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Making Healthy Stem Cells: The New Role of TPO

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Thrombopoietin (TPO) attracts much attention as an effective stimulus for blood cell formation in patients with hematopoietic disorders. In this issue of *Cell Stem Cell*, de Laval et al. (2013) show that TPO can also promote "healthy" hematopoietic stem cells when administered before radiotherapy to minimize HSC injury and mutagenesis in mice.

Thrombopoietin (TPO) has a well-described role as a principal regulator of platelet production, which is stimulated through binding to its receptor Mpl on megakaryocytes. More recently, studies have revealed important roles for TPO/Mpl signaling in hematopoietic stem cells (HSCs) as well (reviewed in Chou and Mulloy, 2011). Mpl is expressed in HSCs and progenitors, and TPO cooperates with other cytokines to promote expansion of HSCs in culture. Mice deficient in TPO and Mpl signaling have reduced numbers of HSCs with impaired repopulating ability as well as the defective platelet formation one would expect. Clinically, inactivating mutations of Mpl in humans cause thrombocytopenia and multilineage marrow failure, while activating Mpl mutations are involved in myeloproliferative disorders. These observations demonstrate an important role of TPO/Mpl signaling in HSCs and progenitors. As a mechanism for TPO-mediated HSC regulation, it was shown that TPO regulates HSC quiescence and interaction with the osteoblastic niche (Qian et al., 2007; Yoshihara et al., 2007).

Cells in the human body are continually exposed to DNA stresses. Physiological stresses as well as environmental agents, such as ionizing radiation (IR) and other genotoxic chemicals, induce DNA damage including double-strand breaks (DSBs). DNA repair is essential for cell survival, and the long lifespan of HSCs suggests that they need an effective DNA repair process to maintain a "healthy" state. DNA damage is repaired through two main pathways: homologous recombination (HR) and nonhomologous end joining (NHEJ). Recent data showed that quiescent HSCs preferentially use NHEJ repair mechanism, and DSB repair through NHEJ is necessary for HSC maintenance (Mohrin et al., 2010; Nijnik et al., 2007; Rossi et al., 2007).

In this issue of *Cell Stem Cell*, de Laval et al. (2013) discovered a new function of TPO in DNA damage response in HSCs and progenitors. They found that Mpl-deficient HSCs and progenitors had significantly increased numbers of γH2AX foci (a marker of DSB formation) after exposure to IR or topoisomerase-II inhibitors relative to wild-type cells. A neutral comet assay (another technique for the detection of DSBs) also showed IR-induced DNA damage was greatly enhanced in Mpl-deficient cells. Consistent with these findings, HSCs treated in the absence of TPO showed similar DSB repair defects and, conversely, TPO injection into mice just before IR reduced the number of γH2AX in HSCs in vivo. No differences in cell cycle and apoptotic status of HSCs were apparent under these experimental settings, implying the TPO/Mpl signaling was affecting DNA damage in HSCs through a direct effect on the DNA repair process. Other hematopoietic cytokines such as SCF and FLT3 ligand did not show such effects, suggesting that the DNA repair activity is a specific function of TPO. de Laval et al. (2013) found that TPO increased phosphorylation of the DNA-PK catalytic subunit (a central player in NHEJ), and pharmacological or genetic inhibition of DNA-PK abolished TPO-mediated DNA repair. TPO did not increase IR-induced Rad51 foci formation nor did it improve repair of DSBs induced by replicative stress, indicating HR was not involved in this DSB repair. Taken together, these results indicate that TPO/Mpl promotes NHEJ-mediated DNA repair by stimulating DNA-PK activity in HSCs. Importantly, TPO treatment before IR limits IR-induced HSC injury and increases HSC function for long-term hematopoietic reconstitution, raising the potential clinical application of TPO agonists in patients receiving radiotherapy or chemotherapy. The findings also imply that agonists of TPO/mpl may be effective in patients suffering from inefficient hematopoiesis caused by damaged HSCs.

Much effort has been devoted to identifying clinically useful drugs that mimic the effects of TPO. After the cloning of human TPO, two recombinant TPO molecules were developed and tested in clinical trials. These first-generation recombinant TPOs showed clinical efficacy in treating idiopathic thrombocytopenic purpura (ITP) patients, platelet apheresis donors, and patients receiving nonmyeloablative chemotherapy. Unfortunately, the appearance of neutralizing antibodies to some forms of recombinant TPO ended these clinical efforts. Since then, a number of nonimmunogenic second-generation molecules have been developed, including TPO peptide mimetics (e.g., romiplostim), TPO nonpeptide mimetics (e.g., eltrombopag), and TPO agonist antibodies. Among these, romiplostim and eltrombopag have shown impressive clinical benefits, increasing the platelet counts in >80% of ITP patients without significant adverse effects (reviewed in Kuter, 2007). More recently, eltrombopag showed significant effects not only in platelet but also in erythroid and neutrophil lineages in 44% of patients with severe aplastic anemia (Olnes et al., 2012). This observation suggests that eltrombopag stimulates hematopoiesis at the level of primitive HSCs, consistent with the findings of de Laval et al. (2013) showing that TPO increases HSC function.

However, some concerns have arisen regarding the clinical use of TPO agonists. The experience of the first-generation molecules suggested that administration of recombinant TPO was not effective in patients with high levels of endogenous TPO, including those receiving stem cell transplantation or induction therapy for acute leukemia (Kuter, 2007). Such predictions may not be true for the TPO nonpeptide mimetics, such as eltrombopag, because they bind to Mpl in a region not occupied by TPO and have an additive effect to that of TPO. Indeed, patients with aplastic anemia showed clinical response to eltrombopag despite their high levels of endogenous TPO (Olnes et al., 2012). Long-term administration of TPO may cause several complications, including thrombosis and bone marrow fibrosis. Ongoing clinical studies should establish the incidence of these complications. Perhaps the most serious concern is that enhanced TPO/mpl signaling can promote clonal evolution toward leukemia. In fact, progression from myelodysplastic syndrome (MDS) to acute myeloid leukemia has been observed in a clinical trial of romiplostim, although it is not yet clear whether the progression rate is truly higher than expected. Enhanced TPO/Mpl signaling has been shown in many hematopoietic neoplasms, and the results of de Laval et al. (2013) further indicate the possibility that TPO could enhance the DNA repair in leukemia stem cells to promote leukemia progression. Thus, caution is required when TPO mimetics are used for MDS and leukemia patients. Nevertheless, the mechanism of action of eltrombopag may make it particularly suited for applications associated with protecting normal HSC from DNA damage, if future studies show eltrombopag does in fact enhance DNA repair similarly to TPO. Studies have recently shown that eltrombopag works through both mpl-dependent and mpl-independent mechanisms, and eltrombopag additionally exhibits significant antileukemia effects (Kalota and Gewirtz, 2010; Roth et al., 2012; Sugita et al., 2012). These properties are especially desirable if eltrombopag were to be utilized during induction chemotherapy and were able to impart protective effects in normal HSC while exerting antileukemia effects on malignant cells.

Specific blood cell growth factors, such as erythropoietin for red cells and granulocyte colony-stimulating factor for granulocytes, have shown impressive clinical benefits. It seems that we now have a method to expand primitive HSCs with the ability to replenish all blood cell types. TPO agonists, as critical factors to promote "healthy" HSCs, show great promise for treating hematopoietic disorders caused by damaged HSCs, and protecting HSCs in patients receiving DNA-damaging therapy. As with these other cytokine regimens, however, it will be critical to determine the specific signaling cascades downstream in order to enhance the desirable outcomes while minimizing any unwanted effects of receptor activation.

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