Endometrial Receptivity: Cell Biological Aspects

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The cell biological basis of endometrial receptivity and trophoblast invasiveness, the two preconditions for implantation ("implantation window"), still remains enigmatic. Recently, however, the application of modern concepts of cell biology and developmental biology concentrating on cell adhesion and cell polarity seems to open promising new views of these phenomena. At implantation initiation, the trophoblast has to attach via its apical plasma membrane to the apical plasma membrane of the uterine epithelium. This presents a cell biological paradox since apical plasma membranes of epithelia are normally non-adhesive. 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 recently 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. While the trophoblast aspect has been discussed in the light of recent literature by Denker (10), we will concentrate here on the uterine epithelium.

The uterine epithelium appears to play a central role in mediating endometrial receptivity or non-receptivity. It seems to be a unique property of this epithelium in contrast to other epithelia to be able to enter a state of receptivity under steroid hormone control. The uniqueness of the changes in behaviour of the uterine epithelium as seen at receptivity is demonstrated by the fact that other epithelia (with the exception of mesothelia and endothelia) do not seem to allow trophoblastic cells to attach: these obviously include the tubal epithelium which the trophoblast cannot penetrate in any hormonal state, at least in animals (25; 28).

In a number of investigations it has been tried to define molecular changes in the composition of the apical plasma membranes of the uterine epithelium at receptivity. Very consistently, a reduction in the thickness of the glycocalyx of uterine epithelial cells and in cell surface charge has been observed in various species (1; 3; 11; 22; 23; 24; 26).
On the other hand, the biosynthesis and expression of new cell surface proteins has also been observed (2; 15; 19). However, a large number of 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 the apical, lateral and basal aspects 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 (9; 10; 12).

Recently endometrial expression of the prominent gene family of cell surface molecules, the integrins, which are known to mediate cell-matrix and cell-cell-interactions, is attracting interest (6; 20). Using an in vitro assay, we have identified specific human endometrial cell lines that allow (RL95-2) or do not allow (AN3 CA) attachment of trophoblastic cells (16; 17). We are now reporting some first results of studies on the expression and localization of alpha 6, beta 1 and beta 4 integrin subunits on RL95-2 cells and AN3 CA cells cultured in lysine coated culture dishes (FIG. 1.). Using immunohistochemistry we could demonstrate that all three integrins are present on both cell lines. The patterns of immunostaining, however, showed significant differences. RL95-2 cells which allow attachment of trophoblast showed strong staining of lateral, basal and apical surface membrane regions. Staining was localized both in contact and non-contact regions of RL95-2 cells. In contrast, AN3 CA cells which do not allow attachment of trophoblast showed no staining of apical and basal surface membranes and only weak staining of lateral membranes (in particular for the alpha 6 subunit). Further experiments will have to show whether alpha 6, beta 1 and beta 4 integrin subunits are directly involved in the attachment of trophoblastic cells.

Interestingly, values of integrin expression changed in these cell lines depending on the growth conditions of the cells obtained by adherent and non-adherent culturing. As shown above adherent cultures of RL95-2 cells exhibited stronger expression of integrins than AN3 CA cells. In suspension culture, however, AN3 CA cells showed stronger integrin staining than RL95-2 cells. The data suggest regulation of these integrins by cell-cell and/or cell-matrix contact.

In addition to the in vitro studies we also investigated the expression of these integrins in uterine epithelial cells of human endometrium in vivo from the early proliferative phase through the late secretory phase of menstrual cycle (FIG. 2.). During the proliferative phase alpha 6 and beta 4 integrin subunits were expressed at the basal membranes of uterine epithelial cells, beta 1 subunits at the basal plus lateral membranes. In tissue of the secretory phase beta 4 subunits were expressed at the basal membranes of uterine epithelial cells, alpha 6
and beta 1 subunits at the basal plus lateral membranes, i.e. alpha 6 expression newly appeared in the lateral membrane domain all the way up to the subapical region at this time point. These studies indicate that changes of cell phenotype throughout the menstrual cycle might be associated with changes in the distribution of certain integrins. Taken together, our data are consistent with the concept that changes in polar organization might be involved in the receptivity of the uterine epithelium. Furthermore, the alpha 6 integrin subunit expression in the various membrane domains of these cells may not only be of diagnostic value but also a functionally important determinant of receptive or non-receptive properties.

The presented data may be of interest in the context of differentiation physiology/embryology that recently attracts much attention: the epithelial-mesenchymal (E-M) transformation. During development cells are able to switch (even several times) from an epithelial to a mesenchymal phenotype and back, a process that profoundly influences cell behaviour (e.g. invasiveness) and that is thought to be governed by master genes which still have to be identified (14). 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 (10) 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 alpha 6 and beta 4 integrin subunits and acquisition of alpha 5 and beta 1 integrin subunits is found in E-M transformation, but is not seen in the uterine epithelium (this communication). However, the latter does show changes in expression of other integrin subunits (appearance of alpha 1, alpha v and beta 3 ; 20) and changes in the polar distribution (alpha 6 ; this communication) that may be indicative of such switches. Up-regulation of vimentin is found in E-M transformation and receptive uterine epithelium (for references see 10). Uvomorulin was reported to be down-regulated in E-M transformation as well as in invasive tumor cells (5; 7; 13; 21). Such down-regulation of uvomorulin is, however, not seen in the uterine epithelium at receptivity (10). Data on other relevant parameters (laminin vs. fibronectin, type IV vs. type I collagen) are still very incomplete for uterine epithelium or as in case of syndecan and perlecan, partially contradictory (8; 27).

Therefore many questions remain open when comparing endometrial receptivity with E-M transformation. This is in particular true with respect to the question what master gene-regulated switches in the general genetic programs transcribed may be involved. However, there are reports on steroid-dependent changes in activity of regulatory
genes coding for transcription factors in endometrium (4; 18; 29). Clearly data on genes that control major switches in differentiation pathways are urgently needed for the uterine epithelium at receptivity.

FIG. 1.
Immunolocalization of alpha 6 (A, B), beta 1 (C, D) and beta 4 (E, F) integrin subunits in monolayer cultures of human endometrial cells, i.e. RL95-2 (A, C, E) and AN3 CA (B, D, F). Cells were cultured on lysin coated coverslips for 24 h. Monolayer cultures were fixed in -20°C ethanol, incubated with monoclonal antibodies to integrin subunits, labeled with appropriate FITC-conjugated second antibodies and viewed by laser scanning confocal microscopy on vertical sections. Note that integrin expression is strong and predominantly membrane-associated in RL95-2 but low and cytoplasmatic in AN3 CA. The apical cell pole is labelled strongly in the former but does not stand out in the latter (only lateral membranes with alpha 6 in AN3 CA). Bar = 10 μm
FIG. 2.
Immunolocalization of alpha 6 (A,B), beta 1 (C,D) and beta 4 (E,F) integrin subunits in human proliferative endometrium (day 7 of menstrual cycle; A,C,E) vs secretory endometrium (day 20 of menstrual cycle; B,D,F). Cryostat sections were fixed in -20°C ethanol, incubated with monoclonal antibodies to integrin subunits, labeled with appropriate FITC-conjugated second antibodies and viewed by conventional fluorescence microscopy. Apical side of epithelium is marked by arrow. Note the change of alpha 6 expression on the lateral membrane in proliferative vs secretory endometrium (A,B). Bar = 20 μm
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References


