suggesting that the contribution of Stat3 in creating a poised state for reprogramming is achieved via regulation of additional yet-to-be-identified target genes. Future studies aimed at uncovering the molecular and biochemical nature of this primed intermediate state may be highly relevant for demystifying the black box of reprogramming.

Smith and colleagues (Yang et al., 2010) extended these findings further in different in vitro reprogramming experimental settings. Somatic neural precursor cells expressing the chimeric Gcsf receptor were generated and shown to be reprogrammed more efficiently after exogenous expression of Oct4 and Klf4 when in the presence of Gcsf. Similarly, the effect of Gcsf-induced Stat3 activation was analyzed in a partially reprogrammed intermediate cell line derived from embryonic fibroblasts (Silva et al., 2008). This intermediate line has not reactivated endogenous pluripotency genes nor silenced the Oct4, Sox2, Klf4, and c-Myc transgenes. Cooperative action of Gcsf and 2i accelerated the kinetics and yielded an 8-fold increase in the efficiency of generating Oct4-GFP$^+$ cells from the partially reprogrammed iPSC line. Naive pluripotent cells derived throughout the study shared traits that define naive mouse ESCs, including gene expression patterns, cell signaling dependence, activation of both X chromosomes in female cell lines, and competence to generate high-contribution chimeric animals (Nichols and Smith, 2009). Notably, although Stat3 activation dramatically facilitated the induction of pluripotency in the different experimental settings applied, Stat3 signaling was not found to be a "roadblock" for this process, because iPSCs did arise, albeit at reduced efficiency, even when this signaling pathway was actively inhibited via specific small molecules.

The findings reported by the Smith group (Yang et al., 2010) provide conclusive evidence that exogenous signaling stimuli and paracrine factors play a direct role in positively or negatively regulating the induction of murine naive pluripotency (Figure 1). Further, these results corroborate recent reports that characterized the involvement of different signaling pathways in regulating ESC growth and iPSC reprogramming (Hanna et al., 2009a; Silva et al., 2008) and collectively underscore striking redundancy and similarities between the determinants involved in (1) maintaining naive pluripotent cells in vitro (ESCs or iPSCs) and inducing naive pluripotency (2) in somatic cells or (3) in primed EpiSCs (Figure 1) and provocatively insinuate that the molecular pathways and rate limiting step underlying these processes (Hanna et al., 2009b) may be highly similar, if not identical.

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Hypoxia Signaling in Hematopoietic Stem Cells: A Double-Edged Sword

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DOI 10.1016/j.stem.2010.08.006

Although hematopoietic stem cells (HSCs) reside in hypoxic niches, the significance of hypoxia signaling in HSCs remains unclear. In this issue of *Cell Stem Cell*, Takubo et al. (2010) and Simsek et al. (2010) demonstrate that hypoxia regulates the metabolic state of HSCs and protects their integrity by controlling HIF-1α.

Multipotent hematopoietic stem cells (HSCs) reside in niches within the bone marrow (BM) and have a unique capacity to sustain life-long multilineage hematopoiesis (Orkin and Zon, 2008). HSCs face tightly orchestrated cell fate decisions between quiescence, self-renewal, apoptosis, and differentiation. Although the precise integrated mechanisms that underlie HSC fate decisions are poorly understood, it is generally accepted that their choices are regulated by both

intrinsic factors and extrinsic cues provided by their niches. Recent studies have suggested that the microenvironment harboring HSCs exhibits low oxygen levels (Parmar et al., 2007). These findings raise some fundamental questions: how do HSCs adapt to and utilize chronic local hypoxia and how might these processes impact HSC fate decisions? In this issue of *Cell Stem Cell*, two groups use complementary approaches to provide important insights into the role of hypoxia and its signaling pathways in regulating HSC functions (Simsek et al., 2010; Takubo et al., 2010).

Cellular responses to hypoxia are mediated by hypoxia-inducible factors (HIFs), which regulate gene expression in order to facilitate an adaptation to the hypoxic conditions (Kaelin and Ratcliffe, 2008). Simsek et al. (2010) demonstrate that adult HSCs have elevated levels of HIF-1α (an alpha subunit of HIF-1) and increased expression of hypoxia-inducible genes, including those controlling glycolysis. As a consequence, HSCs alter their metabolism and exhibit increased rates of glucose consumption and lactate production and decreased rates of mitochondrial oxidative phosphorylation and oxygen consumption compared to mature BM cells. This study therefore provides evidence that HSCs adapt to the hypoxic microenvironment within stem cell niches by utilizing glycolysis instead of mitochondrial oxidative phosphorylation. Whether or not a functional Krebs cycle is required for normal HSC functions remains unclear. This question could be addressed by using existing mouse models that harbor mutations in mitochondrial enzymes.

In the second paper, Takubo et al. (2010) conditionally delete *HIF-1α* and observe a loss of quiescence specifically in the HSC compartment. *HIF-1α*-deficient HSCs have an increased cell cycling rate and show progressive loss of long-term repopulation capacity in serial transplantation assays. Interestingly, although HIF-1α is critical for HSC maintenance under conditions of hematopoietic stress (i.e., transplantation or myelosuppression), under physiological conditions, *HIF-1α*-deficient HSCs sustain hematopoiesis for a prolonged period. It would be of interest to investigate mechanisms through which HSCs lacking *HIF-1α*, an essential regulator of metabolic adaptation to hypoxia, survive and function under hypoxic conditions before they eventually lose their activity.

Takubo et al. also demonstrate that monoallelic deletion of Von Hippel-Lindau (VHL) protein, an E3 ubiquitin ligase that normally mediates HIF-1α degradation (Kaelin and Ratcliffe, 2008), and the consequent HIF-1α stabilization increases the fraction of quiescent HSCs and results in their expansion in transplanted recipients. Paradoxically, ablation of both alleles of *VHL* and overstabilization of HIF-1α enhances quiescence of HSCs, but instead of HSC expansion it leads to a striking loss of HSC activity upon transplantation. These experiments therefore indicated that levels of HIF-1α that are either too low or too high are deleterious for HSC functions. Collectively, Takubo et al. provide genetic evidence that HIF-1α is an essential regulator of HSC quiescence and that strict regulation of HIF-1α is pivotal for the long-term maintenance of HSCs.

Although little is known about the regulation of HIF-1α functions in stem cells, it is becoming clear that HIF-1α is kept in check at multiple levels. Takubo et al. elegantly show that HSCs residing within the hypoxic zone of the BM maintain intracellular hypoxia and stabilize HIF-1α. It is likely that the stabilization of HIF-1α in vivo is caused by local hypoxia and cytokine signaling, because both of these stimuli stabilize the HIF-1α protein. Simsek et al. shed light on the transcription of *HIF-1α* in HSCs by demonstrating that the homeodomain protein Meis1, which is essential for hematopoiesis (Hisa et al., 2004), directly activates *HIF-1α* expression. It will be of interest to investigate whether hematopoietic defects observed in *Meis1*-deficient mice, such as the competitive disadvantage of *Meis1* mutant HSCs, are mediated by defective *HIF-1α* expression. Finally, the regulation of the transcriptional activity of HIF-1α may also play critical roles in HSC maintenance. For example, we showed that conditional deletion of *Cited2*, a negative regulator of HIF-1α-mediated transcription, results in dramatic loss of HSCs (Kranc et al., 2009). The findings by Takubo et al. showing that enhanced HIF-1α function (observed in HSCs lacking both *VHL* alleles) has a detrimental impact on the HSC pool and can, at least in part, explain the phenotype observed in *Cited2*-deficient HSCs. Further studies are clearly required to provide insights into HIF-1α regulation in HSCs.

The identification of hypoxia and the HIF-1 system as major regulators of HSCs provides important insights into HSC functions. In the emerging model, quiescent HSCs residing in hypoxic niches sense the hypoxic microenvironment and stabilize a key regulator of stem cell maintenance, HIF-1, which activates transcription of target genes including those required for the adaptation to glycolysis as the main source of energy. Consistent with this hypothesis, hypoxic in vitro conditions maintain the quiescent phenotype of mouse HSCs (Eliasson et al., 2010). Although HSCs require a strict regulation of HIF-1α to sustain quiescence, the mechanisms through which HIF-1α promotes quiescence remain unexplained. Hypoxia-induced quiescence may be a consequence of slow metabolic rates in HSCs. Alternatively, HIF-1 may activate the expression of genes controlling HSC quiescence. In fact, a direct target gene of HIF-1, *Foxo3a*, is essential for the quiescent phenotype of HSCs (Miyamoto et al., 2007). Furthermore, studies in other systems have revealed that in addition to genes regulating energy metabolism, HIF-1 controls transcription of more than 100 genes involved in multiple cellular processes including the cell cycle, survival, differentiation, and autophagy (Kaelin and Ratcliffe, 2008), suggesting that hypoxia signaling may also be critical for HSC fates other than quiescence.

Hypoxia signaling may be an important and general feature of other stem cell niches. Indeed, it is proposed that HIF promotes the generation of cancer stem cells and regulates dedifferentiation and self-renewal. In this context, HIF has been shown to activate Notch signaling and to regulate Oct4 and c-Myc (Mohyeldin et al., 2010). These processes may also be relevant to cancers in individuals harboring mutations that inactivate the Krebs cycle enzymes, where, similar to the HSC metabolism described by Simsek et al., HIF and glycolytic metabolism are constitutively active.

We are only beginning to understand the role of hypoxia and its signaling pathways in stem cell functions. Considerable effort needs to be invested in

addressing important questions surrounding stem cell niches and the impact of their hypoxic microenvironment on HSC fate decisions in vivo. Are all quiescent BM-resident HSCs localized in a hypoxic microenvironment? How do different levels of HSC oxygenation (e.g., during HSC mobilization from the BM to the peripheral blood) impact on their functions? Which stem cell fates does hypoxia promote? What are the key HIF target genes essential for the maintenance of the HSC pool? The findings presented in this issue of *Cell Stem Cell* set the stage for further investigations in this fascinating and expanding field.

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