Analysis of the Mechanisms of Intravenous Immunoglobulin-Resistant Kawasaki Disease Using iPS Cell Technology


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Introduction
Although the treatment of intravenous immunoglobulin (IVIG) significantly resolves inflammation, 10-20% of Kawasaki disease (KD) patients have persistent or recurrent fever after the administration of IVIG, and IVIG-resistant patients have a particularly high risk of developing coronary artery abnormalities. The mechanisms of IVIG-resistant KD have been analyzed using the patients’ leukocyte samples. However, vascular endothelial cells (ECs), closely related to the vasculitis of KD, have not been examined in the previous reports. We propose a hypothesis that ECs are mainly involved in the etiology of IVIG-resistance.

Methods
The purpose of this study is to establish new in vitro disease models of vasculitis using induced pluripotent stem cell (iPSC) technology, and clarify the mechanisms of IVIG-resistance in KD. Dermal fibroblasts or T cells from 2 IVIG-resistant and 2 IVIG-responsive KD patients were reprogrammed by episomal vectors encoding Oct3/4, Sox2, Klf4, L-Myc, LIN28, and p53 shRNA. The iPSC lines were then differentiated into ECs and smooth muscle cells (SMCs) by using a previously-reported differentiation method, and the EC and SMC samples were subjected to the microarray analyses.

Results
The KD patient-derived iPSCs could be differentiated into ECs and SMCs. The gene expression profiles were compared between iPS-derived ECs (iPS-ECs) generated from IVIG-resistant and IVIG-responsive KD patients, and between iPS-derived SMCs (iPS-SMCs) generated from two group patients. We found the expression of chemokine X, which stimulates migration of monocytes and T-lymphocytes through its receptors, was significantly up-regulated both in iPS-ECs and in iPS-SMCs from IVIG-resistant KD patients compared with those from IVIG-responsive patients. The Principle Component Analysis (PCA) was performed, but the gene expression levels showed no significant differences between the groups. The Gene Set Enrichment Analysis (GSEA) revealed that the gene sets related to IL-6, NRAS (a member of the RAS oncogene family) and breast cancer were up-regulated in iPS-ECs from IVIG-resistant KD patients.

Conclusions
Taking into account that the concentration of IL-6 has been reported to be elevated in acute phase of IVIG-resistant KD, our results suggest that the up-regulation of IL-6 related genes in ECs might be involved in the pathogenesis of IVIG-resistant KD.