Enhancing Stem Cell Transplantation with "Nutri-technology"

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It is necessary to employ myeloablative irradiation or chemotherapy to deplete the HSC niche to optimize hematopoietic stem cell transplantation. In a recent issue of *Science*, Taya and colleagues provide evidence for an alternative to the toxic chemoirradiative procedure by showing that a valine-restricted diet is sufficient to empty the bone marrow niche.

Amino acids are not only essential for protein synthesis, but can act as potent regulators of signaling pathways and the epigenetic mechanisms that underlie tissue homeostasis and regeneration. Diets that provide low or no proteins and glucose alternated with diets that provide high levels of these macronutrients have been shown to promote HSC self-renewal and protect from both chemotherapy and aging-dependent deficiencies in white blood cells and other cell types (Brandhorst et al., 2015; Cheng et al., 2014). Calorie restriction also stimulates self-renewal of intestinal stem cells and neural regeneration (Yilmaz et al., 2012; Lee et al., 2000). Restriction of specific amino acids represents a powerful method to regulate the levels or activity of IGF-1 and mTOR. For example, methionine, cysteine, and tryptophan regulate IGF-1 levels and activity and leucine, glutamine, and arginine regulate mTOR. Notably, both reduction of IGF-1 and mTOR signaling have been proposed to be central for enhanced stem cell activity (Mihaylova et al., 2014).

In this context, the new study by Taya et al. (2016) published in *Science* investigated the role of amino acid restriction in the proliferation and self-renewal of hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs). Taking a cue from an article by Kornberg (1946) showing that the administration of amino acids in rats that are fed a low-protein diet reversed anemia and granulocytopenia, Taya et al. identified amino acids that are indispensable for human and murine HSC and HPC growth or survival. A screen for amino acids whose deficiency blocked HSC proliferation in culture pointed to valine as a regulator of self-renewal in both human and murine HSCs. The screen additionally revealed that leucine and cysteine support the proliferation of human HSCs. It is interesting to note that the absence of valine did not have an effect on the proliferation of HPCs, and rather inhibited self-renewal assayed by competitive repopulation of HSCs following in vitro culture in selective conditions. This effect on self-renewal was also observed in HSCs cultured in the absence of cysteine. These in vitro observations were corroborated by in vivo studies of mice fed complete diets or a diet lacking either cysteine or valine (−Cys or −Val) for 4 weeks.

In vivo, dietary restriction of valine in mice reduced the number of HSCs and the populations of B lymphocytes and progenitor cells in the bone marrow, while leaving unchanged the populations of T lymphocytes, granulocyte/monocyte progenitor cells, and megakaryocyte/erythroid progenitor cells. Importantly, the restrictive diet had no obvious effects on the non-hematopoietic organs and tissues evaluated, with the exception of some minimal effects associated with reduced hair follicle density and increased brown adipose tissue. Only the –Val diet led to these hematopoietic phenotypes, pointing to a specific role for valine deficiency. The lack of in vivo effects of a –Cys diet was attributed to the body's ability to produce cysteine through catabolism of methionine.

To assess effects on long-term HSCs, the authors competitively transplanted HSCs from mice that were valine restricted or fed a normal diet. Although competitive repopulation was relatively similar at early time points, by 12 weeks a severe defect in the –Val group HSC numbers was observed, suggesting a specific effect of valine restriction on long-term HSCs rather than on short-term progenitors. This preferential effect on HSCs led the authors to hypothesize that a valine-restricted diet could potentially be used to generate a receptive bone marrow niche for HSC transplantation (HSCT). Successful transplantation of donor bone marrow cells into non-irradiated mice that were fed a valine-restricted diet confirmed the potential of this strategy. Importantly, the mice remained healthy for over 1 year after transplantation, which contrasts sharply with the adverse consequences observed after standard radiation. Similar results were achieved when human CD34+ cord blood was xenografted into immunodeficient mice that were fed a valine-free diet. Taken together, these results indicate that dietary conditioning could be used as an alternative method to standard myeloablative conditioning regimens, with potentially less toxic side effects for patients. However, it is important to note that the return to a normal diet will need to be controlled carefully, as some adverse effects, including a high mortality rate of recipient mice, were observed when normal diet was immediately resumed. Instead, gradual addition of

valine avoided refeeding syndrome in transplanted mice.

Recently new and safer antibody therapies were successfully employed in allogeneic HSC transplants in immunocompetent mice. In particular, antibodies specific for the hematopoietic-specific cell surface antigen CD45, conjugated to saporin toxin (Palchaudhuri et al., 2016), or the combination of antibodies blocking c-Kit and CD47, a myeloid-specific immune checkpoint protein (Chhabra et al., 2016), could deplete HSCs from their niche without the side effects caused by chemotherapy and irradiation. Although dietary interventions are much less expensive and possibly safer than antibody-based therapies, it might be interesting to study the combination of these two approaches to also identify new pathways that can enhance efficiency of HSCT.

The mechanism through which valine exerts its effects on HSC self-renewal remains elusive; however, the lack of valine recapitulates partially the HSC phenotype observed in mice bearing conditional deletion of mTOR in the hematopoietic cell population. In fact, specific deletion of hematopoietic mTOR leads to a reduction of white blood cells, platelets, and red blood cells in the peripheral blood, and a reduction of the B cell and myeloid progenitor cell number in the bone marrow. These effects are due to increased apoptosis in myeloid, erythroid, and B cell lineages (Guo et al., 2013).

Further studies are needed to clarify the effects of valine on the metabolism and epigenome of HSCs and on the regulation of HSC self-renewal, and to establish the impact of a diet alternating valine restriction and availability on HSC proliferation and stem activity. It will also be important to understand how other nutrients and nutrient-signaling pathways may contribute to the effects observed in this study.

Although these recent results appear to be counterintuitive and go in the opposite direction than the above studies on the effect of calorie restriction and stem cell self-renewal/activation, they may be aligned with them. In fact, deficiencies in IGF-1 and PKA or Tor can promote the activation of stem cells, but these genes are also centrally involved in cell and/or organismal growth. Thus, general nutrients and growth factor deficiencies appear to prepare HSCs for self-renewal but valine and other essential amino acids are required for stem cells to proliferate and differentiate. Taya et al. showed that the concentration of all 20 amino acids in the bone marrow is about 100-fold higher than that measured in the peripheral blood, underscoring the importance of these nutrients in the growth and survival of HSCs and HPCs. These findings also raise the possibility that the lack of valine combined with an excess of other amino acids may provide a mixed signal to HSCs, which may contribute to their death. Thus it will be interesting to investigate whether the specific valine deficiency may fail to achieve the coordinated reduction in white blood cells accompanied by the activation of HSCs caused by fasting. This is possible, since fasting periods were very common during the evolution of both mice and humans, but it is difficult to imagine how a specific and severe restriction of only valine would have occurred. Thus, it will be important to understand whether valine deficiency accompanied by glucose and other restrictions may turn its negative effect on stem cell self-renewal into a positive one.

Dissection of the role of various nutrients in regulating the complex cell signaling network that governs the function of stem, progenitor, and differentiated cells in various systems and organs is allowing the development of "nutri-technology"-based therapeutic approaches for the prevention and treatment of diseases either in combination with pharmaceuticals or in their absence. These nutritech interventions have the potential to be safe but also to provide coordinated responses that can trigger sophisticated but dormant programs, such as those activated in response to starvation and refeeding.

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