signaling pathways that turn on PRC2-silenced genes may not be sufficient to reliably induce expression of their targets. When PRC2 activity is low, as in Eed or Suz12 mutants, target genes are no longer reliably silenced, and such a result can lead to incorrect execution of developmental programs (Chamberlain et al., 2008; Pasini et al., 2007). Indeed, Eed mutant ESCs are prone to differentiation in culture, suggesting that PRC2 is necessary for ESCs to robustly carry out the self-renewal program. Thus, one possibility is that the ratio of different PRC2 complexes occupying each target gene may be important in determining whether a signaling pathway will trigger maintenance of or release from silencing, thus allowing cells to silence or upregulate a subset of PRC2 targets in response to particular developmental cues.

The near-perfect overlap between Jarid2 and PRC2 in ESCs argues that it is not simply the presence or absence of Jarid2 that regulates PRC2 target gene activity. However, in addition to Jarid2 and Mtf2 and other Pcl homologs, there are several other sources of variability between PRC2 complexes (Simon and Kingston, 2009). Specifically, there are two related genes encoding H3K27 KMTs, Ezh2 and Ezh1, both of which associate with Eed and Suz12 and there are four isoforms of Eed that differ in their N-termini because of alternative translation-start-site usage. Thus, there is potential for considerable combinatorial complexity of PRC2 complexes. Once the composition and activities of different PRC2 complexes is determined, and the factors that direct these different complexes to their target genes are identified, it may be possible to fully understand how PRC2 is utilized to control so many genes in such a wide variety of developmental contexts.

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Regulating Cancer Stem Cells the miR Way

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A recent study in *Nature Cell Biology*, Wellner et al. (2009) identifies ZEB1, a known promoter of tumor invasion, as a negative regulator of miRNA clusters that target stem cell factors. These findings provide new insight into the network of transcription factors and miRNAs that regulate cancer stem cells.

Treatment of human cancers is complicated because the majority of cancers become metastatic and/or develop resistance to therapy. Two concepts that may explain tumor progression and acquisition of a therapy-resistant phenotype have received wide attention. In the first concept, tumor cells gain an invasive phenotype by a process that resembles the epithelial to mesenchymal transition (EMT) found during embryonic development and wound healing. During EMT, cells change morphology, lose polarity, and become mobile. The second concept is based on the recognition that many human cancers contain a rare population of cells that exhibit stem cell properties and drive neoplastic growth. Recent data have connected the two concepts in breast cancer by demonstrating that induction of EMT in both mammary epithelial cells as well as in breast cancer cells causes upregulation of stem cell markers (Mani et al., 2008). Conversely, EMT markers were found to be enriched in stem cells isolated from either mammary glands or mammary carcinomas.

miRNAs have emerged as powerful regulators of differentiation, and a number of miRNAs have been shown to be either highly expressed or excluded from stem cells. miRNAs that are highly expressed in stem cells include the miR-302∼371 cluster; expression of miR-302 alone in human skin cancer cells induced a phenotype that included properties of pluripotent embryonic stem cells (Lin et al., 2008). Examples of miRNAs excluded from embryonic stem cells are let-7 (Yu et al., 2007) and miR-145, which targets

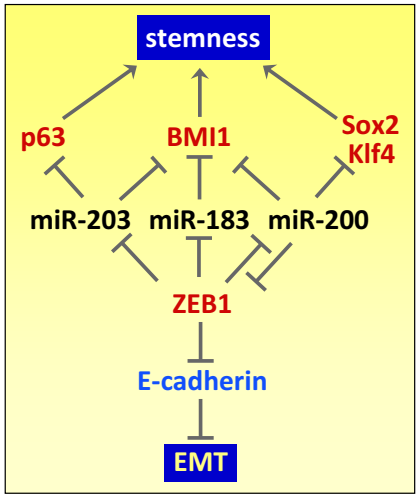

Figure 1. Network of miRNAs Regulating EMT and Stemness

Three miRNAs/miRNA clusters (shown in black) regulate expression of stem cell factors and of ZEB1 (miRNA targets are shown in red). ZEB1 is a central regulator functionally connecting EMT with stem cell maintenance because of its ability to bind to E-boxes in the promoters of E-cadherin and all three miRNA gene clusters.

Sox2, Oct4, and Klf4 (Xu et al., 2009). These data provide evidence that miRNAs, some of which are deregulated in many human cancers, are also markers and powerful regulators of stem cells and cancer stem cells.

Recently, members of the miR-200 family of miRNAs (miR-200a, -200b, -200c, -141, and -429) were recognized to be regulators of the epithelial phenotype and, hence, to be regulators of EMT (Gregory et al., 2008a; Park et al., 2008). miR-200 was shown to target two E-box-binding inhibitors of E-cadherin, ZEB1 and ZEB2, raising the question as to what mechanism regulates expression of miR-200 family members. Subsequently, both ZEB1 and ZEB2 were reported to be part of a double-negative feedback loop, in that they participate in the repression of the expression of all five miR-200 family members (Gregory et al., 2008b). Given that miR-200 is a powerful regulator of EMT, and because EMT has been connected to the regulation of stemness, it seemed merely a question of when, not if, someone would demonstrate that miR-200 regulates the emergence of stem cells and cancer stem cells, in addition to EMT.

To this end, Clarke and colleagues recently identified a set of miRNAs that are not expressed in either mammary epithelial stem cells or in enriched breast cancer stem cells (Shimono et al., 2009). Further, the authors identified and validated that the stem cell factor BMI1 is a target for the miR-200 family of miRNAs. Overexpression of miR-200c, either in normal stem cells or in cancer stem cells, reduced their clonogenic and tumor-initiating activities.

The Shimono et al. findings raise additional questions. First, is BMI1 the only stem cell-relevant target of miR-200? It seemed unlikely that miR-200 would exert its function by targeting only one gene, especially given that both ZEB1 and ZEB2 were known to be efficiently targeted by miR-200. Second, what prevents miR-200 expression in stem cells, but not more differentiated progeny?

In a report from a recent issue of *Nature Cell Biology*, Wellner et al. (2009) offers some answers to these questions and provides further evidence of an miRNA-mediated connection between EMT and cancer stem cells. The authors demonstrated that ZEB1 is preferentially expressed in the invasive front of pancreatic cancer samples, similar to their previous finding in colon cancer (Spaderna et al., 2006). Consequently, knockdown of ZEB1 in pancreatic cancer cell lines reduced both invasion and metastasis in an orthotopic mouse xenograft model. In addition, knockdown of ZEB1 also caused a reduction in tumorigenicity of the cancer cells, based on the size and number of tumors that formed in recipients, even under serial dilution conditions. Other traits attributed to cancer stem cells were also diminished, including the frequency of CD24-positive cells (a marker for pancreatic cancer stem cells), the ability to form spheres in culture, and chemoresistance. Furthermore, expression of a number of miRNAs was affected in these cells. Most notably, members of three miRNA gene clusters were upregulated in ZEB1 knockdown cells. They included two members of the miR-200 family, miR-183 and miR-203. Interestingly, all three miRNA clusters carry E-boxes in their promoters and were found to be repressed by ZEB1. In line with the predicted function, expression of both miR-203 and miR-183 decreased the sphere-forming capacity of pancreatic cancer cells. Consistent with the recent work by Shimono et al. (2009) on breast cancer and normal stem cells, both miR-200 and members of the miR-183-96-182 cluster were also identified as regulating cancer stem cells in pancreatic cancer, suggesting a mechanism that is not restricted to one cancer type. BMI1 was confirmed as a major target of miR-200 regulating cancer stem cells. However, BMI1 was also found to contain seed matches for two other miRNAs in its 3′ UTR, and it was experimentally validated as a target for miR-200, miR-183, and miR-203. Remarkably, evidence was provided to suggest that other pluripotency factors such as Sox2 and Klf4 are also targets of miR-200, pointing at a complex network of multiple miRNAs that influence stem cell regulators, with ZEB1 at the center of multiple negative feedback loops (Figure 1). Although the Wellner study focused on ZEB1, it is likely that ZEB2 has similar activity, given that both ZEB1 and ZEB2 bind to E-boxes in the promoters of the two miR-200 gene clusters (Gregory et al., 2008b).

Not all human cancers contain cancer stem cells. However, the data on the parallels on the roles of miRNAs in normal tissue stem cells and cancer stem cells are consistent with the existence of cancer stem cells in breast, colon, and pancreatic cancer and point at widely overlapping signaling pathways that regulate differentiation and maintenance of both populations. In addition, this work has reinforced the notion that miRNAs often act as part of negative feedback loops. Thus, targeting a combination of protein and RNA components of these loops could open up novel therapeutic opportunities for many cancers that resist conventional treatment.

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TRRAP and the Maintenance of Stemness in Gliomas

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Resistance of brain tumors to therapy and their eventual recurrence is attributed to stem-like cells. In this issue of *Cell Stem Cell*, a high-throughput kinome-wide screen (Wurdak et al., 2010) has identified TRRAP, a kinase-related protein, as being required for stem cell character in gliomas.

The prognosis for treatment of glioblastomas remains dismal (a little more than 1 year), and research toward a better understanding of the biology of therapeutic resistance for this disease is actively ongoing. One proposed reason for the poor response to treatment is the existence of a small subpopulation of resistant cells within these tumors that survive treatment and repopulate the tumor. These resistant cells have properties similar to stem cells, and isolation of this subpopulation, referred to as brain tumor-initiating cells (BTICs) or brain tumor stem-like cells, was first achieved by utilizing the hematopoietic stem cell marker CD133 (Singh et al., 2004). In their study, the authors showed that this cell population from human glioblastoma samples could regenerate tumors with phenotypes comparable to those from the patient more efficiently than their CD133-negative counterparts. These stem-like cells contribute to the glioma's resistance to therapy (Bao et al., 2006). The data suggest that the stem cell-like properties of BTICs are important factors mediating tumor resistance and recurrence and that novel strategies that specifically target this population may be essential for improved therapeutic outcomes. Several signaling pathways including Notch, Shh, and PI3K are known to regulate the activity of normal neural stem cells (Stiles and Rowitch, 2008), implying that kinases regulate this phenotype. These signaling pathways similarly regulate the activity of BTICs and may represent one approach to directly target BTICs. The bone morphogenic proteins (BMPs) induce differentiation of neural stem cells into mature astrocytes and BMP4 induces differentiation of BTICs in gliomas (Piccirillo et al., 2006). By enforcing the differentiation of BTICs in this manner, one might predict that tumor recurrence would be less likely, because the stem cell properties of the tumor cells should be lost in the more mature progeny. This approach was successfully employed in the treatment of promyelocytic leukemia (Wang et al., 1998).

In this issue of *Cell Stem Cell*, a manuscript from the Schultz laboratory (Wurdak et al., 2010) provides further insight into this phenomenon and important experimental evidence to justify targeting of BTICs with differentiating factors. The authors used a phenotypic high-throughput shRNA library screen targeting the kinome to identify genes involved in the maintenance of "stemness" in gliomas, with BMP4 treatment as a positive control. The authors identified many expected kinases such as IGFR, as well as MAP and SRC family kinases. In addition, they identified TRRAP, an adaptor protein with homology to the PIKK kinases but lacking intrinsic kinase activity, as playing a role in the differentiation phenotype in glioblastoma stem-like cells. TRRAP is thought to regulate many biologic functions including chromatin remodeling, embryonic development, oncogenic transformation through c-Myc and E2F, as well as cell cycle progression. The authors report that knockdown of TRRAP in BTICs (grown as a monolayer on laminin) resulted in many phenotypic changes consistent with loss of stem cell characteristics (Figure 1) including decreased neurosphere formation in culture, decreased tumor formation upon transplantation into recipient mice, increased sensitivity to temozolomide and radiation therapy, and alteration of markers associated with differentiation such as loss of Nestin and Sox2 with gain of GFAP and TuJ1. The data imply that TRRAP loss depletes the stem-like pool of BTICs in vitro and mirrors the effects first reported for BMP4 (Piccirillo et al., 2006). Given the presented data on loss of function, one might expect overexpression of TRRAP to promote stem cell character and tumorigenic potential in these BTICs; in fact, TRRAP gene expression was elevated in the gliomas analyzed in this study.

The effects on proliferation and differentiation induced by TRRAP knockdown appeared to be mediated in part by suppression of transcriptional activity for the mitotic cyclin A2 with associated epigenetic modifications at the cyclin