transcription factor MITF and the acquisition of ZEB1 are reminiscent of EMT-like phenotypic changes described in other mouse models and reflect analyses of transcriptomic profiles previously reported in human melanoma cells (Caramel et al., 2013).

Together, these two papers provide *in vivo* evidence of how microenvironmental cues shape the transformation potential of pro-oncogenic signals. In particular, the refractory nature of the MCSCs in the bulge, and the melanomas generated from adult mature melanocytes in the tail, suggest that stemness may not be an obligate requirement for malignant transformation in this disease. Perspectives for future studies are exciting. Considering the plasticity of the neural stem cell precursors that give rise to melanoblasts and mature melanocytes during development (Mort et al., 2015), it appears to be necessary to perform comprehensive kinetic and functional analyses of transcriptomic/proteomic profiles in melanocytic cells at different anatomical locations and in response to intrinsic and extrinsic stimuli (including, but not limited to, oncogenes, UV radiation, or pro-inflammatory signals). In this context, it should be noted that the quest is still open for the identification of human melanocytic stem cells and the characterization of their role in dormant versus proliferative melanocytic lesions (Merlino et al., 2016). In any case, results in these two papers raise an important note of caution on the need for standardization of experimental procedures in the *Tyr::CreERT2* mice. Users should be aware of the markedly differential impact that timing and site of depilation and/or tamoxifen administration may have on the malignant potential of (epi)genetic alterations. After all, location matters.

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Vitamin C: C-ing a New Way to Fight Leukemia

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Metabolic cues and (epi-)genetic factors are emerging regulators of hematopoietic stem cell (HSC) potency. Two new studies in *Nature* and *Cell*, from Agathocleous et al. (2017) and Cimmino et al. (2017), respectively, show that vitamin C regulates HSC function and suppresses leukemogenesis by modulating Tet2 activity.

DNA methylation is an epigenetic modification that plays a critical role in hematopoiesis, controlling proper hematopoietic stem cell (HSC) self-renewal, and lineage differentiation (Jeong and Goodell, 2014). Dysregulation of DNA methylation leads to aberrant stem cell function and cellular transformation. Tet proteins have been identified as key players of DNA demethylation by acting as Fe^{2+} and α-ketoglutarate-dependent dioxygenases (Tahiliani et al., 2009). These enzymes catalyze the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), which leads to DNA demethylation mediated by either replication-dependent dilution or base excision repair (BER). TET2 recurrently undergoes loss-of-function mutations in a wide range of myeloid and lymphoid malignancies (Jan et al., 2012). These lesions are early events in leukemogenesis and are associated with DNA hypermethylation, tumor progression, and poor patient outcome. In homeostasis, the stability and activity of Tet proteins are regulated at multiple levels. Acting as a cofactor, vitamin C (also known as ascorbate) promotes the activity of Tet enzymes (Blaschke et al., 2013). Now, two new studies published in *Nature* and *Cell* provide mechanistic insights on how vitamin C regulates HSC frequencies and leukemogenesis by augmentation and restoration of Tet2 function, respectively (Figure 1) (Agathocleous et al., 2017; Cimmino et al., 2017).

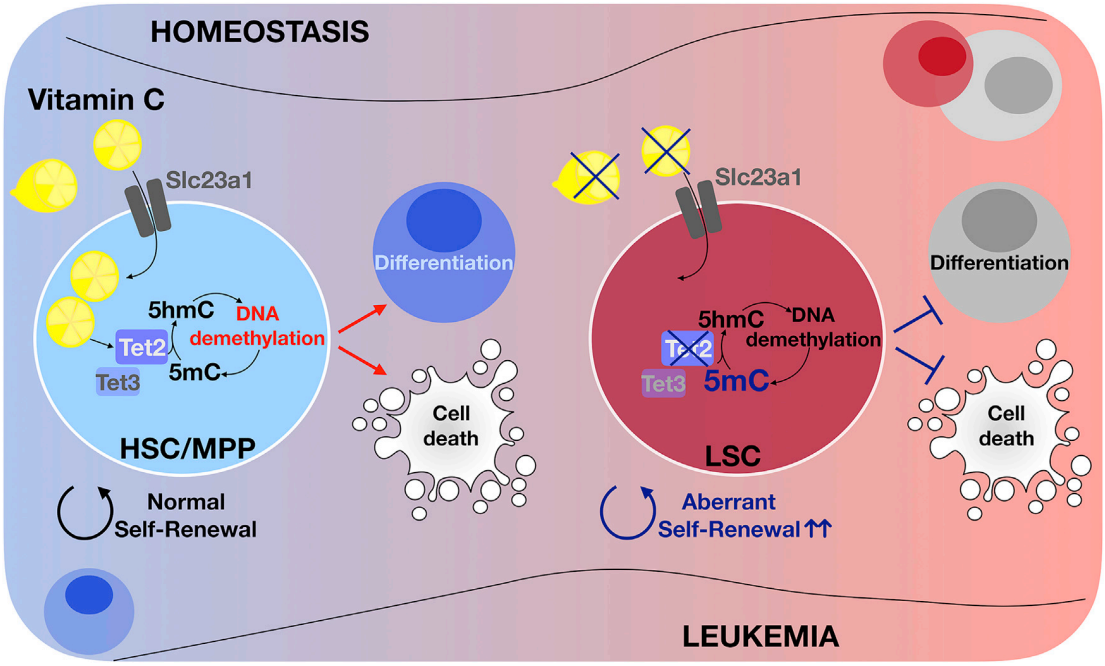

Figure 1. The Role of Vitamin C in Homeostatic HSC/MPPs and Aberrant LSC Function
Tet proteins catalyze the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), a rate-limiting step toward DNA demethylation. In homeostasis, vitamin C regulates the balance between self-renewal, differentiation, and cell death of hematopoietic stem cells and multipotent progenitors (HSC/MPPs) by promoting Tet2 activity. In contrast, vitamin C depletion accelerates leukemogensis of Tet2-mutant leukemic stem cells (LSCs) and leads to increased aberrant self-renewal capacity.

In *Nature*, Sean J. Morrison's laboratory reports that vitamin C limits HSC frequency and suppresses leukemogenesis by acting through Tet2-dependent and -independent mechanisms (Agathocleous et al., 2017). Agathocleous and colleagues combine extensive metabolomics analyses with a remarkable set of genetic mouse models. To unravel the metabolic signatures of distinct murine hematopoietic cell types, the authors establish a highly sensitive metabolomics workflow applicable to rare cell populations. This comprehensive analysis identified ascorbate/vitamin C to be highly enriched in HSCs and multipotent progenitor cells (MPPs) compared to more committed cell types of the blood system. In addition, the ascorbate-transporter Slc23a2 was also highly expressed in HSC/MPPs. Consistently, ascorbate and the SLC23A2 transporter were enriched in human HSCs.

To address the role of ascorbate in HSCs, the authors generated mice lacking Gulo, the enzyme responsible for its synthesis. Strikingly, ascorbate-depleted cells showed increased HSC frequencies and associated lineage reconstitution capacity in transplantation assays. These results provide genetic evidence that vitamin C is a negative regulator of HSC function. This phenotype resembles mice carrying a homozygous Tet2 deletion (Moran-Crusio et al., 2011). Indeed, ascorbate-depleted HSC/MPPs displayed decreased levels of 5hmC, predominantly mediated by reduction of Tet2 function (Agathocleous et al., 2017).

TET2 mutations together with $FLT3^{ITD}$ mutations can cooperatively cause acute myeloid leukemia (AML) with adverse patient outcome (Shih et al., 2015). Strikingly, ascorbate depletion in combination with $Flt3^{ITD}$ overexpression fostered myelopoiesis, partially mediated by reduced Tet2 activity (Agathocleous et al., 2017). This effect was reversed by repletion of dietary vitamin C. Finally, Agathocleous and colleagues showed that $Flt3^{ITD}$ together with Slc23a2 depletion led to the highest *in vivo* reconstitution capability of HSCs when compared to single mutants. The authors concluded that vitamin C is cell-autonomously metabolized by HSC/MPPs and involved in regulating $Flt3^{ITD}$-driven myelopoiesis.

In an independent study published in *Cell*, the laboratories of Iannis Aifantis and Benjamin G. Neel find that treatment of Tet2-mutated HSCs with vitamin C blocked their aberrant self-renewal activity, mimicking the effect observed upon Tet2 restoration (Cimmino et al., 2017). To assess the role of Tet2 deficiency in the maintenance of leukemic stem cells, the authors generated an elegant, reversible, transgenic RNAi mouse model to restore endogenous Tet2 expression. As previously described, Tet2 knockdown led to a myeloid differentiation bias and a competitive advantage in transplantation experiments (Moran-Crusio et al., 2011). Strikingly, restoration of Tet2 was sufficient to reverse leukemic self-renewal capacities by inducing genome-wide DNA demethylation, differentiation, and cell death. These findings indicate that persistent Tet2 deficiency is required to maintain leukemic self-renewal.

To pharmacologically restore Tet2 activity, Cimmino and colleagues treated Tet2-deficient HSCs with vitamin C *in vitro*. This approach mimicked the effects observed upon Tet2 restoration, increasing Tet2 as well as Tet3 activity.

Further, treatment of Tet2-deficient mice with high doses of vitamin C led to genome-wide increased 5hmC levels and decreased white blood cell counts and myeloid cells. Strikingly, treatment of human-patient-derived leukemic cell lines, including non-Tet2-mutated leukemia with vitamin C, caused increased 5hmC levels and decreased clonogenicity and cell viability. The authors concluded that supra-physiological vitamin C levels could prevent myeloid disease progression.

An exciting observation of this study is the effect seen upon combinatorial application of vitamin C together with a poly-(ADP-ribose) polymerase (PARP)-inhibitor. As an essential mediator of BER, PARP is involved in DNA damage repair mechanisms. Its inhibition has been demonstrated to increase tumor sensitivity to DNA damage. The authors now showed that ascorbate treatment enhanced the efficacy of PARP inhibition and suppressed leukemogenesis *in vitro* (Cimmino et al., 2017). It will be of great interest to investigate the efficiency of this treatment strategy *in vivo*.

Both groups thus identify vitamin C as a novel metabolic tumor suppressor involved in epigenetic remodeling (both in mouse and human) and highlight a putative innovative treatment strategy for leukemia (Agathocleous et al., 2017; Cimmino et al., 2017). Although previous reports did not detect positive effects of vitamin C intake in the context of leukemia, patients suffering from hematological diseases often display low ascorbate levels (Huijskens et al., 2016). These two studies point now to supra-physiological concentrations of vitamin C potentially impeding or even reversing leukemogenesis (Agathocleous et al., 2017; Cimmino et al., 2017). Hence, an adequate intake of vitamin C might be highly beneficial for patients with clonal hematopoiesis. Additional work will be needed to investigate whether this approach is also applicable to other types of leukemia, solid tumors, or even metastases harboring a partial or complete loss of TET2 function.

The apparent link between metabolic cues and epigenetic activity has become a highly active and exciting area of research offering the potential to address many unmet medical needs. It is now evident that specific vitamins are key regulators of transformed as well as normal HSCs (Agathocleous et al., 2017; Cabezas-Wallscheid et al., 2017; Cimmino et al., 2017), highlighting the relevance of dietary habits to maintain a healthy stem cell pool. For instance, metabolites from the retinoic acid pathway (vitamin A) have recently been shown to be involved in the *in vivo* modulation of stem cell features (Cabezas-Wallscheid et al., 2017). The great advances in metabolomics (Agathocleous et al., 2017) and other -omics analyses significantly extend the possibilities in the field of stem cell research and other rare cell types for prevention, diagnosis, and treatment of diseases. This may represent the start of a new era of innovative treatment strategies that combine genome-wide epigenetic analyses and specific metabolites or diets with standard therapies to fight hematological diseases and possibly other types of cancer.

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