

# Genetic disruption of the PI3K regulatory subunits, p85 alpha, p55 alpha, and p50 alpha partially normalizes gain-of-function *PTPN11*-induced hypersensitivity to GM-CSF in hematopoietic progenitors

Charles B Goodwin, Zhenyun Yang, Fuqin Yin, and Rebecca J. Chan

Departments of Pediatrics and Medical and Molecular Genetics, Indiana University School of Medicine

Indiana University – Purdue University Indianapolis

## Abstract

Juvenile Myelomonocytic Leukemia (JMML) is a lethal myeloproliferative disorder (MPD) of children, characterized by hyperproliferation of myelomonocytic cells and hypersensitivity to Granulocyte-Monocyte Colony-Stimulating Factor (GM-CSF). JMML is frequently associated with gain-of-function mutations in *PTPN11*, which encodes the protein tyrosine phosphatase, Shp2, and which is known to positively regulate Ras signaling. The role of MAPK signaling in gain-of-function mutant Shp2-induced leukemogenesis is well established. In addition, phospho-Akt levels are elevated in the presence of gain-of-function Shp2 mutations, suggesting a role for Phosphatidylinositol-3-Kinase (PI3K) signaling (Yang, et al, 2008). Class IA PI3K is a lipid kinase heterodimer composed of one of two regulatory subunits—p85 alpha or p85 beta—and one of three catalytic subunits—p110 alpha, p110 beta, or p110 delta. PI3K mediates proliferative and anti-apoptotic signals. We have found that there is increased interaction between the p85 alpha regulatory subunit and the p110 alpha catalytic subunit in gain-of-function mutant Shp2-expressing cells compared to WT Shp2-expressing cells. The p85 alpha regulatory subunit, along with its splice variants, p55 alpha and p50 alpha, is encoded by the gene *Pik3r1*. To investigate the hypothesis that p85 alpha-dependent PI3K signaling contributes to gain-of-function mutant Shp2-induced GM-CSF hypersensitivity, WT and *Pik3r1*<sup>-/-</sup> fetal liver-derived hematopoietic progenitor cells were transduced with WT Shp2 or gain-of-function mutant Shp2 E76K. Ablation of all the *Pik3r1* isoforms resulted in a significant, but incomplete, correction of GM-CSF hypersensitivity in Shp2 E76K-expressing cells. Consistently, upon genetic disruption of *Pik3r1*, Akt phosphorylation was reduced in both WT Shp2- and Shp2 E76K-expressing cells compared to that seen in *Pik3r1*<sup>+/+</sup> cells, but was not completely absent. Additionally, Erk activation was reduced in *Pik3r1*<sup>-/-</sup> cells expressing Shp2 E76K compared to that in *Pik3r1*<sup>+/+</sup> cells, indicating that interruption of Shp2-mediated PI3K signaling affects the MAPK pathway as well, which likely contributes to the reduction in GM-CSF-stimulated proliferation in the *Pik3r1*<sup>-/-</sup> cells. Finally, treatment with the PI3K inhibitor, LY294002

resulted in complete abrogation of Akt phosphorylation in *Pik3r1*<sup>-/-</sup> cells transduced with Shp2 E76K, indicating that residual PI3K activity in the absence of *Pik3r1* likely contributes to the incomplete correction of GM-CSF hypersensitivity and suggesting that although p85 alpha plays an important role in gain-of-function mutant Shp2-induced hyperactivation of PI3K signaling, additional p85 alpha-independent mechanisms contribute as well.