

Lis1 is required for the expansion of hematopoietic stem cells in the fetal liver

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Dear Editor,

Hematopoietic stem cells (HSCs) are responsible for the lifelong production of all blood cells [1]. Like many other tissues and organs, the hematopoietic system develops during embryogenesis [2]. One of the challenges of this developing process is to generate a functional HSC pool, which is a prerequisite for seeding bone marrow and thereafter lifelong hematopoiesis [3]. HSC expansion is critical for the establishment of such HSC pool [2]. To accomplish the expansion, the fetal liver HSCs (FL-HSCs) are continuously undergoing symmetric cell division, which requires precise collaboration of multiple cellular processes [2], especially those that serve to maintain the genomic stability of the dividing HSCs. Many factors have been shown to be involved in FL-HSC expansion. Growth factors such as insulin-like growth factor 2 (IGF2) and stem cell factor (SCF) stimulate FL-HSC proliferation [4, 5], and key transcription factors such as Gata2 and PU.1 regulate the proliferation and maintenance of fetal HSCs by regulating multiple downstream target genes [6, 7]. However, the intrinsic factors that directly regulate FL-HSC expansion remain to be investigated.

Lis1 is identified as the first lissencephaly gene, and is well-known for its function in regulating the microtubule (MT)-associated proteins [8]. By interacting with different partners, *Lis1* participates in several critical cell functions, including mitosis, actin cytoskeleton regulation and membrane protein trafficking [8, 9]. Notably, *Lis1* controls mitotic spindle orientation and MT plus-end dynamics to ensure precise cell division via the *Lis1*-NDEL1-dynein complex [10]. Together, the primary function of *Lis1* in mammalian cells is to regulate MT-associated processes and facilitate cell division.

In the present study, we are interested in whether *Lis1* is involved in the regulation of FL-HSC expansion. To this end, we conditionally deleted *Lis1* from prenatal hematopoietic cells by breeding *Lis1^{LoxP}* mice with the *Vav1-cre* strain. *Lis1* mRNA level was significantly downregulated since 12.5 days post coitum (dpc) (Supplementary

information, Figure S1A). *Vav1-cre⁺Lis1^{LoxP}* mice (designated *Lis1^{-/-}* mice) could not be born due to embryonic lethality. *Lis1*-deficient embryo displayed normal morphology, but pale body and small-sized fetal liver (Figure 1A). And *Lis1*-deficient mice showed a decrease in fetal liver cell number (Figure 1B) and a rapid decline in all hematopoietic cells (Supplementary information, Figure S1B and S1C) compared to control littermates (*Lis1^{LoxP}* or *Lis1^{LoxP/+}*).

These phenotypes inspired us to evaluate the hematopoietic stem and progenitor cells. Indeed, *Lis1*-deficient mice showed significant reduction of myeloid-restricted progenitors (lineage⁻ cKit⁺ Sca1⁻, LK; Supplementary information, Figure S1D and S1E) and HSC-enriched population (lineage⁻ cKit⁺ Sca1⁺, LSK, which includes the hematopoietic stem and progenitor cells, and hereafter referred to as HSC [11]; Figure 1C and Supplementary information, Figure S1F) after 12.5 dpc and 14.5 dpc, respectively. Moreover, *Lis1*-deficient highly purified HSCs (lineage⁻ cKit⁺ Sca1⁺ Mac1⁺ CD150⁺ CD48⁻) stayed at a barely detectable level since 12.5 dpc (Figure 1D and Supplementary information, Figure S1G). Altogether, these results indicate that *Lis1* is critical for establishing the HSC pool in the fetal liver.

Considering that a single HSC has the ability to repopulate the whole hematopoietic system [12], we wondered whether *Lis1*-deficient FL-HSCs have functional defects. To test this hypothesis, we carried out colony-forming unit (CFU) assays. Whole fetal liver cells from *Lis1*-deficient mice formed significantly fewer (Figure 1E) and smaller (Supplementary information, Figure S1H) colonies. Besides, neither multilineage (CFU-GEMM) nor myeloid (CFU-GM) colonies were formed by 100 *Lis1*-deficient LSKs (Supplementary information, Figure S1I), which indicates that no functional multipotent progenitors could be found in *Lis1*-deficient LSKs. To further analyze the function of *Lis1*-deficient FL-HSCs, 1 000 *Lis1*-deficient LSKs (CD45.2⁺) derived from a single fetal liver were transplanted into lethally irradiated recipient (CD45.1⁺) mice together with 500 000 recipient competitor cells. *Lis1*-deficient LSKs failed to multilineage

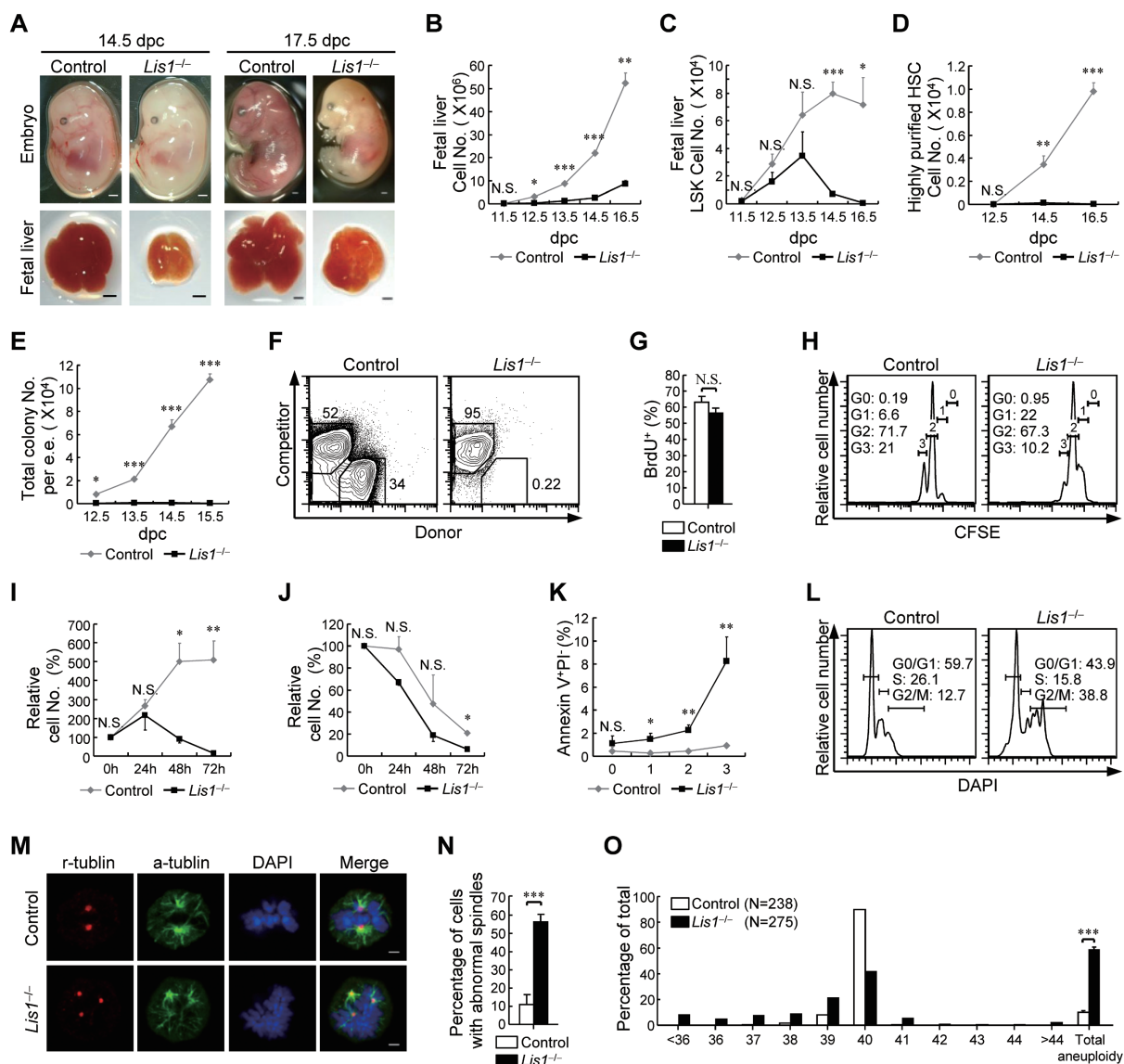


Figure 1 Lis1 is required for the expansion of FL-HSCs. **(A)** Representative images of embryos (top) and fetal livers (bottom) from control and *Lis1*^{-/-} mice at 14.5 dpc (left) and 17.5 dpc (right). Scale bar, 1 mm. **(B)** Absolute cell number of fetal livers from control littermates and *Lis1*^{-/-} mice at different gestational ages (*n* = 2-8 for each genotype for each gestational age). **(C, D)** Absolute cell number of LSKs (**C**) and highly purified HSCs (**D**) from control and *Lis1*-deficient fetal liver cells at different gestational ages (*n* = 2-8 for each genotype for each gestational age). **(E)** Number of colonies generated from control and *Lis1*-deficient fetal livers in CFU assays (*n* = 2-3 for each gestational age, data are represented as number per embryo equivalent). **(F)** Flow cytometry analysis of donor chimerism in recipients transplanted with 1 000 sorted LSKs from control or *Lis1*-deficient 12.5 dpc fetal livers (*n* = 7 for control LSKs and *n* = 6 for *Lis1*-deficient LSKs from 3 independent experiments; data were obtained by analyzing the peripheral blood mononuclear cells from each recipient at week 4 post-transplantation). **(G)** Percentage of the LSKs entering S phase in control and *Lis1*-deficient 12.5 dpc fetal livers (*n* = 3 for control littermates, *n* = 4 for *Lis1*^{-/-} mice). **(H)** Representative CFSE staining profiles of sorted CFSE⁺ LSKs cultured for 20 h (*n* = 5). **(I, J)** Relative survival rates of the LSKs from control or *Lis1*-deficient 12.5 dpc embryos cultured with **(I)** or without **(J)** SCF stimulation for the indicated periods of time. Relative survival rate was calculated as the ratio of surviving cells (Annexin V⁻ PI⁻) relative to the onset of the culture (*n* = 3 for stimulated samples and *n* = 2 for unstimulated samples). **(K)** Percentage of CFSE⁺ LSKs undergoing apoptosis (Annexin V⁺ PI⁺) after 10 h (for generations 0 and 1) or 20 h (for generations 2 and 3) culture (*n* = 5). **(L)** Representative histograms showing the cell cycle profiles of control and *Lis1*-deficient LSKs at 12.5 dpc (*n* = 3). **(M)** Representative metaphase images of control and *Lis1*-deficient LSKs that exhibited multipolar mitotic spindles. α -tubulin (green) marks mitotic spindles, γ -tubulin (red) marks centrosomes, and DAPI (blue) marks chromosomes (*n* = 4). Scale bar, 2 μ m. **(N)** Frequency of control and *Lis1*-deficient LSKs that exhibited multipolar mitotic spindles (*n* = 56 for control LSKs and *n* = 60 for *Lis1*-deficient LSKs from 4 independent experiments). **(O)** Percentages of the LSKs that contained the indicated number of chromosomes as well as the total aneuploidy (data were obtained from 5 independent experiments). The data are mean \pm SEM. For all panels: N.S., no significance. **P* < 0.05, ***P* < 0.001, ****P* < 0.0001 by Student's *t*-test.

age reconstitute the irradiated mice compared to control LSKs (Figure 1F). These data suggest that FL-HSCs fail to sustain normal hematopoiesis in the absence of *Lis1*.

The defects of *Lis1*-deficient FL-HSCs prompted us to evaluate their proliferative capacity and cell viability. To our surprise, the LSKs from *Lis1*-deficient fetal livers could normally enter cell cycle (Figure 1G) and undergo certain rounds of division (Figure 1H). However, in *in vitro* culture system, *Lis1*-deficient LSKs depleted rapidly after 48 h, though normal expansion was observed before depletion (Figure 1I). Interestingly, *Lis1*-deficient LSKs declined similarly compared to control LSKs in the culture that did not trigger cell proliferation (Figure 1J). This exhaustion triggered by continuous cell division highlighted that *Lis1* might regulate FL-HSC survival in a cell division-dependent manner. To test this possibility, the apoptosis ratio of *Lis1*-deficient LSKs was analyzed. Notably, *Lis1*-deficient LSKs showed significantly higher apoptosis ratio since the first division and this ratio rose stepwise as the division progressed (Figure 1K). This division-dependent cell death provided an explanation for the fewer progenies generated after multiple rounds of division seen in Figure 1H. Interestingly, by overexpression of the anti-apoptotic Bcl-2 family members, the LSK proportions from *Lis1*-deficient fetal livers were partially recovered compared to those transduced with vector (Supplementary information, Figure S1J). Together, these data reveal that *Lis1* deficiency leads to a rapid cell death of the FL-HSCs in a cell division-dependent manner, and such a severe survival defect cannot be fully rescued by overexpression of the Bcl-2 family members.

To understand the mechanism by which *Lis1* regulates FL-HSC survival, especially in a cell division-dependent manner, we first examined whether *Lis1* deficiency leads to a dysregulation of cell cycle progression. In spite of the normal cell-cycle entry and proliferation capacity (Figure 1G and 1H), *Lis1*-deficient LSKs harbored a significantly larger proportion of cells that were in the G2/M phase (Figure 1L). This indicates that *Lis1*-deficient FL-HSCs might have a blockage in metaphase processing, which might be due to the dysregulation of centrosome formation and mitotic spindle assembly. Indeed, in the absent of *Lis1*, many of the dividing LSKs had supernumerary centrosomes and formed multipolar mitotic spindles (Figure 1M and 1N). As a result, they could not properly segregate their chromosomes and might eventually undergo apoptosis or produce aneuploid progeny. As expected, *Lis1*-deficient LSKs were often ($58.4\% \pm 2.1\%$) aneuploid, though control LSKs rarely ($10.3\% \pm 1.4\%$) had abnormal chromosome numbers (Figure 1O). Collectively, *Lis1* regulates the survival of continuously dividing FL-HSCs through guiding precise mitotic spin-

dle assembly and ensuring the genomic integrity of the expanding FL-HSCs.

In summary, our findings first identify *Lis1* as a key regulator that sustains FL-HSC expansion. In the absence of *Lis1*, FL-HSCs form multipolar mitotic spindles after several rounds of division. As a result, they fail to properly segregate their chromosomes, become aneuploid and therefore trigger apoptosis. Owing to the property that FL-HSCs are continuously dividing, the cell division-dependent apoptosis eventually leads to a total failure in FL-HSC expansion and embryonic lethality.

Interestingly, a recently published study shows that *Lis1* deficiency results in FL-HSC loss and embryonic lethality due to accelerated differentiation rather than survival defect of the stem cell compartment [13]. Our research here emphasizes that *Lis1* is required for the expansion of FL-HSCs by ensuring their genomic stability and therefore promoting their survival. *Lis1* deficiency causes severe survival defect in a cell division-dependent manner owing to the dysregulation of centrosome formation and mitotic spindle assembly. Together, these two studies reveal the critical functions of *Lis1*, albeit in different aspects, in regulating the hematopoietic system.

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)