Hematopoietic Stem Cell Quiescence: Yet Another Role for p53

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p53, sometimes referred to as the "guardian of the genome," helps regulate cell-cycle arrest, DNA-damage repair, apoptosis, and senescence. Adding to this list, in this issue of *Cell Stem Cell*, Liu et al. (2009) show that p53 also plays a role in regulating hematopoietic stem cell quiescence.

The tumor suppressor p53 is a key transcription factor that functions at the convergence of several signaling pathways involved in the cellular stress response. Upon stress-induced activation, p53 accumulates and mediates the expression of target genes designed to protect the genetic integrity of the cell. These protection mechanisms include the arrest of cell-cycle progression at the G_1/S checkpoint (mediated by p21), activation of DNA repair machinery, induction of apoptotic cell death, or initiation of senescence or differentiation programs.

Now, it appears that p53 has yet another important function that is independent of its role as a regulator of the stress response. In this issue of *Cell Stem Cell*, Liu and colleagues (2009) show that p53 is essential for maintaining hematopoietic stem cell (HSC) quiescence during steady-state hematopoiesis. Hematopoiesis is critically dependent on the ability of at least a subset of HSCs to maintain a quiescent state, which confers resistance to radiation, cytotoxic insults, and oxidative stress and ensures that the population is sufficiently long-lived to maintain lifelong blood cell production. While several transcription factors have already been identified as regulators of HSC quiescence, including HoxB4, STAT5, Gfi-1, SMAD4, c-*myc*, and Mef (reviewed in Zon, 2008), p53 was not known to be involved. Liu and colleagues (2009) show that p53-deficient mice have an increased HSC pool size, consistent with a previous report (TeKippe et al., 2003), but a decreased proportion of that pool exhibits quiescence. In addition, the authors demonstrate that p53 mediates HSC quiescence independently of p21, and they identified *Gfi-1* and *Necdin* as alternate, direct targets of p53 in this context. Gfi-1 has been previously reported to mediate HSC quiescence (Hock et al., 2004), and although Necdin has been shown to be a negative cell-cycle regulator in postmitotic neurons (Yoshikawa, 2000), it has not been previously associated with HSC homeostasis.

In an earlier study, also reported by the Nimer group, *Mef* deficiency was shown to lead to increased HSC quiescence and enhanced self-renewal and conferred a competitive advantage upon HSC transplantation (Lacorazza et al., 2006). In the current report, the authors demonstrate that the HSC quiescence phenotype induced by *Mef* deficiency requires p53 but that the enhanced self-renewal phenotype and competitive transplant advantage exhibited by *Mef* knockout cells are p53 independent. Collectively, the results suggest that the role of p53 in HSC quiescence is independent of its classical role conducted in response to cellular stress (Figure 1).

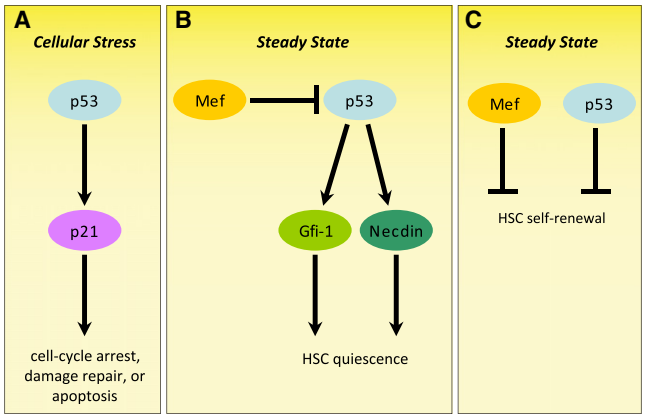

Figure 1. Independent Roles of p53 in the Cellular Stress Response, HSC Quiescence, and HSC Self-Renewal
(A) The cellular stress response of p53 is mediated by p21 and can lead to cell-cycle arrest, DNA-damage repair, cellular senescence, or apoptosis.
(B) Although quiescence and self-renewal are related processes, the data presented by Liu et al. suggest that they are (at least partly) independently regulated by Mef and p53. It should be noted that increased self-renewal was measured as an increase in the number of (transplantable) stem cells and not as an increased number of stem cells generated by division of a single stem cell. Concerning quiescence, Mef acts to inhibit the action of p53 on *Gfi-1* and *necdin*, which normally help maintain quiescence.
(C) Concerning self-renewal or the size of the stem cell pool, Mef and p53 exert independent functions through unknown intermediates.

Quiescence and self-renewal are two of the most essential characteristics of HSCs. Therefore, it is somewhat puzzling why *Mef*-deficient HSCs exhibit increased self-renewal in combination with an elevated proportion of quiescent cells, while *p53*-deficient mice display increased self-renewal and low levels of quiescence. Further, without the ability to assay HSC function after serial transplantation, it remains possible that increased self-renewal as reported by Liu et al. and Lacorazza et al. is merely a reflection of an increase in the number of stem cells and not an increased repopulation potential per (individual) stem cell. Therefore, the expansion

of HSCs observed in *p53*-deficient mice, taken in context of the corresponding decrease in the quiescent fraction, could indicate that HSC function would be exhausted after multiple rounds of stress-induced expansion. Unfortunately, the tumorigenicity of *p53*-deficient cells makes this a difficult, if not impossible, hypothesis to test. Nonetheless, the varied phenotypes observed between the single- and double-mutant HSCs suggest that both Mef and p53 are involved in regulating HSC expansion as well as quiescence, but whether or not downstream targets Gfi-1, Necdin, or p21 are involved in the regulation of self-renewal remains unclear. Downmodulation of Necdin diminished the quiescence-inducing effect in *Mef* null mice, but data on self-renewal are not available. These results combined with the preferential expression of *Necdin* by primitive stem cells also identify Necdin as an important protein in hematopoietic stem cell regulation.

The mechanism by which Mef regulates p53 is unclear. Possibilities include a direct effect on *p53* expression levels, an alteration of p53 stability or activation state, or even effects on p53 coregulators such as Mdm2. Since the authors have found that *p53* is upregulated in *Mef*-deficient fibroblasts, the first option seems plausible, but the authors did not report on the relative *p53* expression levels in *Mef*-deficient HSCs, so this question remains unresolved. Liu et al. also show convincingly that the level of quiescence determines HSC radiation sensitivity and that the increased radiation resistance observed in Mef-deficient HSC is not dependent on p53/p21. Curiously, the sensitivity of double-knockout HSC to radiation seems to be even higher than expected from the single knockouts (see Figures 4C and 4D in the paper). This result would not be predicted by the simple model described in Figure 1 and suggests that additional mechanisms may be involved.

The relationship between reversible stem cell quiescence and irreversible stem cell senescence, and how this balance changes during aging, is an area of intense interest. Constitutive expression of *p53* has been associated with premature aging (Tyner et al., 2002). Since p53 is a downstream target of Mef, it is expected to be continuously activated in *Mef* null mice. Therefore, one may wonder how HSC aging might be affected in the case of Mef deficiency, in either the absence or presence of p53. It seems possible that the large pool of quiescent stem cells that accumulates in the absence of Mef could overcome the decline in HSC function observed during the aging process. However, the larger pool of HSCs combined with their higher self-renewal capacity might also provide fertile ground for the development of leukemia. This hypothesis is supported by the observation that *Mef* is expressed in a significant proportion of ovarian carcinomas, and in ovarian cancer cell lines, but not in normal ovarian surface epithelium. (Yao et al., 2007). Therefore, *Mef* might be classified as a (proto)oncogene. A molecular balance between self-renewal and proliferation may prevent cancer, but signaling pathways that regulate normal stem-cell self-renewal may cause neoplastic proliferation when not properly regulated (reviewed by Pardal et al., 2003). During aging, maintenance of HSC quiescence may be impaired by unfaithful replication of epigenetic marks, resulting in aberrant gene expression. Genes involved in regulation of such epigenetic marks, including *BMI-1*, *Ezh2*, *Mel-18*, *Suz12*, and *Rae28*, have all been shown to affect stem cell maintenance and self-renewal (reviewed in Zon, 2008). Aberrant gene expression of these and other genes may directly lead to transformation or may indirectly induce low levels of cellular damage, which in turn may upregulate genes such as *p16* but also *p53*. Unfortunately, a role of p53 in stem cell aging is likely to remain obscured by its well-known function as a tumor suppressor gene.

Finally, with the discovery of p53 as yet another protein involved in HSC maintenance, the commonly used statement that the mechanisms involved in stem cell regulation remain unknown no longer seems appropriate or fully accurate. In reality, the list of genes involved in HSC regulation is ever expanding, and each new discovery contributes to a growing appreciation for the complexity of the system. Some of these candidate genes seem to affect self-renewal and others quiescence, two related and vital characteristics of HSC. Interestingly, *Mef* is not a stereotypical stem cell gene, as it is expressed at very low levels in HSCs. However, the low expression of Mef in HSCs compared to more differentiated cell types is in line with its proposed role as an inhibitor of p53-induced quiescence during steady-state hematopoiesis (Figure 1). The paper by Liu and colleagues identifies *p53* as an important player in the maze of genes involved in regulation of HSC self-renewal and quiescence. Undoubtedly, more genes will be identified, and further research should be directed to investigate molecular interactions between the different gene products to uncover the full regulatory network involved in HSC regulation.

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