Cubilin is expressed in rat and human glomerular podocytes

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Abstract

Background. The bulk of proteins filtered in the glomeruli are reabsorbed in the proximal tubule by endocytosis mediated by two multiligand receptors operating in concert, megalin and cubilin. Podocytes can also internalize protein and megalin; this was initially reported in rat proximal tubular and glomerular epithelial cells and has recently also been demonstrated in human podocytes. Cubilin, crucial for albumin reabsorption in the proximal tubule, has not been identified in glomerular epithelial cells.

Methods. In the present study, we used immunocytochemistry and reverse transcription-polymerase chain reaction on laser-captured glomeruli to demonstrate synthesis and expression of cubilin in rat and human glomeruli. In parallel experiments, the expression of cubilin was studied in cultured podocytes.

Results. This study identifies cubilin in rat and human glomeruli according to a pattern similar to that reported for megalin. Cubilin revealed a surface expression but also intracellular expression in the podocytes.

Conclusion. Our findings show that the podocytes display the two endocytic receptors which are responsible for the only documented process for protein reabsorption in proximal tubule cells.

Keywords: albumin; cubilin; endocytosis; megalin; podocytes

Introduction

The podocyte is a central player in a variety of glomerular diseases [1]. Podocytes can internalize proteins in vivo [2, 3], in particular albumin, [4] (Figure 1) but the receptors involved are not or poorly documented. Megalin and cubilin, which together are responsible for protein uptake in the proximal tubule [5–9], are obvious candidates. When megalin was initially identified as gp330 [10], it was reported as predominantly expressed in the proximal convoluted tubule but also in the glomerular epithelial cells of Lewis rats where it was the target antigen in a model of glomerulonephritis. In man, megalin was only detected in the proximal tubule [11]. Using more sensitive techniques, including reverse transcription–polymerase chain reaction (RT–PCR) on laser microdissected glomeruli, we recently reported that megalin was expressed in human podocytes [12]. In the present work, we use a similar approach to demonstrate the expression in the glomeruli of cubilin which, together with megalin, is essential for albumin uptake in the proximal tubule [9]. The expression of both cubilin and megalin indicates a potential for endocytic uptake by podocytes.

Materials and methods

Antibodies

Rabbit anti-rat cubilin has been described earlier [13]. A specific polyclonal rabbit anti-human cubilin was a kind gift from Dr. S. K. Moestrup, Aarhus University, Aarhus, Denmark. This antibody gave a specific band for cubilin when tested by western blot on human kidney homogenate. Rabbit polyclonal anti-podocin (P0372) was purchased from Sigma–Aldrich (Saint Louis, MO). Polyclonal rabbit anti-rat albumin, monoclonal mouse anti-human Wilms’ tumour 1 (WT1; clone 6F-H2) protein and peroxidase-conjugated secondary antibodies were purchased from Dako A/S (Glostrup, Denmark). Fluorescence-conjugated secondary antibodies were purchased from Molecular Probes (Eugene, OR). Controls for unspecific binding were performed with non-specific rabbit, mouse or sheep IgG from Dako.

Immunohistochemistry

Lewis, Wistar and Sprague–Dawley rats were perfused retrograde through the abdominal aorta with 4 or 8% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4. Human kidney (8 autopsy material) was routinely fixed and embedded in paraffin. Processing of tissue for light and electron microscope immunocytochemistry has been previously reported [12, 14].

Tissue preparation and laser capture microdissection

Adult (>8-week-old) male Wistar rats were anaesthetized with Mebumal (50 mg/kg intraperitoneally). Kidneys were harvested after perfusion with phosphate-buffered saline, immediately snap frozen and stored at −80°C. All experiments were carried out in accordance with the Danish National Animal Experiments Inspectorate. Human kidneys were obtained from renal carcinoma patients. Ethical approval for the human studies was granted by the Local Research Ethics Committee and informed consent was given by the patients. Immediately after surgical removal, carcinoma free parts of the kidneys were excised, embedded in Tissue-Tek OCT Compound, immediately snap frozen and stored at −80°C until use. Cryosectioning and laser capture microdissection (LCM) of glomeruli (∼600 rat or ∼200 human) using RNase-free techniques, preparation of RNA and RT–PCR were as previously reported [12]. PCR was performed using the primers given in Table 1.

Immunofluorescent microscopy

Conditionally, immortalized human podocyte cell line was previously characterized in detail [15]. The podocytes were maintained as previously described [12], and podocyte differentiation was induced under...
non-permissive conditions by thermo shifting the cells to 37°C for 14 days.

Immunofluorescence localization of proteins in permeabilized and non-permeabilized human podocytes was performed as described previously [12].

Results

Immunocytochemistry

Cubilin was found in podocytes of all rat strains tested (Figure 2) and in human podocytes (Figure 3). In the rat, immunoperoxidase staining is detected in typical glomerular epithelial cells, and in man, immunofluorescence demonstrates colocalization with the podocyte marker WT1. Ultrastructural localization could only be carried out on rat tissue for technical reasons. Cubilin was detected at the plasma membrane especially in coated areas (Figure 2b–d), but much more concentrated intracellularly in vesicles and in multivesicular bodies (Figure 2b and d). The receptors were found both at the cell body of the podocytes and also at the foot processes, but not especially on the plasma membrane facing the basement membrane.

Laser capture and RT–PCR

In order to avoid contamination with especially proximal tubule cells, we selected only the largest cross sections of the glomeruli to be close to the equator and avoid contamination. Furthermore, the dissection was performed with a safe distance (Figure 4A) to the glomerular parietal epithelium. To further confirm that proximal tubules did not contaminate the purified glomeruli, we demonstrated that the proximal tubule marker aminopeptidase N (APN) [16, 17] was not present in the laser-dissected glomeruli, but only in whole rat kidney cortex (Figure 4B). We have previously shown that no APN messenger RNA (mRNA) was detected in isolated human glomeruli [12]. The expression of mRNA for cubilin, megalin, podocin and nephrin is shown in Figure 4B for rat glomeruli. Figure 4C shows the expression of mRNA for cubilin in human glomeruli. Expression of megalin, nephrin and podocin on the same material has previously been reported [12].

Immunofluorescence microscopy of cultured human podocytes

Immunofluorescent studies demonstrated that cubilin is expressed at the cell surface and intracellularly in cultured human podocytes under non-permeabilized and permeabilized conditions, respectively (Figure 5). The phenotype of podocytes was characterized by labelling for podocin, a specific podocyte marker (Figure 5).
The present study was prompted by our recent work on cubilin and/or megalin knockout mice [9] showing that cubilin together with megalin was essential for albumin uptake in the proximal tubule. Albumin being the most abundant protein in the ultrafiltrate, we felt that it was important to show if, in addition to megalin, cubilin was synthesized by glomerular podocytes under physiological conditions. As reported for megalin [10], cubilin in rat podocytes is expressed at the cell membrane mainly in coated pits but also in various domains of the endocytic apparatus, including endosomes, multivesicular bodies/lysosomes. Odera et al. [18] reported a very slight reaction for cubilin in glomeruli of 30 month old rats. The present report, based on both immunostaining of podocytes and RT–PCR on LCM glomeruli, is the first demonstration that cubilin is transcribed from the podocytes. Furthermore, we report that cubilin is also expressed in human podocytes, as previously shown for megalin [12]. Our observations, showing that glomerular visceral epithelial cells are equipped with both megalin and cubilin, suggest that the podocyte can be actively involved in receptor-mediated endocytosis of proteins filtered in the glomeruli. Podocytes from heavily proteinuric patients [19] or animals display vacuolization of glomerular epithelial cells suggesting that this process might be of significance in pathology. It is possible that many/some of the conclusions derived from studies on tubular cells may be applicable to the podocyte. For instance, in proximal tubule cell lines, it appears that—via the PI-3K/PKB pathway—megalin acts as a sensor to determine the effect of albumin on cell
survival: low concentrations of albumin inhibiting apoptosis, whereas high concentrations favour apoptosis. Similarly, it has been proposed that handling by the tubular cells of pathological quantities of protein (and in particular albumin) results in toxic and mitogenic effects, triggering inflammatory processes leading to scarring [20]. Since megalin and cubilin are essential for this process, a similar sequence of events could occur in the podocytes influencing podocyte density and function particularly in cases in which there are no extraneous sources of inflammation. It is beyond the objective of the present paper to list the proteins internalized by podocytes but it is likely that, in addition to albumin, they can take up the various ligands of cubilin and megalin, many of which, because of their size, are expected to be present under physiological conditions in the initial ultrafiltrate. Further studies are needed to determine the significance of our observations in pathophysiology. Podocyte-derived cell lines [21], expressing megalin and cubilin, may be useful tools.

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Conflict of interest statement. None declared.

References

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Table 1. Primers for RT-PCR

<table>
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<tr>
<th>Target gene</th>
<th>Sequence (5′–3′)</th>
<th>Position in cDNA* sequence</th>
</tr>
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<tr>
<td>Rat cubilin (NM_053332.2)</td>
<td>GCCTGGCAATGGAACTAGCA</td>
<td>4436–4456</td>
</tr>
<tr>
<td>Rat megalin (NM_030827.1)</td>
<td>TGATCCAGGAGCACTCTTG</td>
<td>4618–4598</td>
</tr>
<tr>
<td>Rat podocin (NM_130828.2)</td>
<td>TGGAGCTCCTCCTTGACCTTG</td>
<td>5396–5416</td>
</tr>
<tr>
<td>Rat nephrin (NM_022628.1)</td>
<td>TGGTGTGGTGTGGTCAG</td>
<td>5555–5536</td>
</tr>
<tr>
<td>Rat aminopeptidase N (NM_031012.1)</td>
<td>CACCTCCGGCACCTAACA TC</td>
<td>3247–3227</td>
</tr>
<tr>
<td>Human cubilin (NM_001081.3)</td>
<td>CCCCCACACCACCTTGGTTGT</td>
<td>645–625</td>
</tr>
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* cDNA, complementary DNA.