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Expression and localization pattern of ABCA1 in diverse human placental primary cells and tissues

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ABSTRACT

The ATP-binding cassette transporter A1 (ABCA1) mediates the transport of cholesterol, phospholipids, and other lipophilic molecules across cellular membranes. Recent data provide evidence that ABCA1 plays an important role in placental function but the exact cellular sites of ABCA1 action in the placenta remain controversial. To clarify this issue, we analyzed the cellular and subcellular localization of ABCA1 with immunocytochemistry, immunofluorescence and subsequent confocal or immunofluorescence microscopy in different types of isolated primary placenta cells: cytotrophoblast cells, amnion epithelial cells, villous macrophages (Hofbauer cells), and mesenchymal cells isolated from chorionic membrane and placental villi. After 12 h of cultivation, primary cytotrophoblast cells showed intensive membrane and cytoplasmic staining for ABCA1. After 24 h, with progressive syncytium formation, ABCA1 staining intensity was markedly reduced and ABCA1 was dispersed in the cytoplasm of the forming syncytial layer. In amnion epithelial cells, placental macrophages and mesenchymal cells, ABCA1 was predominantly localized at the cell membrane and cytoplasmic compartments partially corresponding to the endoplasmic reticulum. In these cell types, the ABCA1 staining intensity was not dependent on the cultivation time. In conclusion, ABCA1 shows marked expression levels in diverse placental cell types. The multitopic localization of ABCA1 in diverse human placental cells not all directly involved in materno–fetal exchange suggests that this protein may not only participate in transplacental lipid transport but could have additional regulatory functions.

1. Introduction

The ATP-binding cassette (ABC) transporter A1 (ABCA1), a member of the ABC transporter superfamily, is a membrane-spanning protein that mediates the transport of cholesterol, phospholipids, and other lipophilic molecules across cellular membranes and removes excess cellular cholesterol from peripheral cells by the transfer onto lipid-poor apolipoprotein (apo) A1 [1]. Additional functions of ABCA1, such as regulation of macrophage-mediated immune reactions, phosphatidylserine translocation in the cell membrane and its potential implication in the apoptosis cascade have been reported [2,3].

ABCA1 is highly expressed in rodent and human placental tissues [4,5,6]. The placenta supplies the growing fetus with nutrients and oxygen and serves as a source of hormones, cytokines, growth factors and other biologically active substances to maintain the pregnancy. The precise function and molecular mechanisms of ABCA1 in the placenta are still poorly understood. Studies of ABCA1 knockout mice revealed dramatic pathologic changes during pregnancy, such as fetal growth restriction and disrupted placental architecture with hemorrhage and cell debris [7]. In other studies, a 30% decrease of transplacental cholesterol transfer in ABCA1−/− embryos was shown [8]. Concomitantly, the *in vivo* treatment with a liver X receptor (LXR) agonist induced upregulation of ABCA1 expression and significantly increased materno–fetal cholesterol transport, suggesting that ABCA1 could be a potential target for prenatal therapy of the rare Smith–Lemli–Opitz syndrome (inborn deficiency of 7-dehydrocholesterol reductase). Furthermore, our previous data revealed that expression of ABCA1 in the placentas of women with antiphospholipid syndrome was downregulated in comparison to healthy controls [5].

Cholesterol and its derivatives have multiple biological functions. During intrauterine fetal development, they play a crucial role as energy sources, essential components of cell membranes, precursors of steroid hormone synthesis, metabolic regulators [9],

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and propagators of hedgehog signaling, which is responsible for growth and development [10]. Therefore, cholesterol transport from the maternal circulation to the placenta and fetus is highly important during pregnancy. The role of ABCA1 in this context is not yet well understood. Studies performed in placental explants, trophoblast cell cultures, and cultures of other polarized cells suggest that there are different routes by which cholesterol can exit the fetal (basolateral) side of trophoblasts via lipoproteins [11]. It was furthermore demonstrated that the placenta secretes apo A-1, apo B and apo E-containing lipoproteins [11]. Schmid et al. [12] suggested, however, that cholesterol efflux in BeWo trophoblast cells occurs not via the apo A-I route but from the basolateral membrane by aqueous diffusion or via the SR-BI receptor. The exact underlying mechanism of ABCA1-mediated cholesterol efflux in trophoblast cells still needs to be clarified. Furthermore, Stefulj et al. [13] recently provided evidence of ABCA1-associated lipid efflux from the placenta to the fetal circulation via human placental endothelial cells. It is thus assumed that ABCA1 may be responsible for cholesterol transport in the placenta at various cellular levels including trophoblast and endothelial cells. However, to date, information on ABCA1 localization in the placenta has been primarily derived from observations obtained from immunohistochemical staining of placental tissues, and data are incomplete and in part conflicting. A recent study reported localization of ABCA1 in the basal and apical membrane of cytotrophoblast cells in early and term placental tissues but not in the syncytiotrophoblast layer [14]. These data are not in agreement with previous observations [5,15]. In addition, novel localization data of ABCA1 in placental membranes were demonstrated [15].

Moreover, the subcellular localization of ABCA1 in the human placenta is not yet clear. Neufeld et al. [1] reported intracellular localization of ABCA1 in endocytic compartments in HeLa cells. It has been suggested that intracellular trafficking of ABCA1 is important for modulating ABCA1 transporter activity and cellular cholesterol homeostasis [1,16]. Intracellular localization of ABCA1 was demonstrated for other human cell lines and primary cells [17], but no studies of ABCA1 localization in primary placenta cells have been performed. Therefore, in the present study we analyzed the cellular and subcellular localization of ABCA1 in different primary placenta cells, and compared these data with results obtained by immunohistochemistry in formalin-fixed, paraffin-embedded placental tissues.

2. Methods

2.1. Tissue samples

Ethical approval of the study was obtained by the local institutional review board. Placental tissues were collected after obtaining informed consent from pregnant women at the Department of Obstetrics and Gynecology, University Hospital Bern, Switzerland. First trimester placentas (7–12 wk) were collected after elective, legal termination of normal pregnancy for psychosocial reasons ($n = 5$). Term placentas (38–40 wk) were collected from uncomplicated pregnancies after elective caesarean section without prior labor ($n = 5$). For primary cell isolation, villous tissue and placental membranes from term placentas were collected and processed within 2 h after delivery. For immunohistochemistry, placental tissue samples were fixed in 4% formalin for 24 h and embedded in paraffin. From term placentas, full-thickness tissue blocks were taken from the central, paracentral, and peripheral, but not marginal placenta portions. One representative block per case was used for immunohistochemical staining.

2.2. Isolation and cultivation of primary placenta cells

Villous cytotrophoblast cells were isolated from term placentas using a Trypsin-DNase/Percoll method described by Kliman et al. [18] with modifications, and thereafter immunopurified according to Petroff et al. [19]. Briefly, about 40 g of villous tissue were washed in 0.9% NaCl solution and underwent three consecutive digestion steps with 0.25% Trypsin (cat. No 15090/046, Invitrogen)/DNase I 300 U/ml (cat. No D5025-150KU Sigma). Digested tissues were centrifuged through fetal calf serum (FCS) (Invitrogen), followed by density gradient centrifugation in a non-continuous Percoll (Sigma) gradient. The cell fraction from the band corresponding to 1.048–1.060 density was collected, and further purified by negative immunomagnetic purification using 40 μg/ml mouse anti-human HLA-ABC (clone W6/32, Sigma) antibody and goat anti-mouse IgG microbeads (Miltenyi Biotec). The cell purity was evaluated by flow cytometry (FACScan, BD Biosciences) using non-labeled mouse anti-human cytokeratin-7 antibody (DAKO) and secondary goat anti-mouse FITC (Sigma). After magnetic separation, cells showed 92–95% purity. Cytotrophoblast cells were seeded into CellBind 6-well plates (Corning Life Science) in DMEM high glucose containing 10% FCS, antibiotic/antimycotic solution (100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B; Sigma) at a density of 2×10^6 cells per well, and incubated for 12 and 24 h.

Villous mesenchymal cells from term placentas (TVMC) and *villous macrophages (Hofbauer cells*, HC) from term placentas were obtained during the cytotrophoblast cell isolation procedure. After the magnetic cell separation step the cell mixture, positive for HLA class I, which mostly consists of mesenchymal cells was cultivated in α-MEM (Gibco) 20% FCS, antibiotic/antimycotic solution (Sigma). HC were obtained in a mixture together with lymphocytes from the band corresponding to 1.065–1.078 density of the Percoll gradient. One portion of cells was taken for immediate characterization with FACS, and the rest was frozen and stored for immunocytochemical analysis.

Human amnion epithelial cells (HAEC) and *human chorionic mesenchymal cells (HCMC)* were isolated as described previously [20]. Briefly, amnion and chorion were manually separated. Amnion membrane was incubated with 0.05% trypsin in Ca^{2+}- and Mg^{2+}-free PBS containing 0.02% EDTA (Invitrogen). HAEC were gently scratched off with a cell scraper and cultivated in DMEM high glucose (Gibco), 10% FCS, antibiotic/antimycotic solution (Invitrogen). Chorionic membrane was incubated with 2.4 U/ml dispase II (Sigma). The trophoblastic layer was peeled off the chorionic stroma with a cell scraper. The minced chorionic stroma was incubated with 270 U/ml collagenase II (Sigma). The digested tissue was filtered through a 100 μm nylon mesh and cells were cultivated in α-MEM (Gibco) 20% FCS, antibiotic/antimycotic solution (Invitrogen).

To isolate *villous mesenchymal cells* (VMC) from first trimester placentas, minced villous tissue was incubated with 0.05% trypsin in Ca^{2+}- and Mg^{2+}-free PBS containing 0.02% EDTA (Invitrogen). Digested tissue was filtered through a 100 μm nylon mesh. Cells were cultivated in α-MEM (Gibco) 20% FCS, antibiotic/antimycotic solution (Invitrogen).

2.3. Flow cytometry

Mesenchymal cells were trypsinised and resuspended in cell culture medium (10% FBS). After incubation on ice for 10 min, cells were washed twice and resuspended in Dulbecco's phosphate-buffered saline (DPBS, pH 7.2, Invitrogen), 10% FBS (Invitrogen). Primary isolated cytotrophoblast cells and macrophages were immediately resuspended in DPBS, 10% FBS and incubated on ice for 10 min. Primary antibodies were added to the cell suspensions for 1 h at 4 °C. For labeling of macrophages, an additional blocking step in DPBS, 10% FCS and 10% human AB serum (Sigma) was performed for 10 min at 4 °C prior to the incubation with the primary antibodies. The following antibodies were used as mesenchymal stem cell markers: CD105 FITC (Serotec), CD90 FITC (Acris), and CD73 PE (Biolegend). The following antibodies were used as hematopoietic stem cell markers: CD14 FITC (Biolegend), CD34 FITC (Biolegend), and CD45 FITC (Biolegend). Antibodies against immunological markers were: HLA-ABC FITC (Biolegend) and HLA-DR FITC (Biolegend). As macrophage and cytotrophoblast cell markers, anti-CD14 FITC (Biolegend) and anti-cytokeratin 7 (Dako) antibodies were applied, respectively.

For CK7, a secondary FITC-conjugated antibody (1:500, Sigma) was applied. Isotype controls were obtained by incubating cells with primary mouse immunoglobulins and a secondary FITC-conjugated antibody (1:500, Sigma). Cells were analyzed by flow cytometry (FACScan, BD Biosciences, CellQuest software); quantification was performed with FlowJo Software (Ashland, OR, USA).

2.4. Immunohistochemistry

Tissue sections were cut at 5 μm thickness and mounted on Superfrost plus slides (Menzel, Germany). Slides were dewaxed and rehydrated; antigen retrieval was performed in sodium citrate buffer (pH = 5.5) in a microwave oven at 450 W for 10 min. Staining was performed with an UltraVision LPValue Detection System kit (LabVision), according to the manufacturer's instructions. Slides were blocked with peroxidase blocking solution (LabVision) for 12 min and washed with tris-buffered saline, containing 0.03% Tween-20 (TBS-T). Slides were then incubated with UV block (LabVision) containing 10% human AB serum (Sigma) for 7 min and incubated overnight with the primary antibody diluted in PBS containing 4% BSA (MP Biomedicals) in a humid chamber at 4 °C. Rabbit polyclonal anti-human ABCA1 antibody (Novus Biologicals, 1:100, 10 μg/ml) was used as the primary antibody and rabbit immunoglobulins (Sigma, 1:1000, 10 μg/ml) as the isotype control. The next day, slides were washed with TBS-T and incubated with Antibody Enhancer solution (LabVision) for 20 min. After an additional washing step with TBS-T, the HRP polymer (LabVision) was added for 30 min, the slides were washed again and AEC chromogen (LabVision) was applied for 9 min in the dark. Sections were washed in distilled water, counterstained with hematoxylin (Sigma) and mounted with an