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Title

EMT transcription factor Zeb1 regulates T-cell differentiation from haematopoietic stem cells during ontogenesis

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Introduction

We have identified Zeb1, a member of the zinc finger homeobox E-box binding transcription factors, as a key player in normal hematopoietic stem cell function and multilineage differentiation fates. Previous data has shown that Zeb1 is essential for T cell development, yet little is known about the function of Zeb1 in T-cells throughout ontogeny.

Methodolgy

To explore this issue, we employed a conditional genetic approach using the VaviCre system, which deletes gene expression at d11 of embryonic development. Several macroscopical, immunophenotypic and histological experiments were conducted on Zeb1-/- (KO) or Zeb1+/+ (WT) thymus initially. We obtained thymus samples from the 83.87% Zeb1-/- mice that survived after weaning, aged from 8- 12 weeks

Results

Young adult mice engineered to be deficient in Zeb1 using this system displayed reduced thymus weight characterised by hypocellularity which leads to thymus atrophy, and reduced frequency of double positive (DP) cells and a reduction of T cells in peripheral blood (PB), Spleen (SP) and Bone Marrow (BM). Total cell numbers were altered in double negative (DN), DP, CD4+ and CD8+ cells from Zeb1 ablated mice. In particular, T cell subset development was impaired in the thymus. There was a significant increase of immature DN cells contrasting with a significant reduction in the proportion of DP cells, which can be ascribed to impaired positive selection and cell survival of thymocytes. Immunophenotypic analysis showed an incremental differentiation block in DN1 and the opposite pattern in the transition of DN2/DN3 and DN4.

In mature T-cell subsets, loss of Zeb1 causes a pronounced expansion in effector and memory T-cells frequency and a dramatic reduction in naive T-cells in the thymus while the absolute cell counts reveal that there is a depletion of naive, central and effectors CD4+ and CD8+ cells leading to an increase of PD-1 (programmed cell death protein 1) frequency. This reduction in naive CD4+ and CD8+ cells is also observed in BM, SP and PB and leads to an altered ratio of CD4:CD8. RNA-Seq analysis of transcriptome differences reveal that there is an increase in cell adhesion molecules in Zeb1-deleted mice, associated upregulation of Cdh1, EpCAM and Itgb4 and a dysregulation of epithelial markers. In the thymus, Zeb1 deletion leads to a reduction of the receptor CXCR4, associated with cellular migration. These changes leadingto T cell dysfunction imply that Zeb1 malfunction may contribute to premature immunological aging, characterized by immunosenescence (including decrease of naive T cells and CD62L) and exhaustion of T-cells (increased chemokine expression and high levels of PD-1 and FAS).

Conclusion

Altogether, we identify Zeb1 as an indispensable regulator of transcriptional programming for T cell development and survival throughout ontogenesis. This data may have implications for understanding Zeb1 mediated regulation of lymphoid malignancies, immunosurveillance of solid cancers and cancer immunotherapy.