Regulation of Human NK Cell Activation by Expression of HLA Class I Molecules in Pig Endothelial Cells

Benjamin Obando[1], Arthur Cross-Najafi[1], Kevin Lopez[2], Deepthi Thadasina[1], Wenjun Zhang[2], A. Kadir Isidan[2], Yujin Park[2], Gonzalo Campana[2], Ping Li[2], Burcin Ekser[2]

[1]Indiana University School of Medicine, [2] Division of Transplant Surgery, Department of Surgery Indiana University School of Medicine

Background:

Pig-to-human xenotransplantation (XTx) is a promising solution to the organ shortage. Genetically engineered pigs lacking major xenoantigens have reduced hyperacute rejection and prolonged xenograft survival. Despite these advancements, acute xenograft rejection (AXR) remains a major barrier to clinical XTx. AXR is mediated by multiple immune cells, of which natural killer (NK) cells play a crucial role. Previous studies have shown that human HLA-E suppresses NK cell activation through the inhibitory receptor NKG2A. We seek to improve pig-to-human compatibility by expressing HLA-E in a genetically modified pig endothelial cell (pEC) line. This cell line 5GKO/ HLA-G^+ has mutations in five genes encoding for xenoantigens and expresses HLA-G, an inhibitory ligand of the NK cell receptor KIR2DL4. In this study, the 5GKO/HLA-G^+/HLA-E^+ pEC line was established to examine whether co-expression of inhibitory ligands promotes NK cell tolerance.

Methods:

The HLA-Eα/pCDNA3.1 plasmid containing the HLA-E α-chain (HLA-Eα) cDNA driven by a CMV promoter was linearized and introduced into 10^6 cells of the 5GKO/HLA-G^+ pEC line by electroporation. After 48 hours, HLA-E expression was analyzed by flow cytometry. HLA-E^+ pECs were isolated by flow cytometry sort and co-cultured with human peripheral blood mononuclear cells (PBMCs) stimulated by IL-2. NK cell degranulation was compared between the 5GKO/HLA-G^+ and 5GKO/HLA-G^+/HLA-E^+ pEC lines by measuring CD107a expression in the $CD3^-$ $CD56^+$ cell population.

Results:

HLA-E molecules were successfully expressed on the pECs surface, indicating the HLA-E α chain can pair with the existing β2-microglobulin (B2M). The transfection efficiency was 38.2%. Three weeks later, the 5GKO/HLA-G$^+$/HLA-E$^+$ pEC was successfully established, confirming via flow cytometric analysis. The analysis of NK cell degranulation (CD107a) is underway.

Conclusion:

We established a 5GKO/HLA-G$^+$/HLA-E$^+$ pEC line, which is a valuable tool to study human-to-pig xenoreactive immune response *in vitro*, with the goal of improving pig-to-human xenograft immunotolerance.