

Genetic disruption of the PI3K regulatory subunits, p85 α , p55 α , and p50 α , normalizes mutant *PTPN11*-induced hypersensitivity to GM-CSF

Charles B. Goodwin,^{1,2} Zhenyun Yang,^{1,3} Fuqin Yin,^{1,3} Menggang Yu,⁴ and Rebecca J. Chan^{1,2,3}

¹Herman B. Wells Center for Pediatric Research; ²Department of Medical and Molecular Genetics; ³Department of Pediatrics, ⁴Department of Medicine and Division of Biostatistics, Indiana University School of Medicine, Indianapolis, IN, USA

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Online Design and Methods

Mice

Timed matings were performed between *Pik3r1*^{+/-} mice,¹ and fetal liver cells from Day 14.5 WT and *Pik3r1*^{-/-} embryos were cultured in Minimal Essential Media α , 20% fetal bovine serum (FBS, Hyclone, Logan, UT, USA), 1% penicillin/streptomycin, and 100 ng/mL each G-CSF, SCF and TPO (Peprotech, Rocky Hill, NJ, USA), followed by retroviral transduction with WT Shp2 or Shp2 E76K, as described previously.^{2,3} All mice were maintained under specific pathogen-free conditions at the Indiana University Laboratory Animal Research Center (Indianapolis, IN, USA). This study was approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine, USA.

PI3K assay and immunoblot analysis

As JMML is a disease of macrophage overproduction, biochemical studies were performed using murine fetal liver or bone marrow-derived macrophages, generated as previously described.³ Cells were serum- and

growth factor-starved for 12-16 h followed by stimulation with 10 ng/mL GM-CSF. Total cellular protein extracts were prepared as previously described⁴ and assayed using Bradford Reagent and a standard curve. PI3K activity per 100 μ g total cellular protein extract was assayed by measuring [³²P] incorporation into lipid substrates separated by thin layer chromatography.⁵ For immunoblot analysis, blots were probed with anti-phospho-Akt (S473, 587F11 or T308, 244F9), anti-Akt, anti-phospho-Erk, anti-Erk, anti-p110 α (C73F8) (Cell Signaling Technology), anti-p110 β (S-19), anti-Shp2 (C-18) (Santa Cruz Biotechnology), anti-p110 δ (BD Transduction Laboratories), or anti-p85 α (AbD Serotec). Densitometry was performed using Image J (NIH, Bethesda, MD, USA).

Thymidine incorporation assay

Proliferation was measured by [³H]-thymidine incorporation assay performed on retrovirally transduced, sorted, EGFP⁺ fetal liver or bone marrow LDMNCs with indicated concentrations of murine GM-CSF; LY294002 (Calbiochem), GDC-0941 (Axon MedChem, Groningen, The Netherlands), IC87114 (Selleck Chemicals, Houston TX, USA), or tipifarnib (R115777, Selleck Chemicals), as previously described.⁵

References

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