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Derivation of trophoectodermal cells from rhesus monkey embryonic stem cells

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Introduction:

Embryonic stem cells (ESCs) of murine and human origin have been shown to be able to differentiate into derivatives of all three germ layers and also into extraembryonic trophoectoderm. ESC-derived trophoblasts may represent an extremely useful in vitro model to investigate placental morphogenesis and implantation events during embryonic development. Therefore, the aim of our study was to investigate, whether monkey ES cells are able to form trophoectoderm in vitro similar to mouse and human ESCs.

Materials and Methods:

Rhesus monkey ESCs (RESCs) were maintained and passaged using standard protocols. To initiate differentiation, non-differentiated RESC colonies were detached and transferred into agarose-coated culture plates to form embryoid bodies (EBs). After 2d of suspension culture in 80% αMEM, 20% FCS, 1 mM Glutamine, 0.1 mM β-Mercaptethanol and 1% non-essential amino acids, the EBs were plated onto tissue culture plates coated with 0.1% Gelatin in 80% IMDM, 20% FCS, 1 mM Glutamine, 0.1 mM β-Mercaptethanol and 1% non-essential amino acids. In the course of 2-3 weeks the expression of trophoblast markers was assessed by means of semi-quantitative RT-PCR, immunofluorescence and electron microscopy.

Results:

Based on the experimental conditions used to differentiate human ES-cells into cardiomyocytes, we developed a protocol, aiming at the differentiation of RESCs into trophoblasts. Using an EB-based approach, effective differentiation into trophoblasts was achieved. To characterize the trophoblast-like cells, semi-quantitative RT-PCR analyses were performed. The expression of several trophoblast marker genes including human chorionic gonadotrophin α (αHCG), caudal type homeobox transcription factor 2 (cdx2), eomesodermin (eomes) fibroblast growth factor receptor 2 (FGFR-2) and HAND-1 could be detected. Immunofluorescence
staining showed expression of cdx2 and cytokeratin Endo-A (Troma-1), whereas the endodermal marker α-fetoprotein was not expressed. The ultra structural analysis of cystic structures, most likely formed due to the expression of fluid-pumping channels as typical for early trophoblasts, showed that the differentiated cells resembled cells of the trophoectoderm. More detailed analyses of the gene expression and of the ultra structures are ongoing.

**Discussion and Conclusion:**

This is the first study demonstrating the successful differentiation of RESCs towards cells of the trophoblast lineage. RESC-derived trophoblasts represent a valuable tool to investigate differentiation and function of early human trophoblast.