PUM2 PROTEIN SUPPORTS MAINTENANCE AND SUPPRESSES DIFFERENTIATION OF MULTIPOTENT HEMATOPOIETIC PROGENITORS BY REGULATING THE FUNCTION AND ACTIVATION OF C-KIT RECEPTOR

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INTRODUCTION. Pumilio (Pum) RNA-binding proteins function as translational repressors during cell fate specification, and are necessary for germline stem cell self-renewal in \textit{Drosophila} and \textit{C. elegans} (1) The proposed primordial function of Pum protein family is to support maintenance and self-renewal of stem cells in diverse species and tissues (1, 2). We have characterized mouse and human Pum1 and Pum2 genes, which are abundantly transcribed in hematopoietic stem cells (HSC) (3, 4). To study its role in HSC, Pum2 was over-expressed in a SCF-dependent multipotent progenitor cell line EML, which can differentiate into erythroid, myeloid, and lymphoid lineages \textit{in vitro} (5).

RESULTS AND DISCUSSION. Over-expression of Pum2 leads to SCF-independent maintenance of EML cells, and attenuates their multilineage differentiation. This uncoupling of the survival and differentiation signals in EML cells is accompanied by (a) an increased expression of the full-length and novel truncated forms of c-kit receptor, p48 and p30 (Fig. 1A), and (b) cell intrinsic, SCF-independent activation of the c-kit (Figs. 1B and 1C), and its downstream MAPK, PI3K and PLC\textsubscript{γ} signaling pathways (not shown). In addition, Pum2-EML cells are resistant to treatment with blocking α-c-kit antibody (ACK2) and c-kit inhibitor STI-571, but not to treatment with MAPK, PI3K and PLC\textsubscript{γ} inhibitors.

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\caption{Fig. 1. A. Western}
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analysis with the rabbit $\alpha$-c-kit Ab shows that Pum2-EML cells express increased amounts of the full-length 160 kDa c-kit as well as truncated 48 and 30 kDa forms of c-kit. **B.** Both full-length c-kit and p48 c-kit are phosphorylated (pY730) in Pum2-EML cells in the absence of SCF. **C.** Western analysis with $\alpha$-c-kit pY730 antibody after serum and SCF starvation for 6 hours.

The p30 c-kit form EML cells represents a new truncated form of c-kit receptor called tr-kit (6), which is expressed preferentially in HSC, multipotent progenitors and testis (Fig. 2). Tr-kit could play a critical role in the SCF-independent activation of the full-length c-kit. In the absence of SCF, tr-kit signaling prevails over the canonical SCF-dependent c-kit signaling, leading to an increased maintenance and attenuated differentiation. Taken together, these findings suggest a model in which the maintenance of HSC and multipotent hematopoietic progenitors could be mediated through SCF-independent tr-kit signaling, whereas their differentiation depends on the canonical SCF-induced c-kit signaling.

Fig. 2. RT-PCR analysis of the tr-kit and c-kit expression in (A) wt EML, vector control and Pum2-EML cells, and (B) mouse fetal liver HSC (FL Sca-1$^+$AA4.1$^+$) and progenitors (FL AA4.1$^+$), and bone marrow HSC (BM Rho-123$^{low}$ Sca-1$^+$) and progenitors (BM Lin$^-$ Sca-1$^+$ cells). Amplification of the HPRT and the testis cDNA served as positive controls.

**REFERENCES**