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The Histone H2A Deubiquitinase *Usp16* is Required for Mouse Fetal Hematopoiesis

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Introduction

Hematopoiesis, the process by which all blood cells are generated, is controlled by an interactive network of transcription factors and epigenetic regulators [1-3]. In previous studies, we discovered that *USP16*, a histone H2A-specific deubiquitinase, plays an indispensable role in mouse adult hematopoiesis and is require for hematopoietic stem cell (HSC) to undergo differentiation [4]. In this study, we investigated the function of *Usp16* in mouse fetal hematopoiesis by crossing *Usp16* conditional knockout mice with Vav^{Cre} mice. While $Usp16^{KO}$ E14.5 embryos appear normal, E17.5 $Usp16^{KO}$ embryos are pale. The frequencies of LSK cells are increased and the frequencies of MyePro cells are decreased in these embryos. While the absolute number of long-term HSC is not changed, there is a significant decrease of ST-HSC numbers. This study reveals that *Usp16* is required for mouse fetal hematopoiesis and *Usp16* regulates long-term HSC to ST-HSC transition during fetal hematopoiesis.

Methods

Usp16 hematopoietic system deletion

Usp16 conditional knockout mice were described previously [5]. These mice were backcrossed to C57BL/6 over 6 generations. To delete *Usp16* in the hematopoietic system, $Usp16^{2lox/2lox}$: Vav^{Cre}

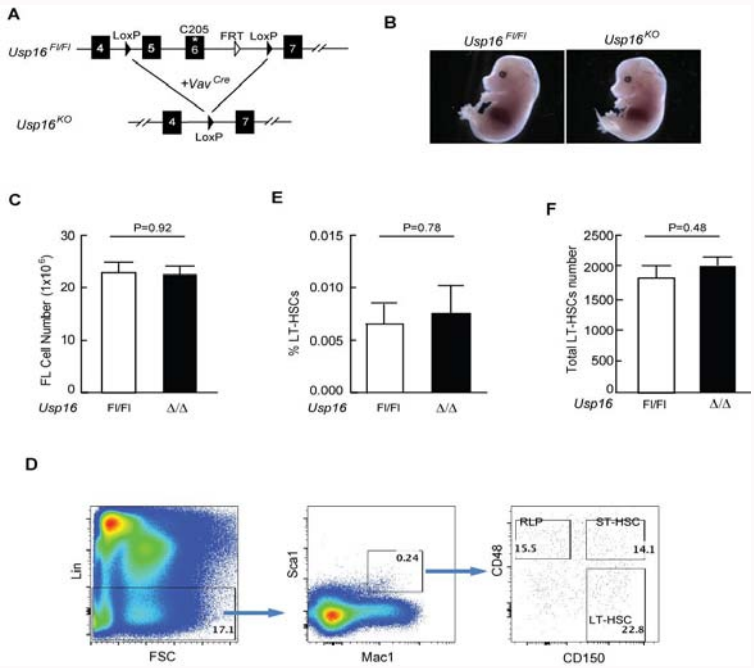

Figure 1: *Usp16* deletion does not affect fetal hematopoiesis at E14.5.
A. Schematic representation of the strategies used to delete *Usp16* in fetal liver. Partial regions of *Usp16* locus (from exon 4 to exon 7) are shown. Exons are shown as filled boxes, introns as black lines, loxp sites as filled triangles, and FRT sites as empty triangles. Position of cysteine 205, an amino acid essential for *Usp16* de-ubiquitination activity, is indicated by a "*".
B. Representative images of the morphology of control and $Usp16^{KO}$ embryos at E14.5.
C. Total cell numbers of E14.5 fetal liver (FL) in control and $Usp16^{KO}$ embryos.
D. Sample plots of FACS analysis for HSC in E14.5 FL.
E-F. Frequencies (E) and total cell number (F) of long-term (LT) HSC in E14.5 FL.

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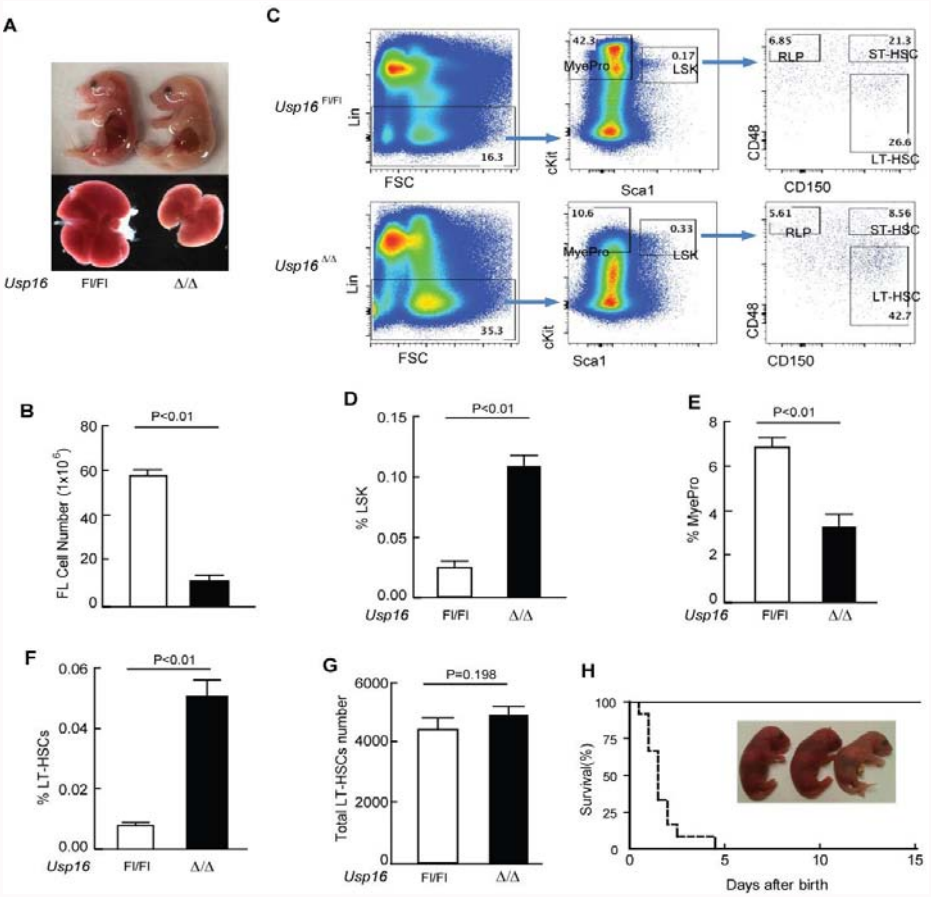

Figure 2: *Usp16* deletion affects LT-HSC to ST-HSC transition in fetal hematopoiesis.
A. Representative images of the morphology of E17.5 embryos and FL. *Usp16*^{KO} embryos are apparently pale as compared to wild-type controls. FL is smaller than wild-type controls.
B. Total cell numbers of E17.5 FL. There is a five-fold reduction of FL cell numbers.
C. Sample plots of FACS analysis for HSC in E17.5 FL. There is a significant reduction of MyePro frequencies and significant increase of LSK frequencies. LT-HSC frequency was also increased while ST-HSC frequency was significantly reduced. RLP frequency was not apparently affected.
D-E. Frequencies of LSK (D) and MyePro (E) in E17.5 FL.
F-G. Comparison of the frequencies (F) and absolute cell number (G) in E17.5 FL of control and *Usp16*^{KO} mice. There is a significant increase of the frequency of LT-HSC but the absolute cell numbers do not change.
H. Kaplan-Meier survival curve of control and *Usp16*^{KO} pups. An image of newborn pups is shown. *Usp16*^{KO} pups are pale.

mice were obtained by crossing *Usp16*$^{2lox/+}$ mice with *Vav*^{Cre} transgenic mice. All animal experiments were carried out according to the guidelines for the care and use of laboratory animals of the University of Alabama at Birmingham under an IACUC-approved protocol.

Flow cytometry

For HSC staining, Fetal liver cells were incubated with PE-conjugated lineage marker antibodies (Ter119 [TER119], B220 [RA3-6B2], Gr-1 [1A8], CD11b [M1/17], CD3e [145-2C11], CD4 [RM4-5], CD8a [53-6.7], CD5 [53-7.3]), APC-conjugated c-Kit (2B8), V500-conjugated Sca-1 (D7), PE-CY7-conjugated CD150 (mShad150), and APC-CY7-conjugated CD48 (HM48-1) antibodies. Dead cells were excluded by staining with 7-amino actinomycin D (7AAD, BD Pharmingen). After washing, labeled cells were run on BD LSR II for analysis or on BD FACSAria for sorting. CD150 antibodies were purchased from eBioscience. All other antibodies were obtained from BD Pharmingen.

Results and Discussion

To study the function of *Usp16* in fetal hematopoiesis, we crossed *Usp16* conditional knockout mice with *Vav*^{Cre} transgene mice, which express Cre recombinase in the hematopoietic system [6]. Cre-mediated recombination resulted in deletion of *Usp16* exons 5 and 6, whereas the catalytic cysteine 205 residue is located (Figure 1A, hereafter referred as *Usp16*^{KO}). At E14.5, *Usp16*^{KO} embryos were morphologically normal (Figure 1B). Total cell numbers in fetal liver (FT) are similar between control and *Usp16*^{KO} embryos (Figure 1C). The frequencies and absolute numbers of long-term (LT)-HSCs (Lin^{-} CD48^{-}CD150^{+}Mac1^{+}Sca-1^{+} cells) between *Usp16*$^{Fl/FL}$ and *Usp16*^{KO} embryos are also similar (Figure 1D-1E). Based on these studies, we concluded that Usp16 does not affect fetal hematopoiesis at E14.5 stage.

We then analyzed fetal hematopoiesis at E17.5. At this stage, *Usp16*^{KO} embryos appeared pale and the size of FL was significantly reduced (Figure 2A). Consistently, we found the total numbers of FL cells reduced by 5-fold (Figure 2B). We then performed detailed analysis of fetal hematopoiesis at this stage (Figure 2C). The frequencies of LSK cells were significantly increased (Figure 2D) and that of MyePro cells were significantly decreased in *Usp16*^{KO} embryos as compared to control *Usp16*$^{Fl/FL}$ embryos (Figure 2E). The absolute number of FL LT-HSC was not changed in *Usp16*^{KO} embryos as

compared to *Usp16*$^{Fl/FL}$ embryos (Figure 2G) but there was a significant increase of LT-HSC frequency in *Usp16*^{KO} embryos (Figure 2F). There was also a significant decrease of ST-HSC frequencies (Figure 1C). *Usp16*^{KO} pups were pale and died within 5 days after birth (Figure 2H). These data indicate that *Usp16*^{KO} FL LT-HSCs are defective in differentiation and *Usp16* regulates the transition from LT-HSC to ST-HSC at fetal hematopoiesis. This is different from the situation in adult hematopoiesis, where Usp16 regulates the transition from HSC to MyePro transition while the transition from LT-HSC to ST-HSC and RLP is not affected [4]. In summary, the histone H2A deubiquitinase appears to regulate hematopoiesis in a stage-specific manner.

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