Research Highlights

Stem Cell INVESTIGATION

Shp2 regulates leukemic stem cell frequency in MLL-rearranged acute myeloid leukemia

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Shp2 is a non-receptor protein tyrosine phosphatase (PTP) encoded by the *PTPN11* gene. It contains two SH2 domains, a PTP domain, and a carboxyl-terminal region with tyrosine phosphorylation sites. The Shp2 PTP activity is autoinhibited by its N-SH2 domain. Shp2 is activated by docking to specific tyrosine phosphorylated sites via SH2 domains or by gain-of-function (GOF) mutations that disrupt the autoinhibition. GOF Shp2 mutations are most frequently detected in juvenile myelomonocytic leukemia (JMML), representing 35% of cases. Animal model studies using conditional knockin of GOF $Shp2^{E76K}$ or $Shp2^{D61G}$ in the hematopoietic compartments have shown that these Shp2 mutants cause JMML-like myeloproliferative disease (MPD), albeit after ~6 months of latency (1,2). The leukemogenic activity of Shp2 mutants is cell autonomous, indicating that these *Ptpn11* mutants are driver oncogenes. These Shp2 mutant-expressing mice have hyperactive hematopoietic stem cells (HSCs) with a decrease of $Linage^-$/$Sca\text{-}1^+$/$c\text{-}Kit^+$ (LSK) cells in the bone marrow and an increase of LSK cells in the spleen (1,2). Besides JMML, GOF *PTPN11* mutations have also been found in other hematologic malignancies and in solid tumors but at lower rates, including acute myeloid leukemia (AML) (3). In pediatric AML, *PTPN11* mutations are most prevalent in the French-American-British (FAB)-M5 subtype, which is monoblastic leukemia. FAB-M4 (acute myelomonoblastic leukemia) and FAB-M5 morphological subtypes of AML correlate with MLL-rearrangement (4). The prevalence of *PTPN11* mutations in a particular AML subtype that displays similar morphologically feature as the *PTPN11*-driven JMML suggests that *PTPN11* mutations are not merely passenger mutations. However, this was not proven experimentally. Since *PTPN11* mutations are present in a fraction of AML cells from each patient, these mutations do not appear to be the primary oncogenic events leading to leukemogenesis (3). Therefore, the role of Shp2 mutations in AML was not clear.

In a new report published online in *Leukemia* (5), Chen *et al.* identified six cases of GOF *PTPN11* mutations among 91 pretreatment AML samples (6.6% mutation rate). These six mutations (E76G, T73I, E76Q, D61Y, F71L, and E76G) are located in exon 3 that encodes the N-SH2 domain. Mutations in these residues are highly predictive of GOF that result in constitutively active Shp2. Based on saturation analysis of cancer genes in AML, these *PTPN11* mutations are predicted to be functionally important. Other genetic alterations in *DNMT3α*, *NPM1*, *WT1*, *CBF*, and *MLL* were found in these *PTPN11* mutated AML samples. This is consistent with the previous observation that *PTPN11* mutations did not associated with any specific genetic lesions in pediatric AML (3).

To begin analyzing the potential function of *PTPN11* mutations in AML, Chen and colleagues determined if a mutant Shp2 could affect the MLL oncofusion in murine bone marrow progenitor cell transformation. $Shp2^{E76K}$ and MLL-AF9 were chosen as models of Shp2 and MLL mutations. $Shp2^{E76K}$ is perhaps the strongest activating Shp2 mutant in exon 3. MLL-AF9 rearrangement is present in approximately 50% of pediatric AML cases (4). $Shp2^{E76K}$ did not show significant transformation activity in their experiments but increased MLL-AF9 transformed colonies in methylcellulose cultures. Intravenous injection of $Shp2^{E76K}$-, MLL-AF9-, and MLL-AF9/$Shp2^{E76K}$-retrovirus transduced lin^-/kit^+ bone marrow cells into lethally

irradiated recipient mice showed that Shp2^{E76K} alone did not cause leukemia in 150 days, whereas it reduced the MLL-AF9-mediated disease latency by 2 folds. To determine if Shp2^{E76K} affects leukemic stem cells, Chen and colleagues performed limiting dilution assay by injecting primary leukemic cells from diseased mice into secondary recipients. The data showed a 5-fold increase in leukemia stem cell frequency in MLL-AF9/Shp2^{E76K} induced leukemia.

The presence of Shp2^{E76K} did not influence MLL-AF9 target genes *Hoxa9* and *Meis1* expression, suggesting that Shp2^{E76K} did not exert its effect on leukemogenesis by affecting MLL-AF9 transcription activity. Instead, hematopoietic progenitor cells transduced with MLL-AF9 together with Shp2^{E76K} showed IL-3 hypersensitivity in methylcellulose colony growth and Erk activation. Hypersensitivity to GM-CSF or IL-3 is the hallmark of biological consequences of activated Shp2 mutants in hematopoietic progenitor cells. Furthermore, cells transduced with MLL-AF9/Shp2^{E76K} had elevated Mcl1 and were resistance to Mcl1 inhibitors as compared with cells transduced with MLL-AF9 alone.

These findings provide evidence of functionality of *PTPN11* mutations in MLL-rearranged AML by demonstrating that Shp2^{E76K} increased leukemic stem cell frequency. Consistent with the observation that *PTPN11* mutations did not associated with particular genetic lesions in pediatric AML, the Shp2 mutant does not act directly on MLL-AF9. It will be interesting to determine if Shp2 mutants also affect leukemic stem cell frequency in cooperation with other genetic alterations that co-exist with *PTPN11* mutations in AML.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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