system is maintained by stem cells with long- and short-term regenerative potential, which are predominantly quiescent and resistant to injury, as well as committed multipotent, oligopotent, and unipotent progenitors, which have increased proliferative potential. Deciphering how environmental and physiological inputs regulate sensing pathways affecting slowly and rapidly cycling ISC populations in the niche during homeostasis and pathological states such as cancer and inflammatory bowel disease will be important directions for future studies.

REFERENCES


The Polycomb repressive complexes (PRC) regulate self-renewal and differentiation in embryonic stem cells (ESCs). In this issue of Cell Stem Cell, Morey et al. (2012) and O’Loghlen et al. (2012) report that dynamic interchange of PRC subunits modulates the balance between self-renewal and lineage commitment in ESCs.

Differentiate or self-renew? This is the principal question faced by all stem cells. The self-renewal of embryonic stem cells (ESCs) is maintained through expression of pluripotency genes and repression of lineage-specific genes. Conversely, differentiation is achieved through repression of the genes required for pluripotency, with simultaneous activation of a cascade of lineage-specific epigenetic and transcriptional changes. First identified in Drosophila, the Polycomb group (PcG) proteins are known regulators of ESC differentiation and do so by maintaining repressive chromatin states. The mammalian genome encodes multiple homologs of PcG components which broadly associate in two functionally distinct complexes, PRC1 and PRC2. PRC2 has been shown to functionally trimethylate lysine 27 on histone H3 (H3K27me3), while PRC1 monoubiquitylates histone H2A on lysine 119 (H2AK119Ub1) (Cao and Zhang, 2004; de Napoles et al., 2004). The prevailing dogma posits PRC2 and PRC1 work as a team to prevent transcription of genes that initiate differentiation. Mechanistically this is thought to occur via a PRC2-mediated deposition of H3K27me3 followed by H2AK119Ub1 catalyzed by PRC1 specifically at these sites. In pluripotent cells, PRC1/PRC2 co-occupy regions which overlap with H3K27me3, and a large proportion of these sites are proximal to genes involved in development and lineage commitment (Ku et al., 2008). Additionally, loss of function of either PRC1 or PRC2 in pluripotent cells does not affect expression of key pluripotency genes, but rather leads to derepression of genes normally upregulated during differentiation (Chamberlain et al., 2008; Leeb and Wutz, 2007).

Although PRC1 functionally targets PRC2 modified chromatin, it is unclear how PRC1 identifies sites of PRC2 catalyzed H3K27me3 and what regulatory mechanisms exist to facilitate derepression of PcG bound chromatin in response to ESC differentiation. Unlike PRC2, the PRC1 complex has been shown to contain a number of Polycomb orthologs (PCs) known as the Cbx family of proteins. Cbx proteins have been shown to interact directly with methylated histone H3 and are enriched at sites of heterochromatin (Bernstein et al., 2006). While PRC1 is functionally important for ESC self-renewal and differentiation, there has been no clear experimental evidence linking Cbx proteins with PRC1
target selectivity (H3K27me3) or regulation of PRC1 complexes in response to differentiation.

In this issue of Cell Stem Cell, reports from Morey et al. (2012) and O’Loghlen et al. (2012) have identified novel roles for several Cbx proteins in maintenance of mouse ESC (mESCs) self-renewal and regulation of differentiation. These studies begin to unravel how PcG complexes are functionally regulated during ESC differentiation and underscore the importance of Cbx proteins in targeting of PRC1 to H3K27me3 in both pluripotent and differentiating cells. Both Morey et al. and O’Loghlen et al. begin their respective studies by identifying Cbx7 as the primary Cbx component of PRC1 in pluripotent cells. The strength of this observation derives from the different methods by which each group arrives at this conclusion. Morey et al. utilized a comparative genome-wide chromatin-immunoprecipitation (ChIP-Seq) approach to assay the binding of PRC1, PRC2, and several methylated histones. Coupled with endogenous communoprecipitation (coIP), they demonstrate that a 97% of Cbx7 binding sites are co-occupied by PRC1, PRC2, of which 86% are also marked by H3K27me3. Several of these sites are associated with early developmental genes which are known to be repressed in pluripotent cells, including the HOX cluster. O’Loghlen et al. take a different approach, utilizing quantitative proteomics to identify proteins which interact directly with H3K27me3 in ESCs versus differentiated cells. In pluripotent cells, Cbx7 is the only Cbx protein found to associate with H3K27me3, while in differentiating cells and fibroblasts, Cbx2 and Cbx8 were the primary H3K27me3 interacting PCs, with no detectable Cbx7 interaction observed. This was the first indication that the Cbx protein composition of PRC1 complexes may be dynamically regulated in pluripotent versus differentiated cells, a phenomenon not previously observed for PC orthologs in pluripotent cells (Figure 1).

Further, ChIP with pluripotent cells revealed strong association of Cbx7/PRC1 localization to Cbx2, Cbx4, and Cbx8, concomitant with transcriptional repression of these genes (Morey et al., 2012, and O’Loghlen et al., 2012). Notably, both Cbx7 and PRC1 localization to chromatin is completely dependent on PRC2-mediated H3K27me3 (Morey et al., 2012). This experiment provides additional support to the idea that Cbx proteins “read” the epigenome to identify appropriate sites for PRC1-mediated deposition of H2AK119Ub1 in pluripotent cells. Transient and stably integrated RNAi-mediated knockdown of Cbx7 in ESCs (Morey et al., 2012; O’Loghlen et al., 2012) leads to increased expression of Cbx2, 4, and 8, spontaneous differentiation, and morphology defects. Conversely, overexpression of Cbx7 leads an increase in several phenotypes associated with self-renewal (O’Loghlen et al., 2012).

These data strongly suggest that the Cbx protein composition of the PRC1 complex may be dynamically altered in response to differentiation and that Cbx7-associated PRC1 is specific to maintenance of self-renewal. To test this possibility, both groups performed a combination of experiments to examine PRC1 composition and chromatin binding in ESCs differentiated into embryoid bodies (EBs). Morey et al. performed ChIP and coIP for Cbx2, Cbx4, and Cbx7, while O’Loghlen et al. examined localization of Cbx8 by ChIP. Both groups found that Cbx7 expression decreased as ESCs differentiated into EBs, while expression of Cbx2, 4, and 8 concomitantly increased. During differentiation into EBs, PRC1 complexes bound to chromatin were found to no longer contain Cbx7, but rather incorporated Cbx2 and
previously associated with shRNA-mediated knockdown of Cbx7 (Gil et al., 2004). Strikingly, when expressed in ESCs, miR-125 and miR-181 downregulated Cbx7 expression and promoted differentiation, providing direct evidence that miR regulation of PcG proteins is a determining factor in maintenance of self-renewal in pluripotent cells.

In their final experiment Morey et al. demonstrate that ESCs depleted of Cbx7, Cbx4, and Cbx2 can form teratomas in vivo. This was not entirely unexpected considering previous work has shown that teratomas can be formed even in the absence of PRC1 (Leeb et al., 2010). The authors assert that the teratoma formation is skewed toward the ectodermal lineage for grafts depleted of Cbx7 and the endodermal and mesodermal lineage for grafts depleted Cbx2 and Cbx4. Although this in vivo data is somewhat qualitative, it is consistent with expression data from Cbx7, Cbx2, and Cbx4 knockdowns. The role of the Cbx/PRC1 complex specificity as it relates to differentiation into the three germ layers needs to be further explored both in vitro and in vivo. Another interesting question not addressed in either paper is how PcG complexes are regulated when differentiated cells are returned to a pluripotent state using iPS technology. It would be interesting and informative to see if Cbx7/PRC1 complexes are reformed with a return to pluripotency and if these complexes function to transcriptionally re-repress Cbx2, 4, and 8. Nonetheless, these reports mark a significant step toward understanding how PcG proteins are regulated in ESCs and how this regulation helps modulate the fine balance between self-renewal and differentiation in pluripotent cells.

REFERENCES


Stem Cells Deployed for Bone Repair Hijacked by T Cells

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DOI 10.1016/j.stem.2011.12.010

Mesenchymal stem cells (MSCs) are a promising source for bone regeneration. Recently, in Nature Medicine, Liu et al. (2011) reported that host lymphocytes secrete IFN-γ and TNF-α to initiate apoptosis of transplanted MSCs and that aspirin can alleviate these effects to improve bone repair.

The skeletal system provides unique niches for maintaining a permanent hematopoietic stem cell (HSC) pool, from which all cells of the immune system derive, and for the differentiation and maturation of immune cells. Osteoblasts, which are derived from mesenchymal stem cells (MSCs), and cells of the myeloid lineages provide key factors for the HSC microenvironment during development and adulthood (Askmym et al., 2009). Lymphocytes are known to continuously migrate through the bone marrow but the precise physiology of this cell traffic is poorly understood. It is, however, well established that certain lymphocyte subsets such as long-lived memory T and B cells reside in specialized niches in the bone marrow (Tokoyoda et al., 2009). Defining cellular interactions between immune cells and the bone tissue in which they reside is an important goal of the newly emerging osteoimmunology field, and such insights may also be helpful for designing improved protocols for MSC-mediated bone repair and/or regeneration.