Intermembrane distances at the feto-maternal interface in epitheliochorial placentation

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Abstract: Introduction In an epitheliochorial placenta, the apical membranes of trophoblast cells and of uterine epithelial cells are in contact to each other (feto-maternal contact). In addition, there are also folds in which the trophoblast membrane is in contact with itself (feto-fetal contact) and areas where apical uterine epithelial membrane is in contact with itself (materno-maternal contact). Methods We use transmission electron microscopy of placental samples from pigs (n = 3), cows (n = 2), sheep (n = 2), goat (n = 2) and roe deer (n = 1) to study the intermembrane distance in these three contact types. Results The measured intermembrane distances vary between 8 and 25 nm. One common feature is that the distance at feto-fetal contact sites is about 6–10 nm wider than at materno-maternal sites and feto-maternal sites show intermediate values. Discussion This finding suggests that the membrane distance at feto-maternal contact sites is determined by heterophilic binding of larger fetal to smaller maternal binding molecules. Homophilic binding of smaller maternal or larger fetal molecules lead to the smaller or wider intermembrane distances at materno-maternal or feto-fetal contact sites respectively. The observation that this similar pattern of membrane distances is present in pigs and in ruminants suggest that an evolutionary mechanism is involved in determining the intermembrane distance in epitheliochorial placentas.

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Intermembrane distances at the feto-maternal interface in epitheliochorial placentation

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ABSTRACT

Introduction: In an epitheliochorial placenta, the apical membranes of trophoblast cells and of uterine epithelial cells are in contact to each other (feto-maternal contact). In addition, there are also folds in which the trophoblast membrane is in contact with itself (feto-fetal contact) and areas where apical uterine epithelial membrane is in contact with itself (materno-maternal contact).

Methods: We use transmission electron microscopy of placental samples from pigs (n = 3), cows (n = 2), sheep (n = 2), goat (n = 2) and roe deer (n = 1) to study the intermembrane distance in these three contact types.

Results: The measured intermembrane distances vary between 8 and 25 nm. One common feature is that the distance at feto-fetal contact sites is about 6–10 nm wider than at materno-maternal sites and feto-maternal sites show intermediate values.

Discussion: This finding suggests that the membrane distance at feto-maternal contact sites is determined by heterophilic binding of larger fetal to smaller maternal binding molecules. Homophilic binding of smaller maternal or larger fetal molecules lead to the smaller or wider intermembrane distances at materno-maternal or feto-fetal contact sites respectively. The observation that this similar pattern of membrane distances is present in pigs and in ruminants suggest that an evolutionary mechanism is involved in determining the intermembrane distance in epitheliochorial placentas.

1. Introduction

The epitheliochorial placenta is characterised by the contact of the trophoblast to the apical surface of the maternal uterine epithelium. In higher mammals an epitheliochorial placenta evolved in several clades of laurasiatheria (cetartiodactyla, perissodactyla and pholidota) and in strepsirrhine primates [1]. Thus, major groups of domesticated animals (e.g. camels, ruminants, equids, pigs) have an epitheliochorial placenta. In the evolution of the placenta in higher mammals, this non-invasive epitheliochorial placenta evolved from an invasive placenta of the endotheliochorial [2] or haemochorial [3] type.

During the development of the epitheliochorial placenta, the connection between the trophoblast and the uterine epithelium passes through stages of apposition, adhesion and further into the formation of the definitive placenta, which is typically characterised by membrane attachment between interdigitating fetal and maternal microvilli [1,4].

Many studies deal with the molecules that are involved in feto-maternal adhesion in the initial stages of pregnancy (reviewed by Refs. [5–7]). Roles for adhesion molecules from different groups (e.g. integrins, cadherins) have been identified for these early stages, but the molecular basis of the attachment in later stages is much less well defined.

Recently, we characterised ultrastructural features of the mature epitheliochorial placenta in several ruminant species, in domestic pigs and in one horse [8]. It was shown that in addition to the contact of fetal to maternal membranes there are also folds in which apical fetal cell membrane is attached to fetal membrane and areas in which apical maternal cell membrane is in contact to maternal membrane. It was also revealed that in the definitive mature epitheliochorial placenta the fetal membrane surface is larger than the maternal surface [8].

In the present study, we present ultrastructural data which show that the inter-cellular membrane distances at areas of fetal-fetal, fetal-maternal and maternal-maternal membrane contacts differ. These data
give important information about the connection between fetal and maternal surfaces in the mature epitheliochorial placenta.

2. Material and methods

2.1. Transmission electron microscopy (TEM)

Placental specimens from cattle (gd 140 and 213) were obtained at routine slaughtering at commercial slaughterhouses. Samples of sheep (gd 120 and 143), goat (gd 100 and 145) and roe deer (fetal crown rump length 8 cm) placenta were epon embedded archival blocks, provided by Dr. Peter Wooding, University of Cambridge, UK. Blocks of porcine placenta (gd 42, 76 and 108) were provided by Dr. Vibeke Dantzer, University of Copenhagen, Denmark. All samples were taken in compliance with the national animal welfare regulation at the time and location of sampling. Specimens from domestic animals were perfusion fixed through the fetal vasculature. The tissues were postfixed with osmium tetroxide and embedded in epon.

Semithin sections (1 μm) were cut with an ultramicrotome (Ultracut E, Reichert, Vienna, Austria), mounted on glass slides (Menzel-Gläser, Braunschweig, Germany), stained with toluidine blue and coverslipped. These sections were used to identify well-preserved tissue regions. Resin blocks with such regions were trimmed and ultrathin sections (70 nm) were cut with the ultramicrotome, mounted on grids and stained with uranyl acetate and lead citrate. A TEM (CM12, Philips, Eindhoven, Holland) was used to identify areas with a well preserved feto-maternal interface. Digital photographs of the feto-maternal interface were taken with a CCD camera (Orius SC1000, Gatan, Pleasanton, California, USA) at a magnification of ×110 000.

Table 1

| Name of Lectin          | Abbreviation | Lectin specificity (according to the product information) |
|------------------------|--------------|----------------------------------------------------------|
| Concanavalin A         | Con A        | Mannose                                                  |
| Dolichos biflorus      | DBA          | N-Acetylgalactosamine                                    |
| agglutinin             |              |                                                          |
| Peanut agglutinin      | PNA          | Galactose                                                |
| Rhus communis agglutinin I | RCA I      | Galactose, N-Acetylgalactosamine                          |
| Soybean agglutinin I   | SBA          | N-Acetylgalactosamine                                    |
| Ulex europaeus agglutinin | UEA I      | Fucose                                                   |
| Wheat germ agglutinin  | WGA          | N-Acetylgalactosamine                                    |

Fig. 1. Transmission electron micrograph of the feto-maternal junction in a pig (gd 108). The feto-maternal junction is a complex zone between the trophoblast (TB) and the uterine epithelium (UE). Towards the trophoblast the tips of maternal microvilli (arrowheads) are embedded in folds of fetal cell membranes (arrows). Closer to the uterine epithelium there is a zone in which fetal and maternal microvilli interdigitate (asterisk). Bar = 1 μm.

Fig. 2. A and B: Transmission electron micrograph (goat gd 100) of maternal microvilli (M) surrounded by fetal trophoblast (F). In this specimen the extracellular surface of the apical cell membrane of the trophoblast shows dark staining. The distance between fetal cell membranes (large arrows in A) is wider than the distance between fetal and maternal cell membranes (small arrows in A and B). The distance between maternal membranes (between arrowheads in B) is smaller. C High magnification of two porcine (gd 42) cell membranes shows a double line (arrowhead) in the middle of the intercellular gap. Bars = 100 nm (in A and B); 20 nm in C.

Fig. 3. Transmission electron micrograph (goat gd 100) of feto-fetal (left), feto-maternal (middle) and materno-maternal (right) contact areas. Bar = 20 nm.
2.2. Measurement of membrane distances

Images were opened with ImageJ (Version 1.0). Regions with distinguishable fetal and maternal membranes were selected. This distinction was based on the knowledge of the ultrastructure of the fetomaternal junction in the species studied [8]. Areas in which adjacent membranes were cut perpendicularly (the pairs osmiophilic leaflets of each membrane were clearly visible) measurements were made between the outer osmiophilic leaflet of the membranes. In each specimen at least 10 measurements were made for feto-fetal, feto-maternal and materno-maternal contact.

2.3. Lectin staining

The lectin stainings were performed as previously described [9]. In brief, biotinylated lectins (Lectin Kit 1, biotinylated, BK-1000 from Vector Laboratories Inc, Burlingame, CA, USA) were used (Table 1). Semithin sections (0.5 μm) of epon embedded tissues (cattle gd 135; 164; 208) were treated with with saturated ethanolic sodium ethoxide for 10 min to remove the resin, rinsed in three changes of ethanol, rehydrated in descending concentrations of ethanol, and rinsed in distilled water. The slides were rinsed in 0.05 M TRIS-buffered saline (TBS), pH 7.6, 1 mM CaCl₂, and incubated for 45 min in a humid chamber at RT with 10 μg/ml biotinylated lectin in TBS. After two washes in TBS, the slides were incubated with streptavidin conjugated to Alexa 633 (Molecular Probes, Eugene, Oregon, USA) for 45 min at RT, rinsed in phosphate-buffered saline (PBS) and after a short wash in H₂O mounted with ProLong Diamond (Life Technologies Corporation, Eugene, Oregon, USA). The samples were analyzed with a Confocal Laser Scanning Microscope SP8 (Leica Microsystems, Wetzlar, Germany).

3. Results

3.1. Transmission electron microscopy

A close attachment of the membranes at the fetomaternal interface was found only in specimens with a very good ultrastructural preservation. In such specimens (Fig. 1) no large gaps of the intercellular space were observed. A complex system of interdigitating fetal and maternal microvilli and folds was found. In other regions with a suboptimal fixation, fetal and maternal tissues were typically separated at this interface. In well preserved regions, adjacent membranes run in parallel at specific distances (Figs. 2 and 3).

In one goat (gd 100) fetal and maternal membranes showed obvious staining differences (Fig. 2 A and B). In this case the outer extracellular leaflet of the fetal membrane was covered by a layer of electron dense material. On the maternal membrane this layer was absent or much less obvious.

In one pig placenta (gd 42) a double line in the middle of the intercellular space could be seen in a feto-fetal contact area (Fig. 2C).

In all specimens a difference between the three distances (feto-fetal, feto-maternal, materno-maternal) was observed (Figs. 3 and 4). The distance was widest at feto-fetal contact areas, intermediate at feto-maternal and smallest at materno-maternal contact areas (Fig. 5).

3.2. Lectin staining

Strong binding of the lectin to the fetomaternal interface was observed with the RCA, SBA, WGA and PNA lectins. This was observed in regions where fetal and maternal membranes were attached to each other and also in regions where they were separated. In such regions where fetal and maternal surfaces were separated, differences in the staining intensity were observed (Fig. 6). While in the RCA and WGA stained specimens the staining of both surfaces did not show obvious differences in intensity, staining with PNA and SBA was stronger on the
Fig. 5. Intercellular distances (nm) in areas of feto-fetal (FF), feto-maternal (FM) and materno-maternal (MM) contact.
endotheliochorial or hemochorial placenta, this apical contact is later replaced by contact of the trophoblast to deeper layers of endometrial tissue or to maternal blood. In the epitheliochorial placenta the apical contact between trophoblast und uterine epithelium remains intact and is further developed into the definitive mature placenta. But what actually keeps the membranes in contact in the developed epitheliochorial placenta is not well characterised.

Our study does not provide the identity of the molecules involved in feto-maternal attachment, but it gives some information about them. The difference in membrane distance indicates that the molecules that determine the distance are larger at the fetal surface and about 3–4 nm smaller at the maternal surface. Thus, homophilic interaction could lead to the small distance in materno-maternal contact and the about 6–8 nm larger distance in feto-fetal contact. The intermediate distance in feto-maternal contact areas might result from heterophilic interaction between the fetal and maternal molecules.

The feto-maternal interface is strongly glycosylated and these glycans may be involved in membrane attachment [15]. Our lectin staining shows that there is a difference between fetal and maternal surfaces. The stronger fetal staining intensity of SBA and PNA lectin suggest that specific N-acetylgalactosamine and galactose residues, respectively, differ between these surfaces. Further investigation would be needed to identify the structure of these glycans.

It has been suggested that integrins and osteopontin might be involved in the feto-maternal attachment, not only in early pregnancy but also in the definitive placenta [16]. In that model extracellular osteopontin serves as a bridging molecule between integrin molecules of the trophoblast and the uterine epithelium. Different types of integrins and different activation stages could lead to differences in the intercellular distance as observed in our study. Integrins occur in different conformations, which can be upright or bent. In the bent conformation the ligand binding site is close to the cell membrane which would allow the relatively small intermembrane distances measured in our study. In the sheep placenta, osteopontin was detected apical between trophoblast and maternal caruncular epithelium [17] and thus may act as a bridging molecule as suggested [16]. In pigs a more diffuse intracellular localisation of osteopontin was observed in the definitive placenta [16,18]. This may indicate that osteopontin bridging between fetal and maternal apical integrin molecules is not a general mechanism of feto-maternal attachment in epitheliochorial placentas.

One further possibility is that cadherins are part of this attachment mechanism. The intercellular distances that we measured are in the range of those described in the cadherin mediated zonula adherens (15–25 nm) [1,19]. The two lines observed in the porcine placenta (Fig. 2C) are very similar to the observations in adherens junction, visualised by cryo-electron microscopy [20]. Cadherins are expressed at the feto-maternal junction in cattle [21–24], sheep [25] and pigs [26]. Beta-catenin, which is involved in the linkage of actin to the intracellular domain of cadherins, is also expressed in bovine feto-maternal junction [23]. The ability of cadherins to undergo heterophilic and homophilic binding [27] would also permit intercellular distance differences, as observed in our study.

In summary, we show that the membrane distances in the epitheliochorial placenta differ between feto-fetal, feto-maternal and materno-maternal contact sites. Feto-fetal distances are 6–8 nm wider than materno-maternal distances and feto-maternal values are intermediate. This suggests size differences between the molecules that determine these distances. The fact that these distance differences occur in ruminants and in the domestic pig may indicate that conserved molecular mechanisms are involved in membrane attachments in the epitheliochorial placentation.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence
the work reported in this paper.

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