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研究課題名	The study of primed-umbilical cord-derived mesenchymal stromal cells to improve immunosuppressive properties in the treatemnet of GVHD	
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Annual Report

Report

Background

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), 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, upregulates 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). But TPL alone could not induce the He et al challenged another Chinese herb, Asarinin derived from Asarum, which display the similar immunosuppressive effects like cyclosporine and seems less toxic than TPL. Similar with TPL, Asarinin could not induce IDO-1 alone, but Asarinin-primed UC-MSCs showed the improvement of aGVHD compared with IFN-γ-primed UC-MSCs (He et al., Inter. Immunopharmacology, 2021;100,108054). IFN-γ is well-known as the strong inducer of indoleamine 2,3-dioxygenase (IDO-1: Tryptophan to kynurenine transfer), PDL-1 in UC-MSCs (Nagamura-Inoue et al, Int J Hematol., 2022) and some clinical trials have been reported, but residual IFN-γ may influence on the immune system in patients. We firstly evaluate the difference of immunomodulation of UC-MSCs primed by TLP and IFN-γ, because we could not obtain the Asarinin to compare with TPL this year, due to the company affair and we have not yet compared the immune modulation by UC-MSCs primed with TPL and IFN-γ (priming means the addition of TPL or IFN-γ, in the UC-MSCs culture followed by washing out the reagents.)

The objectives of this study are to elucidate the mechanisms of activation of UC-MSCs by TPL priming, which exaggerate the immunosuppressive and anti-inflammatory potency.

Method

1 To investigated the HLA-ABC expression in TPL-primed UC-MSCs treated with IFN-γ by flow cytometry.

2 To check the TPL-primed UC-MSCs immunosuppressive effect in MLR using different UC-MSCs samples by FACS analysis using three different UC-MSCs sample. (n＝3).

3 To check SOD1, SOD2, TGF-β, IDO1 gene expression in TPL-primed UC-MSCs in the presence of IFN-γ/TNF-α by RT-PCR using three different UC-MSCs sample. (n＝3).

4 To analysis the genes expression on TPL-primed UC-MSCs with or without IFN-γ using micro-RNA method;

Results

In 2024, we firstly compared the effects of TPL and IFN-γ-primed UC-MSCs on Th1 (IL-2, IFN-γ, TNF-α, TNF-β) and Th2 cytokines (IL-4, IL-5, IL-6, IL-10) in a mixed lymphocyte reaction (MLR), respectively. We also compared the polarization of macrophages (M1 and M2) in the TPL and IFN-γ-primed UC-MSCs. ELISA analysis showed that IL-2, IFN-γ, TNF-α, and TNF-β were not elevated in supernatants of the co-culture of TPL-primed UC-MSCs with MLR, where IFN-γ-primed UC-MSC failed to suppress those cytokines, especially TNF-α and IL-4, IL-5, and IL-10 (Th2 cytokines) are significantly more elevated in the co-culture of TPL-primed UC-MSCs with MLR than in those primed with IFN-γ.

RT-PCR analysis showed that TPL primed UC-MSCs could upregulated the expression of Arg1 and YM1 in M2 macrophages and downregulated the expression of INOS in M1 macrophages. TPL primed UC-MSCs can downregulate CXCL2 in M1 /M2 macrophages. However, it seems to weaken the effect of UC-MSC on the downregulation of CXCL2 in M1 macrophages. These outcomes indicate that TPL pre-stimulation will affect the role of UC-MSCs in macrophage polarization.

Conclusively, we found that TPL priming showed the different action on the immune modulation compared IFN-γ priming. IFN-γ priming induces IDO-1 strongly, but upregulates unfavorite inflammatory cytokine, such as TNF-α.

In the similar previous studies, we have done the microarray analysis of UC-MSCs primed with or without TPL and we looked back the gene expression data. According to the results of microarray, the keratin-associated genes KRTAP2-3 and KRTAP1-5 were elevated significantly in TPL-primed UC-MSCs, although the function of these genes remained to be resolved. KRTAP2-3 and KRTAP1-5 are reported to have the F-actin depolymerization. Transcription factor prediction identified NKX2.5 as the potential transcription factor to control KRT19, KRT34, KRTAP1-5, and KRTAP2-3 in the decreased size of MSCs (Biomaterials, 2014, 35,3934). Recently, Baek et al reported that autophagy and KRT8/keratin 8 protect degeneration of retinal pigment epithelium under oxidative stress in the ophthalmology field. In 2025FY, we will elucidate whether the KRTAP/ KRT are associated with immunomodulation.