**LETTER TO THE EDITOR**

**Reciprocal suppression between Zbtb1 expression and IL-7Rα signalling during T-cell development**

Dear Editor,

The BTB-ZF (broad-complex, tramtrack and bric-à-brac–zinc finger) proteins play essential roles in the development of the immune system. Transcriptional repressor Zbtb1 is one of the BTB-ZF members essential for lymphocyte development and NKp46+ ROR-gamma-T+ innate lymphoid cell (ILC3) development. Although the mechanisms by which Zbtb1 promote lymphoid development have been investigated, many questions are still unsolved, especially for T-cell development. T cells require IL-7 signalling throughout their life, including maturation, differentiation and survival in peripheral lymphoid tissues. Both B and T cells overexpression were deprived of IL-7 overnight and re-stimulated with various concentration of IL-7 for different period of time. Surface IL-7R expression in each condition was detected by flow cytometry. The representative data of D1 cells re-stimulated with 1 ng/mL IL-7 after starvation were shown in (F). H, I, The surface IL-7Rα expression of different subpopulation of thymocytes in wild-type and ScanT mice were analysed by flow cytometry. J, The overexpression of Zbtb1 in the D2 line of hCD2-Zbtb1 transgenic mice was confirmed by RT-qPCR and Western blot. K, The total numbers of thymocytes between wild-type and Zbtb1 transgenic mice were comparable. The surface IL-7Rα expression of different subpopulation of thymocytes in wild-type and ScanT mice were evaluated by flow cytometry. L, The total numbers of CD4 and CD8 T cells in spleen were compared between wild-type and Zbtb1 transgenic mice. The surface IL-7Rα expression in CD4SP and CD8SP thymocytes of wild-type and Zbtb1 transgenic mice were evaluated by flow cytometry.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited. © 2018 The Authors. Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.
pared to wild-type counterpart (Figure S1E). Interestingly, we found that ScanT thymocytes expressed higher level of IL-7Rα compared to wild-type thymocytes during T-cell development, including ETP, intermediate DN1 to DN2 stage (intDN1-DN2) and DN2 stage (Figure 1H). The cell size of ETP, intDN1-DN2 and DN2 thymocytes seemed to be smaller in ScanT mice, indicating that they are less proliferative than that of wild-type (Figure 1D). The IL-7Rα level of TCRβ hi thymocytes in ScanT mice was also higher than that of their wild-type littermates, although the percentage of TCRβ hi thymocytes in ScanT mice was severely reduced (Figure 1I). Furthermore, we generated Zbtb1 transgenic mice in which expression of Zbtb1 was driven by T-cell specific hCD2 promoter. Among four transgenic mouse line generated, only D2 line successfully overexpressed Zbtb1 in CD4 T cells compared to C57BL/6 in both mRNA and protein level (Figure 1J). The total number of thymocytes was comparable between wild-type and hCD2-Zbtb1 transgenic mice. However, the IL-7Rα level in CD4SP and CD8SP thymocytes of transgenic mice was lower than that of wild-type mice (Figure 1K). In the spleen, both CD4SP and CD8SP cells from transgenic mice expressed lower level of IL-7Rα than that of wild-type mice. Surprisingly, the number of CD8SP spleenocytes in hCD2-Zbtb1 transgenic mice was greatly reduced, while the number of CD4SP spleenocytes was comparable (Figure 1L). The reduction in the number of CD8SP spleenocytes in ScanT mice was not due to unregulated expression of Bcl2, although the percentage of TCRβ hi thymocytes in ScanT mice was also higher than that of their wild-type littermates, indicating that they are less proliferative than that of wild-type (Figure 1J).

Altogether, our results suggest that Zbtb1 and IL-7Rα signalling can regulate each other during T-cell development. IL-7Rα signalling negatively regulate Zbtb1, and vice versa. Detailed molecular mechanisms by which Zbtb1 regulate IL-7Rα expression in T cells are still under investigation.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (31700763, 81760287), The Science and Technology Fund Program of Gansu Province for Young Investigators (17JR5RA277), Gansu Provincial Science and Technology Grant (1504WKCA094), Innovative Research Team in University (IRT_17R88), Ministry of Science and Technology Assistance Project Grant (KY201501005). The State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China.

Correspondence

Kui-zheng Cai, Zhong-ren Ma

Emails: ckl000@126.com (KC) and maxiaoxia956@163.com (ZM)

Xin Cao and Xiao-xia Ma equally contributed to this study.

REFERENCES

1. Siggs OM, Beutler B. The BTB-ZF transcription factors. Cell Cycle. 2012;11:3358-3369.

2. Siggs OM, Li X, Xia Y, Beutler B. ZBTB1 is a determinant of lymphoid development. J Exp Med. 2012;209:19-27.

3. Punwani D, Simon K, Choi Y, et al. Transcription factor zinc finger and BTB domain 1 is essential for lymphocyte development. J Immunol. 2012;189:1253-1264.
4. Lu Y, Zhang X, Bouladoux N, et al. Zbtb1 controls NKp46+ RORγ+ innate lymphoid cell (ILC3) development. Oncotarget. 2017;8:55877-55888.
5. Cao X, Lu Y, Zhang X, Kovalovsky D. Zbtb1 Safeguards genome integrity and prevents p53-mediated apoptosis in proliferating lymphoid progenitors. J Immunol. 2016;197:1199-1211.
6. Zhang X, Lu Y, Cao X, Zhen T, Kovalovsky D. Zbtb1 prevents default myeloid differentiation of lymphoid-primed multipotent progenitors. Oncotarget. 2016;7:58768-58778.
7. Fry TJ, Mackall CL. The many faces of IL-7: from lymphopoiesis to peripheral T cell maintenance. J Immunol. 2005;174:6571-6576.
8. Peschon JJ, Morrissey PJ, Grabstein KH, et al. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J Exp Med. 1994;180:1955-1960.
9. Akashi K, Kondo M, von Freeden-Jeffry U, Murray R, Weissman IL. Bcl-2 rescues T lymphopoiesis in interleukin-7 receptor-deficient mice. Cell. 1997;89:1033-1041.
10. Maraskovsky E, O’Reilly LA, Teepe M, Corcoran LM, Peschon JJ, Strasser A. Bcl-2 can rescue T lymphocyte development in interleukin-7 receptor-deficient mice but not in mutant rag-1/- mice. Cell. 1997;89:1011-1019.

SUPPORTING INFORMATION
Additional Supporting Information may be found online in the supporting information tab for this article.