A single copy of large tumor suppressor 1 or large tumor suppressor 2 is sufficient for normal hematopoiesis

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Abstract

Background: Hematopoietic stem cells (HSCs) have the ability to differentiate into all subsets of blood cells and self-renew. Large tumor suppressor 1 (LATS1) and large tumor suppressor 2 (LATS2) kinases are essential for cell cycle regulation, organism fitness, genome integrity, and cancer prevention. Here, we investigated whether Lats1 and Lats2 are critical for the maintenance of the self-renewal and quiescence capacities of HSCs in mice.

Methods: Quantitative reverse transcription-polymerase chain reaction was used to determine the expression levels of Lats1 and Lats2 in subsets of progenitor cells and mature bone marrow cells. A clustered regularly interspaced short palindromic repeats system was used to generate Lats1 or Lats2 knockout mice. Complete blood cell counts were used to compare the absolute number of white blood cells, lymphocytes, monocytes, neutrophils, and platelets between Lats1 or Lats2 heterozygotes and littermates. Flow cytometry was used to assess the size of hematopoietic progenitor cells (HPCs) and HSC pools in Lats1 or Lats2 heterozygotes and littermates. The comparison between the two groups was analyzed using Student’s t test.

Results: Lats1 and Lats2 were widely expressed in hematopoietic cells with higher expression levels in primitive hematopoietic cells than in mature cells. Lats1 or Lats2 knockout mice were generated, with the homozygotes showing embryonic lethality. The size of the HPC and HSC pools in Lats1 (HPC: wild-type [WT] vs. heterozygote, 220,426.77 ± 54,384.796 vs. 221,149.4 ± 42,688.29, P = 0.988; HSC: WT vs. heterozygote, 2498.932 ± 347.856 vs. 3249.763 ± 370.412, P = 0.152) or Lats2 (HPC: WT vs. heterozygote, 425,540.52 ± 99,721.86 vs. 467,127.8 ± 89,574.48, P = 0.527; HSC: WT vs. heterozygote, 4760.545 ± 1518.01 vs. 3327.437 ± 873.297, P = 0.050) heterozygotes were not impaired. Moreover, the depletion of Lats1 or Lats2 did not affect the overall survival of the heterozygotes (Lats1: P = 0.654; Lats2: P = 0.152).

Conclusion: These results indicate that a single allele of Lats1 or Lats2 may be sufficient for normal hematopoiesis.

Keywords: Hippo pathway; Hematopoietic stem cells; Stem cell self-renewal

Introduction

Hematopoietic stem cells (HSCs) are able to self-renew and differentiate into all subsets of blood cells, which is essential for maintaining hematopoietic homeostasis. At any point of time, most HSCs are found in a resting state, which is essential for their life-long hematopoiesis and the preservation of their self-renewal capacity. Investigating the molecular mechanisms of the regulation of HSC quiescence could improve our understanding of the molecular mechanisms underlying tissue regeneration and provide an insight into how these are disorganized under stress conditions. The quiescent state of the HSCs is precisely regulated by both extrinsic signals and intrinsic molecular mechanisms. Many cell-cycle regulators are critical in the transcriptional regulation of hematopoiesis, the regulation of oxidative stress, and chromatin modification, which have been previously reported to be regulated by intrinsic mechanisms.

The Hippo tumor suppressor pathway is a major regulator of the critical balance between proliferation, progenitor cell renewal, and differentiation during embryogenesis. Large tumor suppressor 1 (LATS1) and large tumor suppressor 2 (LATS2) are key mediators of Hippo signaling, which negatively regulate the activity of transcriptional co-activator with PDZ-binding motif...
(TAZ) and the oncogenic transcriptional co-activators Yes-associated protein (YAP).[6,7] The LATS1 and LATS2 kinases are critical for cell cycle regulation, genome integrity, cancer prevention, and organism fitness.[3] The depletion of Lats2 before E12.5 is embryonic lethal, and has been suggested as the result of accumulating genomic instability, mitotic defects, and abnormal proliferation.[9] However, a small number of Lats1 knockout mice are viable, although they suffer from various developmental defects and oncogene-induced sarcomas.[10] The dysregulation of LATS1 or LATS2 is sufficient to promote tumorigenesis in mice due to the hyperactivation of YAP/TAZ.[11,12] Moreover, the loss of LATS2 is associated with malignant mesothelioma.[13,14] Although the majority of the studies agree with the tumor suppressor model of the Hippo pathway, the functions of the Hippo pathway in cancer biology indicate that YAP may play a tumor suppressive role in hematologic malignancies.[15,16] In summary, these results indicate that LATS1 and LATS2 function as tumor suppressors and stem cell regulators.

While Hippo signaling and LATS1 and LATS2 have been extensively studied in many tissues, the role of LATS1 and LATS2 in the hematopoietic system remains unclear. In this study, we investigated the role of the Hippo pathway in hematopoiesis by generating Lats1 or Lats2 knockout mice using a clustered regularly interspaced short palindromic repeats (CRISPR) system. We demonstrate that a single allele of Lats1 or Lats2 is sufficient for normal hematopoiesis.

Methods

Ethical approval

All animal experiments and methods were performed with the approval of the Animal Committee of the Institute of Zoology, Third Military Medical University (No. AMU-WEC20191324).

Animals

C57BL/6 wild-type mice were obtained from the Third Military Medical University Center for Animal Experiment (Chongqing, China; License No. SCXK[Yu]2017-0002). Heterozygotes of Lats1 or Lats2 were obtained from Cyagen Biosciences (Suzhou, China; License No. SCXK [Su]2018-0003). All data were generated from mice at 8 to 12 weeks of age.

Plasmid constructs

LentiCRISPRv2-small guide RNA (sgRNAs) were constructed as described previously.[17,18] Briefly, sgRNAs against Lats1 or Lats2 were designed on the CRISPR design website (http://crispor.tefor.net/crispor.py). sgRNAs were synthesized with a BsmBI (Fermentas, Waltham, MA, USA) sticky-end, and the lentiCRISPRv2 plasmid was digested with the same enzyme. The sgRNAs were denatured for 10 min at 95°C and annealed by slowly cooling down to room temperature, then ligated into the sgRNA expression plasmid. In vitro transcription plasmids for CRISPR-associated protein 9 (Cas9) and sgRNAs were obtained from Addgene (Watertown, MA, USA; #44758 and #51132).

Generation of Lats1 and Lats2 knockout mice and genotyping

Cas9 messenger RNA (mRNA) was generated from a linearized Cas9 expression plasmid using the mMACHINE T7 kit (Fisher Scientific, Goteborg, Sweden), according to the manufacturer’s instructions. sgRNAs were transcribed from linearized sgRNA expression plasmids using the MEGAshortscript T7 kit (Fisher Scientific). The mRNA obtained were purified using a commercial transcription Clean-Up Kit (Fisher Scientific) and stored at −80°C for microinjection. Zygotes were obtained from C57BL/6J mice. Cas9 mRNA (150 ng/mL) and sgRNAs (100 ng/mL) were mixed together and microinjected into the zygotes in M2 culture medium (Sigma, St. Louis, MO, USA). The zygotes were then transplanted into pseudopregnant surrogate mice.

For the screening of the F0 founder of Lats1, polymerase chain reaction (PCR) products were generated from PCR genotyping using primers listed in Table 1, whereby the knockout allele generated a 630 bp band. The amplicons were then purified for DNA sequencing analysis. The DNA sequencing primers used are listed in Table 1. The DNA sequencing results revealed that Mouse-ID#3 was missing 2255 bases and had five-point mutations (GCAAG>TGTGC) in one strand; Mouse-ID#12 was missing 2251 bases and had one point mutation (C>A) in one strand. For the screening of the F0 founder of Lats2, the PCR products were generated from PCR genotyping using the primers listed in Table 1, whereby the knockout allele generated a band of 680 bp. The amplicons were then purified for DNA sequencing analysis. The DNA sequencing primers are listed in Table 1. The DNA sequencing results revealed that Mouse-ID#19 was missing 13,108 bases in one strand and Mouse-ID#22 was missing 13,130 bases in one strand.

The genotyping of adult mice tails was conducted by combined multiplex PCR using a mixture of three primers: Lats1-F, Lats1-R, and Lats1-Wt/He-R. The wild-type alleles produced a 421 bp band and the knockout allele generated a 630 bp band. Similarly, for the genotyping of the Lats2 knockout mice, Lats2-F, Lats2-R, and Lats2-Wt/He-R were used. The wild-type alleles generated a 530 bp band and the knockout allele yielded a 680 bp band. All of the primers used are listed in Table 1.

RNA extraction and quantitative reverse transcription PCR

Total RNA was isolated using RNAsiso Plus (Takara, Shiga, Japan), according to the manufacturer’s instructions. Complementary DNA (cDNA) was obtained using the PrimeScript RT Reagent Kit (Takara). Then, cDNA was subjected to real-time PCR with TB Green® Premix Ex Taq™ II (Takara) in a real-time PCR detection system (Bio-Rad, Hercules, CA, USA). All of the primers used are listed in Table 1. Experiments were conducted in triplicate for all samples. The results were analyzed using the 2−ΔΔCt (Ct: cycle threshold) method, and the actin, beta gene was used as a control.
Blood cell counts

Samples of peripheral blood were obtained from the tail vein of the mice and placed into anticoagulant tube with ethylenediaminetetraacetic acid (EDTA). The complete blood counts were determined using a Hemavet 950FS (Drew Scientific, Dallas, FL, USA).

Flow cytometry

Single-cell suspensions were prepared from bone marrow (BM) (femurs and tibiae). Red cells were lysed with red blood cell lysis buffer (Solarbio, Beijing, China) and then incubated with the corresponding antibodies for 15 min on ice. The following biotin- or fluorochrome-conjugated antibodies obtained from Biolegend (San Diego, CA, USA) were used: anti-Gr-1 (catalogue No. 108404; dilution 1:2000), anti-TER-119 (116204; 1:1000), anti-B220 (103204; 1:1000), anti-CD19 (115504; 1:1000), anti-IgM (408903; 1:1000), IL-7R (135006; 1:2000), and CD3 (100304; 1:2000) for lineage markers; streptavidin-phycocerythrin-cyanine-5 (PE-Cy5) (405205; 1:500), PE-Sca-1 (108108; 1:200), allophycocyanin-cyanine-7 (APC-Cy7)-c-Kit (105826; 1:200), PE-Cy7-CD48 (103424; 1:200), and APC-CD150 (115910; 1:100) for the analysis of HPCs, LSK cells, and HSCs; streptavidin-APC-Cy7 (1:200, 405208), PE-anti-Sca-1 (108108; 1:200), APC-anti-c-Kit (105812; 1:200), PE-Cy7-anti-CD16/32 (101318; 1:200), and BV421-anti-CD34 (152208; 1:200) for the analysis of lymphoid-primed multipotent progenitor (LMPPs); streptavidin-APC-Cy7 (1:200), PE-anti-Sca-1 (108108; 1:200), APC-anti-c-Kit (105812; 1:200), PE-Cy7-anti-CD16/32 (101318; 1:200), and BV421-anti-CD34 (152208; 1:200) for the analysis of common myeloid progenitors (CMPs), granulocyte-monocyte progenitors (GMPs), and megakaryocyte-erythroid progenitors (MEPs).[19,20]

Statistical analysis

All quantitative data are presented as the mean ± standard deviation of n = 5–6 mice per genotype. Data are either representative of three experiments or from three experiments. GraphPad Prism 6 software (Graphpad Software, San Diego, CA, USA) was used for all statistical analyses. Statistical comparisons were conducted using Student’s t test. P values of <0.05 were considered statistically significant.

Results

The core components Lats1 and Lats2 of the Hippo signaling pathway are expressed in HSPCs

To explore the role of Lats1 and Lats2 in hematopoietic cells, we first investigated the expression of Lats1 and Lats2 of the Hippo signaling pathway in the subsets of...
primitive and mature murine hematopoietic cells. In general, \( \text{Lats1} \) and \( \text{Lats2} \) were widely expressed in hematopoietic cells, with higher expression levels found in primitive hematopoietic cells than in mature cells, such as CD4\(^+\) or CD8\(^+\) T cells and B220\(^+\) B cells [Figure 1]. Notably, the expression of \( \text{Lats1} \) and \( \text{Lats2} \) was higher in hematopoietic progenitor cells (HPCs: Lin-Sca-1-c-Kit\(^+\)) than in long-term HSCs (LT-HSCs) (Lin-Sca-1\(^-\)c-Kit\(^-\)).


CD48−CD150+, LSK (Lin−Sca-1−c-Kit+) cells, CMPs (Lin−Sca-1−c-Kit+CD16/32−CD34+), MEPs (Lin−Sca-1−c-Kit+CD16/32−CD34−), and GMPs (Lin−Sca-1−c-Kit+CD16/32+CD34+). In addition, Lats1 had higher expression levels than Lats2 in hematopoietic cells, suggesting that Lats1 plays a more important role in hematopoietic cells.

Generation of mouse models for the Lats1 or Lats2 knockout

To investigate the function of Lats1 and Lats2 in normal hematopoiesis, Lats1 or Lats2 knockout mice were obtained using the CRISPR/Cas9 system. Two sgRNAs for each gene were designed to target exon 4 of Lats1 or exon 2 of Lats2. Two knockout lines for each gene (#3 and #12 for Lats1 and #19 and #22 for Lats2) were obtained [Figure 2A]. For Lats1 knockout mice, deletions of 2255 bp plus five point mutations (#3) and deletions of 2251 bp (#12) plus one point mutation led to frameshift mutations. Analogously, deletions of 13,108 and 13,130 bp resulted in frameshift mutations in the Lats2 knockout mice [Figure 2B]. The following studies were conducted with mice derived from two independent lines. Lats1 or Lats2 homozygous mutants were generated by inter-crossing heterozygous mice. However, no homozygotes were born from the Lats1 or Lats2 knockout lines [Figure 2C]. Heterozygous mice were viable and fertile and did not show any visible differences compared with their wild-type littermates, although the expression level of Lats1 and Lats2 was found to be halved in the BM [Figure 2D]. These results were identical to those obtained by previous studies, which reported that the Lats1 or Lats2 homozygous mutation resulted in embryonic lethality.\[9,10\]

Normal hematopoiesis in Lats1 or Lats2 knockout heterozygotes

As mentioned above, the expression level of Lats1 and Lats2 decreased by half in the BM [Figure 2D]. As such, we investigated whether the single allele of Lats1 or Lats2 was sufficient for normal hematopoiesis. To determine the consequences of the Lats1 or Lats2 heterozygous mutation within the hematopoietic system, several key hematological arguments were analyzed in 8 to 12 week-old mice. No difference was found in numbers of circulating white blood cells, neutrophils, lymphocytes, monocytes, platelets, and red blood cells, as well as hemoglobin concentration [Figure 3A and 3B]. The total number of BM cells was similar between heterozygous mice and their littermate controls [Figure 3C and 3D]. Similarly, flow cytometry analyses revealed no obvious differences in the frequency or total number of early HPCs (HPC, CMP, GMP, MEP, LMPP, and LSK) or HSCs (LT-HSCs and CD34 negative LSK [CD34−LSK]) [Figures 4 and 5]. Overall, based on the analysis of the expression of the cell markers and hematopoietic stem and progenitor cell assays, the hematopoietic profiles were normal in Lats1 or Lats2 heterozygotes. These results suggest that a single allele of Lats1 or Lats2 is sufficient for normal hematopoiesis.

![Figure 3: Peripheral blood cell count. (A, B) Absolute total number of WBC, LY, NE, MO, and EO (left), as well as RBC and concentration of Hb (middle) and PLT, in peripheral blood from 8-week-old Lats1 or Lats2 heterozygotes and their littermates. (C, D) Total BM cells in Lats1 or Lats2 heterozygotes and their littermates. BA: Basophils; BM: Bone marrow; EO: Eosinophils; Hb: Hemoglobin; Lats1: Large tumor suppressor 1; Lats2: Large tumor suppressor 2; LY: Lymphocytes; MO: Monocytes; NE: Neutrophils; PLT: Platelets; RBC: Red blood cells; WBC: White blood cells.](image-url)
Depletion of Lats1 or Lats2 did not affect the overall survival of heterozygotes.

To further evaluate the effects of the depletion of Lats1 or Lats2 on the hematopoietic reconstitution of mice, we treated Lats1 or Lats2 heterozygous and control mice weekly with injections of 5-FU,[21] which killed rapidly proliferating cells and replenished the hematopoietic system. These mice were monitored on a daily basis for 3 weeks. Both heterozygotes and wild-type control mice died gradually after the second injection of 5-FU, with the remaining mice dying after a third 5-FU injection [Figure 6]. There were no significant differences in overall survival between heterozygotes and wild-type control mice. These results indicated that the depletion of Lats1 or Lats2 did not affect the long-term self-renewal of HSCs.

Discussion

Hippo is a key regulator of organ size and stem cell function in the majority of tissues.[22] Lats1 and Lats2 are key mediators of Hippo signaling, which function to negatively regulate YAP and TAZ. To identify whether Hippo signaling is essential for hematopoiesis and the regulation of HSCs, we investigated two knockout mouse models of Lats1 and Lats2 mice. The knockout of Lats1 or Lats2 resulted in early embryonic lethality, which is in line with the results of previous studies.[9,10] We demonstrate that hematopoiesis or HSC function is normal in the heterozygotes of Lats1 and Lats2. Lats1 and Lats2 are considered key stemness genes; however, Lats1 and Lats2 are not haploinsufficiency genes. The functions of Lats1 and Lats2 are not dosage dependent and the remaining Lats1 or Lats2 are sufficient for normal hematopoiesis and HSC function.

Haploinsufficiency describes the situation in which a single functional copy of a gene is insufficient to preserve normal function, which is the main cause of dominant diseases. It has been reported that many genes are haploinsufficient in normal hematopoiesis, such as Apc, Npml, and macroH2A1.1.[23-25] The haploinsufficiency of Apc expands the HSC populations and reduces the capacity to regenerate hematopoiesis.[23] Our findings...
Figure 5: The size of the HPCs and HSCs pools were not impaired in the Lats2 heterozygotes. (A) Flow cytometry analysis of bone marrow cells from Lats2+/− and Lats2−/− mice. The numbers indicate the frequency of LSK (Lin−c-kit+Sca−1−) cells or HPCs (Lin−c-kit+Sca−1−) (left), LT-HSCs (Lin−c-kit+Sca−1−CD48 CD150−) (middle) or CD34 LSK cells (right). (B) The total number of LSK, LT-HSCs, and CD34 LSK cells in the bone marrow of 8-week-old Lats2+/+ and Lats2−/− mice. (C) Flow cytometry analysis of myeloid progenitor cells and HPCs from Lats2+/+ and Lats2−/− mice. The numbers indicate the frequency of MEPs (Lin−c-kit+Sca−1−CD16/32−CD34−) (bottom left), CMPs (Lin−c-kit+Sca−1−CD16/32−CD34+) (bottom right), GMPs (Lin−c-kit+Sca−1−CD16/32−CD34+) (top right), or LMPPs (Lin−c-kit+Sca−1−Flk2+) (right). (D, E) Total HPCs, CMPs, GMPs, and MEPs (D), and LMPPs (E) in the bone marrow of 8-week-old Lats2+/+ and Lats2−/− mice. CD34 LSK, CD34 negative LSK; CMP: Common myeloid progenitor; GMP: Granulocyte-monocyte progenitor; HPC: Hematopoietic progenitor cell; HSC: Hematopoietic stem cell; Lats2: Large tumor suppressor 2; Lin−: Lineage negative cell; LMPP: Lymphoid-primed multipotent progenitor; LSK: Lin−c-kit+Sca−1−; LT-HSC: Long term hematopoietic stem cell; MEP: Megakaryocyte-erythroid progenitor; SS Log: Side scatter log value.

Figure 6: (A and B) Survival curves of Lats1 or Lats2 heterozygotes and their wild-type littermates (n = 11−13 for each genotype) after three injections (arrowheads indicate 5-FU injection time: 0, 7, and 14 days) of 5-FU, presented as Kaplan-Meier curves. 5-FU: 5-Fluorouracil; Lats1: Large tumor suppressor 1; Lats2: Large tumor suppressor 2.
have indicated that the size of the HPC and HSC pools were not affected in the Lats1 and Lats2 heterozygotes. One possibility is that the downstream effectors of Hippo signaling, such as Yap1 and Taz, are not essential in normal hematopoiesis. The ectopic expression of Yap1 does not affect the number and function of HSCs neither in the steady state nor under stress conditions. Moreover, the double knockout of Yap1 and Taz does not affect physiological and malignant hematopoiesis.[27] As such, it seems that the Hippo signaling pathway is not essential in the hematopoietic system. Lats1 and Lats2 are considered to be stemness genes in many tissues, while the functions of Lats1 and Lats2 are clearly dependent on the context.[28] The Hippo signaling pathway is associated with various upstream regulatory molecules, the majority of which are still poorly characterized in mammalian cells. Hippo signaling plays a critical role in the cell-cell interactions that regulate cell proliferation through contact-mediated inhibition.[29] However, the cells in the hematopoietic system reside in a liquid environment, blood, and BM, which are distinct from solid organs. In solid organs, the contact-mediated inhibition of proliferation is critical for regulating tissue homeostasis, while this mechanism may not be effective in liquid tissue. This may explain why the depletion of Lats1 or Lats2 leads to overgrowth and aberrant cell proliferation in solid tissue, but not in hematopoietic cells.

Neurofibromatosis2 (NF2) is a well-studied upstream regulator of Hippo signaling in mammalian cells. NF2 is essential for contact-mediated inhibition in mammalian cells and inhibits Yap1 activity in the liver.[29] Loss of NF2 in the liver leads to an overgrowth phenotype in line with that observed in the livers of macrophage stimulating 1 (Mst1) and macrophage stimulating 2 (Mst2) deletion mice and mice with enforced expression of Yap1.[31] However, the loss of NF2 in hematopoietic cells does not affect the functions of progenitor cells and HSCs, which is consistent with our observations of Lats1 or Lats2 knockout.[30] In contrast, the depletion of NF2 has obvious effects on many non-hematopoietic cells of the BM microenvironment, resulting in secondary effects on the function and localization of HSCs.[30] One possibility is that NF2 functions through the Hippo signaling pathway in this specific context; therefore, it would be interesting to study the role of Lats1 and Lats2 within BM microenvironments.

In conclusion, our results show that the inhibition of the Hippo signaling pathway by the depletion of Lats1 and Lats2 does not affect hematopoiesis or HSC function. Our study has some limitations. This is a preliminary result on the role of Lats1 and Lats2 in normal hematopoiesis. The mRNA levels of Lats1 and Lats2 were found to decrease by half; however, the protein level and Lats1 and Lats2 kinase activity level should be studied further. Although it is clear that the HSC function in the Lats1 and Lats2 heterozygotes is not influenced during steady and regeneration states, we cannot exclude the possibility that the HSC function can change under conditions of drastic hematopoietic stress, such as serial transplantsations.[19] In addition, whether the depletion of Lats1 or Lats2, in the context of serial transplantsations, would affect the size of the HSC pool remains to be investigated further. More extensive and detailed work will be required to elucidate the function of Lats1 and Lats2 in a hematopoietic system-specific knockout mouse model.

Funding

This work was supported by grants from the National Key R&D Program of China (No. 2017YFA0106700), National Natural Science Foundation of China (No. 81670096), National Science Foundation of Chongqing (No. CSTC2016JCYJA0354), Army Major Scientific Research Projects (No. AWS17J007), Military Medical Innovation Program (No. SWH2018QNKJ-09, No. SWH2018LJ-07, and No, SWH2016ZDCX1010), and the Hubei Natural Science Foundation (No. 2018CFB434).

Conflicts of interest

None.

References

1. Laurenti E, Gottgens B. From haematopoietic stem cells to complex differentiation landscapes. Nature 2018;553:418–426. doi: 10.1038/nature25022.
2. Pinho S, Frenette PS. Haematopoietic stem cell activity and interaction with the niche. Nat Rev Mol Cell Biol 2019;20:303–320. doi: 10.1038/s41580-019-0103-9.
3. Pietras EM, Warr MR, Parkague E. Cell cycle regulation in hematopoietic stem cells. J Cell Biol 2011;195:709–720. doi: 10.1083/jcb.2010213.
4. van Velthoven CTJ, Rando TA. Stem cell quiescence: dynamism, restraint, and cellular idling. Cell stem cell 2019;24:213–225. doi: 10.1016/j.stem.2019.01.001.
5. Moya HM, Halder G. Hippo-YAP/TAZ signalling in organ regeneration and regenerative medicine. Nat Rev Mol Cell Biol 2019;20:211–226. doi: 10.1038/s41580-018-0086-y.
6. Zhang H, Liu CY, Zha ZY, Zhao B, Yao J, Zhao S, et al. TEAD transcription factors mediate the function of TAZ in cell growth and epithelial-mesenchymal transition. J Biol Chem 2009;284:13335–13362. doi: 10.1074/jbc.M900843200.
7. Zhao B, Li L, Tumaneng K, Wang CY, Guan KL. A coordinated phosphorylation by Lats and CK1 regulates YAP stability through SCF(beta-TRCP). Genes Dev 2010;24:72–85. doi: 10.1101/gad.1843810.
8. Furth N, Avlon T. The LATS1 and LATS2 tumor suppressors: beyond the Hippo pathway. Cell Death Differ 2017;24:1488–1501. doi: 10.1038/cdd.2017.99.
9. Yabuta N, Okada N, Ito A, Hosomi T, Nishihara S, Sasayama Y., et al. Lats2 is an essential mitotic regulator required for the coordination of cell division. J Biol Chem 2007;282:19259–19271. doi: 10.1074/jbc.M900843200.
10. St John MA, Tao W, Fei X, Fukumoto R, Carcangiu ML, Brownstein DG, et al. Mice deficient of Lats1 develop soft-tissue sarcomas, ovarian tumours and pituitary dysfunction. Nat Genet 1999;21:182–186. doi: 10.1038/35965.
11. Zhou D, Conrad C, Xia F, Park JS, Payer B, Yin Y, et al. Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. Cancer Cell 2009;16:423–438. doi: 10.1016/j.ccr.2009.09.026.
12. Nishio M, Sugimachi K, Goto H, Wang J, Morikawa T, Miyachi Y, et al. Dysregulated YAP1/TAZ and TGFB signaling mediate hepatocarcinogenesis in Mobl1/-deficient mice. Proc Natl Acad Sci U S A 2016;113:E71–E80. doi: 10.1073/pnas.1517188113.
13. Bueno R, Stawiski EW, Goldstein LD, Durinck S, De Rienzo A, Modrusan Z, et al. Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. Nat Genet 2016;48:407–416. doi: 10.1038/ng.3520.
14. Murakami H, Mizuno T, Taniguchi T, Fuji Y, Ishiguro F, Fuku T, et al. LATS2 is a tumor suppressor gene of malignant mesothelioma.
15. Barry ER, Morikawa T, Butler BL, Shrestha K, de la Rosa R, Yan KS, et al. Restriction of intestinal stem cell expansion and the regenerative response by YAP. Nature 2013;493:106–110. doi: 10.1038/nature11693.

16. Cottini F, Hideshima T, Xu C, Sattler M, Dori M, Agnelli L, et al. Rescue of Hippo coactivator YAP1 triggers DNA damage-induced apoptosis in hematological cancers. Nat Med 2014;20:599–606. doi: 10.1038/nm.3562.

17. Sanjana NE, Shalem O, Zhang F. Improved vectors and genome-wide libraries for CRISPR screening. Nat Methods 2014;11:783–784. doi: 10.1038/nmeth.3047.

18. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelson T, et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. Science (New York, NY) 2014;343:848–847. doi: 10.1126/science.1247005.

19. Hou Y, Wang X, Li L, Fan R, Chen J, Zhu T, et al. FHL2 regulates hematopoietic stem cell functions under stress conditions. Leukemia 2015;29:615–624. doi: 10.1038/leu.2014.254.

20. Hou Y, Li W, Sheng Y, Li L, Huang Y, Zhang Z, et al. The transcription factor Foxm1 is essential for the quiescence and maintenance of hematopoietic stem cells. Nat Immunol 2015;16:810–818. doi: 10.1038/nimm.3204.

21. Cheng T, Rodrigues N, Shen H, Yang Y, Dombkowski D, Sykes M, et al. Hematopoietic stem cell quiescence maintained by p21cip1/waf1. Science (New York, NY) 2000;287:1804–1808. doi: 10.1126/science.287.5459.1804.

22. Yu FX, Zhao B, Guan KL. Hippo pathway in organ size control, tissue homeostasis, and cancer. Cell 2015;163:811–828. doi: 10.1016/j.cell.2015.10.044.

23. Wang J, Fernald AA, Anastasi J, Le Beau MM, Qian Z. Haploinsufficiency of Apc leads to ineffective hematopoiesis. Blood 2010;115:3481–3488. doi: 10.1182/blood-2009-11-251835.

24. Raval A, Kusler B, Pang WW, Weissman IL, Mitchell BS, Park CY. Effect of nucleophosmin1 haploinsufficiency on hematopoietic stem cells. Leukemia 2012;26:853–855. doi: 10.1038/leu.2011.270.

25. Bereshtenko O, Lo Re O, Nikulenkov F, Flamini S, Kotaskova J, Mazza T, et al. Deficiency and haploinsufficiency of histone macroH2A1.1 in mice recapitulate hematopoietic defects of human myelodysplastic syndrome. Clin Epigenetics 2019;11:121. doi: 10.1186/s13148-019-0724-z.

26. Jansson L, Larsson J. Normal hematopoietic stem cell function in mice with enforced expression of the Hippo signaling effector YAP1. PLoS One 2012;7:e32013. doi: 10.1371/journal.pone.0032013.

27. Donato E, Biagioni F, Bisso A, Caganova M, Amati B, Campaner S. YAP and TAZ are dispensable for physiological and malignant hematopoiesis. Leukemia 2018;32:2037–2040. doi: 10.1038/s41375-018-0111-3.

28. Pan WW, Moroishi T, Koo JH, Guan KL. Cell type-dependent function of LATS1/2 in cancer cell growth. Oncogene 2019;38:2595–2610. doi: 10.1038/s41388-018-0610-8.

29. Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, et al. Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. Genes Dev 2007;21:2747–2761. doi: 10.1101/gad.1602907.

30. Larsson J, Ohishi M, Garrison B, Aspling M, Janzen V, Adams GB, et al. Nf2/merlin regulates hematopoietic stem cell behavior by altering microenvironmental architecture. Cell stem cell 2008;3:221–227. doi: 10.1016/j.stem.2008.06.005.

How to cite this article: Li ZG, Fu XM, Chai CY, Sun FF, Xiao FF, Huang YX, Yao K, Chen JP, Hou Y. A single copy of large tumor suppressor 1 or large tumor suppressor 2 is sufficient for normal hematopoiesis. Chin Med J 2020;133:1943–1951. doi: 10.1097/CM9.0000000000000934