A Web of Imprinting in Stem Cells

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Imprinted genes are the prototypical epigenetically regulated genes. On the basis of findings in adult lung stem cells, Zacharek et al. (2011) suggest in this issue of *Cell Stem Cell* that epigenetic silencing of imprinted genes is a common requirement for maintaining self-renewal in adult stem cell populations.

Maintenance of stem cells represents a balance between self-renewal and the need to provide precursor cells for differentiation and tissue homeostasis. Small pools of multipotential stem cells exist in many adult tissues for the continuous replacement of differentiated cells or for repair following injury. Epigenetic mechanisms are thought to be crucial in determining the transcriptional networks of multipotential stem cells and in holding at bay expression of the differentiation program. In a study published in this issue of *Cell Stem Cell*, Zacharek et al. (2011) investigate epigenetic silencing in an adult lung stem cell population, bronchioalveolar stem cells (BASCs), and suggest that the maintenance of stem cell character involves the repression of a network of imprinted genes.

The adult lung is thought to harbor a variety of multipotential stem cell populations with regional and functional specificity, although their precise identity continues to be a matter of debate (Fine, 2009). BASCs have been described as a stem cell pool of the distal lung that, in response to lung cell injury, gives rise to Clara cells, the progenitors of ciliated epithelial cells, and alveolar type 2 (AT2) cells, progenitors of the alveolar type 1 (AT1) cells that perform gas exchange (Kim et al., 2005). Previously, Kim and colleagues showed that BASC function depends upon the epigenetic modifier *Bmi1*: mice deficient in *Bmi1* show impaired expansion of BASCs in a model of lung adenocarcinoma and defects in self-renewal assayed in culture (Dovey et al., 2008). Bmi1 is a member of the Polycomb Repressor Complex 1 (PRC1), whose major function is the monoubiquination of the core histone H2A (at lysine residue 119), a modification associated with gene repression. PRC1 is thought to consolidate gene silencing, being largely dependent on prior activity of the PRC2 to trimethylate H3K27, which serves as a docking site for PRC1 (Sauvageau and Sauvageau, 2010). Bmi1 has a track record in repressing *Hox* genes and cell-cycle regulators such as $p16^{Ink4a}$ and $p19^{Arf}$ encoded at the *Cdkn2a* locus. *Cdkn2a* had been identified as a target of Bmi1 in BASCs, and correcting *Cdkn2a* overexpression in *Bmi1* mutant BASCs was able, at least partially, to correct their self-renewal defect (Dovey et al., 2008). In the current report, Zacharek and colleagues further identify *Cdkn1c*, which encodes the cell-cycle regulator $p57^{Kip2}$, as being controlled by Bmi1 in BASCs and as having a major role in self-renewal. Of particular interest is that *Cdkn1c* is an imprinted gene, which introduces an extra level of epigenetic regulation in the control of its expression.

Imprinted genes continue to possess an element of mystique. During mammalian evolution, these genes have attracted a specific form of regulation, wherein one copy (allele) is silenced according to parental origin (Ferguson-Smith, 2011). There are roughly equal numbers of paternally expressed imprinted genes (PEGs) and maternally expressed imprinted genes (PEGs) among the hundred or so imprinted genes so far characterized in the mouse or human genomes (with perhaps more to follow; Gregg et al., 2010). Imprinting of these genes comes about because of a decision taken in germ cells to epigenetically mark them, with distinct states of DNA methylation being established in the egg and sperm, and these marks are faithfully maintained in somatic cells after fertilization as a permanent memory of parental origin. Imprinted genes often reside in clusters and individual imprint marks instruct the monoallelic silencing of multiple imprinted genes in *cis*. Silencing is accomplished by a variety of mechanisms, including the action of long noncoding RNAs, recruitment of histone modifiers such as the PRC2 complex, and deposition of repressive histone modifications such as H3K27me3 (Ferguson-Smith, 2011). The role of the PRC1 complex in imprinted gene regulation has been much less explored. Imprinted genes are important in a variety of developmental and physiological processes in mammals, an overarching theme being the control of offspring growth (Ferguson-Smith, 2011). They act in common pathways, often with PEGs and MEGs fulfilling antagonistic functions. A number of imprinted genes have been implicated, like *Cdkn1c*, in controlling cell turnover and differentiation.

Using coimmunofluorescence, the authors show that $p57^{Kip2}$ undergoes a highly dynamic pattern of expression in the lung after injury. Exposure to naphthalene induces proliferation of BASCs and the proportion of cells expressing $p57^{Kip2}$ peaks five days after injury, specifically in BASCs and Clara cells. In lungs of *Bmi1*-deficient mice, $p57^{Kip2}$ expression is not appropriately downregulated from this peak. To demonstrate that persistent expression of $p57^{Kip2}$ was functionally involved in the self-renewal defect, BASCs from *Bmi1* mutant lungs were tested ex vivo for secondary colony formation. Mutant cells show very poor self-renewal, but could be rescued by restoring *Cdkn1c* mRNA expression to wild-type levels by shRNA knockdown. Intriguingly, knocking down *Cdkn1c* in wild-type BASC cultures also reduced their capacity for self-renewal, suggesting that optimal self-renewal depends upon an exquisitely controlled dose of *Cdkn1c* expression; imprinted

genes are considered to be highly dosage sensitive. The sustained expression of *Cdkn1c* in the absence of Bmi1 could reflect aberrant regulation of the gene or a defect in imprinting control. Experiments in primary embryonic fibroblasts depleted for Bmi1 showed that despite substantial upregulation imprinted expression of *Cdkn1c* remained intact. Whether *Cdkn1c* is a direct target of PRC1 remains to be definitively shown.

As a potent cell-cycle regulator necessary for correct development of several lineages, a role for *Cdkn1c* in stem cell maintenance may not be altogether unexpected. Strikingly, however, imprinted genes as a class were among the most highly deregulated genes in *Bmi1* mutant lung, including many with no previous hint of an involvement in cell-cycle regulation. Is deregulation of multiple imprinted genes in BASCs functionally significant? Zacharek and colleagues show that knocking down several imprinted genes, both PEGs and MEGs, singly or as pools, restores self-renewal in *Bmi1*-deficient BASC cultures; however, it is not known whether all these genes exhibit imprinted expression in BASCs. With their predominant role in growth control, many imprinted genes are collectively downregulated as the organism approaches full size (Lui et al., 2008), and this shared function and regulation underpin the concept of an "imprinted gene network" (Varrault et al., 2006). It appears that this coordinated developmental extinction, rather than imprinted expression, is controlled by Bmi1, because DNA methylation of imprint control regions was not altered by the absence of Bmi1. This would place Bmi1 high within the hierarchy of the imprinted gene network, and the authors report that deregulation of members of the network occurs in other cells lacking Bmi1, raising the possibility that the network operates in many adult stem cell populations. Reliance of adult stem cells on imprinted genes could also pose some vulnerability; given that these genes are normally monoallelically expressed, they have no back-up copy to safeguard against mutation. Equally, could loss of imprinting and consequent overexpression, which occurs in a number of imprinted gene disorders, many of which are characterized by growth and developmental abnormalities, impair adult stem cell self-renewal in other tissues and organs? On the other hand, a network is meant to provide resilience in the face of genetic and environmental change, so there could be compensatory changes if expression of one member of the network were perturbed. Detailed analysis of imprinted gene knockouts, singly and in combination, will be needed to test fully the significance of the network and how it operates in adult stem cells. Why should imprinted genes have a major role in adult stem cells? Considering that one of the drivers for the evolution of imprinting is thought to be parental genome conflict over control of offspring growth, a continued role in adult stem cells is not obvious, but could reflect an ancestral function of these genes that predated the imposition of imprinting. Alternatively, imprinted monoallelic expression may help to ensure tightly controlled dosage of expression necessary to maintain the balance between self-renewal and differentiation. Intriguingly, another recent report has identified a key role of the imprinted gene *Dlk1*, which encodes a member of the Notch/Delta/Serrate family of signaling molecules, in adult neurogenesis (Ferrón et al., 2011). In this case, *Dlk1* expression undergoes an epigenetic switch, such that there is a developmentally programmed loss of imprinting, associated with altered methylation of its imprint control region. This study, as well as that of Zacharek et al., should energize further analysis of the impact of specific imprinted genes and their deregulation in adult stem cell populations.

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