Identification of epithelial sodium channel (ENaC) in endometrial pipelle biopsy samples

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Introduction and Objectives

The epithelial sodium channel (ENaC) family is important in electrolyte homeostasis and blood pressure regulation. (1,2) Type I pseudohypoaldosteronism (PHA1B) is caused by mutations in genes coding for subunits of ENaC, which play a role in the regulation of electrolyte concentration and blood pressure. (3)

Methods

The samples were obtained from four normal control subjects and a PHA1B patient. The samples were fixed in formalin and then reacted with anti-ENaC antiserum. After reaction with secondary antibodies and DAPI (as identified nuclei), sample immunofluorescence was visualized by confocal microscopy. The study was approved by the ethics committee at the E. Wolfson Medical Center.

Results

The samples showed strong ENaC immunofluorescence along the luminal border of the epithelial cells in pipelle samples from four healthy subjects (Fig. 3). In contrast, none of the samples from the PHA1B patient showed ENaC immunofluorescence. In contrast to endometrium where ENaC is localized in the apical membrane of the epithelial cells (Fig. 4), in keratinocytes ENaC is expressed in cytoplasmic pools. Thus, we examined ENaC immunofluorescence in the apical side of the epithelial cells.

Discussion

In contrast to samples from healthy women, the endometrial epithelial cells showed a uniform distribution along the apical walls of the gland, which is consistent with previous studies.(2,3) Our findings suggest that the expression of ENaC, on the endometrial luminal surface is drastically reduced and hardly detectable (Fig. 4), which could explain the lack of ENaC immunofluorescence in the PHA1B patient. ENaC immunofluorescence was abundant in the keratinocytes except for the outermost single layer of cells (Fig. 5). These results are consistent with our hypothesis that the Angiotensin II receptor antagonists increase ENaC expression and function.

Conclusions

1. The renin-angiotensin-aldosterone axis plays a major role during pregnancy.
2. The major cause of the failure of embryo implantation in the PHA1B patient is severely reduced expression of ENaC in the reproductive tract.
3. Pipelle biopsy can be used for the identification of key proteins by immunofluorescence.

Fig. 1. A schematic view of epithelial sodium channel (ENaC) function in tight epithelia.

Fig. 2. Apical localization of α-ENaC in human endometrial glands. 3D image showing uniform distribution of ENaC along the apical walls of the gland.

Fig. 3. Confocal microscopic imaging of immunofluorescence of endometrial Pipelle samples from a healthy subject.

Fig. 4. Confocal microscopic imaging of immunofluorescence of endometrial Pipelle samples from a patient with PHA1B.

Fig. 5. Confocal microscopic imaging of ENaC immunofluorescence in a cross-section of a hair-follicle from a healthy subject and the PHA1B patient.

References

4. Enuka Y, Hanukoglu I, Edelheit O, Vaknine H, Hanukoglu A. Epithelial sodium channels (ENaC) are uniformly distributed on the ciliary surface of all epithelial cells with motile cilia including the fallopian tubes. J Cell Biol. 1998;141:1313-25. https://doi.org/10.1083/jcb.141.5.1313