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POSTER ABSTRACTS

501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

Vitamin C Limits Multipotent Progenitor Self-Renewal and Clonal Expansion

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A fundamental question is whether physiological variations in diet-derived metabolite levels *in vivo* influence stem and progenitor cell self-renewal. Ascorbate (vitamin C) is enriched in hematopoietic stem cells (HSCs) and multipotent progenitors (MPPs) compared to all other hematopoietic cells, and ascorbate depletion increases HSC function by reducing Tet2 function (Agathocleous et al., 2017). However, whether ascorbate regulates the self-renewal of other hematopoietic progenitors downstream of HSCs is still unknown.

To answer this question, we conditionally deleted *Slc23a2*, the gene that encodes the main hematopoietic ascorbate transporter, in murine hematopoietic cells using *Mx1-Cre*. Deletion of *Slc23a2* substantially increased the reconstituting potential of bone marrow cells upon competitive transplantation into irradiated mice. *Slc23a2* deletion only mildly increased donor chimerism in HSCs (Lin- Kit+ Sca1+ CD150+ CD48-) in the reconstituted mice, but significantly increased donor chimerism in MPPs (Lin- Kit+ Sca1+ CD150- CD48-) and downstream progenitors, suggesting that ascorbate depletion enhanced MPP self-renewal. Deletion of the ascorbate transporter also increased the long-term reconstituting potential of purified MPPs in irradiated mice, but not the long-term reconstituting potential of purified HPC1 (Lin- Kit+ Sca1+ CD150- CD48+) or HPC2 (Lin- Kit+ Sca1+ CD150+ CD48+) progenitor cells. These data showed that ascorbate depletion conferred long-term reconstituting potential upon MPPs.

To understand how ascorbate depletion enhanced MPP self-renewal, we measured the composition and proliferation rate of MPPs. *Slc23a2* deletion significantly reduced MPP proliferation, and it increased the frequency of the more quiescent MPP1 (CD229- CD244-) subpopulation while decreasing the frequency of less quiescent MPP2 (CD229+ CD244-) and MPP3 (CD229+ CD244+) subpopulations (Oguro et al., 2013). Single-cell RNA-Seq (scRNA-Seq) analysis revealed that *Slc23a2* deletion increased the frequency of MPPs with high expression of self-renewal gene signatures (Rodriguez-Fraticelli et al., 2020). Taken together, these data suggested that ascorbate depletion promoted MPP self-renewal through the expansion of a subpopulation of quiescent MPPs. To test this, we evaluated the division history of wild-type and *Slc23a2*-deficientMPPs using *H2B-GFP* reporter mice (Foudi et al., 2009). *Slc23a2* deletion significantly increased the percentage of *H2B-GFP* ^{High} quiescent MPPs, while reducing the percentage of *H2B-GFP* ^{Neg} proliferating MPPs as compared to controls. Competitive transplantation of purified *H2B-GFP* ^{High}or *H2B-GFP* ^{Neg} MPPs revealed that *Slc23a2* deletion increased the reconstituting potential of *H2B-GFP* ^{High} quiescent MPPs as compared to controls, but not the reconstituting potential of *H2B-GFP* ^{Neg} proliferating MPPs. Our data thus showed that ascorbate depletion enhanced MPP long-term self-renewal by expanding a subpopulation of quiescent MPPs.

Overall, we showed that ascorbate cell-autonomously limited MPP self-renewal potential by negatively regulating MPP quiescence. Our study thus points to a central role for diet-derived nutrients in the regulation of HSC and hematopoietic progenitor self-renewal abilities. Additionally, our data suggest that nutrition might directly impact the expansion of mutated cells found in clonal hematopoiesis.

Disclosures No relevant conflicts of interest to declare.

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