Imaging of a three dimensional model system for invasive growth of trophoblasts

G. Moser, N. Flieser, M. Gauster, K. Orendi, M. Siwetz and B. Huppertz

Institute of Cell Biology, Histology and Embryology, Center for Molecular Medicine, Medical University of Graz, Austria

g.moser@medunigraz.at
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Introduction

The details of the processes of human implantation and subsequent trophoblast invasion into maternal tissues still remain mostly unknown. Due to ethical reasons live-imaging of the process of blastocyst implantation in humans is impossible and knowledge can only be obtained with different kinds of model systems. Here we present three dimensional double tissue co-culture confrontation *in vitro* model systems to study invasion of placenta derived trophoblast cells into maternal tissues as a model system for human implantation.

Methods

First trimester placental tissues (6 – 12 weeks of gestation) were collected from terminations of pregnancy. Maternal derived parts (decidua parietalis) and embryo derived parts (placental villi) were selected. In one set of experiments pieces of decidua parietalis were confronted directly with villous explants (direct confrontation). In a second set of experiments pieces of decidua parietalis and villous explants were separately precultured for 72h and confronted afterwards (indirect confrontation). During preculture decidua pieces re-epithelialized and thus during indirect confrontation villous explants were confronted with an epithelial rather than a stromal surface of the decidua. All confrontations were harvested after 72h, cryosectioned and processed for microscopy. Various fluorescent or immunohistochemical stainings were performed to describe the properties of the model systems. Sections were analyszed with a Zeiss Axiophot. Confocal laser scanning microscopy was performed on a Leitz/Leica TCS-SP2 microscope.

Results

Co-culture of embryonic and maternal tissues resulted in adhesion of both tissues. First attachment was already seen after 8 hours of culture. Immunohistochemical staining for HLA-G and cytokeratin 7 revealed trophoblast migration along as well as invasion into maternal tissues (Figure 1). Staining with antibodies against different proteins (e.g. nidogen, cytokeratin 7, Ki 67) showed that the invasive behavior of extravillous trophoblast cells changed depending on presence or absence of epithelium (direct versus indirect confrontation). Immunofluorescent staining for nidogen as a marker for basal laminas confirmed rebuilding of a decidual epithelium during indirect confrontation.

Conclusions

The presented model systems represent useful tools for studying implantation and invasion as well as signaling in implantation and invasion. Due to the usage of human tissues this model system comes closer to the *in vivo* situation than any cell line based model or any animal model.

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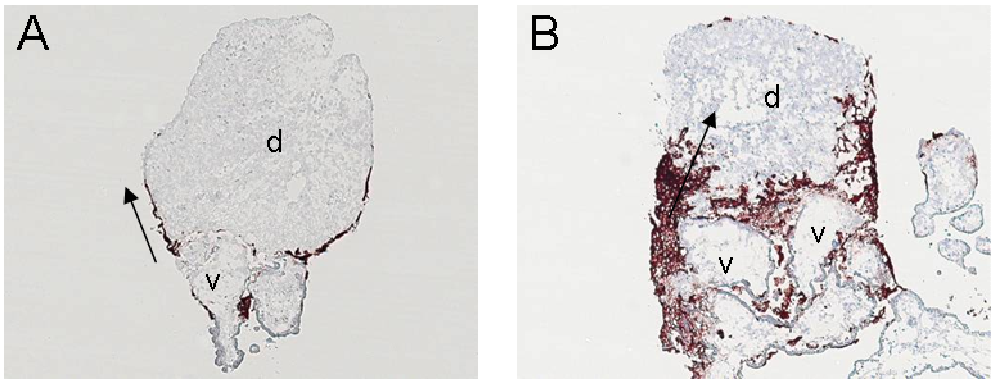

Figure 1: Sections of a double tissue confrontation assay. Cryosections were stained immunohistochemically with an antibody against HLA-G as marker for invasive extravillous trophoblasts.
(A,B) Villi (v) attach to decidual tissues (d).
(A) Trophoblasts migrate along the decidual surface during indirect confrontation (with epithelium)
(B) Trophoblasts invade into decidual tissues during direct confrontation (without epithelium). Magnification x50.

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