No.	K24-1115	
Project Title	The study of primed-umbilical cord-derived mesenchymal stromal cells to improve immunosuppressive properties in the treatemnet of GVHD	
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(Form 2)

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IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Project Completion)

Project Completion Report

Report

Background and objectives

Mesenchymal stromal cells (MSCs) are activated to suppress the immune system and repair tissue damage only after encountering inflammatory stimuli and/or tissue damage. In acute graft-versus-host disease (aGVHD) after hematopoietic stem cell transplantation (HSCT), a Th1-based cellular immune response develops and is primarily responsible for the pathogenesis of aGVHD. The immune regulation of MSCs is easily influenced by external factors and shows plasticity. We recently reported that triptolide (TPL), a diterpene triepoxide purified from a Chinese herb, upregulated PD-L1 in umbilical cord-derived MSCs (UC-MSCs), resulting in the enhancement of the immunosuppressive potency of TPL-primed UC MSCs on the proliferation of activated T cells (He et al, Front Immunol. 2021,12:686356).

In extent to the previous IMSUT project, we aim to elucidate how TPL-primed UC-MSCs affect immune cells, including T cells, NK cells and macrophages, compared to IFN- γ .

Results and Discussions

In 2025 FY, we focused on the immunomodulatory effects of the TPL-primed UC-MSCs in patients with aGVHD. We used flow cytometry to analyze differences in key cytokines between three healthy volunteers and three acute leukemia patients with Grade III-IV aGVHD after allogeneic HSCT. Cytokines included Th1 cytokines (IL-2, IFN-γ, TNF-β), Th2 cytokines (IL-4, IL-5, IL-6, IL-10), chemokines (IL-8), and pro-inflammatory cytokines (IL-1β and IL-17F/A). Additionally, we co-cultured UC-MSCs and TPL-primed UC-MSCs with peripheral blood mononuclear cells (PBMCs) from healthy volunteers and patients for 24, 48, and 72 hours, observing changes in cytokine levels in the supernatant and the proportions of CD3+CD4+ and CD3+CD8+ T lymphocyte subsets. We found that, compared to healthy individuals, patients with grade III–IV aGVHD exhibited an imbalance in cytokine homeostasis, characterized by elevated IL-6, IL-10, increased IFN-γ, IL-8, but decreased IL-2. It was observed that pretreatment with TPL resulted in the suppression of IL-6 and IL-8 expression in UC-MSCs. Correspondingly, a decrease in the secretion levels of IL-6 and IL-8 was also detected in patient PBMCs under UC-MSC co-culture conditions. Furthermore, TPL seems to promote the inhibitory effect of UC-MSCs on the CD3+CD4+ T cells. This study demonstrates that TPL-primed UC-MSCs exert immunomodulatory effects by adjusting the balance of inflammatory factors and suppressing T lymphocyte proliferation. Furthermore, RT-PCR showed that co-culture with TPL-primed UC-MSCs induced M2 macrophage expressing Arg-1 and CXCL2. M2 macrophages play an important role to control the excess immune reaction and repair the damaged tissues.

Conclusively, These results may suggest that TPL-primed UC-MSCs may provide the means for a novel immunosuppressive cell therapy, although some mechanisms have not been clarified and we need to prove the safety dose in clinic.

Future directions/plans:

In our previous microarray studies, TPL-primed UC-MSCs showed a significant increase in the expression of the keratin-associated gene KRTAP2-3 compared to unprimed UC-MSCs. However, the significance of this upregulation remains unclear. Recent papers have shown that TGF-β induces KRTAP2-3, which retains cell growth in the G1 phase of the cell cycle but increases cell mobility. TPL-primed UC-MSCs produce more TGF-β than non-primed UC-MSCs(He et al, Front Immunol. 2021,12:686356). We are going to investigate the influence of TPL priming on the migration of UC-MSCs in relation to TGF-β and KRTAP2-3.