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Cleaning up the environment in juvenile myelomonocytic leukemia

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Juvenile myelomonocytic leukemia (JMML) is a commonly fatal myeloid leukemia occurring in very young children bearing germline or somatic mutations in genes within the RAS-ERK signaling pathway including *NF1*, *CBL*, *KRAS*, *NRAS*, and *PTPN11* (1). Leukemia relapse following allogeneic stem cell transplant is uncharacteristically high at 40–50% for JMML patients, and findings from the recently published manuscript by Dong *et al.* (2) provide some clues that might account for this high relapse rate. The authors found that *Nestin-Cre*-mediated knock-in of *Ptpn11*^{E76K/+}, resulting in mutant Shp2 expression in bone marrow (BM) mesenchymal stem/progenitor and neural cells, induced activation of otherwise normal hematopoietic stem and progenitor cells to differentiate into myeloid cells. The resulting leukemia recapitulated the features of JMML, with splenomegaly, myelocytosis, and increased myelopoiesis in the BM. While *Mx1-Cre*- and *LysM-Cre*-mediated knock-in of *Ptpn11*^{E76K/+} have previously been shown to cause a JMML-like myeloproliferative neoplasm (MPN) in mice (3), this is the first study demonstrating that BM niche-restricted expression of *Ptpn11*^{E76K/+} is capable of producing disease. The elegance of the present study lies in the use of five tissue-specific Cre promoters which identified mesenchymal stem/progenitor cells and osteoprogenitors as the critical mutated BM microenvironment cell types with the capacity to promote myeloid disease, ruling out differentiated osteoblasts and endothelial cells.

Relevant to human disease and hematopoietic stem cell (HSC) transplantation as the standard treatment for JMML, the authors found that lethally irradiated *Ptpn11*^{E76K/+}*Nestin-Cre*⁺ mice transplanted with wild-type (WT) BM cells develop a donor-derived MPN, demonstrating the mutant BM microenvironment's pathogenic effect on transplanted normal cells. A potential mechanism accounting for the development of the donor WT cell-derived

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MPN is the overproduction of the CC chemokine, CCL3, resulting in recruitment of inflammatory monocytes to the HSC niche, production of inflammatory cytokines such as IL-1 β , and displacement of HSCs from their normal quiescence-promoting niches. These findings imply an explanation for the poor engraftment and relapse seen in JMML patients after HSC transplantation (4,5). The commonly observed resurgence of the JMML leukemic clone following stem cell transplantation may be due to the inability of the donor normal HSCs to engraft and remain in a quiescent state in the aberrant BM microenvironment, permitting outgrowth of residual leukemic cells and patient relapse. The findings from Dong et al. that the abnormal microenvironment is antagonistic to HSC self-renewal and quiescence further imply that HSCs and progenitors are more likely to mobilize into the peripheral organs, which is a common cause of mortality for JMML patients (6).

In the reciprocal experiment, the authors found that transplantation of BM cells from *Ptpn11^{E76K/+}Nestin-Cre⁺* mice with chronic phase MPN did not produce disease in recipient WT mice, however, transplantation of BM cells from moribund *Ptpn11^{E76K/+}Nestin-Cre⁺* mice produced donor-derived disease in the WT recipients. These findings indicate that over time, the aberrant microenvironment induced the HSCs to acquire alterations, which conferred increased HSC self-renewal. Notably, treatment of *Ptpn11Osx1-Cre⁺* mice with chronic phase MPN with CCL3 receptor antagonists decreased and even reversed MPN development, suggesting that once the abnormal niche signal is suppressed by the antagonists, the HSCs are able to resume their normal function. Therefore, the timing of intervention is potentially crucial, and perhaps prophylactic anti-inflammatory drugs targeting the BM niche may be beneficial for JMML patients post-transplant.

The findings that an abnormal microenvironment by itself can induce JMML give researchers the rationale to further explore the role of oncogenes specifically in the stromal cells, with their expression under the control of *Nestin-Cre*. Although there are already high throughput methods to screen drugs *in vitro* (7), the authors have validated *Ptpn11^{E76K/+}Nestin-Cre⁺* mice as an *in vivo* model for testing drugs that target altered microenvironments. The importance of verifying results *in vivo* is highlighted in this study, as the authors found no hyperactivation of HSCs when co-cultured with *Ptpn11^{E76K/+}* stromal cells or with exogenous CCL3 *in vitro*, yet the HSCs were hyperactivated by the *in vivo* mutant microenvironment. In the future, the exploration of putative interactions between the microenvironment and hematopoietic cells, their mutual influence on each other, and resultant contribution to leukemogenesis will be of great interest. Further work into the underlying mechanisms, such as chemokine receptor downstream signaling, which includes phospholipase C and activation of the MAP kinase pathway (8), will also be highly rewarding to find other targets for inhibition.

It is important to keep in mind that while the authors' findings are clearly applicable to the development/progression of germline mutant *PTPN11*-induced syndromic JMML (due to the described stromal expression of *Ptpn11^{E76K/+}*), these findings are also relevant to somatic mutant *PTPN11*-induced sporadic JMML. Since some components of the BM microenvironment are hematopoietic-derived, such as the megakaryocyte niche (9), a clean separation between the hematopoietic cell-derived and stromal cell-derived BM microenvironment does not exist. Thus, the BM microenvironments of JMML patients

bearing somatic as well as germline mutations are likely similarly abnormal, and the authors' findings in mice of poorer engraftment in hyperinflammatory niches are applicable to patients with sporadic and syndromic JMML alike. Given the harsh nature of allogeneic stem cell transplant as well as the unfortunate high relapse rate in JMML, further investigation of microenvironment-targeting therapies in both syndromic and sporadic JMML is merited.

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