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In Translation

Saying No to Drugs: Fasting Protects Hematopoietic Stem Cells from Chemotherapy and Aging

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Aging and chemotherapeutics damage hematopoietic stem cells (HSCs), leading to dysregulation of asymmetric division and subsequent immunosuppression and blood-related diseases. In this issue, Cheng et al. (2014) use prolonged fasting as a medical intervention to decrease IGF-1/PKA signaling and protect HSCs against chemotherapeutic toxicity and promote rejuvenation.

Chemotherapeutic strategies for treating blood-based and solid cancers using genotoxic alkylating agents such as cyclophosphamide (CP) have a number of highly undesirable side-effects, including depletion of circulating white blood cells (WBC) and loss of bone marrow (BM) cellularity (Mauch et al., 1995). Over 20% of cancer-related deaths are hastened, or even caused, by toxic effects of chemotherapy rather than the cancer itself (Mort et al., 2008). These acute toxicities reduce overall efficacy by limiting the dosage and schedule frequency of chemotherapeutic interventions. In cancer/chemotherapy survivors, DNA damage to normal cells can promote pro-oncogenic mutations, leading to an increased risk of secondary cancers. It can also irreversibly alter hematopoietic stem cell (HSC) and progenitor cell function in the BM, resulting in dysregulation of asymmetric division and a decrease in the lymphoid to myeloid (L/M) ratio, leading to eventual immunosuppression, anemia, and BM failure (Bartucci et al., 2011; Ding et al., 2014).

Even in the absence of exogenous genotoxic chemotherapeutics, normal aging takes a heavy toll on stem cell homeostasis, including reduced HSC self-renewal capacity and function as well as a decreased lymphoid/myeloid ratio. Endogenous DNA damage and changes in circulating factors have both been implicated in this process (Pang et al., 2011). Currently there are no therapies available to mitigate off-target chemotherapeutic effects on immunosuppression or BM depletion, nor any interventions to prevent HSC dysfunction with aging.

In this issue of *Cell Stem Cell*, the Longo group explores the ability of a simple dietary intervention—periodic fasting—to combat both chemotherapy-induced and aging-related changes in HSC function in mice (Cheng et al., 2014). The current study extends their prior work on the ability of short-term nutrient/energy restriction to increase resistance to genotoxic stress in noncancerous cells and mice (Raffaghello et al., 2008). Here, they tested the ability of cycles of fasting in combination with CP administration to protect against chemotherapy-induced mortality in mice over a total of six cycles (12 weeks). Each cycle consisted of a 2 day water-only fast immediately prior to CP treatment, then a return to unlimited food access for the remainder of a 2 week period. Fasting cycles also facilitated a rebound from CP-mediated peripheral WBC loss and preservation of a healthy L/M ratio by the sixth cycle relative to continually fed animals. Similar protection against peripheral WBC loss and preservation of the L/M ratio was seen in human patients fasted for a single 72 hr period prior to platinum-based chemotherapy as part of a Phase I clinical trial for safety and feasibility.

In order to explain these protective effects, the authors looked to the BM, where they found evidence of reduced DNA damage, reduced apoptosis and increased numbers of HSCs, including omnipotent LT-HSCs, after the sixth fasting/CP cycle. As a result, competitive BM transplantation with total BM from periodic fasted versus continually fed mice favored the fasted group.

To further explain fasting-induced preservation of HSC function upon CP treatment, the authors tested the hypothesis that fasting can stimulate HSC renewal independent of chemotoxicity altogether. Strikingly, even a single bout of fasting significantly increased HSC numbers. This improvement was not simply due to a change in HSC abundance relative to other cell types in the BM, or even the potential stimulatory effects of refeeding, as BrdU incorporation increased specifically in HSCs during the fasting period itself. Nonetheless, the potential contribution of refeeding after prolonged fasting to BM regeneration, and effects of fasting on individual cell function, remain to be fully characterized.

By what mechanism does nutrient/energy restriction promote HSC renewal and promote BM health? Previous work by the Longo group demonstrated the importance of reduced IGF-1 in resistance to chemotoxicity. Here, they confirmed the importance of reduced IGF-1 signaling specifically in BM using mice deficient in growth hormone receptor (GHR) signaling upstream of IGF-1 production. These mice had reduced IGF-1 in circulation as well as in the BM and displayed properties similar to WT mice subject to fasting cycles upon PC treatment, including reduced BM DNA damage and preservation of circulating WBC numbers and L/M ratios, and increased numbers of cycling HSCs and preservation of L/M ratios as a function of age in the absence of PC treatment.

Finally, the authors identified PKA as a relevant downstream target of IGF-1R signaling involved in fasting-mediated HSC self-renewal. Inhibition of IGF-1R or PKA via siRNA in ex vivo BM cultures increased HSC proliferation independent of nutrient/energy availability, and promoted efficient BM reconstitution in vivo. While PKA and its target, CREB, can negatively regulate FoxO1, a critical

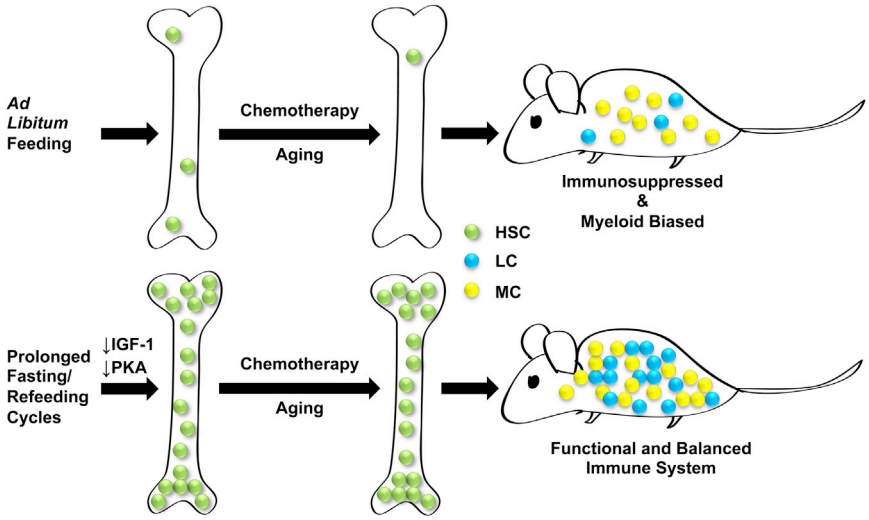

Figure 1. Fasting Ameliorates Effects of Chemotherapy and Aging on Bone Marrow
Prolonged fasting boosts bone marrow hematopoietic stem cell (HSC) self-renewal and increases resistance to chemotherapeutic toxicity and aging-related loss of homeostasis through decreased IGF-1/PKA signaling. This results in maintenance of lymphoid cell (LC) and myeloid cell (MC) numbers and ratios and improved immune function.

regulator of stress resistance and stem cell pluripotency, future experiments will be required to test genetic requirements of these factors in fasting-based HSC renewal. A model summarizing the mechanism by which fasting promotes HSC self-renewal and BM homeostasis via inhibition of IGF-1 signaling and PKA/CREB activity is presented in Figure 1.

The current work raises a number of fascinating questions. First, why is increased mitotic activity of HSCs associated with improved survival upon genotoxic stress? Generally, dividing cells are thought to be more susceptible to killing by genotoxic agents than nondividing cells. Future studies will be required to determine whether cycling HSCs induced by fasting or IGF-1R/PKA inhibition are themselves more or less sensitive to genotoxic stress or whether HSCs driven to proliferate upregulate DNA repair pathways as suggested by recent data from the Rossi group (Beerman et al., 2014).

Second, how do cancer stem cells, which are thought to utilize oxidative metabolic pathways more like HSCs than differentiated cancer cells (Lagadinou et al., 2013), respond to fasting cycles? Moving forward, it will be important to determine whether the concept of differential stress resistance between cancer cells and normal cells (Raffaghello et al., 2008) applies in the context of normal HSCs versus cancer stem cells, which already appear to be highly resistant to chemotherapeutic interventions.

Finally, this work suggests a potential way to improve the efficacy of BM transplants. At face value, these data suggest that fasting the healthy donor may increase the number, and possibly the functionality, of HSCs in the graft, facilitating regrowth of a balanced and healthy BM in both donor and recipient.

The benefits of DR and/or fasting in a wide range of preclinical models have been known for a long time (Robertson and Mitchell, 2013), yet corresponding dietary interventions are rarely tested in clinical trials. Instead, as underlying molecular pathways are revealed, attempts are generally focused on mimicking the effects of diet with pharmacological agents. To date, this approach has not produced effective fasting/DR mimetic drugs for aging or stress-related indications. This failure may be due to difficulties in delivering appropriate doses at the right time and place. However, it may also be that no single drug can effectively mimic the coordinated effects on gene expression, hormones and metabolism that can be achieved by simple yet pleiotropic dietary interventions such as fasting. Time will tell whether this potentially transformative work by Cheng and colleagues is better remembered for elucidating a druggable target pathway in HSC renewal or for demonstrating a viable dietary means to achieve the same end.

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