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TITLE: Simultaneous expression of novel combinations of graft protective human genes in porcine endothelial cells

BACKGROUND AND AIM. Porcine vascular endothelium is the first cell type that is in direct contact with human blood in xenotransplantation settings. Endothelial activation and injury has been demonstrated to be the basis of several inflammatory and immunological mechanisms occurring during the rejection of xenogeneic tissues. Our aim was to investigate the potential protective roles of new combinations of human genes against endothelial activation, inflammation and procoagulant changes in a relevant in vitro model of xenotransplantation.
METHODS AND RESULTS. A porcine endothelial cell line (Porcine Iliac Artery Endothelial cells, PIEC) have been transfected with F2A technology-based multicistronic plasmids in order to obtain the simultaneous expression of up to three human genes or a combination thereof. PIEC cells over-expressing Heme Oxygenase 1 (hHO1), Ecto 5' Nucleotidase (hE5'N or hCD73) and Ecto Nucleoside Triphosphate Diphosphohydrolase 1 (hENTPD1 or hCD39) or hCD73 and hCD39 were produced. As a control, PIEC cells have been mock-transfected or transfected with single gene expressing vectors. PIEC stably transfected cell lines (hHO1/hCD73/hCD39-, hCD73/hCD39-, hCD73-, hCD39- transgenic cell lines) have been enriched for the expression of hCD73 and/or hCD39 markers by fluorescence activated cell sorting (FACS). On the other hand, to enrich by FACS, transfected cells expressing only hHO1, which has a cytoplasmic localization, an EGFP coding sequence had been added to the transgenic construct. For each transgenic PIEC cell line it was obtained a range of 96% - 98% cells positive for the selected transgenic marker(s), as evaluated by post-sorting FACS analyses.
The expression of hHO1 in hHO1/hCD37/hCD39- and hHO1- transfected cells has been verified by immunoblotting. The correct subcellular localization of hCD73 and hCD39 in the plasma membrane or the cytosolic and perinuclear distribution of hHO1 have been confirmed by immunofluorescence and confocal microscope analyses.
Evaluation of apoptotic cell death induced by 10ng/ml human TNF alpha treatment showed that the expression of human genes protects PIEC cells from caspase 3 and 7 activity. In fact, the hHO1/hCD73/hCD39 transfected cells has a 35% reduction in caspase activity after 16 hours of TNF treatment as compared to wild type cells.

CONCLUSIONS. PIEC cells over-expressing selected combinations of human genes were produced by using F2A-based technology and characterized. This porcine endothelial cells model that co-express two to three human genes will be used to investigate the protective role, at molecular level, of the combination of genes against the endothelial activation, inflammation and coagulation dysregulation following xenogeneic injuries.