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Epithelial cell polarity and embryo implantation in mammals

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ABSTRACT At embryo implantation we are confronted with the fact that uterine and trophoblast epithelium make contact via their apical cell membranes. This epithelium-epithelium adhesion leading to definitive attachment of the embryo to the uterine wall, however, is far from being trivial and has been called a cell biological paradox. It has been proposed that some of the molecular events involved in epithelium-to-mesenchyme transformation might play a role in the interaction between uterine cells and trophoblast. As a mechanism to achieve uterine epithelium adhesiveness for trophoblast it is postulated that uterine cells partially modulate their epithelial phenotype. Data from recent in vitro experiments give support to this hypothesis and suggest that loss of apical-basal cell polarity might prepare the apical cell pole of uterine epithelium for cell-to-cell contact with trophoblast *in vivo*.

KEY WORDS: *mammalian embryo implantation, epithelial phenotype, polarity, adhesion, uterus*

A cell biological paradox

The central event of embryo implantation is the formation of a morphologically and functionally specialized cell-to-cell contact between the endometrium and the blastocyst. This review will focus on cell biological aspects of the initial phase of embryo implantation when the apical cell pole of the uterine epithelium interacts with the apical cell pole of the trophoblast.

The cell-cell interaction between uterine and embryonic tissues is far from being trivial. A fundamental property of simple epithelia like uterine and trophoblast epithelium is to possess a polarized organization and, as one aspect of this, three distinct membrane domains, i.e. the apical, the lateral, and the basal plasma membrane domain (Hay, 1985; Rodriguez-Boulan and Nelson, 1989; Simons and Fuller, 1985). While basal and lateral membranes are studded with adhesion molecules so that they can mediate cell-to-cell and cell-to-matrix adhesion, apical plasma membranes normally lack most of these molecules and lack adhesive properties. Consequently, the adhesive interaction between uterine and trophoblast epithelium can be initiated only if both partners have entered a specific physiological state, i.e. the receptive state in case of the endometrium and the invasive state in case of the trophoblast. Implantation can be initiated only when both partners enter these states in synchrony. Receptivity of the endometrium is maintained only for a limited period of time, which defines an implantation window. This window is regulated by ovarian steroid hormones, notably progesterone and changes in progesterone/oestrogen ratio (Psychoyos, 1994).

Thus, at implantation initiation we are confronted with the fact that uterine and trophoblast epithelium make their first contact exactly via their apical cell membranes, and this is what may be called a cell biological paradox (Denker, 1986, 1990, 1993, 1994). Solutions for the paradox are found when taking a side view to processes in embryology that involve interaction of two epithelia, typically combined with epithelium-to-mesenchyme (E-M) transformation, a process that is also being discussed to be involved in tumor cell invasion. It has been proposed that some of the molecular events involved in E-M transformation can also be found in both, the acquisition of receptivity by the uterine epithelium and the expression of the invasive phenotype by the trophoblast. We will concentrate here on the uterine epithelium.

Properties of uterine epithelium at receptivity

In several investigations it has been tried to define molecular changes in the composition of the apical plasma membranes of the uterine epithelium at receptivity. Consistently, a reduction in the thickness of the glycocalyx of uterine epithelial cells and in cell surface charge has been observed in various species (Anderson *et al.*, 1990; Enders and Schlafke, 1977; Morris and Potter, 1990). On the other hand, the biosynthesis and expression of new cell surface proteins has also been observed (Anderson *et al.*, 1988; Hoffman *et al.*, 1990; Kimber and Lindenberg, 1990; Lampelo *et al.*, 1985). However, recent data suggest that much more than the expression of apical membrane-associated molecules is changed in the uterine epithelium at acquisition of receptivity, i.e. changes are seen in apical, lateral and basal features of these cells. These observations have led to the concept that receptivity represents a change and/or a loss in the expression of the general epithelial phenotype of the uterine epithelial cells (Denker, 1986, 1990, 1994; Glasser and Mulholland, 1993).

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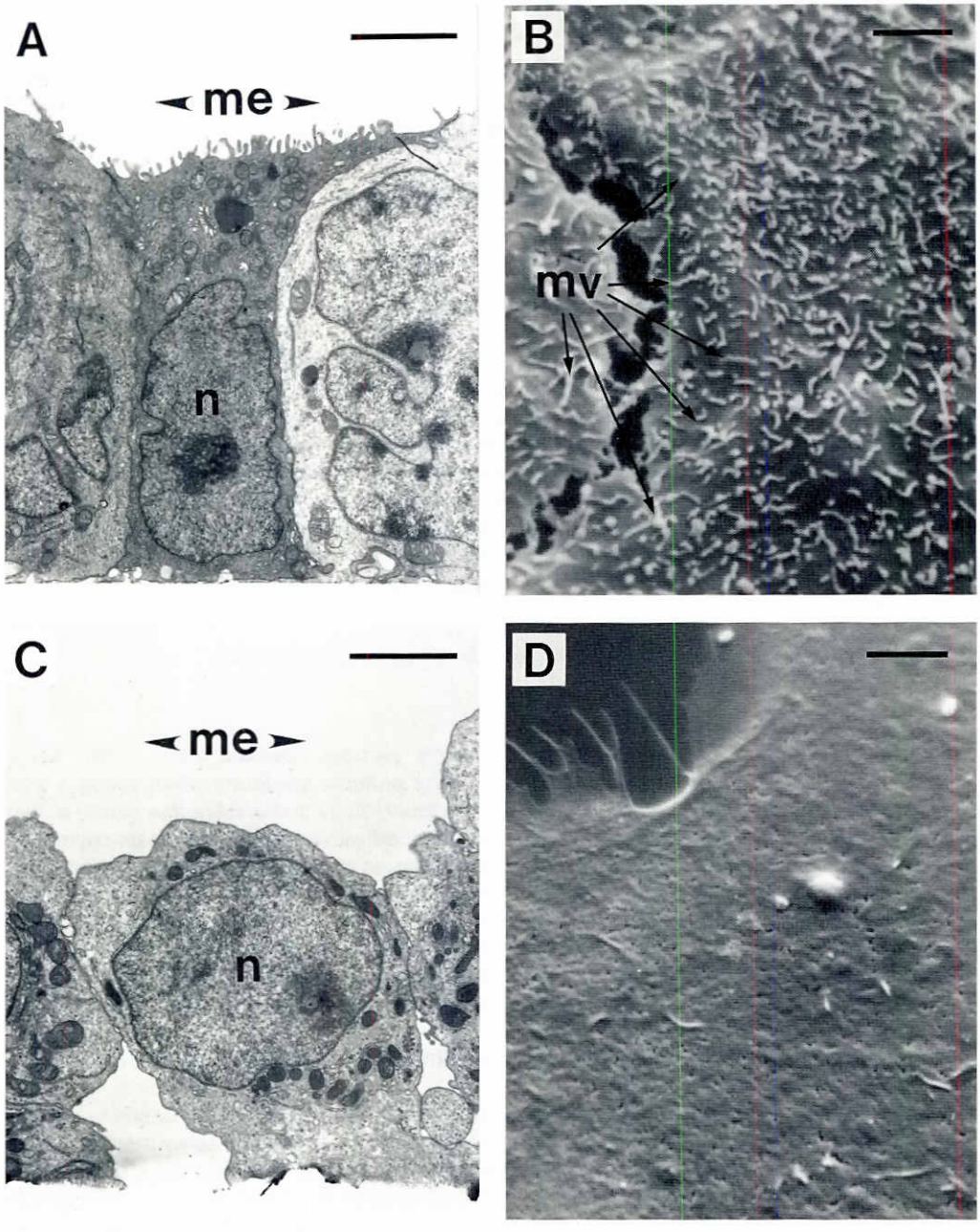

Fig. 1. Transmission (A,C) and scanning electron micrographs (B,D) of HEC monolayers (A,B) and RL monolayers (C,D) cultivated on poly-D-lysine coated glass. *HEC cells exhibit a highly polarized phenotype* **(A)** *with numerous microvilli at the apical cell pole* **(B)**. *RL cells lack structural polarization* **(C)** *and display no microvilli at the upper cell surface* **(D)**. *me: growth medium; mv: microvilli; n: nucleus. Bars, 2.5 µm.*

During development cells are able to switch from an epithelial to a mesenchymal phenotype and vice versa. This process profoundly influences cell behaviour and is thought to be governed by master genes which still have to be identified (Hay, 1990). However, it must be pointed out that application of this concept to uterine receptivity is still very hypothetical. Loss of polar organization along the apico-basal axis appears to be a common theme for all those systems. Changes in molecular parameters appear to be less consistent as far as data are available. As discussed previously (Denker, 1993, 1994) the changes seen in uterine epithelium at receptivity do not seem to comprise the complete set of parameters typical for E-M transformation. For example, loss of α6- and β4-integrin subunits and acquisition of α5- and β1-integrin subunits is found in E-M transformation but is not seen in the uterine epithelium (Thie *et al.*, 1994). However, the latter does show changes in expression of other integrin subunits (appearance of α1, αv, and β3; Lessey *et al.*, 1992) and changes in the polar distribution (α6; Thie *et al.*, 1994) that may be indicative of such switches in parts of the program. Up-regulation of vimentin is found in E-M transformation and receptive uterine epithelium (for reference see: Denker, 1993). E-cadherin was reported to be down-regulated in E-M transformation as well as in invasive tumor cells (Behrens, 1994; Birchmeier *et al.*, 1991; Gumbiner *et al.*, 1988). Such down-regulation, however, is not seen in the uterine epithelium at receptivity (Denker, 1993, 1994). Data on other relevant parameters (laminin vs. fibronectin; type IV collagen vs. type I collagen) are still very incomplete for uterine epithelium or as in case of syndecan and

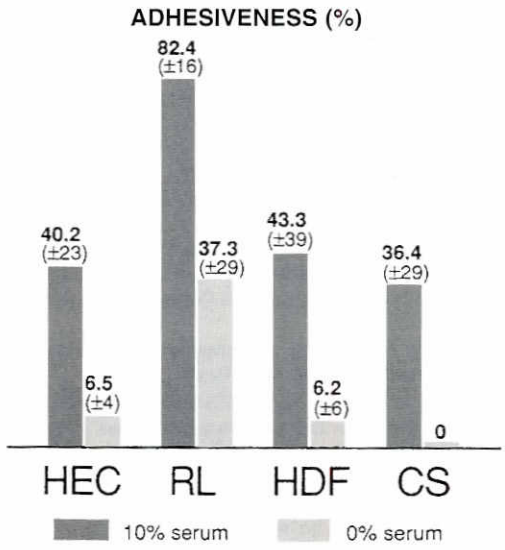

Fig. 2. Adhesiveness of HEC monolayers (HEC), RL monolayers (RL), human dermal fibroblast monolayers (HDF), and poly-D-lysine coated glass coverslips (CS) for human JAR choriocarcinoma spheroids as determined in the centrifugal force-based spheroid adhesion assay. *Adhesiveness in the presence and absence of fetal calf serum is expressed as the percentage of the number of spheroids seeded. The values are mean±SEM.*

perlecan, partially contradictory (Carson *et al.*, 1993; Potter and Morris, 1992).

Uterine epithelial cell polarity – *in vitro* studies

Using *in vitro* studies our laboratory characterized parameters of the epithelial phenotype of certain human endometrial cell lines and correlated these with adhesive or non-adhesive behavior for trophoblast-type cells in an attempt to get insight into the program underlying uterine epithelial cell adhesiveness.

Cell lines established from adenocarcinoma of human endometrium, i.e. HEC cells (Kuramoto *et al.*, 1972) and RL cells (Way *et al.*, 1983), were grown on poly-D-lysine coated glass in medium supplemented with fetal calf serum to confluence (for additional details see: Thie *et al.*, 1995). In HEC monolayers the single cells showed a polarized epithelial phenotype with respect to the distribution of organelles and to membrane organization (Fig. 1A). Nuclei were situated at the base of the cells whereas mitochondria, endoplasmic reticulum and Golgi apparatus were located predominantly at the supranuclear region. The cells showed laterally closely apposed plasma membranes with tight junctions in the subapical region and adherens junctions and desmosomes scattered along the lateral membranes. The apical surface was covered with numerous microvilli which were relatively short (Fig. 1B). In contrast, RL cells showed ultrastructural features indicating lack of epithelial polarization (Fig. 1C). Nuclei were located in the center of the cell and organelles tended to pile up perinuclearly. Cells formed primitive adherens junctions but no tight junctions. The free surface of the cells appeared dome-like and was free of microvilli (Fig. 1D). Although RL cells exhibited a lack of structural polarization these cells expressed proteins associated with the epithelial phenotype as did HEC cells. Immunohistochemically, RL cells were clearly positive for E-cadherin, α6-, β1-, and β4-integrin subunits and cytokeratin but negative for vimentin (data not shown). Thus, we have selected human endometrial cell lines which can be characterized as polarized (= HEC) and non-polarized (= RL) epithelial cells.

The adhesiveness of HEC monolayers and RL monolayers for human trophoblast-type cells (= JAR choriocarcinoma cells) was tested using confrontation cultures. Multicellular spheroids were formed of JAR cells and delivered onto confluent monolayers of HEC cells and RL cells. Subsequently, spheroid adhesion to the monolayers was quantified using a centrifugal force-assisted adhesion assay (John *et al.*, 1993). Attached spheroids were counted, and the results expressed as the percentage of the number of spheroids added initially. JAR cells attached with low efficiency to HEC monolayers but with high efficiency to RL monolayers either in the presence or the absence of serum (Fig.

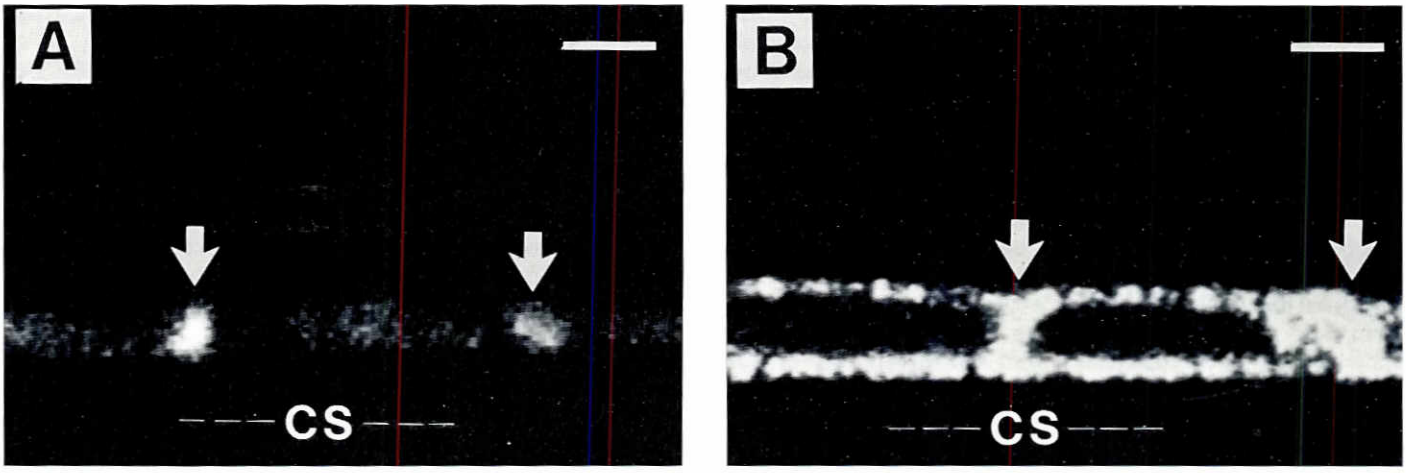

Fig. 3. Confocal images of HEC monolayers (A) and RL monolayers (B) after staining with monoclonal antibody to α6-integrin subunit. *Vertical sections reveal that HEC cells are labelled at sites of cell-to-cell contact* **(A)**, *while RL cells are labelled at the entire plasma membrane* **(B)**. *Similar distribution patterns were obtained after staining with other antibodies mentioned in the text. Arrows mark the position of cell-to-cell contacts. cs: glass coverslip. Bars, 5 μm.*

2). JAR cell attachment to HEC cells was comparable to attachment in the controls, i.e. human dermal fibroblast monolayers and poly-D-lysine coated glass. Therefore, HEC monolayers were classified non-adhesive for JAR cells whereas RL monolayers were classified adhesive for JAR cells. Thus, structural features of non-polarized epithelial cells were correlated with functional features of adhesiveness (= RL). On the other hand, structural features of polarized epithelial cells were correlated with non-adhesiveness (= HEC).

Using confocal laser scanning microscopy the domain-specific localization of cell-cell and cell-matrix adhesion molecules was monitored. We analyzed the localization of E-cadherin and α6-, β1-, and β4-integrin subunits which are also expressed in human uterine epithelium *in vivo* (data not shown). Cell monolayers grown on glass were fixed and permeabilized by incubation in 96% methanol-water. Thereafter cells were incubated with monoclonal antibodies to E-cadherin and integrins, respectively. Fluorescein isothiocyanate conjugated secondary antibodies were used to detect reaction products. In HEC monolayers staining was confined to the sites of cell-to-cell contacts and was absent from the apical cell pole (Fig. 3 A). In RL monolayers, in contrast, staining was evenly distributed over the whole cell membrane (Fig. 3 B). Control monolayers (= human dermal fibroblasts) showed no staining with these antibodies (data not shown). In summary, the confinement of E-cadherin and integrin subunits to the basolateral membrane domains in HEC cells was consistent with their polar epithelial phenotype whereas the random distribution of these proteins in RL cells corresponded with their non-polarized morphology.

Loss of epithelial cell polarity as prerequisite of adhesiveness

These data from *in vitro* experiments give support to the hypothesis that modulation of the epithelial phenotype of uterine cells, i.e. loss of apical-basal polarity, prepares the apical cell pole for cell-to-cell contact with trophoblast *in vivo*. The data extend previous findings on the importance of changes of polar organization of uterine epithelial cells for the switch from non-receptivity to receptivity *in vivo* as indicated by changes in the apical, lateral and basal plasma membrane domains and in the organization of the cytoskeleton (Denker, 1990; Glasser and Mulholland, 1993; Murphy, 1993). Moreover, our data put those findings into a new context. The possibility of modulation of the epithelial phenotype, i.e. the loss of apical-basal polarity by random distribution of cell adhesion molecules like E-cadherin and α6-, β1-, and β4-integrin subunits, suggests that the described endometrial cell lines may be a valuable tool for continuing studies on the role of epithelial cell polarity in embryo implantation in mammals. It will be interesting to see to what extent this approach may give additional insight into the concept of E-M transformation in general.

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