Uvomorulin, Actin and the Shift in Uterine Epithelial Polarity at Embryo Implantation

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POSTER

ABSTRACT

Embryo implantation requires the adhesion of two epithelia, the trophoblast and the endometrium, via their apical membranes. This is very remarkable insofar as apical membranes of polarized epithelia are usually not adhesive for other cells. Therefore we investigated immunohistochemically the distribution of uvomorulin (E-cadherin), a cell adhesion molecule, and of actin, a major constituent of the cytoskeleton, in the uterine epithelium of pregnant rabbits in the pre- and periimplantation phase. Both proteins are possibly involved in the establishment and maintainance of epithelial polarity.

In non-pregnant uteri uvomorulin is located only at the lateral membranes with a subapical maximum in the junctional complex while actin is diffusely distributed throughout the cytoplasm with a subapical maximum in the terminal web. This distribution is typical for polarized epithelia.

In early pregnancy uvomorulin looses its subapical maximum and is more evenly distributed along the lateral membranes while actin remains relatively unaltered. On day 8 p.c. when implantation has just started there is a marked change in the distribution of uvomorulin in the uterine epithelium in certain distinct areas of the implantation chamber: Large amounts of the protein are now located at the basal cell surface of the epithelial cells. Immuno-gold electron microscopy of these areas shows numerous cell projections which contact with each other via uvomorulin. In the same region there is a striking enrichment of filamentous actin in the basal cytoplasm of the cells as shown by TRITC labeled phalloidin.

We suggest a trapping mechanism for uvomorulin that leads to local enrichment of this molecule through the process of establishment of cell-cell-contacts between the basal projections. As uvomorulin is indirectly linked to actin, this cytoskeletal protein becomes enriched in the same basal part of the cell. This shift in the distribution of actin could influence the overall polarity of the cells including the composition of the apical membrane compartment as a prerequisite for successful embryo implantation.
Introduction

The implantation of the mammalian embryo begins with the adhesion of two epithelia, the trophoblast and the endometrium, via their apical membranes. This is very remarkable insofar as apical membranes of polarized epithelia are usually not adhesive for other cells. The uterine epithelium behaves non-typically during the so-called "receptive phase" (controlled by steroids) when it permits the trophoblast to attach. It has already been shown that polarity parameters of the uterine epithelial cells are indeed altered when the endometrium enters the "receptive phase". Here we have investigated the distribution of uvomorulin (E-cadherin), a molecule that has been implicated in the establishment and maintenance of epithelial cell polarity. Because uvomorulin is linked to the actin cytoskeleton (possibly via catenins) we have also investigated the distribution of f-actin as an additional indicator for and an effector of the polar organization of an epithelial cell.
Methods

Rabbit uterine tissues were investigated in the non-pregnant/non-receptive state and in different stages of pregnancy/receptivity (i.e. 0-10 days post coitum, d p. c.).

Uvomorulin (E-cadherin) was detectet with the monoconal antibody 6F9 which was raised against the human molecule and cross-reacts with rabbit tissues. F-actin was stained with TRITC-labelled phalloidin.

Indirect immunofluorescence for light microscopy was performed on native or PLP-fixed cryosections.

For electron microscopic immunohistochemistry a pre-embedding procedure was used: 50μm thick frozen sections of PLP-fixed tissue were incubated with the primary and a peroxydase-labeled secondary antibody, the peroxydase was detected with DAB. These sections were then osmicated and routinely embedded in epon/Araldite and sectioned perpendicular to the cryo-section plane.
Results and Discussion

In non-pregnant uteri and in early (pre-implantation, non-receptive) stages the distribution of uvomorulin and actin was identical to its distribution in other polarized, simple epithelia (e.g. gut): Uvomorulin was demonstrated only at the lateral membranes of the epithelial cells with a more or less obvious maximum in the subapical region of the junctional complex. The staining for f-actin was mainly in the apical cytoplasm in the region of the terminal web. In the peri-implantation phase beginning on the 8th postovulatory day there is a marked shift in the distribution of uvomorulin. It now exhibits a stronger staining than ever before at the lateral membranes, and the subapical maximum is not clearly seen anymore. Remarkably, uvomorulin becomes localized to the basal membranes of the epithelial cells. Many of the epithelial cells have fused at that time to multinucleated symplasms where the uvomorulin accumulation at the basal plasma membrane is particularly prominent but it can also be demonstrated in non-fused cells. With a short delay of about 6 hours a redistribution can be recognized also for f-actin. Double-immunofluorescence shows impressively an increasing staining intensity exactly in the areas of the basally located uvomorulin while most of the apical areas of the cells retain their high actin density. Immuno-electronmicroscopy shows numerous microvilli-like projections of the basal cell membrane which partially penetrate the basal lamina. These projections form between reciprocal contacts, some of which are highly enriched for uvomorulin.

We suggest that this enrichment results from a positive feedback that occurs when these projections come into contact accidentally, a bond is formed between two basolaterally free diffusible uvomorulin molecules and therefore the molecules become trapped. The apparent delay in the change of the actin distribution suggests that the uvomorulin-actin-connection (via catenins) is only formed when the cell-cell-contact is already established. The altered actin distribution will in turn increase/stabilize the change in polarity of the whole cell. The implications of this phenomenon for the cell biology of embryo implantation is currently under investigation.
Abb. 1
Rabbit endometrial epithelium, non-pregnant. Uvomorulin is distributed along lateral membranes with a subapical maximum in the region of the junctional complex. (L: lumen)
Abb. 2
Rabbit endometrium, 9 d p. c., placental folds. Strong expression of uvomorulin along the basal cell membranes of the epithelial cells. (L: lumen)
Abb. 3
Epithelial cell, basal region, 8 2/3 d p. c.
Many irregular projections, some of which penetrate the basal lamina, show uvomorulin-positivity at their contact areas.
Abb. 4
Epithelial cell, basal region, 8 2/3 d p. c.
Multiple finger-like projections, invaginations, and intracellular vesicles exhibit uvomorulin-positivity.
Abb. 5
Endometrium, placental fold, 8 2/3 d p. c.
Double-immunofluorescence staining for uvomorulin and actin. There is colocalization at the lateral and basal membranes; additionally, actin is detected along the apical membrane. (S: stroma; L: lumen)
Abb. 6
Endometrium, paraplacental fold, 8 2/3 d p. c.
Double-immunofluorescence staining for uvomorulin and actin. A large symplasm shows positivity for actin and uvomorulin at the basal membrane. Strong actin staining is also demonstrated in the apical area of the symplasm. (S: stroma; L: lumen)
uvomorulin

actin

orthodox

paradox