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BONE MARROW MYELOPOIESIS INDEPENDENTLY OF CANONICAL NOTCH SIGNALING

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Background: Notch signaling is a highly conserved pathway important in multiple developmental processes. Canonical signaling through all Notch receptors converges on the CsI transcription factor recombination signal binding protein for immunoglobulin kappa J region (Rbpj). In haematopoiesis, Notch is critical for the emergence of definitive hematopoietic stem cells (HSCs) in the embryo and in thymic T cell development. Contrastingly, canonical Notch signaling has been shown to be dispensable for HSC homeostasis in the adult bone marrow (aBM). Recent studies have however suggested a role of Notch in promoting megakaryocyte (Mk) and erythroid (E) progenitor cell development as well as in suppressing granulocyte-macrophage (GM) progenitor expansion and acting as a tumor-suppressor in myeloid malignancies. However, these findings were largely made through genetic approaches potentially also affecting regulatory pathways distinct from canonical Notch signaling.

Aims: To unambiguously investigate the role of canonical Notch signaling in aBM myelopoiesis, in steady-state and following transplantation.

Methods: B6-SJLCD45.1, *Rbpj*$^{fl/fl}$, *Mx1-Cre*, *Vav-Cre* and *Vwf-eGFP* BAC mice were used. Flow cytometry (FACS) was applied for phenotypic analyses. Gene expression levels were measured by real-time reverse transcription-PCR. Mk/E and GM *in vitro* colony forming potentials were applied in mouse colony assays. For transplantation study, lethally irradiated recipients were competitively transplanted (1:1) and reconstitution assessed 7-9 weeks after transplantation.

Results: FACS staging of GM, Mk and E progenitors in aBM of *loxP*-flanked *Rbpj* mice crossed to both *Mx1-Cre* and the pan-hematopoietic *Vav-Cre* strains was applied. As expected, HSCs were unaffected. Not previously investigated, FACS analysis of distinct stages of GM, Mk and E progenitors revealed no defects, at any progenitor stage, in *Rbpj*-deficient mice. To demonstrate that this lack of a phenotype was not due to BM cells escaping *Rbpj* deletion, we FACS purified HSCs and all GM, Mk and E progenitor stages from *Rbpj*-deficient mice and verified a virtually complete deletion of *Rbpj* in all populations. In further agreement with canonical Notch signaling not being required for steady-state generation, maintenance or stepwise differentiation of adult GM, Mk and E progenitors, the number of GM, E and Mk colonies generated from unfractionated aBM cells as well as circulating platelet counts were also unaffected in *Rbpj*-deficient mice. We next sought to address whether we could uncover a role of the Notch pathway in regulation of GM, Mk and E progenitors by establishing BM chimeras in which *Rbpj*-deficient progenitors compete with wild type (WT) progenitors for replenishment and differentiation in lethally irradiated recipients. No deficiencies were observed in the replenishment of HSCs and any stages of GM, Mk and E progenitors in mice competitively transplanted with *Rbpj*-deficient as compared to control WT BM cells. Moreover, transplanted *Rbpj*-deficient and control progenitors contributed equally well to platelet reconstitution. We next investigated whether loss of canonical Notch signaling might nevertheless impact on expression of genes for key regulators the Mk and E lineages at distinct progenitor stages for these lineages, as previously implicated. Notably, transcript levels of genes encoding key Mk/E regulators were unaffected in *Rbpj*-deficient Mk/E progenitors. In previous studies, expression of Notch target genes in Mk and E progenitors in aBM has been implicated as reflecting activation through Notch signaling. However, since the expression of Notch targets might also be regulated by other pathways besides Notch pathway, we investigated whether the expression of key Notch target genes (*Hes1, Hes5, Nrarp* and *Gata3*) in Mk/E progenitors in aBM was dependent on canonical Notch signaling. Neither in HSCs or any Mk/E progenitor was the expression of these Notch genes negatively affected by *Rbpj*-deficiency, demonstrating that their low expression levels in aBM HSCs and Mk/E is independent of canonical Notch signaling.

Summary/Conclusions: Studies implicating canonical Notch signaling as a critical regulator of aBM Mk, E and GM progenitors potentially failed to target only canonical Notch signaling. Herein, we demonstrate that canonical Notch signaling is dispensable for generation and replenishment of Mk, E and GM progenitors in aBM in steady-state as well as following BM transplantation.