Golgi reorganization and decreased endocytosis provide basis for a functional differentiation of urothelial cells

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Introduction: A tight blood-urine barrier is critical to bladder function and metabolic homeostasis; therefore the interdependency of structure and function of the urothelium that lines urinary bladder is of fundamental importance. In the urothelium, the urothelial cells differentiate, temporally and spatially, from the basal cell layer to the superficial cell layer. This process is exemplified in the expression of tight junctional proteins [1] and in the expression of integral membrane proteins uroplakins [2]. The latter are the most significant molecules in the apical plasma membrane of differentiated superficial urothelial cells (SUCs). The detailed mechanisms and regulation of the apical plasma membrane biogenesis in the urothelial cells are still not completely understood. It is hypothesized that uroplakins organize into the asymmetric unit membranes (AUMs) in specific post-Golgi vesicles, i.e., fusiform vesicles in the first place and finally in the apical plasma membrane. Since the GA has been proposed to play a central role in the assembly of uroplakins, we hypothesized that the subcellular organization and localization of the GA in the urothelial cells is highly regulated. Our aim was to examine the organization and subcellular localization of GA during differentiation of the urothelial cells *in vitro* and *in vivo*. Given the role of the microtubules in maintaining the organization and dynamics of the GA, we speculated that microtubular changes might be implicated in GA positioning and differentiation state of the urothelial cells. Finally, our recent study has shown that downregulation of apical endocytosis correlates with differentiation of SUCs [3]. Our aim was therefore to analyse the possible role of uroplakins in regulating endocytotic events.

Materials and Methods: Normal mice and pig urothelial cell cultures, and normal mice bladders or bladders of cyclophosphamide (CP) treated mice were used. For immunolabelling the following antibodies were used: rabbit polyclonals against uroplakins (a kind gift of Dr. T.-T. Sun) and against TGN38 (Abcam), mouse monoclonals against α-tubulin (Sigma), GM130 (BD Transduction Laboratories) and giantin (Alexis Biochemicals) and adequate secondary antibodies (Molecular Probes and Amersham). For morphological studies, urothelial cells were chemically fixed and embedded in Lowicryl HM20 or rapidly frozen, freeze-substituted, and embedded in Epon. 3D tomography was performed on 300 nm thick sections with FEI Tecnai 20 microscope. We have stably expressed the transGolgi/TGN enzyme β1,4-galactosyltransferase (GalT) fused to yellow fluorescent protein (YFT) in the pig urothelial cells and made the fluorescence recovery after photobleaching (FRAP) experiments. Apical endocytosis was followed in live urothelial cells, which were simultaneously incubated with endocytotic markers and antibodies against uroplakins.

Results: By immunofluorescence analyzing of Golgi-related markers (giantin, GM130, TGN38) and urothelial differentiation-related markers (uroplakins), we observed that in non-differentiated, uroplakin-negative urothelial cells, the GA is simple (in ribbon structure), while in differentiated, uroplakin-positive urothelial cells, the GA become complex (enlarged, fragmented) and spread almost throughout the entire cell (Figure 1). The

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FRAP experiments showed that Golgi-resident enzyme cycles between Golgi elements, when GA is simple. When the GA is in complex form, the FRAP is diminished. 3D ultrastructural analysis showed some interconnections between GA elements in complex GA. The expression of α-tubulin was significantly lower in differentiated urothelial cells than in undifferentiated cells. In highly differentiated SUCs, the uroplakin-positive membrane regions were excluded from internalization.

Discussion: These findings provide a new view of Golgi reorganization associated with the process of urothelial cell differentiation. The microtubular rearrangements during urothelial cells differentiation coincided with the GA reorganization. Furthermore, the uroplakins are supposed to hinder endocytosis from the apical plasma membrane and thus maintain optimal permeability barrier function. In conclusion, functional differentiation of urothelial cells is reflected in the specific Golgi reorganization, uroplakin assembly in the apical plasma membrane and greatly diminished apical endocytosis.

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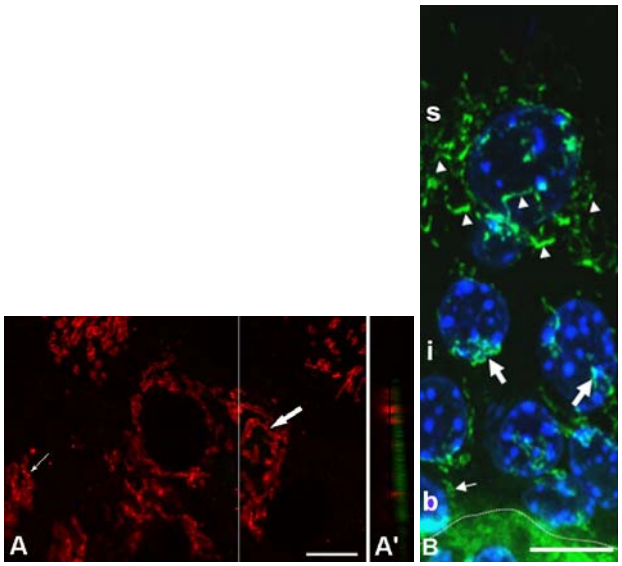

Figure 1. Golgi organization and localization in differently differentiated urothelial cells *in vitro* (**A**) and *in vivo* (**B**). Apical surface (A) and (A') cross section of pig urothelial cell culture. Double immunofluorescence of giantin (red) and uroplakins (green) in normal pig urothelial cell culture. Anti-giantin labelling shows that in undifferentiated, uroplakin-negative urothelial cell the Golgi is simple (small arrow), while in differentiated, uroplakin-positive urothelial cell, the Golgi is in complex form (large arrow). Grey line in (A) indicates the intersection of two perpendicular planes. (B) GM130 distribution (green) in layers of normal mice urothelium. In basal cells (b), GM130 is distributed as dots and small patches (small arrow) close to the nucleus (blue). In intermediate cells (i), GM130 is distributed as in basal cells or form larger patches close to the nucleus (large arrows). In superficial urothelial cells (s), GM130 is spread throughout the cell (arrowheads). Bars, 10 μm.

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