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p53 Regulates Hematopoietic Stem Cell Quiescence

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SUMMARY

The importance of the p53 protein in the cellular response to DNA damage is well known, but its function during steady-state hematopoiesis has not been established. We have defined a critical role of p53 in regulating hematopoietic stem cell quiescence, especially in promoting the enhanced quiescence seen in HSCs that lack the MEF/ELF4 transcription factor. Transcription profiling of HSCs isolated from wild-type and p53 null mice identified *Gfi-1* and *Necdin* as p53 target genes, and using lentiviral vectors to upregulate or knockdown the expression of these genes, we show their importance in regulating HSC quiescence. Establishing the role of p53 (and its target genes) in controlling the cell-cycle entry of HSCs may lead to therapeutic strategies capable of eliminating quiescent cancer (stem) cells.

INTRODUCTION

Hematopoietic stem cells (HSCs) can remain quiescent or they can enter the cell cycle and either self-renew or differentiate into multiple lineages. Although relatively quiescent, the HSC population must give rise to a hierarchy of differentiating progenitor cell populations that can replenish the blood system each day (Attar and Scadden, 2004). Blood cell production must also respond efficiently to hematologic stresses, such as blood loss, infection, or exposure to cytotoxic agents, via expansion of the HSC and/or progenitor cell populations (Passegué et al., 2005). These processes must occur without depleting the stem cell pool (Venezia et al., 2004; Forsberg et al., 2005).

HSC quiescence is likely controlled by both HSC-intrinsic mechanisms and bone marrow microenvironmental factors (Wilson and Trumpp, 2006). Several transcription factors have been shown to play key roles in HSC-fate decisions. Gfi-1 has been shown to restrict HSC proliferation and preserve HSC functional integrity (Hock et al., 2004; Zeng et al., 2004). HOXB4 and GATA-2 regulate HSC self-renewal (Krosl et al., 2003; Ling et al., 2004), whereas the Ets transcription factor MEF/ELF4 regulates both HSC self-renewal and quiescence (Lacorazza et al., 2006). Mef null mice exhibit greater numbers of HSCs (i.e., LSK cells), and Mef null LSK cells are more quiescent than normal. Early studies suggested a role for p21 in restricting HSC entry into the cell cycle and regulating HSC pool size and HSC exhaustion under stress (Cheng et al., 2000). However, the regulation of HSC self-renewal by p21 under steady-state conditions may be minimal (van Os et al., 2007). Several studies have implicated both the Ang-1/Tie2 and the thrombopoietin/MPL signaling pathways in maintaining HSC quiescence (Arai et al., 2004; Yoshihara et al., 2007; Qian et al., 2007).

The p53 tumor suppressor gene may regulate various aspects of hematopoietic cell behavior (Wlodarski et al., 1998; TeKippe et al., 2003; Chen et al., 2008; Akala et al., 2008). Although hematopoiesis in p53 knockout mice appears to proceed normally, numerous studies have identified roles for p53 in the proliferation, differentiation, apoptosis, and aging of hematopoietic cells (Shounan et al., 1996; Shaulsky et al., 1991; Kastan et al., 1991; Lotem and Sachs, 1993; Guzman et al., 2002; Park et al., 2003; Dumble et al., 2007). Moreover, p53 deletions and mutations have been found at high frequency in blast crisis chronic myelogenous leukemia and with some frequency in acute leukemia (Prokocimer and Rotter, 1994). In response to DNA damage, p53 can either elicit cell-cycle arrest or apoptosis, the typical outcome for mature hematopoietic cells (Wu et al., 2005). But p53 has recently been shown to negatively regulate neural stem cell proliferation and self-renewal (Meletis et al., 2006), and given that long-term reconstituting HSCs (LT-HSC) express high levels of p53 transcripts (Forsberg et al., 2005 and our data, see below), we examined the function of p53 during steady-state hematopoiesis. We find an important interdependency between MEF/ELF4 and p53 on HSC quiescence and identify two p53 target genes, *Gfi-1* and *Necdin*, that regulate quiescence in wild-type (and Mef null) HSCs. Our findings identify distinct roles for p53 in resting versus cycling cells.

RESULTS

Maintaining HSC Quiescence by p53

We have recently found that Mef/Elf4 null mouse embryonic fibroblasts (mefs) accumulate p53 protein and undergo premature senescence, which appears to be due to the ability of Mef to directly upregulate Mdm2 expression (G.S., Y.L., S.E.E., Y.M., K. Ohyashiki, S.M., S.D.N., unpublished data). We hypothesized that p53 could play a role in the enhanced stem cell quiescence (or the increased HSC frequency) seen in Mef null mice, so we examined p53 mRNA expression in primitive $Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$ (LSK) cells and in various myeloid progenitor cells (CMP, GMP, and MEP). p53 is most highly expressed in LSK cells (Figure S1 available online), suggesting that it could play an important role in HSC physiology.

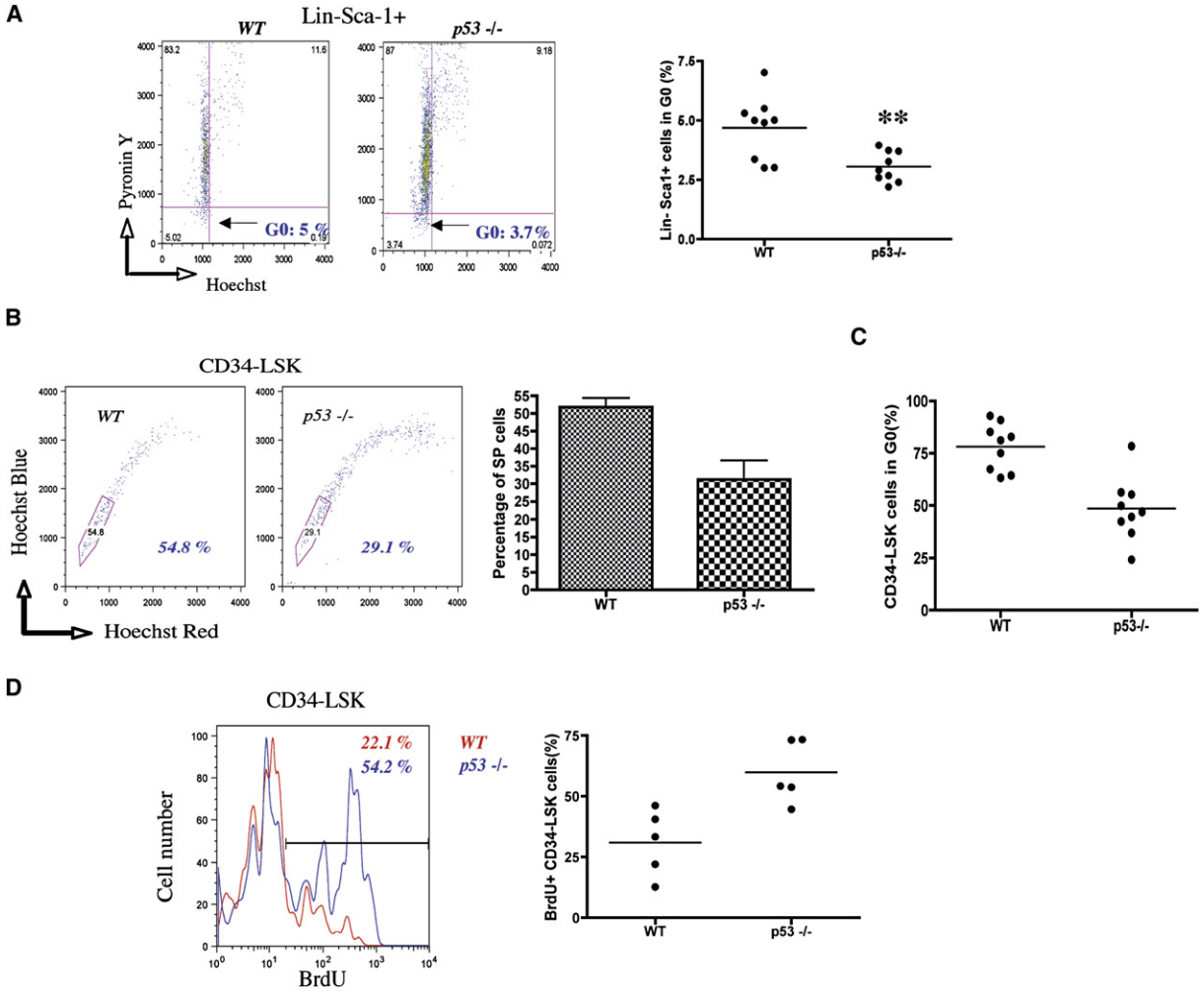

Figure 1. Maintenance of HSC Quiescence by p53

(A) Multicolor flow cytometry was used to determine the percentage of hematopoietic stem/progenitor cells ($Lin^-Sca\text{-}1^+$) in the G0 phase of the cell cycle (defined as cells with low Pyronin Y content that contain 2 n DNA [G0/G1]). Total bone marrow cells from wild-type and $p53^{-/-}$ mice were stained with Pyronin Y and Hoechst 33342. One representative experiment is shown on the left. The graph on the right indicates the mean percentage (±SD) of G0 cells present ($p < 0.005$, $n = 9$).

(B) Side population (SP) cells ($CD34^-$LSKs) from wild-type and $p53^{-/-}$ mice were identified by Hoechst 33342 staining and the use of blue and red filters. The bar graph on the right indicates the mean percentage (±SD) of SP cells present ($p < 0.003$, $n = 7$).

(C) Cell-cycle analysis of $CD34^-$LSK cells was performed by staining with Hoechst 33342 and Ki67 and analyzed by FACS. Data shown are the mean values ± SD ($p < 0.0001$, $n = 9$).

(D) The proliferation of $CD34^-$LSK cells was measured by in vivo BrdU incorporation over 48 hr. Greater proliferation of $p53^{-/-}$ $CD34^-$LSK cells was seen (60% versus 30% for wild-type $CD34^-$LSK cells; $p < 0.009$, $n = 5$).

We then examined the cell-cycle status of $p53^{-/-}$ $Lin^-Sca\text{-}1^+$ cells using Pyronin Y and Hoechst 33342 staining and observed a reduction of Pyronin Y^{low} cells, indicating the presence of fewer quiescent HSCs ($p < 0.005$, Figure 1A). Using the SP phenotype as a marker for quiescent HSCs in adult bone marrow (Goodell et al., 1996; Arai et al., 2004), we also found a 2-fold decrease in the frequency of $CD34^-$LSK SP cells in the absence of p53 ($p < 0.003$, Figure 1B). Staining of p53 null $CD34^-$LSK cells with the proliferation marker Ki67 also showed enhanced HSC proliferation with fewer quiescent cells present ($p < 0.001$, Figure 1C). Furthermore, to determine the proliferative rate of $p53^{-/-}$ $CD34^-$LSK cells in vivo, we administered BrdU to mice orally for 2 days and isolated $CD34^-$LSK cells from the bone marrow. While ~30% of wild-type $CD34^-$LSK cells incorporated BrdU over this period, ~60% of the $p53^{-/-}$ $CD34^-$LSK cells were BrdU positive (Figure 1D, $p < 0.01$; and Figure S2). These data suggest that p53 promotes HSC quiescence, and in its absence, HSCs more easily enter the cell cycle.

Role of p53 in HSCs of $Mef^{-/-}$ Mice

Given the HSC phenotype of the p53 null mice and the upregulation of p53 in Mef null fibroblasts, we generated $p53^{-/-}Mef^{-/-}$ mice to determine both the frequency and the cell-cycle status of the hematopoietic stem (and progenitor) cells. Loss of MEF alone, p53 alone, or both MEF and p53 increased the frequency of LSK cells (2- to 3-fold) compared to wild-type mice (Figure 2A).