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Wdr5 is essential for fetal erythropoiesis and hematopoiesis

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Abstract

WDR5 is a highly conserved protein that performs multiple scaffolding functions in the context of chromatin. However, efforts to understand the function of WDR5 in normal tissues physiologically are quite limited so far. In our study, we explored the function of Wdr5 in erythropoiesis and hematopoiesis by using a hematopoietic-specific *Wdr5* knockout mouse model. We found that loss of *Wdr5* mediated by *Vav-iCre* leads to embryonic lethality with defective erythropoiesis. In addition, Wdr5-deficiency completely impairs the hematopoietic stem and progenitor cells function and might alter the immunophenotype of these stem cells and progenitors by decreasing c-Kit expression. Collectively, we identified the pivotal role of Wdr5 in fetal hematopoiesis and erythropoiesis as the de novo findings.

Keywords Wdr5, Erythropoiesis, Hematopoiesis, Hematopoietic stem cell

To the editor,

The canonical function of WDR5 is as a core component of the MLL histone methyltransferase complexes [1]. While the function of several other subunits of MLL complexes has been well elucidated in hematopoiesis [2–12], little is known about the function of WDR5. Our study aimed to identify the role of Wdr5 in normal hematopoiesis by utilizing hematopoietic lineage specific knockout mouse model.

To investigate the function of Wdr5 in normal hematopoiesis, *Wdr5* was conditionally deleted in the

hematopoietic lineage by *Vav-iCre* transgenic mice (Additional file 1: Fig. S1A). Strikingly, we did not observe any *Wdr5*^{fl/fl}, *Vav-iCre* mice (referred as CKO mice) among 40 offspring from the intercross between the male *Wdr5*^{fl/fl} mice and the female *Wdr5*^{fl/+}, *Vav-iCre* mice at weaning age (Fig. 1A), which urged us to further dissect the effects at the fetal stage. Interestingly, the CKO embryos showed roughly normal morphology, but much paler body color and smaller-sized fetal livers (FLs) compared with the littermate control at E15.5 and E16.5 (Fig. 1B and Additional file 1: Fig. S1B). Consistently, the total cell number of FL from CKO was robustly decreased at E15.5 (Fig. 1C). We further dissected this at E13.5, and similar effect was observed (Additional file 1: Fig. S1C). However, the absolute cell numbers of FLs were comparable between CKO and the littermate control embryos at E12.5 (Additional file 1: Fig. S1D). These indicated that the defective erythropoiesis might occur in *Wdr5*-deficient embryos. Next, we evaluated the erythropoiesis (Additional file 1: Fig. S1E). Compared with the littermate control, CKO embryos showed an increased percentage of the immature population including S0-S2 but a decreased percentage of relative mature population S4, whereas the absolute cell numbers of all stages were reduced at E15.5 (Fig. 1D and Additional file 1: Fig.

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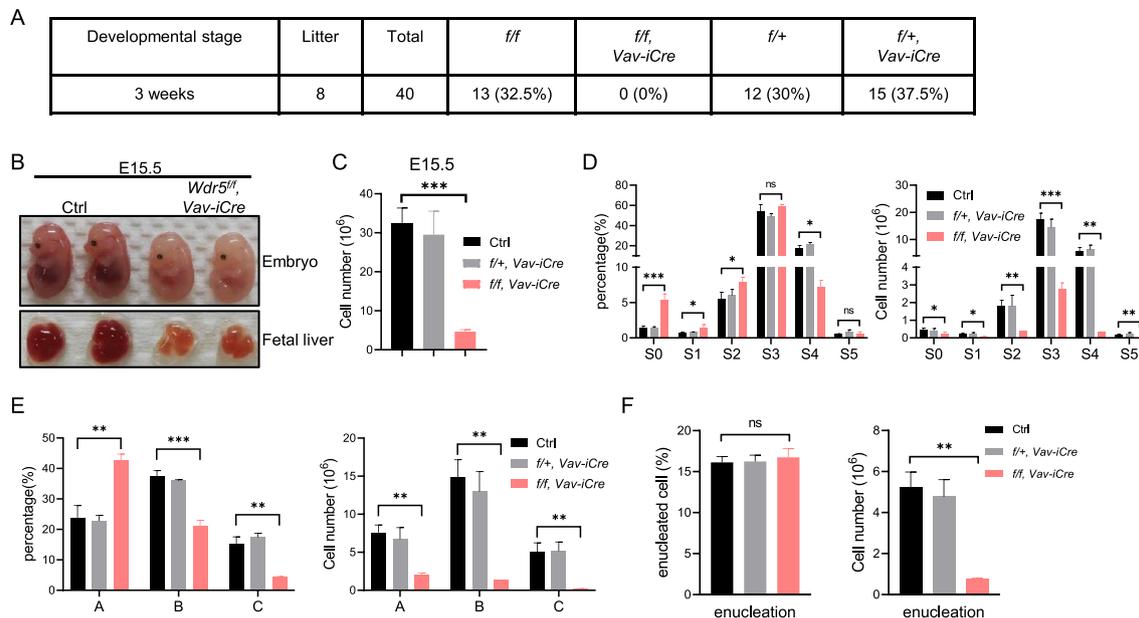


Fig. 1 Loss of *Wdr5* mediated by *Vav-iCre* leads to embryonic lethality with defective erythropoiesis. **A** The offspring from the intercross between the male *Wdr5^{ff}* mice and the female *Wdr5^{ff/+}, Vav-iCre* at weaning age. **B** Representative photographs of the embryos (top) and the FLs (bottom) from CKO and the littermate control at E15.5. **C** The absolute cell number of FLs from CKO, heterozygous, and the littermate control embryos at E15.5 ($n = 2-6$ per genotype). **D** Graph showing the percentage (left) and the absolute cell number (right) of CD71/Ter119 subsets in the FLs from CKO, heterozygous, and the littermate control embryos at E15.5 ($n = 2-6$ per genotype). **E** Graph showing the percentage (left) and the absolute cell number (right) of EryA/B/C subsets in the FLs from CKO, heterozygous, and the littermate control embryos at E15.5 ($n = 2-6$ per genotype). **F** Graph showing the percentage (left) and the absolute cell number (right) of the enucleated cells in the FLs from CKO, heterozygous, and the littermate control embryos at E15.5 ($n = 2-6$ per genotype). Statistical significance is indicated by ns for not statistically significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, or **** $p < 0.0001$. Data are presented as mean \pm SD

S1F). In addition, Ter119^+ cells could be further divided into EryA/B/C based on the FSC parameter (Additional file 1: Fig. 1E). Though the absolute cell numbers of EryA/B/C were reduced, the percentage of EryA was increased and the percentage of EryB/C was decreased in CKO embryos at E15.5 (Fig. 1E and Additional file 1: Fig. S1F). Furthermore, we examined whether the terminal erythroid differentiation is impaired in *Wdr5*-deficient embryos by Hoechst 33342 staining. Although the percentage of the enucleated erythrocytes were comparable in CKO and the littermate control embryos, the total number of the enucleated erythrocytes was decreased robustly in CKO embryos (Fig. 1F) and the enucleated cells from CKO embryos showed a larger size compared with those from the littermate control determined by the FSC parameter (Additional file 1: Fig. S1G). Collectively, these data suggested that loss of *Wdr5* results in the blockade of fetal erythropoiesis.

In addition to the defective erythropoiesis observed in the CKO embryos, we wondered whether the impaired hematopoiesis also contributed to the embryonic lethality by loss of *Wdr5*. We first examined the hematopoietic stem and progenitor cells (HSPCs) in the FLs from CKO or the littermate control embryos at E12.5. The

percentage and the absolute cells number of hematopoietic progenitor cells (HPCs) ($\text{Lin}^- \text{Sca1}^- \text{c-Kit}^+$) or LSK ($\text{Lin}^- \text{Sca1}^+ \text{c-Kit}^+$) were roughly normal in CKO embryos compared with the littermate control at E12.5 (Fig. 2A, B), indicating the initial seeding of HSPCs in the FLs was not influenced in the CKO embryos. We further examined the HSPCs in the FLs from CKO and the littermate control embryos at E13.5 and E15.5 (Fig. 2C, D). We found that the percentage and the absolute cell number of HPCs and LSK cells from FLs were significantly reduced in the CKO embryos compared with the littermate control at E13.5 (Fig. 2E). Intriguingly, the HSPCs underwent rapid expansion in the littermate control FLs but not in the CKO FLs from E13.5-E15.5 (Fig. 2E), indicating the crucial role of *Wdr5* in regulating the expansion of HSPCs in FLs. Notably, the expression of c-Kit on Lin^- population was slightly decreased in the E12.5 CKO embryos, then further decreased in the E13.5 CKO embryos, and almost diminished in the E15.5 CKO embryos (Fig. 2A, C and D), suggesting *Wdr5* might be required for the expression of c-Kit. Surprisingly, the percentage of $\text{cKit}^- \text{Sca1}^+$ population was increased in the CKO FLs at E13.5 and E15.5, so we further dissected this population. Interestingly, a certain

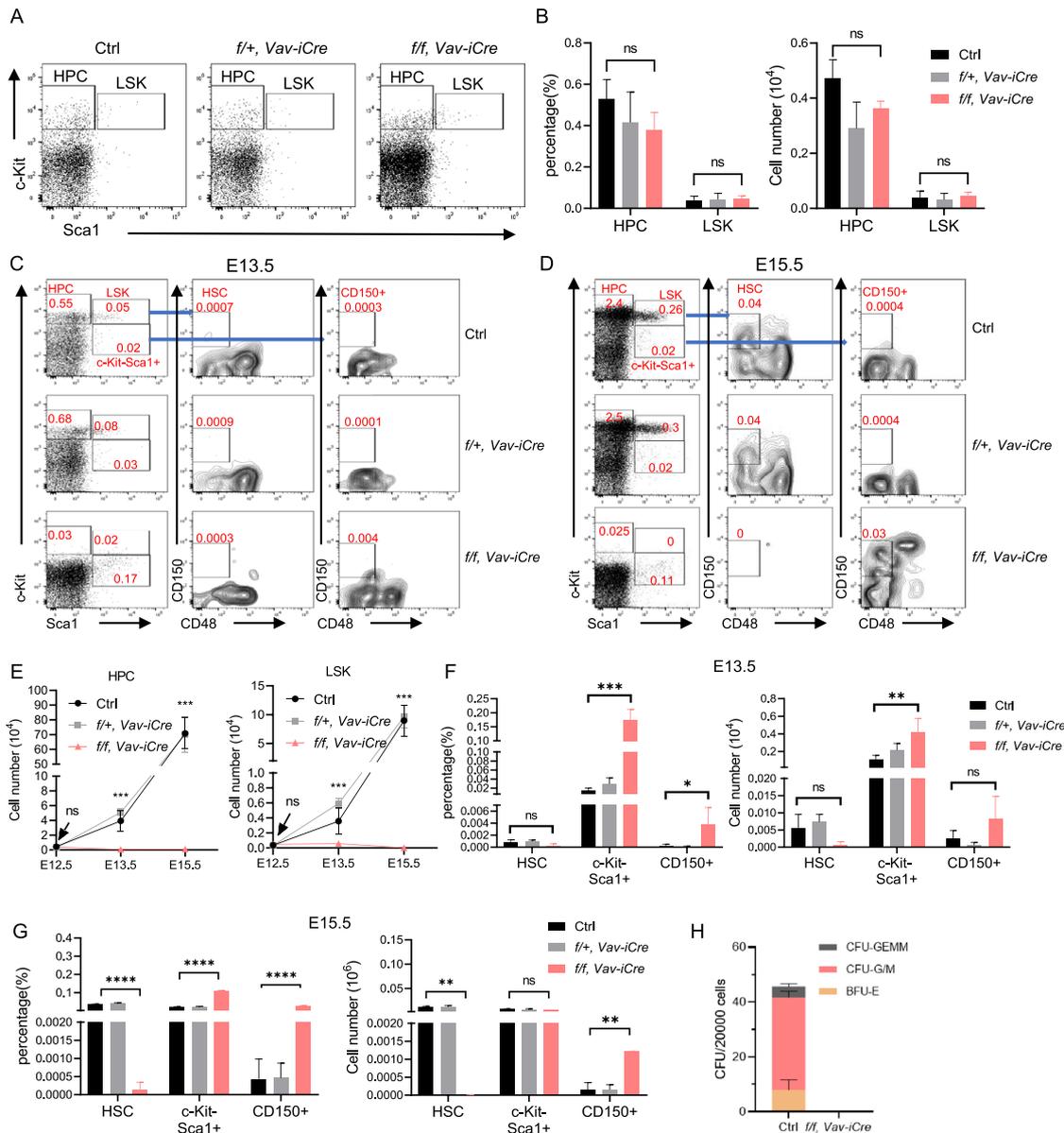


Fig. 2 Wdr5 plays a pivotal role in the maintenance of fetal HSPCs function. **A** Representative FACS profile showing the LSK and HPC populations from CKO, heterozygous, and the littermate control embryos at E12.5. **B** Graph showing the percentage (left) and the absolute cell number (right) of the LSK and HPC populations in the FLs from CKO, heterozygous, and the littermate control embryos at E12.5 (n = 3–6 per genotype). **C** and **D** Representative FACS profiles showing the HSPCs in the FLs from CKO, heterozygous, and the littermate control embryos at E13.5 (**C**) and at E15.5 (**D**). **E** Graph showing the absolute cell number of HPC (left) and LSK (right) in the FLs from CKO, heterozygous, and the littermate control embryos at various developmental stages (n = 2–7 per genotype for each stage). **F** Graph showing the percentage (left) and the absolute cell number (right) of HSC, cKit⁺Sca1⁺, and CD150⁺CD48⁻cKit⁺Sca1⁺ (CD150⁺) subsets in the FLs from CKO, heterozygous, and the littermate control embryos at E13.5 (n = 2–4 per genotype). **G** Graph showing the percentage (left) and the absolute cell number (right) of HSC, cKit⁺Sca1⁺, and CD150⁺CD48⁻cKit⁺Sca1⁺ (CD150⁺) subsets in the FLs from CKO, heterozygous, and the littermate control embryos at E15.5 (n = 2–4 per genotype). **H** Graph showing the colony number derived from CKO and the control FLs in CFU assay. Statistical significance is indicated by ns for not statistically significant, *p < 0.05, **p < 0.01, ***p < 0.001, or ****p < 0.0001. Data are presented as mean ± SD

percent of CD150⁺CD48⁻ population was found from c-Kit⁺Sca1⁺ population in CKO but hardly detected in the littermate control FLs at E15.5 (Fig. 2D, G). By contrast, the CD150⁺CD48⁻ population was only found

in the cKit⁺Sca1⁺ population from the control FLs but not the CKO FLs at E13.5 and E15.5, suggesting loss of Wdr5 might alter the HSPCs immunophenotype. Therefore, we performed the colony forming assay to detect the

function of HSPCs. Consistently, the CKO FL cells failed to give rise to any colonies in the colony forming assay in vitro (Fig. 2H), indicating loss of *Wdr5* impairs the function of HSPCs.

In conclusion, we revealed the crucial role of *Wdr5* in regulating fetal erythropoiesis as well as hematopoiesis as the de novo findings, which would broaden the understanding of *WDR5* function in this field. *WDR5* is a very promising therapeutic target in multiple cancers including MLL-rearranged leukemia with genetic model validation and the substantial efforts have been devoted to developing the inhibitors for *WDR5* [13]. Our findings for the physiological function of *Wdr5* will help to elucidate the safety profile of *WDR5* inhibition.

Abbreviations

KO	Knockout
FL	Fetal liver
HSPCs	Hematopoietic stem and progenitor cells
HPC	Hematopoietic progenitor cells

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40164-023-00385-3>.

Additional file 1: Table S1. List of the commercially available antibodies used in this study. **Figure S1.** Defective erythropoiesis was observed in the CKO embryos, related to Figure 1. (A) Schematic of the genetic targeting strategy to generate *Wdr5* KO mice. (B) Representative photographs of the embryos (top) and the FLs (bottom) from CKO and the littermate control at E16.5. (C and D) The absolute cell number of FLs from CKO, heterozygous, and the littermate control embryos at E13.5 (C) and E12.5 (D) ($n=2-7$ per genotype for each stage). (E) Strategy to analyze fetal erythropoiesis. (F) Representative FACS profile showing the various developmental stages of erythrocytes in the FLs from CKO, heterozygous, and the littermate control embryos at E15.5. (G) Representative FACS profiles to analyze enucleation. Statistical significance is indicated by ns for not statistically significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, or **** $p<0.0001$. Data are presented as mean \pm SD.

Acknowledgements

We thank Dr. Jinyong Wang for providing the *Vav-iCre* mice. We thank Anne Granger, En Li, Rishi Jain and John Tallarico for their support and scientific input, and NIBR Discovery Postdoctoral program for the support of Lulu Liu.

Author contributions

LL, YZ, and HW designed the study and interpreted data. LL performed the experiments and analyzed the data. LL, YF, XD, YZ and HW contributed to the mouse model generation. RT and WZ helped with the interpretation of the mouse study phenotype. HW guided the study and the team collaboration. LL, YZ and HW wrote the manuscript with the input from co-authors. All authors read and approved the final manuscript.

Funding

This study was supported by Novartis, the National Key R&D Program of China (2019YFA0111000) and Science and Technology Commission of Shanghai Municipality of China (19JC1414400) to YZ.

Availability of data and materials

Materials and original data generated in this study are available from the corresponding authors on the reasonable request.

Declarations

Ethics approval and consent to participate

All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) in China Novartis Institutes for BioMedical Research Co., Ltd. and the IACUC of Shanghai General Hospital, China.

Consent for publication

Not applicable.

Competing interests

All the authors affiliated with Novartis Institutes for BioMedical Research are or were employees of Novartis. The remaining authors declare they have no competing interests.

Received: 21 October 2022 Accepted: 9 February 2023

Published online: 15 April 2023

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