

## Balance your folate or the yin and yang of folate in hematopoiesis

Hartmut Geiger

Institute of Molecular Medicine and Aging Research Center, University of Ulm, Germany and Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

E-mail: [hartmut.geiger@uni-ulm.de](mailto:hartmut.geiger@uni-ulm.de) / [hartmut.geiger@cchmc.org](mailto:hartmut.geiger@cchmc.org)

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A new study in mice demonstrates that, in general, both low and high levels of dietary folate compromise hematopoiesis, and affect the B-cell progenitor compartment in particular.<sup>1</sup> Given that there are substantial fractions of the world population at risk for either excessive (*via* fortified diets) or very low folate intake, this study might contribute to novel public policy recommendations on the level of folate intake, specifically in the context of fortified diets.

There has, of late, been a widespread interest in the role of dietary nutrients in hematopoiesis, including specific types of vitamins and amino acids, as, interestingly, dietary factors seem to play a critical regulatory role in blood cell production as well as in leukemia. For example, high-dose vitamin C treatment induced hematopoietic stem cells (HSCs) to mature, and suppressed the growth of Tet methylcytosine dioxygenase 2 (Tet2) deficient leukemia cancer stem cells from human patients implanted in mice, by promoting DNA methylation.<sup>2</sup> It was further demonstrated that in the absence of retinoic acid, a vitamin A metabolite, active HSCs are unable to return to a dormant state, instead maturing into specialized blood cells, and that mice fed with a vitamin A-deficient diet showed a disrupted reentry of HSCs into dormancy following exposure to inflammatory stress stimuli, thus eventually losing their HSCs.<sup>3</sup> The Nakauchi laboratory showed that a diet deficient in the essential amino acid valine, resulted in the selective depletion of HSCs in the bone marrow of mice, which allowed for a successful transplant of HSCs without an additional preconditioning regimen.<sup>4</sup> Ultimately, it has also been reported that multiple cycles of short-term fasting, and the accompanying restricted uptake of vitamins and essential amino acids, leads to signal transduction changes in HSCs and niche cells which promote stress resistance, self-renewal, and regeneration.<sup>5</sup>

We can now add to this list a novel yin/yang role of folate in hematopoiesis. Folate is a water-soluble B vitamin (coenzyme) listed as vitamin B9. Generally, folate coenzymes, such as vitamin B9 and vitamin B12, act as acceptors and donors of one-carbon units in a variety of reactions critical to nucleic acids synthesis (including thymine, but also purine bases), amino acids and DNA methylation reactions, and hence epigenetic mechanisms.<sup>6</sup> An inadequate low folate status during early pregnancy increases the risk of congenital abnormalities. Notably, neural tube defects have been linked to inadequate levels of folate provided to the fetus in the first trimester of development. In general, due to the molecular role of folate in nucleotide synthesis, systems with an overall high number of dividing cells, such as the hematopoietic system, depend on proper levels of folate intake to provide adequate levels of erythrocytes and leukocytes and to prevent anemia.<sup>7</sup> Erythroblasts, for example, require folate for proliferation during their differentiation. Folate further supports the generation of “normal” red blood cells in sickle cell

patients.<sup>8</sup> The negative role of low folate levels with regard to hematopoiesis has therefore been recognized for quite some time.<sup>7,9</sup>

Folate is provided naturally by a whole variety of foods, including green leafy vegetables as well as meat, fish, eggs and grains. Folate can also be chemically synthesized. Due to the low dietary intake of folate in multiple countries, and its vital role in preventing multiple congenital defects, folate has been supplemented in at least one major cereal grain product since the late 1990s in order to reduce, for instance, neural tube defects (which it proved to do) in more than 70 countries worldwide, including North America, while in the EU such a fortification with folate is not mandatory.

In contrast to the well-known positive benefits of folate supplementation, epidemiological data also support a likely negative impact of high levels of folate intake with respect to diseases and cancer;<sup>10</sup> thus, both too low and very high levels of folate in diets might have negative consequences on health. While there are ample experimental studies on the negative consequences of low levels of dietary folate intake, the impact of high levels of folate has not of yet been addressed in great detail. The toxicity of folic acid has, up to now, been universally regarded as being very low, as folate is a water-soluble vitamin and thus an excess will be likely removed from the body through urine, as in the case of vitamin C. Henry *et al.* have now addressed, in an elegant set of well designed experiments, the extent to which distinct levels of dietary folate influence B cell development and hematopoiesis, *via* transplantation experiments in particular.<sup>1</sup> To this end, they simply fed mice diets deficient in folate or diets that contained high levels of folate for up to 12 months. The diet high in folate resulted in folate serum levels that are within the range which are likewise achieved with fortified diets in human serum. Both low and high levels of folate resulted in impaired hematopoiesis, and primarily affected the B cell compartment. High and low levels of folate further reduced the survival rate of animals exposed to an irradiation dose that was not lethal to control animals. These novel studies ultimately confirm that excessively low and high levels of dietary folic acid negatively impact B cell development and hematopoiesis. Interestingly, changes in folate primarily affected the nucleotide synthesis pathway in hematopoietic cells, while other pathways linked to folate seemed to play only a minor role in affecting hematopoiesis. This suggests that hematopoietic cells might be very sensitive to changes in the level of nucleotides, both too low and too high. This work, although inherently less mechanistic than, for example, pure “genetic” approaches, is nonetheless an important contribution to the field of dietary interventions in hematopoiesis and their influence on metabolites in the system. These results will need to be further tested in additional epidemiological studies, focusing

on the high or higher end levels of folate and their connection to the long-term outcome rather than investigating low levels and their connection to disease. Taken together, these data will support the consideration of novel public guidelines on the recommended level of folate that will keep one healthy and strong.

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## Transplantation for therapy-related, TP53-mutated myelodysplastic syndrome – not because we can, but because we should

Corey Cutler

Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

E-mail: corey\_cutler@dfci.harvard.edu

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*To the man who only has a hammer, everything he encounters begins to look like a nail.*

Abraham Maslow, and others

Transplant physicians have often been accused of performing allogeneic hematopoietic stem cell transplant (HSCT) in patients for whom no hope of cure, or even meaningful gain, was thought plausible, simply because HSCT was deemed possible. Exemplifying this is the concept of transplantation for therapy-related myelodysplastic syndrome (tMDS), or MDS associated with the most severe of genetic lesions, *TP53*, for which outcomes have historically been poor.

In this issue of *Haematologica*, Aldoss and colleagues from the City of Hope Medical Center compare the outcomes of patients with tMDS and *de novo* MDS who underwent allogeneic HSCT, and correlate molecular features with outcome.<sup>1</sup> Although the study was limited by small sample size, the authors noted no significant differences in all important post-HSCT clinical outcomes, including survival, between tMDS and *de novo* MDS patients, even when prior chemoradiotherapy was considered in multivariable models. This analysis therefore suggests that allogeneic transplantation for tMDS should be performed whenever it would be considered in the *de novo* MDS setting.

Perhaps even more importantly, the authors examine the impact of molecular lesions on transplant outcome in a subset of the patients. From the original cohort, 60 tMDS patients underwent a comprehensive molecular analysis: 30% had a *TP53* mutation, and the authors found that the presence or absence of a *TP53* mutation had no correlation with outcome among these tMDS

patients, although sample sizes were limited. In this context, grouping a heterogeneous subset of patients with genetic changes associated with adverse outcome in the non-transplant setting did carry prognostic information. In patients with any one of five genes (*TP53*, *EZH2*, *ETV6*, *RUNX1*, *ASXL1*) associated with adverse risk (48% of molecularly characterized tMDS patients) the authors demonstrated an adverse impact on relapse-free survival (hazard ratio: 1.58). This difference was also not statistically significant due to small sample size. It is worth noting that in this single center, retrospective analysis, outcomes were generally favorable, with 5-year overall survival rates approaching 50%.

Numerous studies have now examined the outcomes associated with molecular mutations following allogeneic HSCT (Table 1); however, this is one of the largest to specifically examine tMDS patients and compare their outcomes with those of patients with *de novo* MDS. Initial studies examining molecular prognostic features demonstrated dismal results for patients with mutated *TP53*.<sup>2</sup> In a study of 87 MDS patients who underwent allogeneic HSCT at our institute, there were no long-term survivors in the subset with *TP53* mutations, with the majority of deaths occurring within the first 12 months following allogeneic HSCT. Fifteen of 18 deaths in this group were attributed to disease relapse. While sample sizes were exceedingly small, this result instantly changed the landscape of transplantation, with many centers deciding not to perform HSCT in *TP53*-mutated MDS patients at all.

Other larger series have now reported outcomes of *TP53*-mutated HSCT patients with slightly more promising results. For example, Yoshizato and colleagues