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The scaffold protein PDZK1 modulates expression and function of the organic anion transporting polypeptide 2B1

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ABSTRACT

The protein family of Organic Anion Transporting Polypeptides (OATPs) summarizes various transporters known to facilitate cellular uptake of xenobiotics. One member of this family is OATP2B1. This transporter is ubiquitously expressed and possesses a PDZ-binding motif at the C-terminus. PDZK1 (PDZ domain-containing 1) is a scaffold protein that influences function of different membrane proteins by sorting/stabilization of their membrane localization. It was aim of the herein reported study to investigate whether there is an interaction between OATP2B1 and PDZK1, and to further characterize its impact on transport function. At first expression of both OATP2B1 and PDZK1 was evaluated in liver, kidney and intestine. Based on the existence of a C-terminal PDZ-class I binding motif in OATP2B1 and the co-expression in all tested tissues an interaction was likely. Testing the influence of PDZK1 on OATP2B1 transport function revealed enhanced transport capacity for estrone 3-sulfate, thereby suggesting a change in OATP2B1 amount in the membrane. This assumption was validated by Western blot analysis. Finally, deletion of the C-terminal PDZ-binding motif in OATP2B1 lowered the impact of PDZK1 on transport function. Taken together, we report an interaction of PDZK1 with OATP2B1, which influences localization and function of the transporter. Changes in PDZK1 expression may therefore be one factor contributing to interindividual differences in OATP2B1 mediated pharmacokinetic processes.

1. Introduction

Transfer through the plasma membrane is crucial for drugs to reach their site of action. It is well known that the process of crossing the membrane is facilitated by uptake and efflux transporters (Giacomini et al., 2010; Mandal et al., 2017). Accordingly, changes in membrane transporter expression and/or activity influence transmembrane transfer of substrate drugs and thereby pharmacokinetics, which finally impacts treatment outcome. One mechanism shown to modulate membrane protein expression and activity is the interaction with PDZ adapter proteins (Sugiura et al., 2011). Particularly, the protein family of Na^+/H^+ exchanger regulatory factors (NHERF), which summarizes various PDZ domain-containing scaffold proteins, has been reported to modify the function of membrane proteins (Walsh et al., 2015). The PDZ domains of NHERF proteins directly interact with specific amino acid sequences at the C-terminus of their target proteins thereby influencing their cellular sorting (Kalyoncu et al., 2010). The interplay with PDZ-containing proteins is assumed to not only stabilize the localization of target proteins (Shenolikar et al., 2002; Sugiura et al., 2011), but also to modulate their function due to the spatial arrangement of functional associated proteins in protein complexes (Kato et al., 2005; Sugiura et al., 2011; Weinman et al., 2007).

One member of the NHERF protein family is PDZK1 (NHERF3). This scaffold protein contains four PDZ domains, and has been shown to interact with the C-terminal residues of a variety of transporters involved in uric acid handling (Anzai et al., 2004; Kocher et al., 1998; Park et al., 2014). Accordingly, PDZK1 is assumed to be a determinant of the function of the so called “urate transportosome” (Anzai et al., 2012), which is a multi-protein complex composed of various uptake and efflux transporters localized in the apical membrane of the renal tubular cells (Anzai et al., 2012; van Aubel et al., 2002).

One transporter that is assumed to be part of this tubular transportosome, and that has been shown to be modulated by the direct interaction with PDZK1, is ABCC4 (MRP4) (Park et al., 2014). This member of the ATP-binding cassette (ABC) family exerts energy-dependent cellular efflux of uric acid, but also of nucleotide based drugs

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(Park et al., 2014; Van Aubel et al., 2005). Furthermore, ABCC4 is not only highly expressed in kidney, but also in other tissues of pharmacokinetic relevance including liver and intestine (Giacomini et al., 2010). Another ABC-transporter exhibiting a C-terminal class 1 PDZ-binding motif that has been shown to interact with the fourth PDZ domain of PDZK1 is ABCC2 (Emi et al., 2011; Kocher et al., 1999). Considering that ABCC2 is a major determinant in renal, hepatic and intestinal handling of products of the phase II biotransformation (Jedlitschky et al., 1997; Suzuki and Sugiyama, 2002) further provides evidence that PDZK1, even if highly expressed in kidney, executes its organizational role also in other tissues with polarized cells. This assumption is further supported by the presence of PDZK1 in liver and intestine (Kocher et al., 1998).

Intestinal handling of drugs is modulated by uptake and efflux transporters. In terms of cellular uptake, it is assumed that members of the solute carrier (SLC) superfamily are of relevance. One subfamily of SLC transporters is the family of Organic Anion Transporting Polypeptides (OATPs) (Kalliokoski and Niemi, 2009; Shitara et al., 2013). The first OATP, shown to be modulated by PDZK1 is OATP1A2 (Zheng et al., 2014). Even if OATP1A2 was reported to be present in intestine, its function is assumed to be of highest relevance in the blood-brain-barrier (Glaeser et al., 2007; Meier et al., 2007; Steckelbroeck et al., 2004). Another OATP, reported to be functional in liver and intestine is the ubiquitously expressed OATP2B1 (Kobayashi et al., 2003; Kullak-Ublick et al., 2001). This transporter facilitates sodium-independent uptake of endogenous sulfated anionic conjugates as well as of drugs such as HMG-CoA reductase inhibitors (Grube et al., 2006b; Kullak-Ublick et al., 2001; Tamai et al., 2001).

Most of the clinical studies investigating the intestinal function of OATP2B1 focused on its luminal localization whereby referring to findings showing OATP2B1 in the apical membrane of enterocytes and Caco-2 cells (Kobayashi et al., 2003; Sai et al., 2006). However, a recent report by Keiser et al. states conflicting data suggesting basolateral enrichment of OATP2B1 in jejunum and Caco-2 cells (Keiser et al., 2017). Nonetheless, among the OATPs present in enterocytes, OATP2B1 is assumed to be dominant in drug handling (Tamai, 2012). Consequently, understanding factors modulating transport activity of OATP2B1 would contribute to an improved comprehension of oral drug absorption. One of these modulating factors could be the interaction with PDZ domain-containing proteins, like PDZK1. Considering that OATP2B1 exhibits a carboxyl-terminal class I PDZ-binding motif it was aim of the herein reported study to investigate whether there is an interplay between OATP2B1 and PDZK1.

2. Material and methods

2.1. Materials

Unless stated otherwise chemicals and media were purchased from Sigma Aldrich, Buchs, Switzerland. Bovine serum albumin, Citric acid, DAPI (4′,6-diamidino-2-phenylindole), EDTA, glycerol, glycin, hematoxylin (hemalum solution acid acc. to Mayer), HEPES, H_2O_2, KCl, KH_2PO_4, 2-mercaptoethanol, NaCl, Na_2HPO_4, sodium citrate, SDS, Tris-HCl, Tween 20, Roti-Mount Fluor Care and Rotiszint®eco Plus were obtained from Carl Roth, Arlesheim, Switzerland.

2.2. Cell culture

Madin-Darby canine kidney (MDCKII, ATCC® CRL-2936™) and cervix carcinoma HeLa (ATCC® CCL-2™) cells originally obtained from ATCC were cultured in Dulbecco's modified Eagle's medium (DMEM, D6429) supplemented with 10% fetal calf serum (FCS, AMIMED, BioConcept Ltd., Allschwil, Switzerland) and 1% GlutaMAX™ (Thermo Fisher Scientific, Reinach, Switzerland) at 37 °C in a humidified atmosphere containing 5% CO_2. MDCKII-OATP2B1 cells (Grube et al., 2006b) were cultured in presence of Hygromycin B (Roche, Basel, Switzerland) at a final concentration of 0.4 mg/ml to maintain continuous selection.

2.3. Human tissue samples

Human kidney tissue samples from patients undergoing surgery of renal carcinoma were obtained after written informed consent and approval by the local ethics committee of the Medical Faculty of the University of Greifswald (III UV 12/03). Isolation of RNA and protein of the kidney samples was previously described (Prestin et al., 2017b). The paraffin embedded tissue blocks from kidney, intestine and liver were purchased from AMS Biotechnology (Bioggio-Lugano, Switzerland). Tissue sections (7 µm) were prepared using the microtome HM 340E (Thermo Fisher Scientific, Zug, Switzerland) and mounted on glass slides, dried over night at 37 °C, and stored at 4 °C until further use.

2.4. Quantitative polymerase chain reaction (qPCR)

The total RNA of kidney, small intestine, and liver was purchased from AMS Biotechnology and 1000 ng of each RNA was reverse transcribed with the High-Capacity cDNA Reverse Transcription Kit (LuBioScience). The resulting cDNA (10 ng/µL) was applied to real-time PCR using the commercially available TaqMan® Gene Expression assays (Thermo Fisher Scientific, Reinach, Switzerland) for detection of OATP2B1 (*SLCO2B1,* Hs01030343_m1), PDZK1 (*NHERF3*, Hs00275727_m1) and 18SrRNA (H s4319413E), and the ViiA™ 7 Real-Time PCR System (Thermo Fisher Scientific). The relative gene expression was calculated with the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Here, ΔCt was the difference of the Ct-values (threshold of cycle) of the target gene and the gene used for normalization (18S ribosomal RNA). The ΔCt value of each sample was related to the mean ΔCt-value of the indicated control, resulting in the ΔΔCt-value, which was then potentiated to the base of two ($2^{-\Delta\Delta Ct}$). No template controls were included in all analyses.

2.5. Immunohistochemistry

Staining started with the removal of paraffin in two changes of xylene followed by rehydration in a declining ethanol series. Heat induced epitope retrieval was performed autoclaving the tissue for 20 min in citrate buffer (pH 6.0) or in Tris-EDTA buffer (10 mM Tris base, 1 mM EDTA; pH 9.0). After heating, the samples were transferred to ice-cold distilled water, washed with phosphate-buffered saline (PBS), and the activity of endogeneous peroxidase was quenched by incubation in 3% H_2O_2 in methanol for 20 min. After blocking in 5% FCS - PBS the slides were incubated with the primary antibody at 4C° overnight. The anti-PDZK1 (ab92491; abcam), and the anti-OATP2B1 antibody (Grube et al., 2006a) were used at a dilution of 1:50 in blocking solution. Before and after the incubation with the secondary antibody (diluted 1:200) for 2 h at room temperature, the tissue sections were washed several times with PBS. The slides were then pre-incubated for 10 min with 50 mM Tris-buffer (TB, pH 7.6) at 37 °C before being treated with freshly prepared DAB solution containing 1 mg/ml 3,3′-diaminobenzidine tetrahydrochloride (Thermo Fisher Scientific) in TB supplemented with 0.02% H_2O_2. The nuclei were stained with Mayer's hematoxylin and mounted using Aquatex (Merck & Cie, Schaffhausen, Switzerland). Staining was imaged under bright-field illumination using the Leica DMi8 microscope (Leica, Heerbrugg, Switzerland) equipped with the MC10 HD camera (Leica). Images were acquired and processed with the LAS (Leica Application Suite) software version 4.8 (Leica).

2.6. Immunofluorescence microscopy

The paraffin embedded tissue sections were deparaffinized and rehydratized as described above. After heat induced epitope retrieval and