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Differential effect of cross-linking the CD98 heavy chain on fusion and amino acid transport in the human placental trophoblast (BeWo) cell line

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Abstract

CD98 (otherwise known as 4F2) is an integral membrane protein with multiple functions including amino acid transport, integrin activation, cell fusion and cell activation. The molecular mechanisms coordinating these multiple functions remain unclear. We have studied CD98 heavy chain (hc) function in a human placental trophoblast cell line (BeWo). We show that cross-linking of CD98hc by incubation of cells in the presence of functional monoclonal antibodies causes cellular re-distribution of the protein from the cytoplasm to the plasma membrane as measured by flow cytometry, western blotting and quantitative immuno-electron microscopy. The latter technique also indicated that CD98hc is trafficked between cell surface and cytoplasmic pools in vesicles. Increased cell surface CD98 correlates with increased cellular fusion in BeWo cells. In addition, we show reduced LAT 1 surface expression and neutral amino acid transport in the presence of the CD98 mabs. The results thus suggest that the function of CD98 in cell fusion is distinct from its role in cellular nutrient delivery.

Keywords: CD98; LAT 1; Cell fusion; Amino acid transport

1. Introduction

Syncytialized trophoblast forms during implantation and is then maintained at the villous maternal–fetal interface throughout pregnancy. Understanding the mechanism that leads to formation, maintenance and turnover of the syncytiotrophoblast is clearly important for understanding both normal and abnormal pregnancy (including pre-eclampsia). However, cell fusion is a very rare event in cell biology and in humans is typically observed only in the placenta (syncytiotrophoblast), in skeletal muscle (striated muscle fibre) and in bone formation (osteoclast) as well as during the fusion of gametes at fertilization. It is also recognised pathologically as a mechanism induced or required by some viruses to engage with host cells.

CD98 is a multifunctional transmembrane protein originally found on the surface of activated T cells [1] but now known to be present on virtually all cells, being expressed at particularly high levels in actively proliferating tissue. Recent reports describe the possible role of CD98 in the regulation of cell differentiation, adhesion, growth, apoptosis and cancer development [2–5]. Often these diverse cellular functions require activation of integrin molecules but there is little information about the exact mechanisms underlying them and more generally of CD98 intracellular pathways [6–8].

The CD98 antigen forms a dimeric structure consisting of a type 2 glycosylated integral membrane protein of around 80 kDa (heavy chain) and one of at least six proteins with apparent molecular mass of 40 kDa (light chain) linked by disulfide-bond. Depending on which light chain is part of the heterodimer many amino acids, including several essential amino acids, are transported across the plasma membrane in a Na+-independent (system L, neutral amino acids transport) and Na+-dependent (system y+L, neutral and cationic amino acid transport) manner. The heavy chain is ubiquitous but variously glycosylated according to the tissue of origin while the non-glycosylated light chains are expressed differentially according to tissue origin [9,10]. Transfection studies in mammalian cells have indicated that while CD98hc can be expressed on the plasma membrane on its own, trafficking of the light chain to

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the cell surface is possible only in the heterodimer form and apparently independently of disulfide linkage [7,11].

CD98 has been found to be identical to fusion regulatory protein-1 (FRP-1) and its expression is necessary for virus-induced cell fusion [12] and for osteoclast formation [13,14], it is therefore of particular interest that CD98 is expressed on the cytotrophoblasts and on the plasma membrane of the syncytiotrophoblast, the site of exchange of nutrients, lipids and minerals between the mother and the fetus, which results from the fusion of the underlying cytotrophoblast cells [15,16].

While some antibodies against CD98/FRP-1 are known to suppress virus-induced cell fusion and CD98-mediated cell fusion of monocytes [13,17], others cross-linking CD98 stimulated cell aggregation and growth [3,4,18] indicating that CD98/FRP-1 molecules are able to regulate cell fusion.

Forskolin, a cell permeable diterpenoid isolated from Coleus forskohlii, stimulates adenylate cyclase activity to increase intracellular cyclic adenosine monophosphate (cAMP) thus activating cAMP-dependent protein kinase and other cAMP receptor proteins. BeWo cells, a well established choriocarcinoma cell line, can undergo fusion and morphological differentiation similar to the formation of syncytiotrophoblast by the cytotrophoblast in the placenta. Forskolin is known to increase this cellular fusion in BeWo cells, and in other fusogenic cell lines [19–22].

Our laboratory has previously shown that manipulation of CD98 cellular expression by antisense oligonucleotides [20] has an effect on amino acid transport and on cellular fusion in BeWo cells; in this paper we show that two monoclonal antibodies against CD98 (mab4F2 and mabAHN18) can affect its surface expression, cell differentiation and amino acid transport.

2. Materials and methods

2.1. Primary antibodies

4F2 was a generous gift from Dr. T. Sethi; AHN18 was from Chemicon International. Goat anti-human CD98 (C-20), rabbit anti-human CD98 (H-300), normal goat IgG and normal rabbit IgG (isotype-matched controls) were from Santa Cruz Biotechnology Inc. Rabbit anti-rat LAT 1 (AHP735) and mouse IgG_1 were from Serotec; 98C01 was from Stratech Scientific Ltd. and mouse IgG_{2a} was from Dako.

2.2. Secondary antibodies

Goat anti-rabbit IgG horseradish peroxidase-conjugated was from Dako. Donkey anti-goat IgG fluorescein isothiocyanate-conjugated was from Jackson immuno research laboratories and rabbit anti-goat IgG r-phycoerythrin-conjugated was from Sigma. Protein A–15 nm gold complex was from British Biocell.

2.3. Cell culture

BeWo cells were cultured at 37 °C as monolayers in F-12K Nutrient Mixture (Kaighn's modification) supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine (all GIBCO), 100 U ml^{-1} penicillin and 100 U ml^{-1} streptomycin (Sigma) in an humidified atmosphere of 5% CO_2 and 95% air. Confluent cells were harvested by trypsinisation with trypsin-EDTA in HBSS w/o Ca^2 and Mg^2 (GIBCO), viable cells counted by the trypan blue (Sigma) method, resuspended in serum-free medium and stained with either with 10 μl of vibrant DiO cell labelling solution (1 mM, Molecular probes) per 10^6 /ml cells for 30 min at 37 °C or with MitoTracker Deep Red 633 (Molecular probes) at a concentration of 25 nM per 10^6 /ml cells for 15 min 37 °C in the dark shaking gently. After extensive washing with warm serum-free medium each group of cells was resuspended in complete growth medium and plated either on their own or in a 50% mixture in six well culture plates (BD Falcon). When the cells reached 65–70% confluence forskolin (Sigma) or vehicle (dimethyl sulfoxide, DMSO) was added in fresh medium at a final concentration of 100 μM for 24 h, unless otherwise indicated. In some wells, after 5 h of culture in forskolin or DMSO, 4F2 or AHN18 monoclonal antibody or the respective isotype matched control (mouse IgG_{2a} or IgG_1) was added at a final concentration of 20 μg/ml (19 h).

2.4. Preparation of cell fractions, SDS-PAGE and Western Blotting (WB)

Confluent cultures from six well plates were washed with ice-cold PBS and then lysed by manual agitation at 4 °C in ice-cold buffer containing 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA and 10 μl protease inhibitor mixture, (Sigma). We used classical cell fractionation [23,24] to explore the distribution of CD98hc. Thus the lysed cell homogenate (H) was centrifuged at low speed (800×*g*) for 15 min at 4 °C. Pellet (P1) was suspended in 200 μl buffer containing 50 mM Tris–HCl (pH 7.4), 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA and 10 μl protease inhibitor mixture for 15 min on a rocker and then sonicated three times for 30 s. Using the same buffer, supernatant (S1) was sonicated three times for 30 s (200 μl buffer) and clarified by centrifugation at high speed (17000×*g*) for 15 min at 4 °C, allowing us to collect supernatant S2. WB was then carried out on P1, containing crude plasma membrane and heavy organelles, and S2, containing purified membranes including small intracellular vesicles. For WB, precisely 10 μg of protein was solubilised in NuPAGE sample buffer with reducing agent (Invitrogen), warmed for 10 min at 75 °C and then run on 4–12% Novex Bis-Tris NuPAGE gels with MOPS running buffer (Invitrogen). The proteins were transferred to nitrocellulose membranes, blocked using 5% (w/v) non fat dry milk in 0.01 M PBS (Sigma) with 0.05% (v/v) Tween 20 for 1 h at room temperature and then incubated with rabbit anti human CD98 (H-300, 1:200) overnight at 4 °C. Goat anti rabbit horseradish peroxidase-conjugated antibody was used for secondary labelling. Immunoreactive bands were identified by SIGMA*FAST* 3,3′-Diaminobenzidine tablets (Sigma) according to the manufacturer's instructions. SeeBlue Plus2 pre-stained protein standards were from Invitrogen. As a negative control we used a mouse M1 cell homogenate (10 μg of protein).

2.5. Flow cytometry: surface staining on intact cells

Cells from six wells plates were detached with trypsin-EDTA (GIBCO). Aliquots of 1×10^6 cells were washed in PBS and re-suspended in 250 μl of FACS buffer (PBS, 1% fetal calf serum, 0.1% NaN_3) with goat anti-human CD98 (C-20, 1:20) or isotype control IgG or no primary antibody. Cells were incubated for 45 min on ice, followed by three washes with FACS buffer. Samples were then incubated with donkey anti-goat IgG fluorescein isothiocyanate-conjugated (1:50) or rabbit anti-goat IgG r-phycoerythrin-conjugated secondary antibody (1:20) for 45 min on ice and washed three times. Samples were finally resuspended in FACS buffer and 2% paraformaldehyde (PFA) and analyzed by flow cytometry using FACSCalibur (BD Biosciences) and Cell Quest software and/or EPICS Altra (Beckman Coulter) and EXPO32 software.

2.6. Flow cytometry: surface and intracellular staining

Cells suspensions were fixed in 2% PFA for 20 min at room temperature, washed once in PBS, permeabilised with 1% saponin in FACS buffer for 15 min at room temperature and then stained following the surface staining protocol. After the final wash samples were fixed again in 2% PFA before analysis.

2.7. Immunogold electron microscopy

Cells were prepared for electron microscopy by standard methods [25]. Briefly, cell pellets were post-fixed in osmium tetroxide (1% w/v in 0.1 M sodium phosphate buffer), contrasted with uranyl acetate (2% w/v in distilled water), dehydrated through increasing concentrations of ethanol (70–100%) and embedded in LR Gold resin (Agar Scientific UK). Ultra-thin sections (50–80 nm) were prepared by use of a Reichert Ultracut S microtome and mounted on 200-mesh