MicroRNAs and Parallel Stem Cell Lives

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A new study by Shimono et al. (2009) demonstrates that certain microRNAs that regulate the self-renewal factor BMI1 are downregulated in purified populations of normal mammary epithelial stem cells and breast tumor-initiating cells. These findings have important implications for the regulation of self-renewal and differentiation by microRNAs and suggest new ways of targeting cancer stem cells.

Analyzing tumors may be akin to watching a baseball game from the last row of the bleachers. You easily perceive the hits and big defensive plays, but the essence of the dual between pitcher and batter, the heart of the game itself, is difficult to appreciate when you view the game from a distance. As well, the complex strategies used to get the batter out, involving a knowledge of the batter's weaknesses, and a repertoire of different pitches to prevent hits, may not be readily apparent. A new study in this issue of *Cell* by Mike Clarke and his team (Shimono et al., 2009) reinforces the notion that fresh molecular insights into cancer, at least for some important human tumors, may only come from purifying the cell subpopulations that lie at the heart of tumor growth. The yield of such an analysis is potentially great, as this study shows. Clarke and colleagues reveal the roles of three microRNA (miRNA) clusters in the regulation of self-renewal in both cancer stem cells and normal stem cells. Importantly, they show that the mRNA encoding BMI1, a critical promoter of stem cell self-renewal, is specifically targeted by this miRNA family cluster (Figure 1).

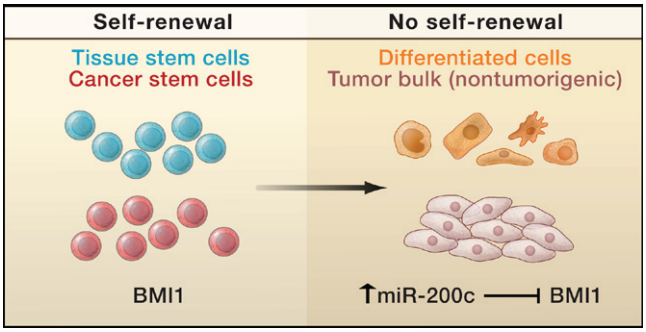

Figure 1. MicroRNAs Target BMI1 to Block Self-Renewal
MicroRNA-200c (miR-200c) shows decreased expression in breast cancer stem cells compared to cancer cells in the bulk tumor. Normal mammary epithelial stem cells show lower expression of miR-200c than differentiated cells. It is possible that miR-200c blocks stem cell self-renewal by targeting the 3′UTR of the self-renewal gene *BMI1*, resulting in loss of BMI1 protein,and attenuation of the ability of cancer stem cells to self-renew and form tumors.

The cancer stem cell hypothesis posits that only a subset of cells, endowed with stem cell functional properties that are not present in the majority of the tumor cells, drives clonal neoplastic growth. It is therefore evident that the molecular pathways operational in these cells must be unique from those in the bulk of the tumor, but such pathways will remain elusive unless these cells can be purified and studied separately from bulk tumor cells. Although not all human cancers may contain rare populations of tumorigenic cells, human breast cancer and genetically engineered mouse models of breast cancer do have relatively rare populations of tumorigenic cells that are responsible for tumor propagation (Al-Hajj et al., 2003; Cho et al., 2008). The emergence of miRNAs, as an additional cellular epigenetic mechanism and layer of control for gene expression (Ventura and Jacks, 2009), has spurred studies to investigate whether miRNAs play an important role in the cancer stem cell phenotype. The exquisite regulation that stem cells must exert to control self-renewal strongly suggests that miRNAs could provide an important additional level of molecular control. Parallels between cancer stem cells and normal stem cells are strong, and miRNA mechanisms could be predicted to be shared.

Following purification of breast cancer-initiating cells from human breast tumor samples, Clarke and colleagues used multiplex real-time PCR to study the expression of 460 miRNAs. After choosing a smaller subset of 37 miRNAs and testing them on a larger number of samples, three miRNA clusters were identified that had decreased expression in freshly isolated $CD44^{+}CD24^{-/low}$ breast cancer cells compared to cells in the tumor bulk. The same miRNA clusters were found to be downregulated in normal mammary epithelial stem cells, and similar seed sequences were shared by two of the three clusters. MicroRNA target prediction, using the tool Targetscan 4.2, identified the 3′UTR of BMI1 as the component linking the function of these miRNAs to molecular control of stem cell self-renewal.

In particular, one of the miRNAs, miR-200c, was shown to interact directly with the 3′UTR of BMI1, in a translation initiation reporter assay, and to decrease production of the BMI1 protein. Most importantly, overexpression of miR-200c was shown to have functional consequences for both normal stem cell and breast cancer stem cell function in vitro and in vivo, restraining clonogenic and tumor-initiating activities. Genetic loss of function of *BMI1* is well known to attenuate the self-renewal properties of normal stem cell and cancer stem cell populations.

These findings make several important contributions. First, they provide additional data showing the critical regulatory roles that miRNAs play in cancer biology (Ventura and Jacks, 2009) and, in particular, stem cell biology. Recently, in human embryonic stem cells, increased expression of miR-145 in clusters of these cells called embryoid bodies was shown to target the 3′UTRs of mRNAs encoding pluripotency factors, resulting in the promotion of differentiation and loss of self-renewal (Xu et al., 2009). In the new study, identical miRNA clusters are suppressed in both normal mammary epithelial stem cells and human breast tumor-initiating cells, demonstrating the close similarities in molecular regulatory controls that are used by stem cells and their malignant counterparts. Again, it is shown that stem cell regulatory pathways are the same as cancer stem cell regulatory pathways. Second, the new data demonstrate an important additional layer of regulatory control that is exerted on BMI1. As the miRNA clusters are located on three distinct chromosomal regions, the authors rightly point out the advantages of such an organization, providing layers of back up to restrain BMI1 activity in inappropriate cell populations.

As with many other miRNA studies and because this is a new and rapidly evolving field, many questions remain. Obviously, many other targets of these three miRNA clusters exist and the importance of these other targets in determining the effects of expression of the miRNA clusters remains to be defined. Mechanisms that regulate the expression of miRNAs during stem cell differentiation are not known. Also, functional data are shown only for one of the miRNA species, and it would be valuable to know the effectiveness of different members of the miR-200c cluster in suppressing *BMI1* translation or effecting *BMI1* mRNA degradation. Although the effects of miR-200c also are shown in a teratocarcinoma (Tera-2) cell line, it is likely that completely distinct milieus exist for miRNA expression and targeting in distinct cell types, and it is not clear whether targeting BMI1 is as important in these cell types. As well, the promotion of differentiation by miR-200c in Tera-2 cells may be a unique effect in this particular cell type. A role for miR-200c in the induction of differentiation in normal mammary epithelial stem cells is less clear, and it is not known whether miR-200c is actually required for the production of differentiated cell types or just blocks self-renewal.

The Shimono et al. study is the first to identify roles for specific miRNAs in parallel populations of stem cells and in their neoplastic counterparts. This field of research is likely to be a rich but complex arena for studying mechanisms of stem cell function, particularly in cancer stem cells. It will be especially interesting to determine whether miRNA genes are directly targeted, either alone or cooperatively, during cancer initiation in normal stem cells or progenitor cells, or whether they are altered later in the neoplastic process and so contribute to tumor progression. The new work also suggests that therapeutic approaches involving manipulation of miRNA expression may be another strategy to target cancer stem cells.

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Alpha Cells Beget Beta Cells

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Understanding the origins of insulin-producing beta cells of the pancreas could lead to new treatments for diabetes. Collombat et al. (2009) now show that in response to injury, a population of pancreatic progenitor cells can give rise to glucagon-expressing alpha cells that then transdifferentiate into beta cells.

Diabetes results from loss of the insulin-producing beta cells of the pancreatic islets. A clear identification of the progenitor cell population that gives rise to beta cells and an understanding of the factors and the cellular mechanisms that govern beta cell regeneration may lead to new and more effective treatments for diabetes. The paired homeobox transcription factor Pax4 has been implicated in the control of endocrine cell fate during pancreas development. Mice lacking Pax4 have diminished numbers of beta cells and instead accumulate alpha cells, a cell type that expresses the hormone