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Transcriptomic signatures of villous cytotrophoblast and syncytiotrophoblast in term human placenta

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ABSTRACT

During pregnancy, the placenta ensures multiple functions, which are directly involved in the initiation, fetal growth and outcome of gestation. The placental tissue involved in maternal-fetal exchanges and in synthesis of pregnancy hormones is the mononucleated villous cytotrophoblast (VCT) which aggregates and fuses to form and renew the syncytiotrophoblast (ST). Knowledge of the gene expression pattern specific to this endocrine and exchanges tissue of human placenta is of major importance to understand functions of this heterogeneous and complex tissue. Therefore, we undertook a global analysis of the gene expression profiles of primary cultured-VCT (n = 6) and *in vitro*-differentiated-ST (n = 5) in comparison with whole term placental tissue from which mononucleated VCT were isolated. A total of 880 differentially expressed genes (DEG) were observed between VCT/ST compared to whole placenta, and a total of 37 and 137 genes were significantly up and down-regulated, respectively, in VCT compared to ST. The 37 VCT-genes were involved in cellular processes (assembly, organization, and maintenance), whereas the 137 ST-genes were associated with lipid metabolism and cell morphology. *In silico*, all networks were linked to 3 transcriptional regulators (PPARγ, RARα and NR2F1) which are known to be essential for trophoblast differentiation. A subset of six DEG was validated by RT-qPCR and four by immunohistochemistry. To conclude, recognition of these pathways is fundamental to increase our understanding of the molecular basis of human trophoblast differentiation. The present study provides for the first time a gene expression signature of the VCT and ST compared to their originated term human placental tissue.

1. Introduction

During pregnancy human placenta plays a central role in fetal and maternal physiology. It allows all the exchanges (oxygen, nutrients, and waste products) between the mother and the fetus, produces hormones required for the establishment and maintenance of pregnancy, adaptation of the maternal organism to pregnancy, fetal growth and wellbeing, and development of the mechanisms involved in parturition. The human placenta is a hemochorial villous placenta. The structural and functional unit of

Abbreviations: aRNA, amplified RNA; DEG, differentially expressed genes; FDR, false discovery rate; HCL, hierarchical clustering; IHC, immunohistochemistry; IPA, Ingenuity pathway analysis; RT-qPCR, real time quantitative PCR; SAM, Significance analysis of microarrays; VCT, villous cytotrophoblast; ST, syncytiotrophoblast.

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human placenta is the chorionic villous, which becomes apparent in its definitive structure as early as day 21 of pregnancy. At term, the mesenchymal core of the villi that derived from the inner cell mass of the embryo is perfused by fetal blood. The trophoblast at the surface of the villi, named villous trophoblast, derives from the trophectoderm and includes the mononuclear villous cytotrophoblast cells (VCT) attached to the villous basement membrane and the syncytiotrophoblast (ST) that covers the entire surface of the villi. *In vivo* and *in vitro* in VCT primary cultures, the multinuclear ST arises from the differentiation, aggregation and fusion of VCT. It is this villous trophoblast that regulates gas and nutrient exchanges, possesses intensive endocrine functions and provides immunological support to the fetus. This villous trophoblast of the floating chorionic villi is bathed in the intervillous space by maternal blood supplied by uterine radial arteries through the basal plate bathes. Therefore, at term, the placenta is a complex organ that includes numerous cellular components of both fetal and maternal origin as well as cells from different embryonic origin. In addition as well demonstrated the placental architecture and blood flow are not uniform across the placental disk. Proximity to the umbilical cord, basal plate or chorionic plate influence perfusion and lead to regional difference within the placenta illustrated in histology [1] but also in gene expression [1,2]. On the other hand at term the placenta is an easily accessible organ. Several pregnancy disorders such as preeclampsia and preterm labor are from placental origin. Numerous studies using microarray technology aimed to characterize the placental gene expression pattern between normal and pathological placentas in order to establish a relationship with physiopathology of pregnancy (as for preeclampsia, reviewed in Ref. [3]). Even if some comprehensive analyses of the human placenta transcriptome have been published [4–6], very few studies have focused on VCT differentiation [7] and none has been done on VCT and ST compared to their own placenta tissues. Indeed, using microarrays, Aronow et al. [7] analyzed the gene expression patterns of term villous cytotrophoblast cultured from day one to day six and defined the 12h-cultured cells as reference. In this study, they did not compare trophoblastic expression to gene expression of corresponding chorionic villi from which VCT were isolated.

The aim of this work was therefore to guide investigators interested in placental physiology and pathology by giving a transcriptomic signature of the villous trophoblast and the syncytiotrophoblast within the human term placenta. In the present study, we isolated mononucleated VCT from term chorionic villi and cultured them to differentiate *in vitro* into ST. We compared the gene expression pattern from the primary cultured-mononucleated VCT and the *in vitro* differentiated-ST to the gene expression of the total placental extract from which VCT were isolated.

2. Materials and methods

2.1. Collection of term placental samples

Six term placentas were obtained immediately after iterative cesarean from healthy mothers with uncomplicated pregnancies. The reasons for caesarean section were as follow: breech delivery or transverse presentation, covering placenta, multi-cicatricial uterus, narrow pelvis. The terms of the pregnancies were 39 weeks of amenorrhea. One sample was taken from each of six placentas (the maternal side facing upwards), half distance from the edge and the insertion of the cord blood, at a depth of 1.5 cm to avoid a sampling contamination by the decidua basalis. Placenta samples essentially constituted of chorionic villi were immediately frozen in liquid nitrogen and stored at −80 °C for total RNA extraction. The study was approved by our local ethic committee.

2.2. Villous trophoblastic cell primary cultures

In vitro studies were performed on villous cytotrophoblasts isolated from the same six different term placentas used for tissue collection. Villous tissue was dissected free of membranes, rinsed, and minced in Ca^{2+},Mg^{2+}-free Hanks' balanced salt solution. Mononucleated cytotrophoblasts were isolated after trypsin-DNase digestion and discontinuous Percoll gradient fractionation, using a slight modification of the method of Kliman et al. [8] and Alsat et al. [9] as previously described [10]. Briefly, the villous sample was subjected to sequential enzymatic digestions in a solution which contains 0.25% (wt/vol) trypsin powder (Difco), 5 IU of DNase I per ml, 25 mM HEPES, 4.2 mM $MgSO_4$, 100 IU/mL penicillin and 100 μg/mL streptomycin (Biochemical Industry) in Hanks' balanced salt solution and monitored under light microscopy. The first and/or second digestion was discarded after light microscopy analysis to eliminate syncytiotrophoblast fragments, and the following four or five sequential digestions were kept. The cells collected during these last digestions were purified on a discontinuous Percoll gradient (5–70% in 5% steps). The purified cytotrophoblastic cells (density, 1.048–1.062 g/mL) were platted in 60 mm diameter-tissue culture plastic dishes (TPP) to a final density of 2.7×10^6 cells in 3 mL of Dulbecco's modified Eagle's medium (DMEM) containing 10% decomplemented fetal calf serum (FCS), 2 mM glutamin, 100 IU/mL penicillin and 100 mg/mL streptomycin. The dishes were maintained at 37 °C in humidified 5% CO_2. After a three-hour platting period, adherent cells were washed three times with complete medium to remove non adherent cells and syncytiotrophoblast debris. About 95% of adherent cells were cytokeratin 7 positive cytotrophoblasts (monoclonal antibody (DAKO), dilution, 1:200). These mononucleated villous cytotrophoblasts (VCT) were cultured for up to three days to obtain a multinucleated syncytiotrophoblast (ST) by cell aggregation and fusion. Total RNA was extracted from mononucleated VCT and ST after 12 h and 72 h of culture, respectively.

2.3. Microarray experiments and analysis

For each placenta (n = 6), total RNA was prepared from 12h-cultured VCT and *in vitro* differentiated ST (72h culture) and from the corresponding placental tissue sample using the RNeasy total RNA Minikit (Qiagen), according to the manufacturer's protocol. The concentration of total RNA was determined using Ultrospec 2000 spectrophotometer (Pharmacia Biotech) and the integrity of the RNA was assessed using a 2100 Bioanalyzer (Agilent Technologies). One microgram of total RNA from each sample preparation was amplified using the MessageAmp RNA kit (Ambion), and 3 μg of amplified RNA (aRNA) was Cy-dye labeled using the 26 CyScribe first-strand cDNA labeling kit (Amersham Biosciences) ([11]; Brown Lab protocols, http://cmgm.stanford.edu/pbrown/protocols/index). To compare the gene expression pattern obtained from each cultured cells (VCT or ST) with the one obtained from corresponding total placental extracts, amplified RNA from cultured cells was labeled with Cy5, while the aRNA from placental tissue was labeled with Cy3. Several quality cross-checks (for total RNA quality, aRNA quality, dye incorporation efficiency) were performed and in final, a total of six (VCT) or five (ST) individual cDNA microarrays were performed in this condition (one ST sample didn't pass the RNA quality check). The labeled mixture (Cy5 and Cy3) was purified and concentrated using Microcon YM 30 column (Millipore) after the addition of human cot-1, yeast tRNA, and poly A. After denaturation of probes, the mixture was hybridized overnight at 65 °C in a sealed humidified hybridization chamber, and then washed in 0.03% SDS, 2XSSC, 1XSSC and 0.2% SDS solutions for 2 min. After washing, the arrays were immediately scanned using a Genepix