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LETTER

Deficiency of Trp53 and Rb1 in myeloid cell lineage spontaneously develops acute myeloid leukemia in a mouse model

Acute myeloid leukemia (AML) is a rapidly lethal blood cancer characterized by aberrant proliferation and differentiation of myeloid progenitors in the hematopoietic tissue. In recent decades, AML patients are significantly increased. In 2019 alone, 21,450 new AML cases were diagnosed in the United States, with an estimated 10,920 deaths due to AML. What's worse, AML occurs with an increasing incidence with advanced age, and the survival rate remains dismal with a median overall survival of only 5–10 months. Notwithstanding great advances in allogenic transplant and chemotherapy, the therapeutic outcomes are unfavorable due to the disease relapse and mortality rates. More importantly, the development of more specific and effective therapies was hampered in part by the lack of proper animal models for AML. Thus, management and elucidation of the pathological mechanism of AML remain an ongoing challenge, and the development of effective AML mouse models and treatment options are urgently needed.

Current leukemia mouse models range from xenograft models, and transgenic animals, to murine leukemia viruses (MuLV)- and carcinogen-induced tumors. Previous evidence showed that carcinogen-induced AML models were usually established in DBA/2 mice exposed to the compound carcinogen 3-methylcholantrene, and abrogation of protein tyrosine phosphatase 1 B (PTP1B) and phosphatase and tensin homolog (PTEN) in myeloid cell lineage by using LysM-Cre transgenic mice contributes to the AML formation. However, these kinds of models are less relevant to the onset and progression of human AML and not useful for developing novel therapeutic drugs.

The aberrations of tumor suppressors have proved to be a critical part of leukemogenesis, which provides potential therapeutic targets for the treatment of AML. Among these tumor suppressors, the mutations of Trp53 and Rb1 are most well-studied in different tumors. By analysis of 1540 AML patients, approximately 86% of AML patients have two or more drivers, and Trp53 and/or Rb1 gene(s) were found to be involved in AML progression.[1] Recent findings showed that Trp53 regulates normal and leukemic hematopoiesis and plays a pivotal role in the complicated network where the signaling pathways are caused by AML.[2,3] Accordingly, low or absent levels of Rb1 were reported in 19%–55% of AML patients, highlighting the dire consequence of Rb1 inactivation.[4] However, whether Trp53 and/or Rb1 deficiency in myeloid lineage results in AML is unknown.

To better understand the role of Trp53 and Rb1 in AML, we first identified their expression in human AML patients from the dataset available in the GEO database under accession number GSE114868.[5] Intriguingly, we found that the expression of Trp53 and Rb1 significantly decreased in human AML patients compared to the controls (Fig. S1A), indicating that Trp53 and Rb1 may be negative drivers of AML formation. To gain further insight into the function of Trp53 and Rb1 in AML, we next generated the conditional knockout mouse models by crossing floxed strains of Trp53 or/and Rb1 with the LysM-Cre mice, in which the Cre recombinase mainly expressed in the myeloid lineage such as monocyte, macrophage, and neutrophils (Fig. S1B). qRT-PCR verified that Trp53 and Rb1 were efficiently extinguished in LysM-expressing cells from LysM-Cre/+; $Trp53^{f/f}$, LysM-Cre/+; $Rb1^{f/f}$, and LysM-Cre/+; $Trp53^{f/f}$/$Rb1^{f/f}$ mice compared to those in controls (Fig. S2). As expected, the LysM-Cre/+; $Trp53^{f/f}$/$Rb1^{f/f}$ mice spontaneously developed AML-like phenotypes with visible development of tumors at 6 months, exhibiting seriously swollen abdomens due to hepatomegaly and splenomegaly with tumors (Fig. 1A), whereas there were no obvious morphological differences and tumor formation in LysM-Cre/+; $Trp53^{f/f}$ and LysM-Cre/+; $Rb1^{f/f}$ single gene KO mice compared to the controls at the observed time point of 12 months (Fig. S1C). Necropsy revealed an aggressive form

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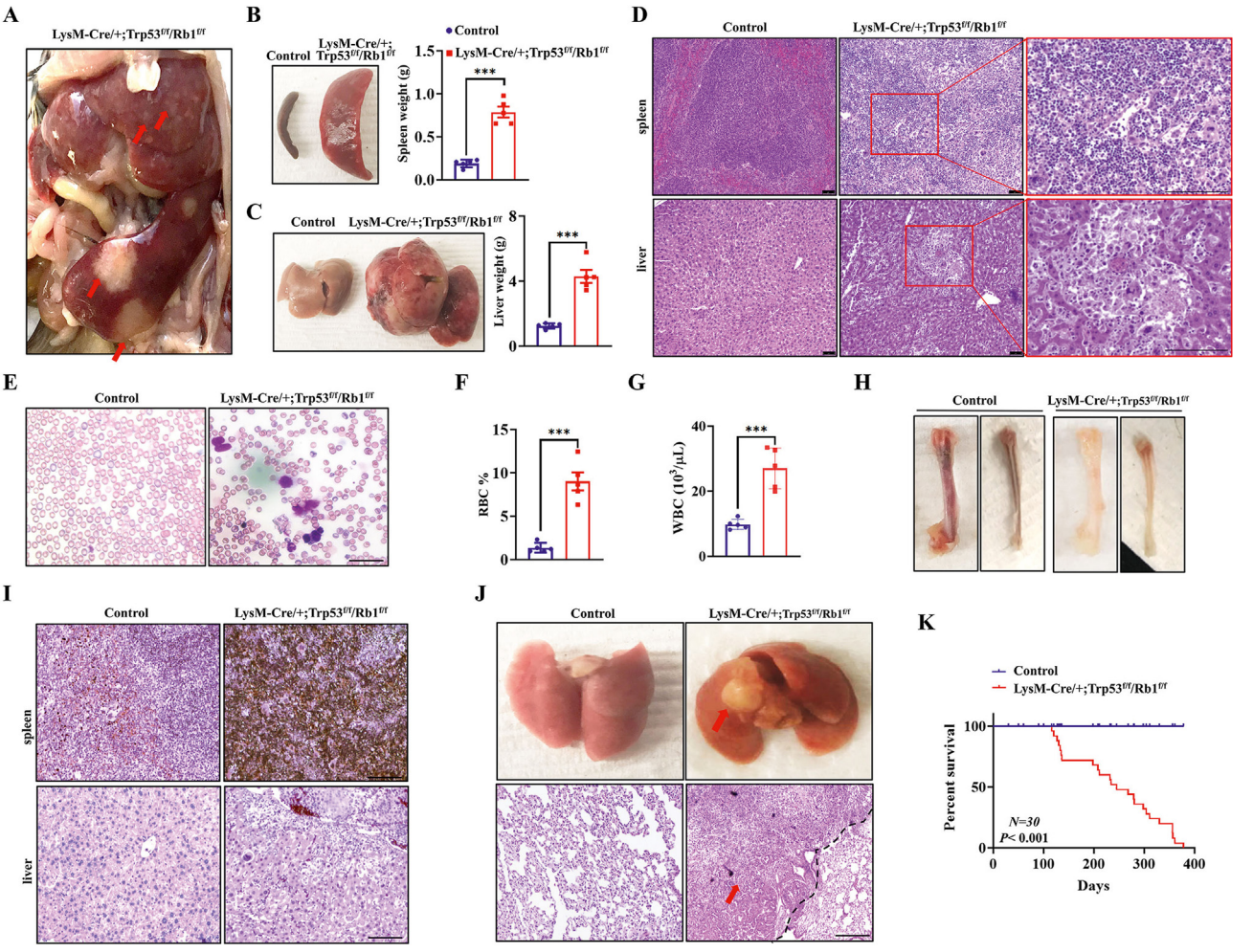

Figure 1 Trp53 and Rb1 deficiency in the myeloid cell lineage spontaneously develops acute myeloid leukemia. **(A)** A representative image of 6-month-old LysM-Cre/+; Trp53$^{f/f}$/Rb1$^{f/f}$ mice. Red arrows point to the tumors in the spleen and liver. **(B, C)** Representative images of spleens (B) and livers (C) from 6-month-old LysM-Cre/+; Trp53$^{f/f}$/Rb1$^{f/f}$ mice and controls. Right panels: analysis of the weight of spleens and livers. $n = 5$. ***$P < 0.001$. **(D)** Representative hematoxylin/eosin-stained images of spleens and livers from 6-month-old LysM-Cre/+; Trp53$^{f/f}$/Rb1$^{f/f}$ mice and controls (LysM-Cre mice) as indicated. $n = 5$. Scale bar = 75 μm. The high-resolution images were shown on the left panel. Scale bar = 25 μm. **(E)** Peripheral blood from 6-month-old LysM-Cre/+; Trp53$^{f/f}$/Rb1$^{f/f}$ mice and controls were stained by Giemsa-stained solution. **(F)** Percentage of immature red blood cells (reticulocytes) relative to total red blood cell count. ***$P < 0.001$. **(G)** Total white blood cell (WBC) counts as indicated. ***$P < 0.001$. **(H)** A representative image of femurs and tibia from 6-month-old LysM-Cre/+; Trp53$^{f/f}$/Rb1$^{f/f}$ mice. **(I)** Representative IHC-stained images of Mac-3 in the tumor-burdened spleen and liver tissue and controls. **(J)** Representative images of lungs from 6-month-old LysM-Cre/+; Trp53$^{f/f}$/Rb1$^{f/f}$ mice and controls. Lower panels: representative hematoxylin/eosin-stained images of AML lung metastasis tissues and normal lung tissue from 6-month-old LysM-Cre/+; Trp53$^{f/f}$/Rb1$^{f/f}$ mice and controls as indicated. The red arrow points to the tumor in the lung. **(K)** Kaplan–Meier survival analysis indicating overall survival of LysM-Cre/+; Trp53$^{f/f}$/Rb1$^{f/f}$ mice ($n = 30$) and controls ($n = 30$) as indicated.

of AML with significantly enlarged livers and spleens infiltrated with tumor cells in LysM-Cre/+; Trp53$^{f/f}$/Rb1$^{f/f}$ mice and the mice may die by the leukemic burden (Fig. 1A; Fig. S3). Of note, the weight of spleen and liver in the LysM-Cre/+; Trp53$^{f/f}$/Rb1$^{f/f}$ mice showed 4.14- and 3.44-fold increase respectively compared to those in the controls (Fig. 1B, C). Moreover, the histologic analysis of spleen and liver sections from LysM-Cre/+; Trp53$^{f/f}$/Rb1$^{f/f}$ mice showed seriously disrupted architecture with infiltration of tumor cells in both spleen and liver (Fig. 1D; Fig. S1B). Besides, our data revealed that loss of Trp53 and Rb1 in myeloid lineage caused a dramatic increase in leukemia cells with prominent nucleoli and severe anemia with abnormal hematopoiesis (Fig. 1E–H), as evidenced by increased expression of leukemia marker (Mac-3) in spleen and liver from LysM-Cre/+; Trp53$^{f/f}$/Rb1$^{f/f}$ mice (Fig. 1I). Additionally, we found the AML could metastasize to lung (Fig. 1J). The Kaplan–Meier survival curves demonstrated a significantly shorter survival rate in the LysM-Cre/+; Trp53$^{f/f}$/Rb1$^{f/f}$ mice compared to that in the control mice (Fig. 1K). These results indicated that the mice with Trp53 and Rb1 deficiency in myeloid cell lineage mimic the human AML phenotype. Given the expression pattern of LysM-Cre and our findings, deficiency of Trp53 and Rb1 in myeloid cell lineage spontaneously may

cause AML-French-American-British (FAB) subtype M5 formation. It may be a good mouse model of AML.

In conclusion, we reveal for the first time that depletion of Trp53 and Rb1 in myeloid cell lineage spontaneously developed AML and provided a new transgenic AML mouse model for unveiling its pathogenetic mechanisms and new drug screening.

Author contributions

Shuying Yang and Yang Li conceived this study and designed experiments. Yang Li and Shu-ting Yang performed experiments and analyzed data. Yang Li and Shuying Yang wrote and edited the manuscript.

Ethics approval

All animal experiments were carried out with the guidelines of the Institutional Animal Care & Use Committee at the University of Pennsylvania.

Conflict of interests

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.12.004.

References

1. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med*. 2016;374(23):2209–2221.
2. Chen S, Wang Q, Yu H, et al. Mutant p53 drives clonal hematopoiesis through modulating epigenetic pathway. *Nat Commun*. 2019;10(1):5649.
3. George B, Kantarjian H, Baran N, et al. *TP53* in acute myeloid leukemia: molecular aspects and patterns of mutation. *Int J Mol Sci*. 2021;22(19):10782.
4. Melo MB, Costa FF, Saad ST, et al. Molecular analysis of the retinoblastoma (*RB1*) gene in acute myeloid leukemia patients. *Leuk Res*. 1998;22(9):787–792.
5. Huang HH, Chen FY, Chou WC, et al. Long non-coding RNA HOXB-AS3 promotes myeloid cell proliferation and its higher expression is an adverse prognostic marker in patients with acute myeloid leukemia and myelodysplastic syndrome. *BMC Cancer*. 2019;19(1):617.

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