

The Relationship between Transmembrane Mucins, Ion Channels and PDZ Adaptor Proteins in the Small Intestine

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien vid Göteborgs Universitet kommer att offentlig försvaras i hörsal Arvid Carlsson, Academicum, Medicinargatan 3, Göteborg, Fredagen den 16 november 2012, kl 09.00

Av

Thaher Pelaseyed

Fakultetsopponent: Martina Gentsch, PhD, Assistant Professor
Cell and Developmental Biology, UNC at Chapel Hill

Avhandlingen baseras på följande delarbeten:

- I. Malmberg, E. K., Pelaseyed, T., Petersson, A. C., Seidler, U. E., De Jonge, H., Riordan, J. R. and Hansson, G. C. (2008) **The C-terminus of the transmembrane mucin MUC17 binds to the scaffold protein PDZK1 that stably localizes it to the enterocyte apical membrane in the small intestine.** *Biochem. J.* 410, 283-289
- II. Pelaseyed, T. and Hansson, G. C. (2011) **CFTR anion channel modulates expression of human transmembrane mucin MUC3 through the PDZ protein GOPC.** *J. Cell. Sci.* 124, 3074-3083
- III. Pelaseyed, T., Zäch, M., Petersson, Å. C., Svensson, F., Johansson, D.G.A., Hansson, G. C. **Unfolding dynamics of the mucin SEA domain probed by force spectroscopy suggest that it acts as a cell protective device**
Submitted.
- IV. Pelaseyed, T., Gustafsson, J. K., Gustafsson, I. J., Hansson, G. C. **Carbachol-induced internalization of human transmembrane MUC17 mucin is concomitant with NHE3 internalization and CFTR externalization in enterocytes**
Manuscript.



UNIVERSITY OF GOTHENBURG

ABSTRACT

Thafer Pelaseyed

Department of Medical Biochemistry, Institute of Biomedicine,
Sahlgrenska Academy at University of Gothenburg

The human body is continuously exposed to challenges from the surrounding world. In analogy with the skin, mucus is a well-organized and highly regulated barrier composed of polymeric and *O*-glycosylated mucins that protects luminal organs such as the gastrointestinal tracts from the outer milieu. As part of a first defensive barrier, the secreted mucins entrap pathogens and act as a network for antiseptic enzymes and proteins. A second barrier is the dense glycocalyx which is composed of transmembrane mucins anchored to the apical membrane of cells.

The focus of this thesis was to identify novel interactions between transmembrane mucins and cytoplasmic PDZ adaptor proteins and to determine the role of these interactions in mucin expression and regulation. Furthermore, the interplay between transmembrane mucins and ion channels expressed in the small intestine was explored. Finally, the stability of the SEA domain in transmembrane mucins was assessed.

Using different techniques in molecular biology and confocal imaging, this thesis proves that the transmembrane mucins MUC3 and MUC17 bind to PDZ adaptor proteins that are interaction partners for two intestinal ion channels, namely CFTR and NHE3. Specifically, MUC17 is retained in the apical surface of enterocytes by PDZK1. In analogy with acute Ca^{2+} -mediated regulation of NHE3 and CFTR, the cholinergic agonist carbachol induces endocytosis of MUC17, concomitant with NHE3 internalization and CFTR recruitment to the cell surface. This thesis also demonstrates that the expression of MUC3 is counter-regulated by CFTR via a *trans*-Golgi-resident PDZ adaptor protein called GOPC. Finally, using atomic force microscopy, it is demonstrated that the SEA domain of transmembrane mucins protects the apical cell membrane by acting as a breaking point upon mechanical stress.

In summary, the results from this thesis deliver new evidence regarding the relationship between transmembrane mucins and ion channels. These novel networks cast light on important cellular processes, involving the formation of physical barriers coupled to fluid and mucin secretion, that occur in response to native and foreign provocations.

Keywords: intestine, mucin, MUC3, MUC17, SEA, PDZK1, GOPC, CFTR, NHE3