Chapter 16
MicroRNAs and Cancer Stem Cells

Zuoren Yu and Richard G. Pestell

Abstract MicroRNAs (miRNAs) are a class of non-coding RNAs that are believed to play important roles during tumorigenesis and cancer metastasis. Growing evidence has shown altered regulation of miRNAs in cancer stem cell populations. In this chapter, the expression profiles of miRNA in embryonic stem cells and cancer stem cells are summarized. The individual miRNAs which may regulate cancer stem cells and their target genes are described. Several miRNAs, including *miR-302* and *miR-181*, function to promote the cancer stem cell phenotype. Conversely, other miRNAs including *let-7, miR-145, miR-200* family, *miR-203, miR-128, miR-34*, and *miR-199b*, suppress stemness and promote differentiation of cancer stem cells. The recent evidence for a role of miRNA in regulating cancer stem cells, epithelial-mesenchymal transition, and cancer metastasis are described. We introduce the potential of miRNA for cancer diagnostics and therapeutics based on current tests and studies of miRNA treatment on cancer. The current challenges to apply miRNA-based cancer therapeutics are also discussed with an emphasis on recent evidence for miRNA-mediated heterotypic signals. The miRNA regulation of factors that are secreted into the blood stream creates an attractive new approach to managing miRNA-driven disease processes.

16.1 Introduction

16.1.1 Definition of the Stem Cell and Cancer Stem Cell

Stem cells are cells that are capable of self renewal, capable of differentiation and thereby giving rise to specialized cell types. This concept has been extended to cancer and a growing body of evidence has indicated a subpopulation of stem-like

R.G. Pestell (✉)
Departments of Cancer Biology and Medical Oncology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA 19107, USA
e-mail: Richard.pestell@jefferson.edu

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cells within tumors, known as cancer stem cells (CSCs). A definition of CSCs has been based on a self-renewal capacity, an ability to differentiate into populations of non-tumorigenic cell progeny, and their ability to seed tumors when transplanted into animal hosts. As recently reviewed, cancer stem cells have specific alterations in their cell cycle compared with normal cells, which may be important in targeting cancer stem cells (Rosen and Jordan 2009; Velasco-Velázquez et al. 2009).

16.1.2 Discovery of the Cancer Stem Cell

The evidence of SCs in cancer originated from a research on human acute myeloid leukaemia (AML) in 1994 by Dick (Lapidot et al. 1994), who identified an AML-initiating cell population by transplantation into severe combined immune-deficient (SCID) mice. These cells homed to the bone marrow and showed a pattern of dissemination and leukaemic cell morphology similar to that seen in the original patients. The leukaemic stem-like cells, named SCID leukemia-initiating cell (SL-IC), possessed differentiative and proliferative capacities and a potential for self-renewal. They were fractioned on the basis of cell surface marker expression ($CD34^+$ $CD38^-$). CSCs have been demonstrated in several solid tumors including human breast cancer and brain cancer (Al-Hajj et al. 2003; Singh et al. 2003), and later in colon, pancreas, lung, prostate, melanoma and glioblastoma cancers. Clarke et al. identified and isolated mammary tumorigenic stem-like cells as $CD44^+CD^{24-/low}Lineage^-$ in eight of nine breast cancer patients. As few as 100 cells with this phenotype were able to form tumors in nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice indicating the CSCs properties of this cell fraction (Al-Hajj et al. 2003).

The initial description of CSCs claimed that CSCs represent only a small fraction of the total cancer cell population in a solid tumor, which had the ability to self-renew and maintain the tumor. However recent findings suggest that as many as 25% of cancer cells may have the properties of CSCs, and co-exist with non-CSCs (Kelly et al. 2007; Quintana et al. 2008). The proportion of CSCs population in tumors requires further experimental clarification and may relate to the original methods of isolation. CSC-specific cell surface markers such as CD44, CD24 and/or CD133 enrich CSCs from cancer cells (Al-Hajj et al. 2003; Singh et al. 2003), but these markers may depend upon the type of tumor. Thus, breast CSCs are characterized as $CD44^+CD24^{-/low}$; colon CSC are isolated by cell sorting with $CD133^+$ (Ricci-Vitiani et al. 2007). Recent reports have extended the CSCs-related cell surface markers to include markers such as epithelial-specific antigen (ESA) and aldehyde dehydrogenase-1 (ALDH-1) (Ginestier et al. 2007; Li et al. 2007). The variety of CSC markers in different tumors or tissue types and even within the same tissue type induced by distinct oncogenes suggests significant complexity of CSCs (Table 16.1).

Table 16.1 Cancer stem cell markers in human cancer

Cancer type	Cancer stem cell markers	References
Breast	$CD44^+/CD24^{-/low}/Lin^-ALDH1^+$	Al-Hajj et al. (2003); Ginestier et al. (2007)
Brain	$CD133^+$	Singh et al. (2003)
Leukemia	$CD34^+/CD38^-$	Lapidot et al. (1994)
Head and neck	$CD44^+$	Prince et al. (2007)
Lung	$CD133^+$	Eramo et al. (2008)
Liver	$CD90^+$	Yang et al. (2008)
Pancreas	$CD44^+/CD24^+/ESA^+$	Li et al. (2007)
Colon	$CD133^+$	Ricci-Vitiani et al. (2007)

16.2 MicroRNA

MicroRNAs (miRNAs) are a class of non-coding RNAs that regulate the stability or translational efficiency of targeted messenger RNAs. Over 1,000 miRNAs have been already identified from human (miRBase Sequence Database Release 16.0 in September 2010). As each mRNA contains binding sites for different miRNAs, and each miRNA is predicted to target as many as hundreds of genes, it is predicted that about one-third of human mRNAs could potentially be regulated by miRNAs. MiRNAs are believed to play important roles in a broad range of biological processes including embryonic development, differentiation, apoptosis, cell division, cell proliferation, tumorigenesis and cancer metastasis (Sotiropoulou et al. 2009; Yu et al. 2010a). Aberrant expression of miRNAs in many types of tumors (below) indicates the involvement of miRNAs in the regulatory net of tumorigenesis.

16.2.1 MiRNA and the Stem Cell

MiRNAs have been linked to stem cells from the original discovery of miRNA as the first two miRNAs, *lin-4* and *let-7*, were involved in regulation of developmental timing in *C. elegans* (Reinhart et al. 2000). A subset of miRNAs was cloned and sequenced from mouse embryonic stem (mES) cells in 2003 (Houbaviy et al. 2003), and later from human embryonic stem (hES) cells (Suh et al. 2004). Subsequent studies indicated the regulation of miRNAs at distinct stages of the onset and progression of tumorigenesis and cancer stem cells. *Let-7* was reported to act as a regulatory switch for stem cell self-renewal and differentiation in breast tumor-initiating cells (Yu et al. 2007); *miR-145* expression was low in self-renewing hES cells and upregulated during differentiation. *miR-145* functioned via stem cell reprogramming factors (*OCT4, SOX2, KLF4*) to regulate stem cell populations (Xu et al. 2009). Given the aberrant expression of miRNAs in tumors and the crucial regulatory function of miRNA in tumorigenesis and cancer stem cells, it is likely that miRNAs will be found to play important roles in diagnosis and therapy of cancers.

16.2.2 MiRNA Expression Profile in ES Cells

A global search of miRNA expression in embryonic stem cells was performed by Dr Sharp and Dr Kim using a small RNA cloning and sequencing method (Houbaviy et al. 2003; Suh et al. 2004). From mES cells 53 small RNAs were identified as miRNAs including 6 embryonic stem cell-specific miRNAs (*miR-290, 291, 292, 293, 294,* and *295*) which were either silenced or down-regulated upon differentiation. From hES cells, 36 small RNAs met the criteria of miRNA including the *miR-302* cluster and the *miR-371-373* cluster. The *miR-371-373* cluster on chromosome 19 is the human homologues of the *miR-290-295* cluster in mES cells. The *miR-302* cluster on human chromosome 4 is a close homologue of the *miR-302* cluster on mouse chromosome 3. The *miR-302* cluster and the *miR-371-373* cluster are expressed in hES, mES and embryonic carcinoma (EC) cells suggesting conserved roles in mammalian pluripotent stem cells. Expression of several mouse and human miRNA clusters (*miR-15/16, let-7* family, *miR-34* family, and *miR-17* cluster) are observed in both mES and hES cells suggesting their function in stem cells may be conserved across species.

MiRNA microarray analysis and miRNA quantitative reverse transcription-polymerase chain reaction (qRT-PCR) are more sensitive than RNA cloning method and have been used to detect the expression profiles of miRNAs. Li et al. (2009) examined 250 human miRNAs expression in a hES cell line hES-T3 using TaqMan real-time PCR assays. They confirmed 10 hES cell-specific miRNAs including *miR-302a**, *302b**, *302c**, *302d, 367, 368, 371, 372*, *373**, *200c*, and predicted their target genes as well. Differences in miRNA expression between undifferentiated hES cells and their corresponding differentiated cells using both microarray analysis and qRT-PCR methods confirmed the identity of a miRNA signature profile in pluripotent cells, which comprised a small subset of differentially expressed miRNAs in hES cells (Lakshmipathy et al. 2007).

16.2.3 MiRNA Expression in Cancer Stem Cells

MiRNAs regulate self-renewal and differentiation of ES cells and adult tissue stem cells, and they also regulate cancer stem cells. The miRNA expression profiles of cancer stem cells are a key starting point. Zhu et al. reported altered expression of miRNAs (*miR-142-3p, miR-451, miR-106a, miR-142-5p, miR-15b, miR-20a, miR-106b, miR-25*, and *miR-486*) in bronchioalveolar "stem-like cells" which were proposed to be lung cancer progenitor cells (Qian et al. 2008). Clarke et al. recently compared miRNA expression profiles between human breast cancer stem cells ($CD44^{+}CD24^{-/low}lineage^{-}$) and the remaining $lineage^{-}$ nontumorigenic breast cancer cells (Shimono et al. 2009). 460 miRNAs were measured. 37 miRNAs were found to be deregulated in breast cancer stem cells. MiRNAs that were upregulated included *miR-199, miR-125b*, and *miR-34b*, whereas down-regulated miRNAs included the *miRNA-200c-141* cluster, the *miR-200b-200a-429* cluster, and the

miR-183-96-182 cluster. Some of these miRNAs regulate cancer stem cells and tumorigenesis (below).

16.3 Individual MiRNAs

16.3.1 MiR-302

The *miR-302* cluster was initially cloned from mES and hES cells (see above). It is highly expressed in the ES cell population but expression decreases rapidly after cell differentiation, and is not expressed in somatic cells (Barroso-del Jesus et al. 2009). The promoter of the *miR-302* cluster is regulated by *Oct4* and *Sox2* (Card et al. 2008), two transcription factors required for pluripotency during early embryogenesis and for maintenance of "stemness". Recent elegant study by Archer identified the key G_1 cell cycle regulator *cyclin D1* as a target of the *miR-302* cluster in ES cells (Card et al. 2008). It has been proposed therefore that *Oct4/Sox2-miR-302-cyclin D1* network is crucial for ES cell pluripotency and self-renewal properties.

MiR-302 over-expression converts cancer cells into ES-like pluripotent stem cells with high expression of several ES cell markers (*Oct3/4, SSEA-3, SSEA-4, Sox2*, and *Nanog*). These *miR-302* induced pluripotent stem cells differentiate into distinct tissue cell types in vitro including neurons, chondrocytes, fibroblasts, and spermatogonia-like primordial cells. The authors concluded that the *mir-302* cluster reprograms cancer cells into an ES-like pluripotent state and maintains this state under feeder-free culture conditions (Lin et al. 2008).

16.3.2 MiR-17 Cluster

The *miR-17* cluster, which encodes six mature miRNAs within a 1 kb genomic region, inhibits tumor growth in breast cancer (Yu et al. 2008, 2010b) and promotes cell growth in both lung cancer and lymphomas (Hayashita et al. 2005; He et al. 2005). These findings suggest that the *miR-17* cluster functions in a cell type-dependent manner. The *miR-17* cluster coordinates the timing of cell cycle and tumorigenesis by targeting multiple transcription factors such as *E2F, c-myc, Rb*, and *cyclin D1* (Yu et al. 2008). Within the *miR-17* cluster, *miR-19* may be the key oncogenic component rather than *miR-17-20* (Mu et al. 2009; Olive et al. 2009). *MiR-19* is required and largely sufficient to recapitulate the oncogenic properties of the entire cluster. It is possible that in a given tumor type, distinct components of the cluster drive the distinct oncogene/tumor suppressor phenotypes. Emerging evidence indicates that the *miR-17* cluster is involved in regulating the stem cell phenotype. During lung development, expression of the *miR-17* cluster is high at early stages, but declines as development proceeds. Transgenic mouse expressing the *miR-17* cluster develop proliferative lung epithelial cells with high levels of *Sox9*, suggesting that the *miR-17* cluster may promote the proliferation of lung epithelial progenitor cells (Lu et al. 2007). The *miR-17* cluster is differentially expressed in

developing mouse embryos and function to control differentiation of stem cells perhaps via its target *STAT3* which is a known ES cell regulator (Foshay and Gallicano 2009).

16.3.3 MiR-181

MiR-181 is upregulated during mammalian skeletal-muscle differentiation to establish the muscle phenotype by down-regulating the homeobox protein *Hox-A11* (a repressor of the differentiation process) (Naguibneva et al. 2006). In B-lymphocytes, *miR-181a* was upregulated in differentiated cells compared with undifferentiated progenitor cells, suggesting it is a positive regulator for B cell differentiation (Chen et al. 2004). In contrast, *miR-181* family members were up-regulated in hepatocellular carcinoma (HCC), particularly within EpCAM-positive hepatic cancer stem/progenitor cells isolated from alpha-fetoprotein $(AFP)^+$ liver tumors (Ji et al. 2009b). Ji et al. (2009b) found that *miR-181* family members were highly expressed in embryonic livers and isolated hepatic stem cells. *MiR-181* may contribute to "stemness" through regulating HCC cell differentiation (via suppressing *GATA6* and *CDX2*) and activating the *Wnt/β-catenin* pathway (by down-regulating the *NLK* gene).

16.3.4 Let-7

Let-7 regulation has been studied during development of *C. elegans* (Reinhart et al. 2000). *Let-7* was undetectable at embryonic, L1 or L2 stages, expressed at low level at the early L3 stage, and highly expressed at the early L4 and adult stages. Loss of *let-7* causes larval cell fates during the adult stage, whereas increased *let-7* gene dosage causes precocious expression of adult fates during larval stages. Several heterochronic genes such as *lin-41* and *daf-12* have been confirmed as targets of *let-7* (Reinhart et al. 2000).

In mouse and human, the *let-7* family is broadly expressed in adult tissues. Expression of mature *let-7* family members is absent in human and mouse embryonic stem cells and pluripotent cell populations, and show increased expression upon differentiation (Büssing et al. 2008; Wulczyn et al. 2007). Collectively these observations suggested a conserved function of *let-7* in regulating self-renewal and cell differentiation. The abundance of *let-7* family members is reduced in several types of cancer including lung and breast cancer (Takamizawa et al. 2004; Yu et al. 2007). *Let-7* over-expression suppressed cell cycle progression and tumorigenesis by inhibiting oncogenes including *Ras* and *HMGA* (Johnson et al. 2005; 2007; Mayr et al. 2007; Tsang and Kwok 2008). Multiple important cell cycle control genes are repressed by *let-7* including *cyclin D1, cyclin D3, cyclin A, CDK 4* (Schultz et al. 2008) and *CCNA2, CDC25A, CDK6*, *CDK8* (Johnson et al. 2007). Yu et al. (2007) compared miRNA expression profiles in self-renewing and differentiated cells from breast cancer lines and in breast tumor-initiating cells (BT-IC)

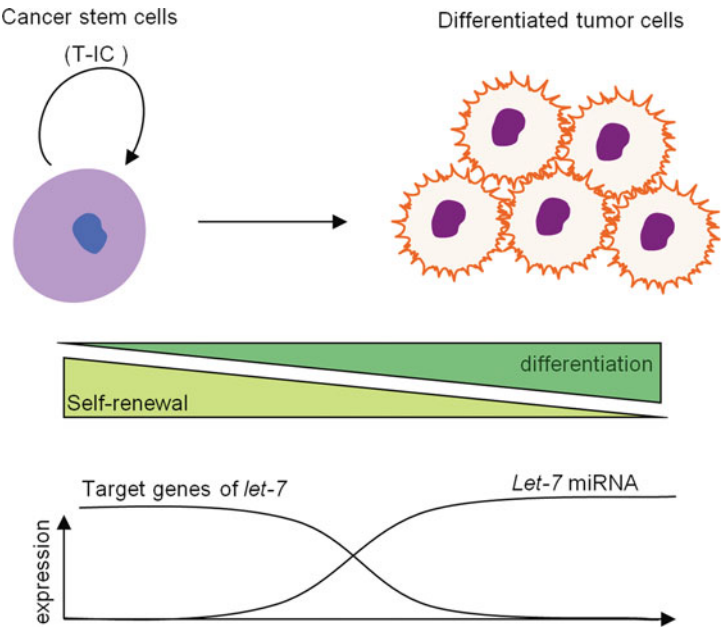

Fig. 16.1 *Let-7* miRNA regulation of cancer stem cell differentiation

and non BT-IC. *Let-7* expression was low in BT-IC and increased with differentiation. *Let-7* over-expression in BT-IC reduced cell proliferation and mammosphere formation. Enforced *let-7* expression in BT-IC interfered with tumor initiation and self-renewing capacity in vivo (Yu et al. 2007). Reduced *let-7* maintains the undifferentiated state and proliferative potential of mammospheres and BT-IC. Two known targets of *let-7*, *H-RAS*, and *HMGA2* were examined for their contribution to the BT-IC characteristics. Silencing *H-RAS* in a BT-IC-enriched cell line reduced self renewal but had no effect on differentiation, while silencing *HMGA2* enhanced differentiation but did not affect self renewal (Yu et al. 2007). Thus *let-7* acts to promote stem cell differentiation (Fig. 16.1).

16.3.5 MiR-145

MiR-145 was reported as a tumor suppressor (Cho et al. 2009; Kent and Mendell 2006). *MiR-145* inhibits human cancer cell growth in vitro via the 3′UTR of the type 1 insulin-like growth factor receptor (*IGF-IR*) and its docking protein, the insulin receptor substrate-1 (*IRS-1*) (Shi et al. 2007). In undifferentiated mES cells *IRS-1* expression is high, but decreases when cells differentiate (Rubin et al. 2007). *IRS-1* over-expression inhibits mES cell differentiation. Since *miR-145* regulates ES cell, *miR-145* regulation of stem cell differentiation may be mediated in part via *IRS-1*. *MiR-145* expression is low in self-renewing hES cells, and upregulated during differentiation (Xu et al. 2009). The pluripotency genes *OCT4, SOX2*, and *KLF4*

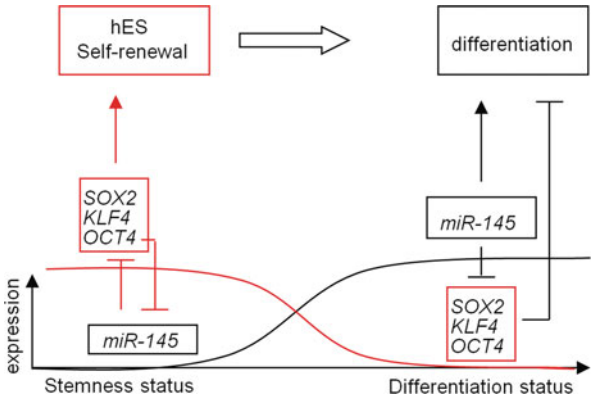

Fig. 16.2 *OCT4-miR-145* regulatory feedback in control of human embryonic stem (hES) cell stemness and differentiation

are the direct targets of *miR-145*. *MiR-145* over-expression inhibits self-renewal of hES cells and induces lineage-restricted differentiation. As a regulatory feedback loop, *OCT4* in turn represses *miR-145* expression (Xu et al. 2009). *MiR-145* regulates cell differentiation in smooth muscle cells (Cordes et al. 2009) where *miR-145* was necessary for myocardin-induced reprogramming of adult fibroblasts into smooth muscle cells. In addition to *KLF4*, *myocardin*, and *Elk-1* are targets of *miR-145*. Like *miR-145*, *miR-143* promotes differentiation, and represses smooth muscle cell proliferation (Cordes et al. 2009) (Fig. 16.2).

16.3.6 MiR-203

MiR-203 functions as a switch between proliferation and differentiation during skin development (Yi et al. 2008). *MiR-203* is expressed poorly in epidermis from embryonic day 13.5 but emerges as one of the most abundant epidermal miRNAs from embryonic day 15.5 onwards. *MiR-203* promotes epidermal differentiation by inhibiting the proliferative potential of epidermal stem cells and inducing cell-cycle exit through suppressing *p63*. *p63* is a transcription factor member of *p53* family which plays an essential role for stem-cell maintenance in stratified epithelial tissues.

In pancreatic cancer stem cells *miR-203* inhibits "stemness" (Wellner et al. 2009). *MiR-203* itself is suppressed by *ZEB1* which is an epithelial-mesenchymal transition (EMT)-activator and necessary for the self-renewing capacity of pancreatic and colorectal cancer stem cells. Knockdown of *ZEB1* led to a reduction of the pancreatic cancer stem cell ($CD24^+/CD44^+$) population, reducing sphere formation in undifferentiated pancreatic cancer cell line. *ZEB1* may thus link EMT-activation and maintenance of "stemness" by suppressing miRNAs (Fig. 16.3) (Wellner et al. 2009).

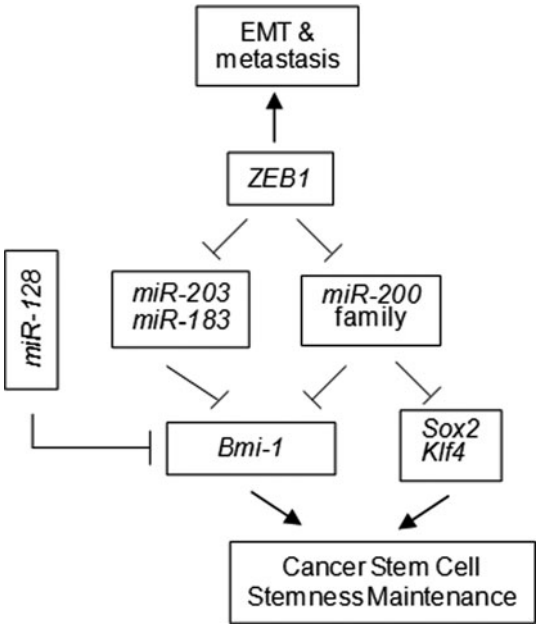

Fig. 16.3 The epithelial-mesenchymal transition (EMT)-activator *ZEB1* regulates cancer stem cell population mediated by microRNAs

16.3.7 MiR-34

The *miR-34* family was initially cloned from hES and mES cells, and is abundant in male germ cells (Yu et al. 2005). *MiR-34* expression induces cell cycle arrest, induces apoptosis and inhibits cell proliferation and colony formation in part via *p53* (Corney et al. 2007; He et al. 2007; Tarasov et al. 2007). *MiR-34* over-expression also reduced pancreatic cancer stem cells, inhibiting tumor-sphere growth in vitro and tumor formation in vivo. Two stem cell regulators, *notch* and *bcl-2*, were identified as targets of *miR-34* (Ji et al. 2009a).

16.3.8 MiR-199b

As a tumor suppressor miRNA, *miR-199b* expression is lost in metastatic medulloblastoma tumors. *MiR-199b* over-expression negatively regulated cell proliferation and impaired the oncogenic potential of brain tumor cell lines (Garzia et al. 2009). Enforced *miR-199b* expression suppressed cancer stem-cell gene expression and decreased the medulloblastoma cancer stem-cell-like ($CD133^+$) subpopulation, which resulted in impairment of medulloblastoma tumor development in the cerebellum xenograft mouse model in vivo. *MiR-199b* was shown to target *HES1*, a transcription factor of the notch pathway, thereby regulating cancer stem cell self-renewal (Garzia et al. 2009).

16.3.9 MiR-128

MiR-128 expression is down-regulated in human glioblastoma. *MiR-128* expression reduced glioma cell proliferation in vitro and inhibited glioma xenograft growth in vivo (Godlewski et al. 2008). Thus *miR-128* is considered a suppressor of glioblastoma, functioning by blocking glioma "stem-like" cell self-renewal via *Bmi-1*. *Bmi-1* is activated in breast cancer stem cells $CD44^{+}CD24^{-/low}Lin^{-}$ (Liu et al. 2006), and *Bmi-1* loss decreased neural stem cell population. In glioblastoma specimens, *Bmi-1* expression was significantly up-regulated while *miR-128* was down-regulated compared with normal brain, which is consistent with the finding that *miR-128* inhibits glioma proliferation and self-renewal by targeting *Bmi-1* (Godlewski et al. 2008) (Fig. 16.3).

16.3.10 MiR-200

The *miR-200* family inhibits epithelial-mesenchymal transition (EMT) in human breast cancer induced by *TGF-β*. *MiR-200* over-expression in mesenchymal cells initiated mesenchymal-epithelial transition by inhibiting expression of *ZEB1* and *ZEB2* (Eger et al. 2005). The *miR-200* family member *miR-200a, miR-200b*, and *miR-200c* were down-regulated in human breast cancer stem cells, normal human and murine mammary stem/progenitor cells, and embryonal carcinoma cells (Shimono et al. 2009; Wellner et al. 2009). *MiR-200c* inhibits stemness of normal mammary stem cell differentiation into mammary duct and tumor formation driven by human breast cancer stem cells in vivo (Shimono et al. 2009). *MiR-200c* inhibits stemness of pancreatic cancer stem cell of (Wellner et al. 2009) and breast cancer stem cell (Shimono et al. 2009). *Bmi-1* is a common target of stem cell-related miRNAs including *miR-200* family, *miR-203* and *miR-183* (Fig. 16.3).

16.4 MiRNA, Cancer Stem Cell, EMT, and Cancer Metastasis

Metastasis is a complex process by which primary solid tumor cells invade adjacent and distant tissues and grow into secondary tumors. EMT is believed to be an essential early step in tumor metastasis. MiRNA regulates EMT and cancer metastasis (Ma and Weinberg 2008; Valastyan and Weinberg 2009; Yu et al. 2010a). Recent reports have linked miRNA on one hand to the regulation of cancer EMT and metastasis and on the other hand to the regulation of the cancer stem cell phenotype in human breast cancer (Shimono et al. 2009; Tavazoie et al. 2008). *MiR-335* suppresses human breast cancer metastasis and inhibits *SOX4* expression (Tavazoie et al. 2008). *SOX4* is known to promote progenitor cell development and migration. *MiR-335* suppression of *SOX4* may thereby decrease breast cancer stem cell expansion, and thereby decrease breast cancer metastasis.

The *miR-200* family (*miR-200a*, *miR-200b*, *miR-200c*, *miR-141*, *miR-429*) and *miR-205* are down-regulated in cells that have undergone EMT (Gregory et al. 2008;

Peter 2010). Enforced expression of the *miR-200* family prevents *TGF-β* induced EMT. *ZEB1* and *ZEB2* are targeted by *miR-200* family, and *ZEB1* represses *miR-200* family. *MiR-200* is down-regulated in breast cancer stem cells and in normal mammary stem cells. *Bmi-1*, a promoter of stem cell self-renewal as noted previously, is targeted by *miR-200c*. *ZEB1* represses a stemness-inhibitor, *miR-203,* in pancreatic and colorectal cancer (Wellner et al. 2009). *Sox2* and *Klf4* are also repressed by *miR-200c*. Collectively, miRNA provide a link between EMT (*ZEB1*) and stem cells (*Sox2/Klf4*) via a *ZEB1/ miR-200/ Sox2/Klf4* loop (Fig. 16.3).

16.5 MiRNA, Cancer Stem Cell, and Cancer Therapy

Cancer stem cells are being targeted by a number of investigators and are discussed elsewhere (Gupta et al. 2009). MiRNAs also represent an excellent target for therapeutic and prognostic action (Cho 2010).

Oncogenic miRNAs are targets for inactivation and for therapy. The tumor suppressor miRNAs may be ideal candidates for the development of new drugs to inhibit tumorigenesis by enhancing their biogenesis, abundance and function. So far synthetic miRNA mimics, miRNA expression vectors and chemically modified anti-miRNAs (antisense oligonucleotides as miRNA inhibitors) have been successfully applied in bench work for the purpose of modifying miRNA abundance and activity in cells and experimental animals. The tumor suppressor function of *let-7* was demonstrated in non-small cell lung cancer by applying miRNA knock-in and knock-down techniques in a mouse model in vivo (Trang et al. 2010). By combining a successful protocol for delivery of small RNAs by intranasal passage with established mRNA antisense technology, they delivered anti-*let-7* into mouse lung increasing tumor burden. Intranasal delivery of *let-7* reduced *K-ras*-dependent lung tumors. Intratumoral delivery of *let-7* reduced lung cancer size in a Xenograft. *MiR-26a*, which is highly expressed in normal tissues and lost in hepatocellular carcinoma cells, was delivered into a mouse model of hepatocellular carcinoma, and protected from disease progression (Kota et al. 2009).

16.6 MiRNA-mediated Heterotypic Signals in Tumorigenesis

Until quite recently a prevailing model of tumorigenesis focused on the epithelial cell rather than the tissue microenvironment. It is now known that the local tissue environment contributes to the onset and progression of tumorigenesis. Similarly it is now known that tumors secrete key factors that promote tumor progression and metastasis. In this regard tumors secrete interleukin-8 (IL-8), and antibodies directed to IL-8 prevent breast tumor metastasis in vivo (Wu et al. 2008).

Recent studies have demonstrated that miRNA also regulates the secretion of key factors that are essential for the migration phenotype in breast cancer cells. In this regard *miR-17/20*, which inhibits breast cancer cell proliferation, suppresses breast tumor invasion and migration through regulating secreted factors, including IL-8

(Yu et al. 2010b). *MiR-17/20* abundance is reduced in highly invasive breast cancer cells and in node positive breast cancer (Yu et al. 2010b). *MiR-17/20* inhibited secreted plasminogen activators and reduced IL-8 production by interaction with the *IL-8* 3′UTR. Of interest, *miR-17/20* inhibited the secreted cytokeratin 8 (CK8) through a mechanism that required cyclin D1 (Yu et al. 2010b), the G1 cell-cycle regulator.

As noted above, miRNAs can regulate cell migration by secreted factors. Ultimately therapies directed to miRNA mediated disease can be directed to the cell making the miRNA, or to the mediator induced by the miRNA such as secreted growth factors or cytokines.

16.7 The Challenges of MiRNA-based Therapeutic

A miRNA-based therapeutic targeting intracellular miRNA faces three technical challenges.

16.7.1 How to Alter the MiRNA Level in Tumors?

An oncogenic miRNA can be reduced via chemically modified antisense oligos (2′-*O*-methyl RNA oligonucleotides, locked nucleic acid oligonucleotides and peptide nucleic acid (PNA)) (Fabani and Gait 2008). PNA oligonucleotides show high affinity and sequence specificity for complementary RNA, and are stable. These three approaches have been successfully used to knock down miRNA in vitro and in vivo (Fabani et al. 2010; Krützfeldt et al. 2005). Two other approaches, named “miR-sponge” and “miR-mask”, were developed to block miRNA-mRNA interaction (Ebert et al. 2007; Choi et al. 2007). Tumor suppressor miRNA can be increased via synthetic miRNA mimics or miRNA expression vectors carrying either a pre-miRNA sequence or an artificial miRNA hairpin sequence.

16.7.2 How to Deliver MiRNA to Patients?

Several small interfering RNA delivery systems for cancer treatment have been developed and applied on humans (Oh and Park 2009). Cationic liposomes are attractive vehicles for small RNA because of their high transfection efficiency, low toxicity and protection of miRNAs from enzymatic degradation. Polymer-based delivery systems include synthetic polymers which have high transfection efficiency but induce cell death, and natural cationic polymers which are non-toxic, biocompatible, and biodegradable such as chitosan which is widely used. Physical delivery via electroporation is used for small RNA delivery in vivo.

Viral vector mediated delivery has high miRNA transfer efficiency with sustained and consistent gene modulation. However the residual viral elements are immunogenic in humans although modification to virus structure and delivery method has

minimized the risk. A modified recombinant adeno-associated virus (rAAV) delivered *miR-26a* to mouse live tumors (Kota et al. 2009). Intranasal administration is ideal for respiratory therapy with no major adverse immune reactions or nasal-mediated RNA detected in other organs (Bitko and Barik 2008). This technique was used to deliver *let-7* miRNA to mouse lung and block lung cancer in vivo (Trang et al. 2010).

16.7.3 How to Locate MiRNA to the Tumor Tissue?

Targeting miRNA delivery to avoid off-target effects is essential for miRNA cancer therapy. Tissue-specific miRNA expressing or tumor-targeting miRNA delivery systems are being established. A tumor-specific, ligand-targeting system for gene therapy of cancer has been developed and patented (DeSano and Xu 2009; Xu et al. 2002) employing transferrin (Tf) or the scFv against the transferrin receptor (TfR) as a tumor-targeting ligand. The transferrin receptor is over-expressed in most human cancers, warranting FDA approval for a clinical trial (DeSano and Xu 2009).

An alternative strategy to restrict gene expression is the use of temporally and spatially controlled gene delivery systems. These systems include tetracycline and ecdysone based delivery systems (Albanese et al. 2002). With the advent of photouncaging, delivery can be directed at a single cell level, improving therapeutic efficiency to a theoretical maximum (Lin et al. 2002).

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