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ORAL ABSTRACTS

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

Iguratimod Regulates CD4+ T-Cell Homeostasis and Function By Restoring PINK1/Parkin-Mediated Mitophagy in Immune Thrombocytopenia

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Introduction

Immune thrombocytopenia (ITP), an acquired autoimmune disease, is characterized by immune-mediated platelet destruction and suppressed platelet production, resulting in isolated thrombocytopenia. Subpopulation imbalance and dysfunction of CD4+ T cells are involved in the pathogenesis of ITP. However, the exact mechanism of CD4+ T-cell abnormalities is not fully understood. Mitophagy, a process that eliminates damaged and dysfunctional mitochondria, has conventionally been considered the primary mechanism responsible for mitochondrial quality control. Mitophagy deficiency has been implicated in a series of inflammatory and autoimmune diseases, such as systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD) and rheumatoid arthritis (RA). However, whether it plays a role in ITP immune abnormalities and its specific mechanism remain unclear. Iguratimod is a small molecule compound that is widely used as a novel antirheumatic drug in the treatment of rheumatoid arthritis. Iguratimod inhibits the production of several inflammatory cytokines, including IL-1, IL-6, IL-8, and tumor necrosis factor (TNF), by enhancing mitophagy. Our previous clinical study demonstrated the efficacy of iguratimod in the treatment of ITP. This study aimed to explore whether iguratimod regulates CD4+ T-cell homeostasis and function by enhancing mitophagy in ITP.

Methods

BM CD4+ T cells were isolated from ITP patients and healthy donors. The subgroup of CD4+ T cells was analyzed by flow cytometry. ELISA was used to evaluate cytokine and chemokine levels in bone marrow plasma. Mitochondrial mass, morphology, quality and mitophagy were evaluated by flow cytometry, confocal microscopy, electron microscopy and western blotting. RNA sequencing and metabolomics were performed. Epigenetic state alteration was assessed by a transposase-accessible chromatin assay with high throughput sequencing (ATAC-seq). Iguratimod-treated BM-derived ITP-CD4+ T cells and an ITP active mouse model were used to explore its regulation of mitophagy and effects on T-cell homeostasis and function.

Results

Th1/Th2 imbalance and Treg reduction were observed in the bone marrow of ITP patients, consistent with a previous report. Flow cytometry and confocal microscopy revealed an increase in mitochondrial content and mtROS in CD4+ T cells but a decrease in mitochondrial membrane potential. Furthermore, electron microscopy revealed structural damage to mitochondria in ITP-CD4+ T cells. These data confirmed an accumulation of damaged mitochondria in ITP-CD4+ T cells. The expression levels of LC3II/LC3I, PINK1 and parkin decreased in CD4+ T cells, and the colocalization of mitochondria and lysosomes decreased, which suggested compromised PINK1/parkin-mediated mitophagy in ITP-CD4+ T cells.

RNA-seq revealed transcriptional changes associated with mitochondrial function, mitophagy and metabolic pathways in ITP-CD4+ T cells. Moreover, metabolomics showed alterations in TCA cycle metabolites. Since epigenetic programs are regulated by metabolites, ATAC-seq was performed to obtain the epigenetic state. We found that ITP-CD4+ T cells altered chromatin accessibility in regions with T cell differentiation, transcription factors and T-cell function.

After in vitro treatment with iguratimod, the Th1/Th2 imbalance was corrected, and the number of Tregs was restored. The content, morphology and membrane potential of mitochondria in ITP-CD4+ T cells were restored after iguratimod intervention. The expression level of PINK1 and parkin and the colocalization of mitochondria and lysosomes were increased, suggesting an improvement in mitophagy. In vivo, iguratimod accelerated platelet recovery in an ITP active mouse model. After treatment, the Th1/Th2 imbalance and the Treg number were restored, and the mitophagy of CD4+ T cells was corrected in the bone marrow of the ITP mouse model.

Conclusion

PINK1/Parkin-mediated mitophagy deficiency contributed to mitochondrial dysfunction and altered the metabolism of ITP-CD4+ T cells, which affected their differentiation and function by regulating the epigenetic state of immune response genes. Iguratimod could restore CD4+ T-cell subpopulations and treat ITP by enhancing mitophagy.

Disclosures No relevant conflicts of interest to declare.

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