Check for updates





Blood 142 (2023) 2565

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

The Alternative Splicing of Lck in Treg Induction in Immune Thrombocytopenia

Nan Jiang¹, Ruxia Zhao¹, Xiang Hu¹, Shuwen Wang¹, Shaoqiu Leng¹, Qi Feng¹, Jun Peng¹

¹Qilu Hospital of Shandong University, Jinan, China

Background:

Immune intolerance, causing the production of autoantibodies, is thought to be one of the main pathogenesis of immune thrombocytopenia (ITP). As regulatory T cells (Tregs) are one of the most important cells to maintain immune tolerance and the phenotype, function, and importance of Tregs have been well defined over the past decade, extensive efforts have been devoted to unveiling the dysregulation of Tregs in ITP. It has been reported that the proportion of Tregs in peripheral blood of ITP patients is reduced and the immunosuppressive function of Tregs is impaired, but the mechanism remains unclear. As peripheral Treg production can be induced from CD4+ naive T cells (NTC) with TCR-stimulated activation and TGF- β treatment, how peripheral Treg induction is impaired in patients with ITP remains to be further investigated. Method:

We isolated and performed high-throughput sequencing of the CD4+ NTC from the peripheral whole blood of ITP patients and healthy controls. After analyzing the differential expression and splicing pattern, we screened several genes of interest. In addition, a minigene plasmid of the target gene was constructed and RNA-chromatin immunoprecipitation (RNA-ChIP) was performed to explore the upstream splicing factor. PCR,Real-time quantitative PCR and Western Blot were used to validate the expression level and splicing pattern of various genes. Results:

Our results revealed the proportion of Tregs induced from CD4+ NTC of ITP patients was decreased in vitro, suggesting the abnormal Treg induction in ITP patients. The differentially expressed genes of the CD4+ NTC between ITP patients and healthy controls were significantly enriched in the alternative splicing pathway based on high-throughput RNA-seq data. Further exploration of the differentially spliced genes revealed significant differences in the exon 8 skipping in the LCK gene between healthy controls and ITP patients, as LCK plays a crucial role in TCR signaling and TCR signaling is essential for Treg induction. Overexpressing the exon 8 spliced LCK, other than overexpressing the exon 8 included LCK in T cells limited the TCR activation. Furthermore, we found splicing factor X could recognize the exon 8 of LCK and promote the inclusion of exon 8 of LCK. Splicing factor X was lowly expressed in the CD4+ NTC of ITP patients. Accordingly, we proposed the hypothesis that the lower expressed splicing factor X in the CD4+ NTC of ITP patients may limit peripheral Treg induction by regulating LCK alternative splicing in ITP. By using RNA-CHIP, we found splicing factor X specifically bound to the exon 8 of LCK. Moreover, the administration of antisense oligos, which could mask the binding site, suppressed the recognition of the exon 8 of LCK and enhanced the exon 8 of LCK transcripts. As expected, ASO transfection inhibited the Treg induction in CD4+ NTCs from healthy controls, which may mimic the Treg induction in ITP patients.

Here we found splicing factor X was downregulated in the CD4+ NTC of ITP patients, which resulted in the exon 8 skipping in the LCK gene and a down-regulated TCR signaling, thereby limiting the induction of peripheral Tregs. This study will explore the pathogenesis of ITP from a novel perspective, and provide new ideas and targets for the clinical treatment of ITP.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-179056