論文の内容の要旨

論文題目　Evi1 is a stem cell-specific regulator of self-renewal capacity in the definitive hematopoietic system.

（Evi1 は造血系における幹細胞特異的な自己複製能の制御因子である。）

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Self-renewal is a defining property of stem cells. Although a number of molecules have been implicated in the regulation of hematopoietic stem cell (HSC) self-renewal, these genes are widely expressed in the hematopoietic system, and their mutations in genetic models are exclusively accompanied with other hematological abnormalities. Thus, a bona fide stem-cell specific regulator of their function has not been identified and the functional identification of HSCs based on their ability to self-renew remains difficult. Ecotropic viral integration site 1 (Evi1) is an oncogenic transcription factor in myeloid malignancies. Evi1 expression is limited to hematopoietic stem/progenitor fraction, and Evi1 is essential for the maintenance of HSCs, but is dispensable for blood cell lineage commitment. To elucidate Evi1 expression within the hematopoietic system, we have generated Evi1-IRES-green fluorescent protein (GFP) knock-in mice, in which GFP was expressed under the endogenous transcriptional regulatory elements of Evi1 gene.

First, I found Evi1 was predominantly expressed in the hematopoietic stem/progenitor fraction (Lin^- $Sca\text{-}1^+$ $c\text{-}kit^+$ (LSK)), but its expression was rapidly extinguished during early stages of lineage commitment. Among the LSK compartment, Evi1 was expressed at the highest level in the long-term HSC (LT-HSC) fraction ($Flk\text{-}2^-$ $CD34^-$ LSK or $CD48^-$ $CD150^+$ LSK). Next, I hypothesized that Evi1 would have the potential to mark long-term repopulating HSCs effectively. To test this issue, I compared GFP^+ and GFP^- cells in the LSK fraction, and revealed that LSK GFP^+ cells were more immature and quiescent with a higher colony-forming capacity than LSK GFP^- cells. In addition, in vivo long-term multilineage repopulating cells were exclusively enriched in the LSK GFP^+ fraction. Furthermore, even within the highly subfractionated LT-HSC fraction ($Flk\text{-}2^-$ $CD34^-$ LSK or $CD48^-$ $CD150^+$ LSK), long-term repopulating HSCs predominantly reside in the GFP^+ fraction, suggesting Evi1 expression can further augment the conventional HSC purification strategy.

In the embryo, Evi1 was highly expressed in the hematopoietic stem/progenitor fraction; that is, $CD45^+$ $CD34^+$ $c\text{-}kit^+$ cells in embryonic day 10.5 (E10.5) aorta-gonad-mesonephros, $CD34^+$ $c\text{-}kit^+$ $CD48^-$ cells in E12.5 placenta, and $Mac\text{-}1^+$ $Sca\text{-}1^+$ Lin^- (MSL) $CD48^-$cells in E14.5 fetal liver. In vivo competitive repopulation assay showed that, in the $CD34^+$ $c\text{-}kit^+$ $CD48^-$ fraction of E12.5 placenta or the MSL fraction of E14.5 fetal liver, GFP^+ cells exclusively had a long-term multilineage repopulating capacity, suggesting a specific relationship between Evi1 expression and HSC self-renewal capacity throughout ontogeny.

These results implied Evi1 plays a more specific role in LT-HSCs than in other

hematopoietic cells. To clarify this, I analyzed heterozygous Evi1 knockout mice (*Evi1*$^{+/-}$ mice). Here I demonstrated LT-HSCs (Flk-2^{-} CD34^{-} LSK or CD48^{-} CD150^{+} LSK cells) from *Evi1*$^{+/-}$ mice exhibited a marked reduction in frequency as compared to *Evi1*$^{+/+}$ controls. However, there were no significant differences in the numbers of lymphoid and myeloid progenitors between *Evi1*$^{+/+}$ and *Evi1*$^{+/-}$ mice. *Evi1*$^{+/-}$ CD34^{+} LSK cells had an equivalent in vitro colony-forming capacity and day 11 colony-forming unit-spleen activity to *Evi1*$^{+/+}$ CD34^{+} LSK cells. However, *Evi1*$^{+/-}$ CD34^{-} LSK cells had a pronouncedly impaired in vivo repopulating capacity. These data suggested the differentiation capacity of *Evi1*$^{+/-}$ HSCs was maintained, but their self-renewal capacity was specifically reduced. In contrast, Evi1 overexpression in HSPCs suppresses differentiation and boosts self-renewal activity in vitro. Re-introduction of Evi1, but not Mds1-Evi1, rescues the HSC defects caused by Evi1 heterozygosity.

Thus, in addition to documenting a specific relationship between Evi1 expression and HSC self-renewal activity, these findings highlight the utility of Evi1-IRES-GFP reporter mice for the identification and sorting of functional HSCs.