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Expression of xenobiotic transporters in the human renal proximal tubule cell line RPTEC/TERT1

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ABSTRACT

The kidney is a major target for drug-induced injury, primarily due the fact that it transports a wide variety of chemical entities into and out of the tubular lumen. Here, we investigated the expression of the main xenobiotic transporters in the human renal proximal tubule cell line RPTEC/TERT1 at an mRNA and/or protein level. RPTEC/TERT1 cells expressed OCT2, OCT3, OCTN2, MATE1, MATE2, OAT1, OAT3 and OAT4. The functionality of the OCTs was demonstrated by directional transport of the fluorescent dye 4-Di-1-ASP. In addition, P-glycoprotein activity in RPTEC/TERT1 cells was verified by fluorescent dye retention in presence of various P-glycoprotein inhibitors. In comparison to proliferating cells, contact inhibited RPTEC/TERT1 cells expressed increased mRNA levels of several ABC transporter family members and were less sensitive to cyclosporine A. We conclude that differentiated RPTEC/TERT1 cells are well suited for utilisation in xenobiotic transport and pharmacokinetic studies.

1. Introduction

The kidney plays a vital role in whole body homeostasis via blood filtration and subsequent reabsorption of essential substances such as glucose, amino acids, water and various ions, while waste products such as urea and ammonia are secreted. A multitude of transporting and metabolising systems have evolved to perform these tasks. As a consequence the kidney handles a wide variety of chemical entities, many of which have the potential to cause cellular injury or stress if present in sufficient concentrations. The proximal tubule is the major site of the nephron for the reabsorption and secretion of endogenous and exogenous substances, which underpins its susceptibility to xenobiotics.

The concerted activities of several classes of transporters, including the SLC and the ABC transporter families, play a key role (El-Sheikh et al., 2008; Koepsell et al., 2007; Lee and Kim, 2004; Perri et al., 2003; Wright and Dantzler, 2004). In the proximal tubule of the human kidney, uptake of organic anions is mainly mediated by basolaterally expressed organic anion transporters (OAT) OAT1 (*SLC22A6*) and OAT3 (*SLC22A8*). On the apical brush border membrane, organic anions are primarily secreted into the lumen by the ABC transporters MRP2 (*ABCC2*), MRP4 (*ABCC4*) and BCRP (*ABCG2*) as well as OAT4 (*SLC22A11*). The major uptake transporters in the proximal tubule at the basolateral membrane side for organic cations are organic cation transporters (OCT) OCT2 (*SLC22A2*) and OCT3 (*SLC22A3*). ABC transporter MDR1 (*ABCB1*, P-glycoprotein), multidrug and toxin extrusion proteins (MATE) MATE1 (*SLC47A1*), MATE2/2-K (*SLC47A2*) and OCTN2 (SLC22A5) meditate the main part of the apical efflux of organic cations. In addition, organic anion polypeptide transporters (OATP, SLCO family), nucleoside (SLC28 family), peptide (SLC15 family) and protein transporters (cubilin and megalin) play a role in drug transport (Daniel and Kottra, 2004; Kuo et al., 2012; Lee and Kim, 2004; Liu et al., 1995; Moestrup et al., 1995; Schmitz et al., 2002). Many of these transporters possess a broad and overlapping substrate specificity, but may also exhibit deviations in their affinity and turnover for distinctive compounds (Lee and Kim, 2004).

Transporters play an important role in absorption and clearance of compounds, which can have serious implications for both efficacy and toxicity. In fact the inclusion of biokinetics was a major aim in the Predict-IV project, where cisplatin (Wilmes et al.,

Abbreviations: 4-Di-1-ASP, 4-(4-(Dimethylamino)styryl)-N-Methylpyridinium Iodide; CsA, Cyclosporine A; primary hPT, human primary proximal tubule cells; MATE, multidrug and toxin extrusion proteins; OAT, organic anion transporter; OATP, organic anion polypeptide transporters; OCT, organic cation transporter; PBPK, physiologically based pharmacokinetic modelling; RFU, raw fluorescence unit; TEER, transepithelial electrical resistance.

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Table 1
Sources of antibodies used and dilutions.

Immunogen	Gene name	Species	Company	Catalogue#	Dilution (μg/ml)
OCT2	SLC22A2	Rabbit	Sigma	HPA008567	2
OCT3	SLC22A3	Rabbit	Abcam	ab124826	4
OCTN2	SLC22A5	Rabbit	Abcam	ab79964	2
OAT1	SLC22A6	Rabbit	Abcam	ab135924	4
OAT3	SLC22A8	Mouse	Abcam	ab69883	4.4
OAT4	SLC22A11	Rabbit	Sigma	AV43994	2
MATE1	SLC47A1	Rabbit	Abcam	ab104016	4
MATE2	SLC47A2	Goat	Sigma	SAB2501835	2

2015), adefovir dipivoxil (Crean et al. 2015) and cyclosporine A (CsA) (Wilmes et al., 2013) were studied in detail in the human renal proximal tubule cell line RPTEC/TERT1. The RPTEC/TERT1 cell line was chosen as they exhibit several characteristics of their *in vivo* counterparts such as well-developed polarisation, responsiveness to parathyroid hormone, pH-dependent ammoniagenesis, γ-glutamyl transferase activity, functional protein uptake and a transepithelial electrical resistance (TEER) similar to primary proximal tubule cells (Wieser et al., 2008). We previously investigated the molecular events during RPTEC/TERT1 monolayer formation. During the maturation process the proliferating cells eventually become contact-inhibited, undergo a cell cycle arrest in G_0/G_1, and exhibit a stabilised trancriptome (Aschauer et al., 2013). Furthermore, we demonstrated that p53 activity is increased in the matured monolayer and the cells exhibited a decrease in glycolysis rates and an increased expression of tissue-typical claudins (*CLDN2, 10*). These are important prerequisites for studying transport processes and exposure to potential harmful substances. However, to date, we have not investigated the expression of xenobiotic relevant organic anion, organic cation or ABC transporters in RPTEC/TERT1 cells. Indeed, this was the aim of this study.

2. Materials and methods

All chemicals were purchased from Sigma–Aldrich unless otherwise stated.

2.1. Cell culture

2.1.1. Routine cell culture

Human primary proximal tubule cells (primary hPT) were prepared as previously described (Jennings et al., 2007). RPTEC/TERT1 cells were obtained from Evercyte GmbH, Vienna (Wieser et al., 2008). Both cell types were cultured in hormonally defined, serum-free medium (HDM) consisting of a 1–1 mixture of Dulbecco's modified Eagle's medium (DMEM, life technologies, cat. No. 11966) and Ham's F-12 nutrient mix (life technologies, cat. No. 21765) supplemented with 2 mM glutamax (life technologies), 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml sodium selenite, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 ng/ml epithelial growth factor and 36 ng/ml hydrocortisone. Cells were cultured routinely in 10 cm dishes (Sarstedt) at 37 °C in a 5% CO_2 humidified atmosphere and subcultured by trypsinisation. Ten millilitres fresh HDM was provided 3 times per week. For 96 wells and 6 wells 100 μl and 2 ml of HDM, respectively, were applied. RPTEC/TERT1 cells were used at passages 76–95. hPT cells were used at passage 3.

2.1.2. Cell culture on microporous filter inserts

Depending on the experiment either 24 or 6 well inserts were used (Millicell® hanging cell culture inserts, PET membrane, 1.0 μm pore size, Millipore, Merck, cat. No. PIRP12R48 and PIRP30R48). Cells were seeded at approx. 5×10^5 cells per ml and allowed to reach confluence. Cells were maintained at confluence for at least 10 days before experiments. HDM volumes applied were 200 μl/500 μl and 1.5 ml/2 ml apical and basolateral, 24 and 6 well respectively.

2.1.3. Undifferentiated vs differentiated

Cells were seeded at approx. 2×10^5 cells/ml and harvested (or treated) on either the day of confluence (undifferentiated) or at least 10 days thereafter (differentiated). These conditions are associated with radically different phenotypes – see Aschauer et al. for more details (Aschauer et al., 2013).

2.2. Quantification of mRNA transcripts

Data from two previous whole genome experiments were utilised. The first employed RPTEC/TERT1 and hPT seeded at low density in 6 well plates and harvested at set intervals over the differentiation/maturation period (Aschauer et al., 2013). The second employed differentiated RPTEC/TERT1 cells cultured on filters (Wilmes et al., 2013). Both studies used the Illumina® Human HT12 v3 BeadChips platform. The relative fluorescent intensities of the probes from three biological replicates were averaged. For the plastic vs filter comparison 6 biological replicates from the last two days of the Aschauer study were compared to 9 vehicle controls from the Wilmes study. Data is presented either as averaged raw fluorescent units (RFU) or relative to the averaged Aquaporin 1 (AQP1) RFU.

2.3. Western blot analysis

Whole cell extracts of undifferentiated and differentiated RPTEC/TERT1 cells cultured on plastic as well as of differentiated RPTEC/TERT1 cells cultured in microporous filter inserts (filters) were harvested by lysis in cold RIPA buffer (Sigma, cat. No. 8340) and 10 μl/ml phosphatase inhibitor (Sigma, cat. No. P0044). Samples were centrifuged at 8000 g for 5 min and protein was determined by BCA (Pierce). Samples (60 μg total protein) in Laemmli buffer were run on 4–12% gradient Bis-Tris Mini-Gels (life technologies) in NuPage MOPS SDS running buffer (life technologies) at 200 V for 50 min. Proteins were transferred onto methanol-activated low fluorescence Immobilon-P membranes (Millipore, Merck) in 2× NuPage transfer buffer (Invitrogen) including 10% methanol using a semi-dry transfer system (life technologies). Membranes were blocked in 5% (w/v) skim-milk/TBST for 1 h and probed with primary antibodies (Table 1) over night at 4 °C. Mouse anti-beta actin (Sigma, A2228) at 0.1 μg/ml was used as an internal loading control. All antibodies were diluted in TBST containing 1–5% BSA (Calbiochem). Blots were incubated with the appropriate secondary antibody conjugated to HRP (goat anti-rabbit IgG 1:5000, life technologies cat. No. 656120; rabbit anti-goat IgG 1:5000, Zymed cat. No. 61-1620; goat anti-mouse IgG Fc 1:2000, cat. No. Pierce 31437) or Cy3 (goat anti-mouse 1:5000, GE Healtcare cat. No. PA43009) for 1 h at RT. Bands were detected by enhanced chemiluminescence applying LumniGlo reagent and peroxide (Cell signaling) or directly by fluo-