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LASER MICRO-DISSECTION MICROSCOPY OF SINGLE CELL-COMPARTMENTS FROM HUMAN PLACENTAL TISSUE: A NEW APPROACH TO STUDY TROPHOBLAST DIFFERENTIATION

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Objectives: Different trophoblast subtypes are responsible for the architecture and function of the human placenta. Terminal differentiation of trophoblast stem cells is contributing to the formation of villous cytotrophoblasts (VT). This subtype forms the syncytiotrophoblast (SCT) during early gestation by cell-cell-fusion and is later responsible for the maintenance of the SCT by cell-SCT-fusion. Furthermore, extravillous cytotrophoblasts (EVT) invade the decidua and spiral arteries and originate also from the trophoblast stem cell. Interstitial EVTs are located within the uterine stromal tissue and have the ability to fuse or replicate without cytokinesis into giant cells (GC). Conventional gene expression approaches by e.g. placental biopsies are often not sufficient to study the function and regulation of these terminally differentiated cytotrophoblasts. We wanted to determine the gene expression profile of terminal differentiated cytotrophoblasts (VT, SCT, GC) from flash frozen placental tissue by laser microdissection microscopy.

Methods: Cells from flash frozen human term placental tissue (>39 week) (n=3) were isolated by laser micro-dissection microscopy (Zeiss) and separated in different tubes. After RNA isolation gene expression profile was determined by the Human Genome U133 PLUS 2.0 chip (Affymetrix).

Results: The RNA of the different cell types (VT, SCT, GC) was of good quality and quantity. All cell compartments were identified by immunohistochemistry by specific protein markers e.g. cytokeratin, vimentin, CD45, Syncytin-1 and HLA-G. This cell type specific profile was identical at the level of gene expression, verifying the high specificity. Overall we detected highly significant ($p<0.005$) changes in the gene expression profile between the three cohorts, e.g. between VT and SCT (1364 genes/EST) or between SCT and GC (3067 genes/EST).

Conclusion: Gene expression profiling of single cell compartments of the human term placenta identified a high number of differentially expressed genes in terminal differentiated trophoblast subtypes.