast	(Korkaya et al., 2009)
	Glioblastoma	(Zheng et al., 2008b)

Table 2. Signaling pathways that are involved in stem cell self-renewal and are dysregulated in cancer stem cells

the proto-oncogene Bmi-1 is required to maintain both the proliferative potential of leukemic stem cells (Lessard and Sauvageau, 2003) and the self-renewal potential of normal hematopoietic stem cells, mammary stem cells, and NSCs (Liu et al., 2006; Molofsky et al., 2003; Park et al., 2003). Similarly, *PTEN* and *TP53* are required for differentiation and to maintain self-renewal not only in normal NSCs but also in neoplastic stem cells of glioblastoma (Zheng et al., 2008a; Zheng et al., 2008b). Notch signaling is also required to maintain self-renewal in normal and glioma stem cells (Hu et al., 2011), and HH signaling is required not only for normal NSC maintenance but also for brain tumor cell proliferation (Balordi and Fishell, 2007).

Whereas some key transcription factors share some of their target genes and participate in autologous feedback loops to control one another's transcription, others directly regulate self-renewal. On the other hand, in addition to key transcription factors and RNA-binding proteins that regulate self-renewal, miRNAs are also involved in this complex regulatory network.

5. miRNAs

Small noncoding RNAs, which include miRNAs, are a new class of gene that do not code mRNA or protein but are post-transcriptional regulators of gene expression. This regulation generally occurs by binding of a small (~22-nucleotide-long) mature miRNA to mRNA via direct canonical base-pairing between nucleotides 2–8 at the 5′ end of the miRNA (the seed region) and the 3′ untranslated region (UTR) of the target mRNA (its complementary seed-match sequence). Mature single-stranded miRNA is unwound by the helicase activity of Dicer and the RNA-induced silencing complex, resulting in the inhibition of translation, destabilization, and localization of target mRNA. miRNAs are not only post-transcriptional regulators of target genes but also play roles in establishing epigenetic programs (Filipowicz et al., 2008; Stefani and Slack, 2008). miRNAs are not translated into protein, rather, their function is to regulate gene expression by binding to other RNAs, particularly mRNA (Bartel, 2004) (Table 3).

The first miRNAs were discovered in *Caenorhabditis elegans* when mutations in *lin-4* (Lee et al., 1993) and *let-7* (Reinhart et al., 2000) were found to result in defective stem cell maturation (Bartel, 2004). Since then, the miRNA field has been explored extensively and miRNAs have been found to be key regulators of many gene expression networks. In humans, thousands of miRNAs regulate thousands of mRNAs, and each miRNA targets and regulates hundreds of mRNAs to either induce their degradation or prevent their translation. Accumulating data have shown that miRNAs are involved in almost every biological process, and therefore dysregulation of miRNAs is involved in many human diseases, most notably cancer (Esquela-Kerscher and Slack, 2006; Yu et al., 2007) (Table 4).

miRNAs play crucial roles as regulators of stem cell function, differentiation, and embryonic development (Filipowicz et al., 2008; Stefani and Slack, 2008), as well as act as oncogenes and tumor suppressor genes (Garzon et al., 2006). Recent discoveries have revealed that a complex regulatory network of miRNAs, transcription factors, and signaling pathways orchestrate cell-renewal and differentiation (Ferretti et al., 2008; Kato et al., 2009; Kennell et al., 2008; Marson et al., 2008b). The switch from pluripotent to lineage-specific cells is characterized by suppression of pluripotency by activation of expression of lineage-specific genes and repression of self-renewal genes in ESCs, and miRNAs are involved in the regulation of genetic programs. For example, miR-145 promotes the switch from the

Target	Effect (positive or negative)	miRNA	Biological process	References
I. Wnt signaling				
β-catenin	–	*miR-200a*	Meningioma	(Saydam et al., 2009)
APC	–	*miR-135a, miR-135b*	Colorectal cancer	(Nagel et al., 2008)
Wnt1		*miR-34a, miR-21*	Dendritic cell differentiation	(Hashimi et al., 2009)
II. Hedgehog signaling				
Smoothened (Smo)	+	*miR-324-5p* *miR-125b* *miR-326*	Neural stem cell proliferation, medulloblastoma	(Ferretti et al., 2008)
Gli1	+ & –	*miR-324-5p*	Neural stem cell proliferation, medulloblastoma	(Ferretti et al., 2008)
Dkk1 Kremen2 SFRP2	+	*miR-29*	Osteoblast differentiation	(Kapinas et al., 2010)
III. Receptor tyrosine kinase signaling				
NRAS, KRAS	–	*let-7*	Cancer stem cell differentiation, tumor formation	(Johnson et al., 2005)
IV. Notch signaling				
HES1	–	*miR-159b-5p*	Medulloblastoma	(Garzia et al., 2009)
JAG1	–	*miR-34a, miR-21*	Dendritic cell differentiation	(Hashimi et al., 2009)
JAG1	–	*miR-200*	Pancreatic adenocarcinoma Basal-type breast cancer	(Brabletz et al., 2011)
Notch1 JAG1	–	*miR-34a*	Cervical carcinoma	(Pang et al., 2010)
LATS	+	*miR-372, miR-373*	Testicular germ cell tumor	(Voorhoeve et al., 2006)
V. p53 signaling				
TP53	+	*miR-125b*	Apoptosis in the brain	(Le et al., 2009)

Target	Effect (positive or negative)	miRNA	Biological process	References
VI. PTEN signaling				
PTEN Bim Prkaa1 PP2A	–	*miR-19*	T-cell acute lymphoblastic leukemia	(Mavrakis et al., 2010)
PTEN	–	*miR-21*	Hepatocellular cancer	(Meng et al., 2007)

Table 3. MicroRNAs that regulate signaling pathways that determine properties of cancer stem cells

pluripotent state to lineage-specific differentiation by supressing pluripotency factors (e.g., Klf4, Sox2, and Oct4) (Xu et al., 2009). Similarly, the switch from multipotent to lineage specific cells is marked by inhibition of self-renewal and proliferation and induction of cell fate decision. For example, miR-124 promotes neuronal differentiation by downregulating Sox9 in adult neural stem cells (Cheng et al., 2009). miRNAs that are involved in stem cell self-renewal and differentiation and thus regulate cell type specification and differentiation are summarized in Table 3.

Recent reports indicate that miRNAs are central players in stem cell biology (Gangaraju and Lin, 2009), and may have a crucial role in future stem cell therapies. Each type of cell has a distinct miRNA signature. For example, Suh and colleagues reported the first miRNA signature in human ESCs and grouped those miRNAs into four classes; (1) miRNAs found to be specific to ESCs (miR-154, miR-200c, miR-368, miR-371, miR-372, and miR-373); (2) miRNAs found in both ESCs and their malignant counterpart, embryonal carcinoma cells (miR-302a, miR-302b, miR-302c, miR-302d, and miR-367); (3) miRNAs found to be rare in ESCs but abundant in HeLa and STO cells (let-7a, , miR-21, miR-29, miR-29b, miR-301, and miR-374); and (4) miRNAs found to be expressed in most of the cell lines tested (miR-16, miR-17-5p, miR-19b, miR-26a, miR-92, miR-103, miR-130a, and miR-222) (Suh et al., 2004).

miRNA	Type of cell	Biological process	References
let-7	Breast cancer stem cells	Self-renewal	(Yu et al., 2007)
let-7	Breast cancer stem cells	Differentiation	(Yu et al., 2007)
let-7a-1 *let-7d* *let-7-f-1*	ESCs	Pluripotency	(Navarro et al., 2009)
let-7a-2 *let-7a-3,* *let-7b*	ESCs	Pluripotency	(Navarro et al., 2009)
miR-92a	ESC	Self-renewal and differentiation	(Sengupta et al., 2009)
miR-124	Adult neuronal stem cell	Differentiation	(Cheng et al., 2009)

miRNA	Type of cell	Biological process	References
miR-200 *miR-205*	ESCs	Epithelial–mesenchymal transition	(Bracken et al., 2008; Gregory et al., 2008)
miR-150	B cells	Differentiation	(Xiao et al., 2007)
miR-1	Myoblasts	Differentiation	(Chen et al., 2006)
miR-430 *miR-427* *miR-302*	ESCs	Repress formation of ectoderm progenitor cells	(Ivey and Srivastava, 2010)
miR-109 *miR-24*	ESCs	Repress formation of endoderm progenitor cells	(Ivey and Srivastava, 2010)
miR-122 *miR-192*	ESCs	Promote formation of endoderm progenitor cells	(Ivey and Srivastava, 2010)
miR-17-92 *miR-15a* *miR-16-1* *miR-21*	ESCs	Self-renewal	(Navarro et al., 2009)
miR-199a	Mesoderm progenitor cells	Repress differentiation into chondrocytes	(Ivey and Srivastava, 2010)
miR-296 *miR-2861*	Mesoderm progenitor cells	Promote differentiation into osteoblasts	(Ivey and Srivastava, 2010)
miR-214 *miR-206* *miR-1* *miR-26a*	Mesoderm progenitor cells	Promote differentiation into skeletal muscle cells	(Ivey and Srivastava, 2010; Chen et al., 2006)
miR-133 *miR-221* *miR-222*	Mesoderm progenitor cells	Repress differentiation into skeletal muscle cells	(Ivey and Srivastava, 2010)
miR-1	Mesoderm progenitor cells	Promote differentiate into cardiac muscle cells	(Ivey and Srivastava, 2010)
miR-133	Mesoderm progenitor cells	Repress differentiation into cardiac muscle cells	(Ivey and Srivastava, 2010)
miR-145	Neural crest stem cells	Promote differentiation into smooth muscle cells	(Ivey and Srivastava, 2010)
miR-203	Ectoderm progenitor cells	Promote differentiation into keratinocytes	(Ivey and Srivastava, 2010)
miR-9 *miR-124a*	Neural stem cells	Promote differentiation into glial cells and neurons	(Ivey and Srivastava, 2010)

miRNA	Type of cell	Biological process	References
miR-223 *miR-181*	Hematopoietic progenitor cells	Promote differentiation into lymphoid progenitor cells	(Ivey and Srivastava, 2010)
miR-223	Hematopoietic progenitor cells	Promote differentiation into myeloid progenitor cells	(Ivey and Srivastava, 2010)
miR-146 *miR-128a* *miR-181a*	Hematopoietic progenitor cells	Repress differentiation into lymphoid progenitor cells	(Ivey and Srivastava, 2010)
miR-128a *miR-181a* *miR-155* *miR-24a* *miR-17*	Hematopoietic progenitor cells	Repress differentiation into myeloid progenitor cells	(Ivey and Srivastava, 2010)
miR-150	Lymphoid progenitor cells	Promote differentiation into T cells	(Ivey and Srivastava, 2010)
miR-223	Myeloid progenitor cells	Repress differentiation into granulocytes	(Ivey and Srivastava, 2010)
miR-17-5p *miR-20a* *miR-106a*	Myeloid progenitor cells	Repress differentiation into monocytes	(Ivey and Srivastava, 2010)
miR-150 *miR-155* *miR-221* *miR-222*	Myeloid progenitor cells	Repress differentiation into red blood cells	(Ivey and Srivastava, 2010)
miR-451 *miR-16*	Myeloid progenitor cells	Promote differentiation into red blood cells	(Ivey and Srivastava, 2010)
miR-355	Mesenchymal stem cells	Repress proliferation and migration	(Ivey and Srivastava, 2010)
miR-92a	ESC	Repress G_1–S transition	(Sengupta et al., 2009)
miR-372 *miR-195*	ESC ESC	Repress G_1–S transition Repress G_2–M transition	(Qi et al., 2009)

Table 4. miRNAs involved in self-renewal and differentiation processes in normal stem cells and cancer stem cells.

Nanog, Oct4, and Sox2 have been found to be key regulators of ESC pluripotency. miR-134, miR-296, and miR-470 have been shown to modulate ESC pluripotency by regulating Nanog, Oct4, and Sox2, which are key regulators of ESC pluripotency (Tay et al., 2008). Recent studies have identified two groups of miRNAs: markers of pluripotency, which are expressed in the undifferentiated state (miR-200c, miR-371, miR-372, miR-302a, miR-320d, miR-373, miR-302c, miR-21, miR-222, miR-296, miR-494, and miR-367) and miRNAs that regulate the differentiation of cells into one of the different lineages (miR-17, miR-92, and miR-93, which are overexpressed in differentiated cells; and miR-154, miR-29a, miR-143, miR-29c, and let-7a, which are underexpressed in differentiated cells) (Lakshmipathy et al.,

2007). miR-302d and miR-372 target the transcription factors TRPS1 and KLF13 and the RNA binding protein MBNL2 to regulate ESC self-renewal (Li et al., 2009).
miRNAs of the let-7 family (let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i and miR-98) are key regulators of self-renewal and proliferation and act as tumor suppressors. Numerous genes that promote the G_1/S or G_2/M transition, such as CDK6, CDC25A, and CCND2, are direct targets of let-7. Let-7 also negatively regulates oncogenes such as NRAS, KRAS, HMGA2, and c-Myc, and pluripotency-regulating genes such as Lin28 (Chivukula and Mendell, 2008). Let-7 modulates self-renewal by targeting HRAS and differentiation by targeting HMGA2 in breast cancer cells (Yu et al., 2007). Expression of the let-7 family of miRNAs has been found to be downregulated both in embryonic lung tissue and in lung tumors (Navarro et al., 2009), colon cancer (Akao et al., 2006), and breast cancer (Iorio et al., 2005). Moreover, let-7 has been shown to be downregulated in ESCs and high during differentiation, in which LIN28 expression is high in ESC, but decreases during differentiation (Marson et al., 2008b). Let-7 and LIN28 form a tight feedback loop that is fundamental for stem cell self-renewal and differentiation (Gunaratne, 2009; Martinez and Gregory, 2010). miR-150 regulates differentiation by targeting c-Myb in B-cells (Xiao et al., 2007), while miR-1 regulates differentiation by targeting Mef2c in myoblasts (Chen et al., 2006). miRNAs regulate self-renewal in ESC by controlling the G1-S and G2-M transition. For example, miR-92a is a negative regulator of G1-S transition by targeting CDKN1C (Sengupta et al., 2009). miR-372 targets CDKN1A to negatively regulate G1-S transition, while miR-195 negatively regulates G2-M transition by targeting WEE1 in ESCs (Qi et al., 2009). miR-125b, miR-504, miR-25 and miR-30d directly target and negatively regulate TP53 (Kumar et al., 2011).
The following miRNAs have been found to be key regulators of EMT: miR-200a, miR-200b, miR-200c, miR-141, and miR-429 (Gregory et al., 2008). The miR-200 family regulates EMT by targeting different genes. For example, miR-200b, miR-141 and miR-205 target ZEB2 (Gregory et al., 2008), miR-141 and miR-155 targets TGF-β2 (Bracken et al., 2008; Burk et al., 2008), miR-200a targets ZEB2 and CTNNB1 (Xia et al., 2010) to regulate EMT. In addition, miR-335 has been found to regulate differentiation, proliferation, and migration in mesenchymal stem cells (Tome et al., 2011).

6. Conclusion

In the past decade, tremendous progress has been made in discovering molecular mechanisms (signaling pathways, transcription factors and miRNAs) that regulate stem cell self-renewal and differentiation, but many questions remain to be answered. For example, which factors and signaling pathways determine which daughter cell of an adult stem cell remains a stem cell and which undergoes differentiation. How do cells decide whether to self-renew? How do cells decide whether to migrate to develop organs during embryogenesis, and how do cells decide when that specific organogenesis process is complete? How do cells decide to stop proliferating? Which regulatory factors are involved in normal cell differentiation, and which factors are aberrantly expressed in cancer?
New discoveries will add to our understanding of the balance between self-renewal and differentiation in normal stem cells and, therefore, provide new insights into development and progression of cancer, which may lead to the development of more effective molecular cancer therapies. Most current cancer therapeutic agents aim to kill cancer cells. These

therapeutic agents kill cancer cells as well as normal cells, but do not kill CSCs. A more effective approach to the treatment of cancer may be to use therapeutic agents that block self-renewal and that induce cell to complete differentiation instead of killing cells. Since miRNAs are key regulators in self-renewal and differentiation, thereby miRNAs can be used as potential therapeutic agents or targets.

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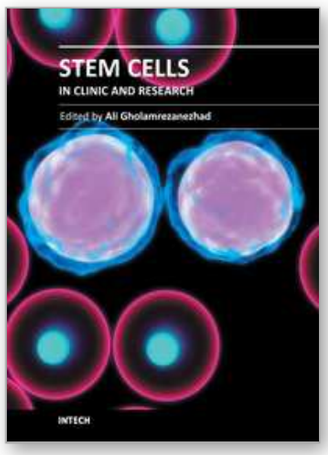

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Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigationally more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

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