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Caki-1 Cells Represent an in vitro Model System for Studying the Human Proximal Tubule Epithelium

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Key Words
Caki-1 · Proximal tubule · Electron microscopy · Differentiation · Human kidney

Abstract
Background/Aims: The human proximal tubule (PT) epithelium is distinguished from other nephron segments via several unique characteristics. Studies assessing PT epithelium increasingly employ cell lines, bypassing the complexity of primary cell cultures. However, few human model systems exist for studying PT cells in vitro. The current work involves an intensive characterization of Caki-1 cells, a commercially available human renal cell line. ***Methods:*** Caki-1 cells were validated as a representative model system for PT cell research via morphological, physiological and biochemical investigations including light and transmission electron microscopy, transepithelial electrical resistance (TER) measurements and the detection of PT markers. ***Results:*** Morphologically, these cells form a polarized monolayer with apical located microvilli and multiple mitochondria per cell. Low TER ranging from 2 to 28 Ω cm^2 was determined for Caki-1 cells, characteristic of the 'leaky' PT epithelium in vivo. Expression of the PT markers: NHE3, GGT, DPP IV, APM and AP were present in Caki-1 cells. Two epithelial markers, E-cadherin and Na^+/K^+-ATPase, were additionally observed. ***Conclusion:*** The current work is a concise summary which confirms that Caki-1 cells represent well-differentiated polarized PT cells in vitro, regardless of its cancerous origin and multiple passaging. They prove to be a significant contribution to the field of PT research.

Introduction

The mammalian kidney in regard to morphology, physiology and metabolism is a dynamic organ composed of various cell types (15–20 cells types) [1, 2]. The tubular segment of the nephron consists of the proximal, distal and collecting ducts, each having its own distinct functional capacities [3]. The human proximal tubule (PT) epithelium in particular is a complex metabolically active epithelial system [4]. The availability of in vitro cell culture model systems of human PT epithelia, whether from primary or immortalized origins, is very limited. Primary PT cell cultures can be routinely obtained from pig, rabbit, mouse, rat and human; human cultures most closely simulating the clinical situation [3]. Differentiated immortalized human PT cell lines which indicate ex-

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tended in vitro growth potential provide a convenient alternative to labor-intensive whole-tissue and primary cell cultures [5]. However, most models with a few exceptions such as the HK-2 cell line stem from non-human origins (e.g. LLC-PK_1 (porcine), OK (opossum) and NRK-52E (rat)) [6–10]. Additionally, immortalization of the HK-2 cell line was accomplished via transfection with a recombinant retrovirus, placing it in the second biosafety (BS) level of the BS classification system, according to the ATCC (www.lgcpromochem-atcc.com). The BS level is often the sole limitation of many researchers, lacking the capacity to work at a higher BS level than BS1. Therefore, the introduction of a standardized cell line belonging to the lowest BS level would be a significant contribution to PT epithelium research. The cells investigated in the current work, Caki-1, are commercially available and therefore avoid the extensive work of isolation and culturing from fresh tissue. Caki-1 cells were originally obtained from a 49-year-old male Caucasian patient, with a clear cell renal carcinoma. They have been employed thus far in various fields of research, where they have been shown to functionally express molecules relevant to the kidney and/or proximal tubule in vivo [11–14].

The isolation of pure cultures of PT cells and the characterization of new PT systems is commonly accomplished via the detection of various morphological, physiological and biochemical unique characteristics [15]. Morphologically characteristic of PT cells is the presence of multiple mitochondria per cell and microvilli isolated to the apical portion of the cell membrane, the latter of which is associated with the polarized nature of the PT epithelium [16, 17]. Physiologically, the PT epithelium forms a 'leaky' low resistance barrier in vivo, which should be reflected in the cell culture model system [6, 18]. Biochemical analysis includes the detection of epithelial and/or PT specific markers such as the Na^+/H^+-exchanger isoform NHE3, alkaline phosphatase (AP), γ-glutamyl transferase (GGT), dipeptidyl peptidase IV (DPP IV), aminopeptidase M (APM) and Na^+/K^+-ATPase [19, 20]. Additionally, members of the epithelial barrier proteins of the tight and adherent junctions (AJ) including the cadherins, catenins, occludins, claudins and zonula occludens are often investigated [18, 21].

The focus of this study was to determine the relevance of the human renal carcinoma cell line, Caki-1, as a representative PT in vitro cell system, on the basis of a palette of morphological, physiological and biochemical characterizations.

Methods

Growth of Cells in Culture

Caki-1 and Caco-2 cells were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ; Braunschweig, Germany). Caki-1 and Caco-2 cells were grown in McCoy's 5A and Dulbecco's modified Eagle's medium, respectively (Biochrom AG; Berlin, Germany), and supplemented with 10% FBS (Biochrom AG), 1.14% penicillin/streptomycin (Biochrom AG) and 1.14% non-essential amino acid solution (Biochrom AG). Caki-1 McCoy's 5A medium was additionally supplemented with 0.7% L-glutamine (Biochrom AG). Primary human proximal tubule cell lots (HPT) were obtained from In vitro Technologies (In vitro Technologies, Baltimore, Md., USA) and were used at passage 0 without further subcultivation. HPT cells were grown in *Invitro*GRO PT complete medium (In vitro Technologies). All cell cultures were routinely grown in 75-cm^2 cell culture flasks and were maintained at 37°C, 5% CO_2 and 95% relative humidity.

Transmission Electron Microscopy (TEM)

Caki-1 cells grown on polycarbonate (PC) filters were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 for 1 h. The tissue was then post-fixed with 2% OsO_4 in cacodylate buffer for 1 h at room temperature (RT), dehydrated in a graded series of ethanol (30–100%), infiltrated 2 times with propylene oxide and with a 1:1 mixture of propylene oxide and araldite resin overnight. For embedding, samples were transferred to pure araldite resin (Plano, Wetzlar, Germany) and polymerized for 48 h at 60°C. Ultrathin sections were cut with a diamond knife on a Leica Ultracut S. Counterstained ultrathin sections were analyzed with a FEI Technai 12 BioTwin transmission electron microscope and imaged with a SIS MegaView III SCCD camera.

Transepithelial Electrical Resistance (TER)

For the routine determination of tightness and integrity of the monolayers, Caki-1 and/or HPT cells were seeded at a density of 100,000 cells per 1.13 cm^2 PC filter, with a filter pore size of 0.4 μm (Transwell™, Corning Costar Corp., Cambridge, Mass., USA). For standard TER measurements, cells were fed and TER was measured every second or third day for a time span of 7 days in a similar manner as has been previously reported [6]. Briefly, TER measurements were conducted at a constant RT (25 ± 2°C) using a 'chopstick' electrode and Millicell-ERS device (Millipore, Schwalbach, Germany). The electrode was pre-calibrated for a minimum of 2 h in incubation medium prior to all measurements. The results were calculated as Ω cm^2 after subtracting the TER value of the membrane filter supports alone. The development of TER in Caki-1 cells was assessed over a time span of 10 days, when grown under routine conditions (see above). The influence of cell passage number and substratum type on the tightness of the Caki-1 monolayers was determined by observing the stability of the TER values between passages 8 and 71 and the employment of various growth substrata. All growth substrata were purchased from Corning Costar.

RT-PCR of NHE3

The RNA was isolated from the cells using the RNA STAT-60™ (Tel-Test Inc., Friendswood, Tex., USA), according to the company's protocol for RNA isolation. The RNA was purified us-

ing a DNA-free™ purification kit (Ambion Ltd., Cambridgeshire, UK). Quantification of isolated RNA was based on spectrophotometric analysis (GeneQuant Pharmacia Biotech, Freiburg, Germany) at a wavelength of 260 nm (RNase-free water served as a blank). The integrity of the isolated RNA was checked by standard gel electrophoresis with 1% agarose in 1×TAE. The total RNA was reverse transcribed into cDNA using a SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen™ Ltd., Paisley, UK), according to the manufacturer's guidance. cDNA was then amplified by PCR. The sense and antisense primer sequences used for the NHE3 detection were 5′-GGAAATCTTCCACAGGACCAT and 3′-CACTCATCTCCTCATCATAGTTGG, respectively (Operon Biotechnologies GmbH, Cologne, Germany). The expected product size was 245 bp. The PCR was carried out using the Expand™ High Fidelity PCR System kit (Roche Diagnostics, Mannheim, Germany). Samples were initially heated for 4 min and 94°C and PCR amplification cycling conditions were the following: 1 min denaturation at 94°C, 2 min annealing at 60°C and 3 min extension at 72°C, for a total of 35 cycles. Subsequently, the amplified PCR products were analyzed by 2% agarose gel electrophoresis with ethidium bromide staining along with a 100-bp DNA ladder (GeneRuler™, Fermentas GmbH, Leon-Rot, Germany).

Immunofluorescent Visualization of Molecules

E-cadherin and Na^+/K^+-ATPase were visualized by indirect immunofluorescence labeling procedures using confluent cells grown on glass coverslips. The samples were fixed and permeabilized at –20°C in methanol for 10 min and subsequently dried at RT for 30 min. The cells were washed twice with PBS and incubated with Tween 20 (0.04% in PBS) for 20 min. After washing with PBS and blocking (0.5% cold-water fish gelatin (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) plus 0.1% ovalbumin (Sigma-Aldrich) in PBS) for 30 min, the cells were incubated with primary antibody, anti-E-cadherin (BD Biosciences, Heidelberg, Germany) or Na^+/K^+-ATPase (Upstate Biotechnology, Lake Placid, N.Y., USA) diluted 1:25 and 1:200, respectively, in blocking solution, at 4°C overnight. The cells were washed with PBS and subsequently incubated with the secondary antibody conjugated to Alexa® 488 (Invitrogen GmbH, Karlsruhe, Germany) (1:400) and DAPI (1:8,000) in blocking solution for 2 h in the dark. Washed cells were mounted in Mowiol 4.88 (Hoechst, Frankfurt, Germany). Mounted sections were examined by fluorescence microscopy with a Leica DMRP fluorescence microscope. Images were obtained with a Hamamatsu Orca ER CCD camera and processed with Adobe Photoshop (Adobe Systems, San Jose, Calif., USA). To assess background labeling, all experiments included a series of samples in which primary antibodies were omitted. No significant labeling was observed for the controls. All experiments were repeated inter- and intra-day at a minimum of three replications with good reproducibility.

Assay of Cellular DPPIV and APM Enzyme Activity

DPPIV and APM enzyme activity was measured spectrophotometrically using Gly-Pro-pNA and L-alanine-nitroanilide hydrochloride as DPPIV and APM substrates, respectively. The confluent cell monolayers in 96-well flat-bottomed microtiter culture plates were washed twice with PBS; 100 μl of either 1 mM Gly-Pro-pNA or 1.66 mM L-alanine-nitroanilide hydrochloride in 0.1 M Tris buffer (pH 8.0) containing Trizma Base were applied and incubated at 25°C for 30 min. Real-time measurements were taken at 2-min intervals at a wavelength of 405 nm in a microplate reader. The enzyme activity was calculated after subtraction of the absorbance values for cell-free controls. Values are displayed as end concentrations of the reaction product *p*-nitroaniline after 30 min. A standard curve was constructed using *p*-nitroaniline. All chemicals were purchased from Sigma-Aldrich.

GGT Staining

Caki-1 cells were grown in 24 multi-well plates with McCoy's 5A complete medium at a seeding density of 12,000 cells/well until confluence. The cells were washed three times with 0.85% saline solution; 0.5 ml of completed reaction mixture (14 ml 0.85% saline, 5 ml 0.1 M Tris buffer pH 7.5, 10 mg Gly-Gly, 10 mg Fast Blue and 1 ml L-glutamic acid γ-(4-methoxy-β-naphthylamide) (GMNA) stock solution (15.2 ml distilled water, 0.4 ml DMSO, 0.1 ml NaOH and 10 mg GMNA)) was added and incubated at RT for 3 h in the dark. The cells were washed with 0.85% saline solution. Subsequently, each well was incubated with 1 ml of a 2.5% $CuSO_4$ solution for 2 min and washed with 0.85% saline solution. The cells were fixed in a 50:50 glycerol:water solution. Controls were carried out with a reaction mixture containing serine borate. All chemicals were purchased from Sigma-Aldrich.

AP Staining

Caki-1 cells were grown in 24 multi-well plates in McCoy's 5A complete medium at a seeding density of 12,000 cells/well. The cells were washed with PBS and fixed in a 4% formaldehyde mixture at RT. The cells were stained using a SIGMA FAST BCIP/NBT substrate solution (Sigma-Aldrich), dissolved in deionized water. Cells were incubated at 37°C for 30 min with the substrate solution. Positive bluish-purple staining was observed via light microscopy for the treated cells.

Results

Cell Morphology

With regard to general morphology, Caki-1 cells grow to form a complete monolayer after approximately 7–8 days in culture. Directly after seeding, up until 4–5 days post-plating, the cells take on the typical elongated shape of healthy (primary) PT cells and after growth into a dense monolayer, the cultures pack tightly to form a regular packed polygonal array of cells, characteristic of immortalized PT cells (fig. 1a and 1b, respectively). Cells grown in transwell plates on PC filters were analyzed by TEM. The ultrastructure of Caki-1 cells revealed an organized polarized epithelium, with apically located microvilli, multiple mitochondria and tight junctions were observed between adjacent cells (fig. 2a–c).

Identification of Marker Molecules

Marker molecules specific to epithelial cells and/or the human PT epithelium were selected for the charac-

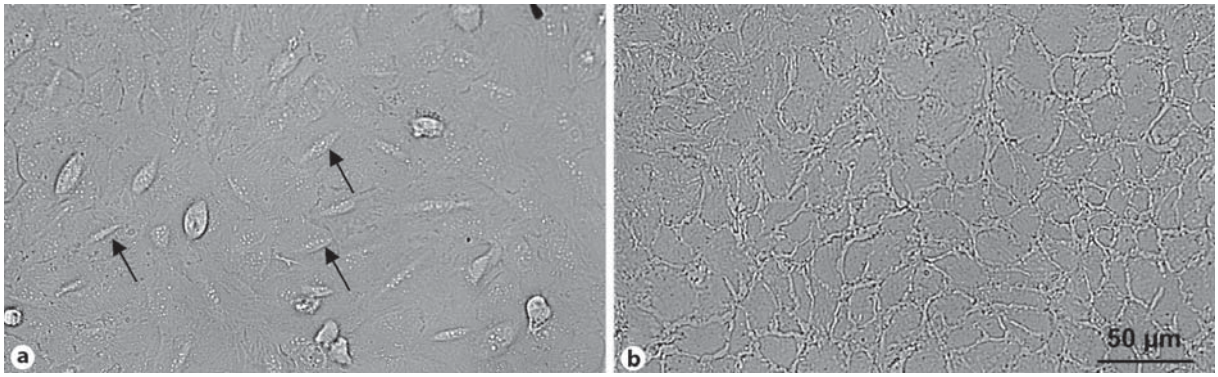
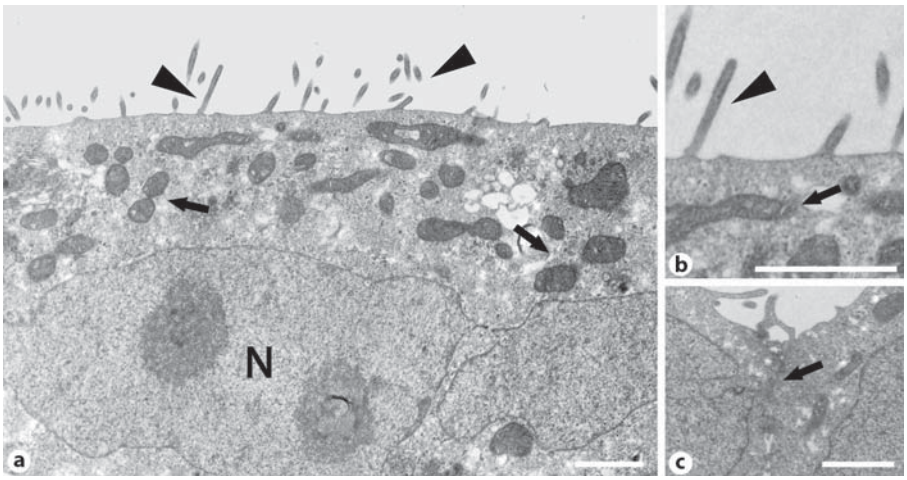


Fig. 1. Morphology of Caki-1 cells. Caki-1 cells 4 days post-plating are elongated and characteristic of healthy PT cells (arrows) (**a**), and after 8 days post-plating a confluent monolayer of Caki-1 cells is formed with typical polygonal-shaped cells, a characteristic of immortalized PT cells (**b**). Caki-1 cells were grown in 75-cm^2 tissue culture flasks and observed via phase-contrast microscopy.

Fig. 2. Transmission electron microscopic analysis of the ultrastructure of Caki-1 cells. Upon confluent monolayer formation on polycarbonate filters, which imparts cell polarity and differentiation, cells were fixed and sectioned for TEM analysis (see 'Methods'). Caki-1 cells showed typical epithelial ultrastructure, including the cell nucleus (N), apically located microvilli (arrowheads) and multiple mitochondria (arrows) (**a**), an enlargement of the microvilli (arrowheads) and multiple mitochondiria per cell (arrows) is depicted (**b**), and the apically located tight junctions with a looser association below the junctional areas (arrow) is shown (**c**). All scale bars represent 1 µm.



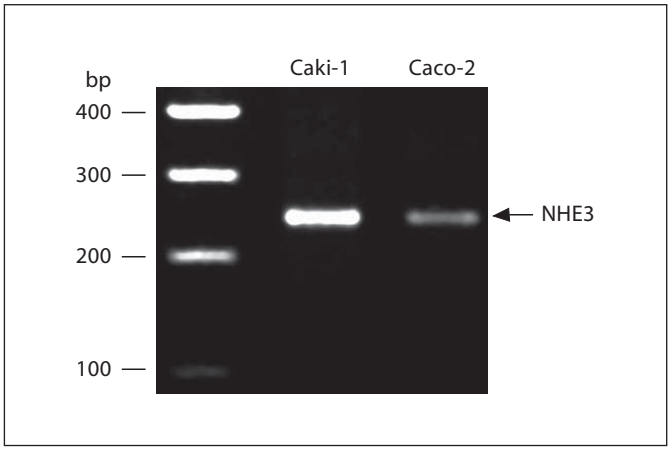


Fig. 3. RT-PCR demonstrating NHE3 isoform mRNA expression in Caki-1 and Caco-2 cells. PCR products were electrophoresed on 2% agarose gel and stained with ethidium bromide. Lanes 1 and 2 represent the 245-bp product determined for Caki-1 and Caco-2 cells, respectively; Caco-2 cells were used as a positive control. Molecular weight markers were a 100-bp molecular ladder.

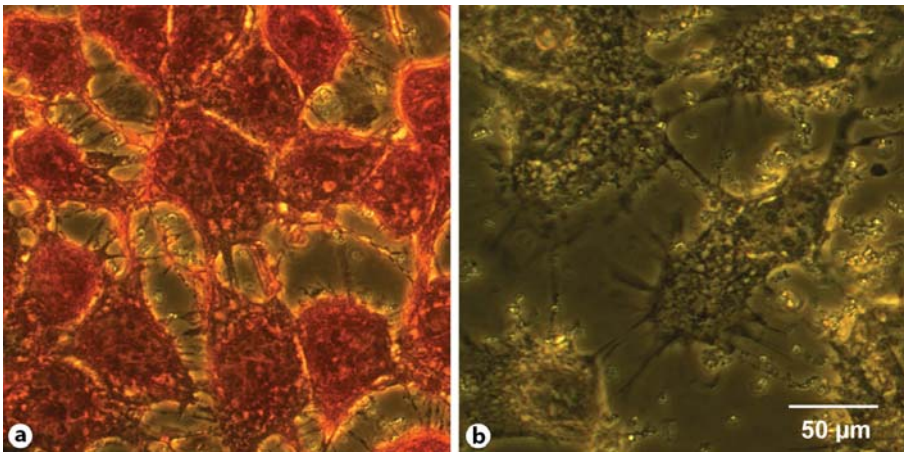


Fig. 4. Cytochemical staining of GGT in Caki-1 cells. Cells grown in 24 multi-well plates were stained for GGT expression as described in the methods section. Positive GGT labeling was observed as a brilliant brownish-red staining (**a**), and negative controls containing serine borate, run in parallel, were unstained (**b**).

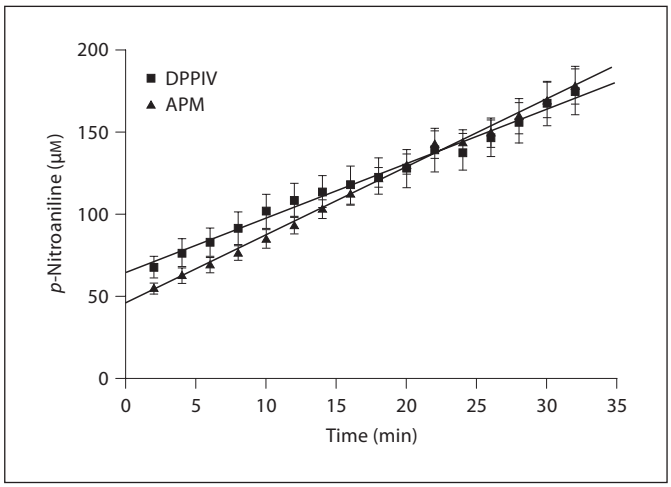


Fig. 5. Determination of DPPIV and APM activity as assessed by *p*-nitroaniline accumulation. DPPIV or APM enzyme activity was measured spectrophotometrically using Gly-Pro-pNA and L-alanine-nitroanilide hydrochloride as DPPIV and APM substrates, respectively, over a time span of 30 min. All enzymatic activity determinations represent the mean of 12 ± SEM.

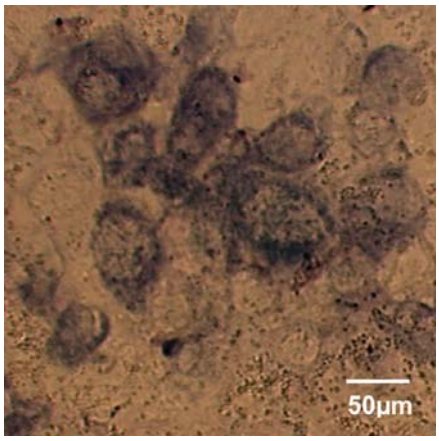


Fig. 6. Cytochemical staining of AP in Caki-1 cells. Caki-1 cells were stained for AP using BCIP/NBT as a precipitating substrate. AP was located to the cell membranes in Caki-1 cells. Negative controls, without BCIP/NBT, were run in parallel; no staining was observed.

terization and confirmation of the epithelial and PT origin of Caki-1 cells. NHE3, GGT, APM, DPPIV and AP are characterized to be selective human PT cell markers. Figure 3 shows the amplification products of NHE3 detected in Caki-1 and Caco-2 cells; an expected band size of 245 bp was observed for both cell types. Caco-2 cells were used as a positive control since it has been previously confirmed to express the NHE3 isoform. The presence of GGT in Caki-1 cells was indicated by the bright red staining profile (fig. 4a). No staining was observed with the negative control containing serine borate (fig. 4b). The enzyme activity of APM and DPPIV were analyzed by the application of specific enzyme substrates (see 'Methods') and the representative end product (*p*-nitroaniline) accumulation. A linear increase in *p*-nitroaniline was observed over a time span of 30 min, with an end concentration of approximately 175 µM being produced by both enzymes/per well (fig. 5). AP expression was visualized as a deep purple staining along the cell membranes in Caki-1 cultures (fig. 6). Epithelial

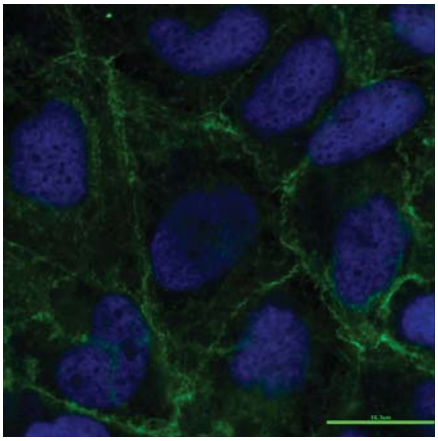


Fig. 7. Expression pattern of E-cadherin in Caki-1 cells. Confluent monolayers of Caki-1 cells on glass coverslips were processed for the visualization of E-cadherin (see 'Methods'). E-cadherin was located to the cell-cell adhesions of Caki-1 cells.

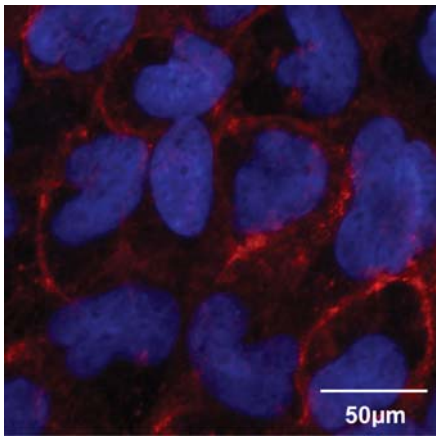
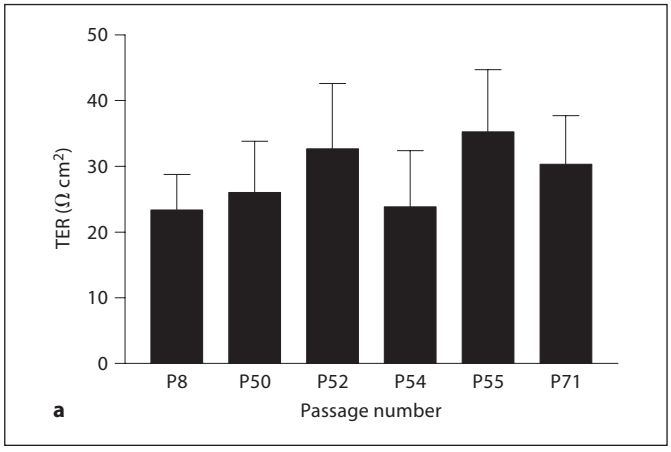
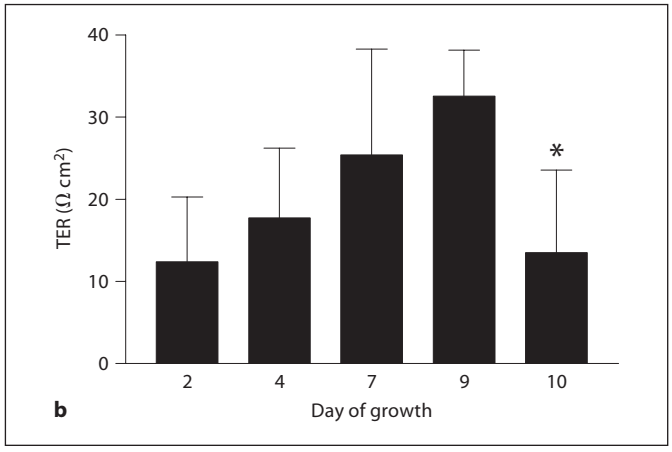


Fig. 8. Expression pattern of the Na^+/K^+-ATPase in Caki-1 cells. Confluent monolayers of Caki-1 cells grown on glass coverslips were processed for the visualization of Na^+/K^+-ATPase (see 'Methods'). Na^+/K^+-ATPase was located to the cell membranes of Caki-1 cells.





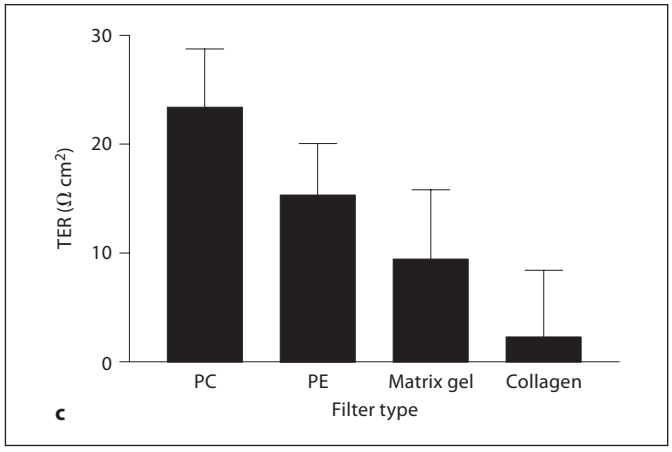


Fig. 9. TER measurements of Caki-1 cells grown under varying conditions. Caki-1 cells were cultivated on polycarbonate (PC) filters between passages 8 and 71 and TER values were measured every second day for a duration of 7 days, no significant differences were observed (**a**), the development of TER in Caki-1 cells was observed for 10 days when cells were grown on PC filters, a significant decrease in TER was observed after day 9 as indicated by an * ($p < 0.0001$) (**b**), and in a last series of experiments Caki-1 cells were cultivated on filters of polyethylene (PE), PC, collagen-coated PC or matrix gel for a duration of 7 days and the changes in TER were observed (**c**). All values are expressed as mean ± SEM of a minimum of 6 independent determinations.

Table 1. TER values were determined with cell monolayers 7 days post-plating

Cell type	TER, Ω cm^2
Caki-1	23.42 ± 1.54
HPT	19.04 ± 4.88

Caki-1 and HPT cells were grown on polycarbonate permeable inserts, at a seeding density of 1×10^5 cells per 1.13 cm^2 filter support. TER measurements were taken every second day using a Millicell-ERS device. The results represent the mean ± SEM of a minimum of 6 independent determinations.

marker molecules included the epithelial barrier protein E-cadherin, known to be the most predominant cadherin protein found in vitro, in PT cells. Accordingly, immunostainings of E-cadherin in Caki-1 cultures indicated a sharp staining pattern located to the cell-cell contacts as observed in figure 7. Na^+/K^+-ATPase known to be present in cells of epithelial origin was located to the cell membranes of Caki-1 cells via immunocytochemical studies (fig. 8).

Transepithelial Resistance (TER) Experiments

To determine the effect of passage number, the time course and extent of tight junction formation and the influence of substrata on tight junction formation in Caki-1 cells, TER values were measured. Several passages of Caki-1 cells between passage 8 and 71 were routinely cultivated (see 'Methods') and were observed for changes in TER; no significant changes were observed with passaging, and TER values consistently remained representative of a 'leaky' epithelium (fig. 9a). In a second experiment, Caki-1 cell monolayers were grown for 10 days on semi-permeable PC filters. A steady increase in TER until day 9 (32.55 ± 1.70 Ω cm^2) was observed and a significant decrease in TER was noticed on day 10 (13.5 ± 2.9 Ω cm^2), as can be seen in figure 9b. In a series of TER measurements, the influence of substrata on tight junction formation was investigated. PC filters revealed the highest TER after 7 days in culture and collagen-coated PC filters resulted in the leakiest monolayer formation. Polyethylene (PE) and matrix-gel filters revealed similar TER values of moderate proportion in comparison to the PC and collagen-coated PC filter TER values. TER values of Caki-1 and HPT cells, after routine cultivation were compared, with no significant differences observable (table 1). Successful completion of monolayers was confirmed via light microscopy.

Discussion

Cell cultures provide researchers with a simplified system to focus directly on the epithelium, uninfluenced by higher-order regulatory systems. The handling of primary cell cultures is tedious, time-consuming, costly and limited with regard to cell life span and cell number in the case that fresh human kidney tissue is not readily available. Therefore, the introduction of new PT cell lines with their unlimited life span would provide valuable tools to the field of PT research. Unfortunately, many of the cell lines used for these purposes thus far have not been critically evaluated and standardized or have not met the necessary requirements. As an example, the human PT cell line HK-2 is the most commonly used human PT cell model system; however, it has been discovered that it expresses abnormalities in the expression pattern of many molecules. It was confirmed to be an inadequate representation of the intact PT barrier [6]. Secondly, HEK cells of embryonic origin are commonly used as an in vitro system for studying the human kidney; however, it may not adequately represent the mechanisms of the adult tissue. Furthermore, LLC-PK1 cells form moderately tight monolayers, reaching TER values greater than 213 Ω cm^2, indicating that they no longer reflect the 'leaky' epithelial characteristics of the human PT [6]. The majority of the information regarding the expression of such marker molecules is scattered among various cell systems originating from different species; therefore, the characterization of a single PT cell line of human origin, covering all aspects would be beneficial. Important to note here, finding an in vitro cell system which completely mimics the in vivo situation is also expecting the impossible since it is common knowledge that functional differentiation is maintained only to a limited degree when cells of malignant tissue are cultured in vitro as monolayers [19].

The key objectives of this study were to confirm the PT origin of Caki-1 cells and to determine how closely this model system reflects in vivo PT epithelium, based on the following three points: (1) morphological characterization, (2) expression and function of epithelial barrier proteins, and (3) the presence or functional activity of a palette of marker PT molecules.

Morphologically, PT cells isolated from healthy tissue grow in the form of a single layer of elongated cells [22]. PT cells of immortalized and cancerous origin have been previously described to grow as a monolayer of polygonal-shaped cells. Caki-1 cells, during the initial phases of growth, take on this typical healthy PT cell

form and revert to a polygonal shape (cobblestone-like pattern) after a dense monolayer has been established, after approximately 7 days in culture. Reversion to the latter form may be a result of competition for space (contact inhibition) and should serve as an indicator that these cells should be allowed to grow to a maximum of 90–95% confluence in order to retain their healthy PT cell shape. Furthermore, a significant decrease in TER of Caki-1 cells was observed after 9 days in cultivation, indicating that deteriorations to the monolayer is occurring, perhaps as a direct result of the detachment of cells from the filter surface. Regarding the cell structure of polarized PT cells, they are often characterized via two observations: the presence of multiple mitochondria per cell and the apically located microvilli; both were observable in Caki-1 cells demonstrating that these tubular cells are able to grow in a functional polarized fashion in vitro [23].

Physiologically, the PT epithelium forms a 'leaky' barrier in vivo, with a TER ranging from 5 to 12 Ω cm^2 [6, 18]. This TER value is representative of the interactions of many adhesion molecules of the tight and adherent junctions located between epithelial cells. These adhesion junctions were visible in Caki-1 cells. Nephrotoxic injury often involves the disruption of barrier function and hence alterations in the permeability and/or polarity of the PT epithelium [6]. The TER values of the Caki-1 and HPT cell systems were approximately 23 and 19 Ω cm^2, respectively, when grown on PC filter supports. Both values are slightly higher than the expected in vivo TER but nevertheless representative of a 'leaky' epithelium. It should be noted that in the current study, a relatively simple volt-ohm meter was used to determine TER measurements. This is a valid and commonly used technique for evaluating changes in relative TER; however, its sensitivity and reliability in determining absolute electrical resistances have been questioned [6]. Nevertheless, the validity of the results can be confirmed by observing the consistency in data across the series of experiments which were conducted in the Caki-1 cells. Significant to the current studies on TER was the demonstration that Caki-1 cells adequately represent a 'leaky' epithelium with resistances in the range of what is expected in vivo. To put the leakiness of the PT epithelium into perspective, one should consider that tight epithelia, such as the human bladder, can reach TER values up to 300,000 Ω cm^2 [18]. E-cadherin was selected as a marker molecule of the epithelial barrier proteins since it has been classified to be one of the most significant AJ molecules involved in the maintenance of the epithelial barrier [6, 24, 25]. In vitro it has been proven to be the most common cadherin found in PT cells, and was strongly visible in the Caki-1 cells via immunocytochemical techniques. However, in vivo it has been suggested that N-cadherin is the most prominent cadherin located in the PTs, indicating that cadherin expression may be altered during in vitro cultivation of immortal PT cell lines [25]. This de-differentiation of Caki-1 cells should be taken into consideration when in vitro research studies are concerned with tight junction formation and/or activity.

The selective isolation of PTs from kidney tissue is most often confirmed by the expression of specific marker molecules [22]. The most commonly used and characterized marker molecules of human PTs are NHE3, GGT, APM, DPPIV and AP [1, 3]. Expression and/or activity of all of these proteins, using previously published techniques, was confirmed for Caki-1 cells, verifying the PT nature of this cell line. The origin and purity of the commercially available HPT cells were also partially confirmed by the aforementioned techniques. Previous studies have stated that a full expression and sorting of apical and basolateral proteins to their respective domains requires a high degree of differentiation [26, 27]. The basolaterally positioned Na^+/K^+-ATPase was located to the basolateral membrane in Caki-1 cells and other proteins to the apical membrane, providing further proof that the Caki-1 cells retain an extended well-differentiated growth pattern in vitro [11, 18, 24, 28].

The Caki-1 cell line has been previously used for various fields of research including nephrotoxicity, general cytotoxicity, physiology, and carrier-mediated drug transport studies. In respect to drug transport, PTs are the major site of active drug reabsorption and secretion in the kidney; therefore, as one example, these data provide support for previous studies which employed Caki-1 cells as a representative model for kidney drug transport [11, 29, 30]. However, to our knowledge no single comprehensive study confirming the PT nature of Caki-1 cells via morphological, physiological and biochemical characterization has been published previously. Caki-1 cells show considerable potential as a PT cell model system for the following reasons: (1) Caki-1 cells possess the typical membrane proteins NHE3, GGT, APM, DPPIV and AP, suggesting that a normal PT phenotype is retained, (2) Caki-1 cells react to antibodies specific to E-cadherin and Na^+/K^+-ATPase, specific to in vitro PT and/or epithelial cells, (3) Caki-1 cells retain functional activity in respect to specific proteins of the PT, includ-

ing drug transport proteins and metabolizing enzymes, (4) Caki-1 cells represent a polarized adherent epithelium, (5) Caki-1 cells are classified BS1, and (6) Caki-1 cells were derived from human adult kidney tissue. Important to note here, certain results present in this work are in contradiction to previous results published regarding Caki-1 cells. In a previous study, it has been stated that Caki-1 cells do not express AP and do not form discrete monolayers [31]. Based on these criteria, Caki-1 cells were not considered to be an accurate representation of the PT epithelium. In contrast, our work indicated a membrane localization of AP, the presence of E-cadherin at the cell-cell adhesions, the constant formation of a 'leaky' monolayer independent of passage number and substrata and additionally formed polarized monolayers as observed via TEM.

In light of these findings, the Caki-1 cell line appears to be a suitable, convenient and reliable new tool for PT epithelium research, which is commercially available and represents the first characterized human PT cell line classified to be BS1. Caki-1 cells have already shown promising results in many fields of research. Important to note is that this cell line has been proven to phenotypically resemble the PT epithelium in many ways, but a full characterization does require further studies and is currently underway. Immortalization may also lead to the acquisition of atypical properties; therefore, methods should be tailored to fit the immediate purpose. The investment into in-depth characterizations of other commercially available cell lines could unveil further interesting and useful information.

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