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Previews

Oncogenic miRNAs and the Perils of Losing Control of a Stem Cell's Epigenetic Identity

Piero Dalerba[1,*] and Michael F. Clarke[1,*]
[1]Stanford Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA 94305, USA
*Correspondence: pdalerba@stanford.edu (P.D.), mfclarke@stanford.edu (M.F.C.)
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Pathways that regulate epigenetic control of stem cell identity are critical to the molecular etiology of cancer. In back-to-back articles in *Cell* and *Cell Stem Cell*, Song et al. identify miR-22 as both a repressor of TET proteins and a powerful oncogene in the mammary epithelium and hematopoietic system.

One of the fundamental traits of malignant tumors is their capacity to grow indefinitely, beyond the natural limits usually observed for most normal, differentiated cells. This property, often referred to as immortality, was among the very first to be recognized by cellular oncologists and is regarded as a fundamental hallmark of the transformed phenotype (Hanahan and Weinberg, 2011). In the eye of the stem cell biologist, however, the capacity for extensive growth and expansion does not constitute, in and of itself, a pathological trait. By definition, stem cells are selectively endowed with the capacity to self-renew: they preserve intact their ability for long-term expansion over multiple rounds of sequential divisions, and serve as a constant source of mature cells throughout the lifetime of an organism. If considered from this perspective, therefore, immortality could be interpreted as a pathological form of self-renewal, in which the normal constraints that regulate stem cell expansion and ensure homeostatic control of tissue size have been disabled as the result of oncogenic mutations (Dalerba et al., 2007). Our ability to test this concept has been limited by an incomplete understanding of the basic molecular circuitry that defines the epigenetic identity of normal stem cells and the degree to which this circuitry is either mirrored or hijacked by cancer cells.

In an impressive tour de force that combines evidence from two independent studies (Song et al., 2013a, 2013b), Song and colleagues have now identified miR-22 to be both a new regulator of the self-renewal machinery and a powerful oncogene that directly targets multiple members of the "ten-eleven-translocation" (TET) protein family, a group of enzymes involved in DNA demethylation. In their first study (Song et al., 2013b), the authors examined the effects of constitutive miR-22 overexpression on the breast epithelium using transgenic mice engineered to achieve constitutive expression of miR-22 in mammary epithelial cells. Their results indicate that miR-22 overexpression causes epithelial cells to acquire biochemical features of the epithelial-to-mesenchymal transition (EMT), such as downregulation of E-cadherin and upregulation of Zeb1. In addition, miR-22 overexpression is associated with upregulation of the Bmi1 oncogene and an increase in the frequency of normal mammary stem/progenitor cells, which can reconstitute full mammary epithelial trees in transplantation assays. These changes are followed by spontaneous neoplastic transformation of normal mouse breast epithelia into metastatic breast carcinomas. When combined with additional oncogenic insults, such as when miR-22 transgenic mice are crossed with MMTV-PyVT or MMTV-neu transgenic mice, constitutive miR-22 overexpression accelerates both tumor progression and metastasis. Finally, high levels of miR-22 expression are associated with high-grade tumors and reduced survival in breast cancer patients.

In their second study (Song et al., 2013a), the authors used a similar transgenic approach to investigate the effects of constitutive miR-22 overexpression on mouse hematopoietic cells. In this second case, however, miR-22 overexpression was not specific to the hematopoietic system and the authors decided to test the effects of increased miR-22 dosage using transplantation assays. Constitutive miR-22 overexpression augmented the proliferative capacity of hematopoietic stem/progenitor cells (HSPCs), causing them to progressively outcompete their wild-type counterparts in cotransplantation experiments. When observed for longer periods of time, transplanted HSPCs overexpressing miR-22 gave rise to a disease reminiscent of a myelodisplastic syndrome (MDS), which subsequently progressed to full-blown acute myeloid leukemia (AML). As observed in the case of breast cancer, high levels of miR-22 expression were associated with reduced survival in human MDS patients.

In both the mammary epithelium and the hematopoietic system, the biological effects of miR-22 were mediated by its capacity to suppress expression of TET family members. TET proteins are DNA hydroxylases that convert 5-methylcytosine into 5-hydroxymethylcytosine to initiate DNA demethylation (Wu and Zhang, 2011). Indeed, genetic inactivation of TET proteins is known to disrupt the epigenetic remodeling that accompanies normal differentiation processes, and TET mutations are commonly observed in human hematological malignancies. Similar to constitutive miR-22 overexpression, genetic inactivation of *Tet2* in mice is associated with a numerical expansion of HSPCs and neoplastic transformation (Cimmino et al., 2011).

In a fascinating set of experiments, Song and collaborators also showed that constitutive miR-22 overexpression is associated with hypermethylation and epigenetic silencing of the miR-200c promoter. This is accompanied by upregulation of Bmi1, a key member of the *Polycomb group* (PcG) protein family and a core element of the self-renewal machinery in both hematopoietic and mammary epithelial stem cells (Park et al., 2003; Pietersen et al., 2008). These

findings are consistent with previous reports that identified human miR-200c as a direct repressor of BMI1, limiting the expansion and tumorigenicity of breast cancer cells (Shimono et al., 2009). Importantly, the effects of miR-22 on the expression of miR-200c and Bmi1 are mediated through a direct interaction of miR-22 with TET mRNAs and can be reproduced in a line of immortalized mammary epithelial cells by shRNA-mediated knockdown of TET2 and TET3. These observations provide fundamental mechanistic insights into developmental biology in that they explain how different arms of the molecular machinery that shapes the epigenetic identity of stem cells work together in an integrated system to control the capacity to self-renew. Members of the TET family act as initiators of DNA demethylation while Bmi1, a member of the *Polycomb* repressor complex 1 (PRC1), regulates chromatin remodeling through specific histone modifications such as ubiquitination of lysine-119 of histone-2A. Both systems oversee the coordinated regulation of multiple gene expression programs during differentiation. Learning how these epigenetic pathways interact is a fundamental step toward understanding how even relatively subtle genetic manipulations (e.g. the constitutive expression of one miRNA) can "ripple" into profound perturbations of stem cell homeostasis and cause cancer.

In our opinion, however, the most compelling finding that emerges from the aggregate work of Song and collaborators is that chromatin-remodeling systems with opposing effects on cell identity (self-renewal versus differentiation) appear to directly antagonize each other through opposing sets of miRNAs (e.g. miR-22 versus miR-200c). A series of theoretical questions thus arises. If chromatin-remodeling systems directly antagonize each other as part of a dynamic equilibrium between self-renewal and differentiation, what tilts the balance toward one fate or the other? Under physiological conditions, what makes changes in stem cell identity (i.e., differentiation) irreversible? The answer to these questions lies in a more advanced, systems-level understanding of these molecular circuitries and in a deeper characterization of their positive and negative feedback loops. For example, are members of the *Polycomb* family able to regulate miR-22 expression? If so, do they positively affect miR-22 expression, thus "locking" the stem cell identity in a self-reinforcing loop, or do they suppress it, thus "limiting" the stem cell identity in a cell-autonomous manner? The challenge for the future will be to develop new experimental approaches, and mathematical algorithms, to model the integrated action of these complex relationships and their impact on cell fate (Sahoo, 2012).

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Nanog Heterogeneity: Tilting at Windmills?

Austin Smith[1,*]
[1]Wellcome Trust-Medical Research Council, Cambridge Stem Cell Institute, University of Cambridge, UK
*Correspondence: austin.smith@cscr.cam.ac.uk
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Fluctuating expression of transcription factors in embryonic stem cells is an alluring observation, but, as outlined by two articles in this issue, appearances can be misleading.

Mouse embryonic stem cells (ESCs) closely approximate pluripotent embryo founder cells resident in the blastocyst. However, it is important to keep in mind that ESC propagation is a cell culture phenomenon. ESCs may be liberated from constraints imposed by the developmental program in vivo, but they are also subject to stimuli and conditions that do not occur in the embryo. Depending on the specific culture setting, ESCs exhibit different morphology, gene expression, epigenetic features, and self-renewal efficiency (Wray et al., 2010). Notably, ESCs on a feeder layer present as homogenous clusters of small, tightly packed cells, whereas without feeders and in the presence of leukemia inhibitory factor (LIF), ESCs are flattened and exhibit heterogeneous morphologies. A suite of transcription factors is expressed in a mosaic