Expression of Human Mucin Genes During Normal and Abnormal Renal Development

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Key Words: Kidney; Fetus; Development; Mucin; MUC6; Cystic malformation

DOI: 10.1309/A9YM1CBQDYFRC2EY

Abstract

Human mucin genes encode large O-glycoproteins, which are expressed in various epithelial tissues. The proteins are the main components of mucus, but also might be involved in morphogenesis of or carcinogenesis in many organs. We studied the expression of human mucin genes during fetal kidney development and in malformed cystic renal diseases in 10 normal fetal kidneys and 12 malformed kidneys by in situ hybridization and immunohistochemical analysis. MUC1, MUC3, and MUC6 were expressed in normal fetal kidney. MUC1 was expressed from 7.5 weeks of gestation in the metanephric blastema and throughout fetal life in the ureteric buds, distal convoluted tubules, and collecting ducts. MUC3 was expressed weakly in immature tubules from 8 weeks of gestation, after which it was expressed weakly and focally in the proximal convoluted tubules. MUC6 was expressed at 9.5 weeks of gestation in the tips of the ureteric buds and later in the collecting ducts. In malformative cystic diseases, only MUC1 expression was retained; no expression of MUC6 and MUC3 was observed. These results implicate human mucin genes (MUC1, MUC3, and MUC6) in renal morphogenesis processes.

Renal development is a complex mechanism that is thought to involve more than 300 genes indexed to date.1,2 The processes of tubulogenesis and branching morphogenesis are under the control of inductive epithelial-mesenchymal interactions stimulated by many growth factors or signaling molecules. Abnormal fetal development may cause cystic diseases such as renal dysplasia or autosomal recessive polycystic kidney disease (ARPKD), leading to renal failure.2

Human mucins belong to a large family of O-glycoproteins, which are the main component of mucus.3,4 The findings of previous studies suggested that some mucins not only are responsible for protection of epithelial surfaces but also are believed to have a role in the development of many organs from the respiratory or digestive tracts (MUC1-4, MUC5AC, MUC5B, MUC6). In these tissues, human mucin genes are expressed during embryonic and fetal development according to differential expression patterns in comparison with adult tissues.5-7 To date, little is known about mucin gene expression during metanephric development; however, 3 of the genes, MUC1, MUC3, and MUC6, are expressed in the adult urogenital tract.8 The aim of the present study was to investigate human mucin gene expression in fetal kidney during the period covering renal development and compare coexpression patterns with those observed in renal cystic malformations.

Materials and Methods

Tissue Selection

Ten normal kidneys were obtained from 2 embryos and 8 fetuses after spontaneous or therapeutic abortion. The
embryos and fetuses ranged in age from 7.5 to 39 weeks of gestation. One normal kidney from a 6 month-old child who died suddenly also was studied. Twelve malformed kidneys also were obtained (from 20 to 26 weeks of gestation): 8 kidneys with renal dysplasia (3 cases of primitive dysplasia, 3 cases associated with urinary tract maldevelopment, and 2 cases associated with other syndromic malformations) and 4 kidneys with ARPKD. All tissues were used after approval of the local ethical committee.

All tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections 3 µm thick were cut and stained routinely with H&E and saffron.

Immunohistochemical Analysis

Immunohistochemical analysis was conducted on formalin-fixed, paraffin-embedded tissues using an automated immunostainer (ES, Ventana Medical Systems, Strasbourg, France). Following deparaffinization, immunohistochemical analysis was performed using a 3-step indirect process based on the streptavidin-biotin complex. The primary antibodies used were directed against MUC1 (monoclonal, M8; microwave, 20 minutes; dilution 1:50), MUC2 (polyclonal, Lum2-3EU; pressure cooker, 1 minute, 30 seconds; dilution 1:1,000), MUC5AC (monoclonal, CLH2; microwave, 20 minutes; dilution 1:2), and MUC6 (microwave, 20 minutes; dilution 1:2; Novocastra Laboratories, Newcastle, England). The European Union consortium (see “Acknowledgments”) provided the primary antibodies.

The sections were incubated for 32 minutes with goat serum to block the nonspecific antibody binding sites. Adding an excess amount of avidin followed by washing and addition of free biotin blocked the endogenous biotin. Slides were counterstained with hematoxylin. Positive and negative controls were added on each automated immunohistochemical run. Negative controls consisted of the following: (1) slides run without the primary antibody and (2) tissues negative for MUC1, MUC2, MUC5AC, and MUC6 by in situ hybridization (eg, vessels, fat). Normal bronchus tissue was used as a positive control for MUC1 and MUC5AC, normal gastric mucosa for MUC2, and normal gastric mucosa for MUC5AC and MUC6.

Two pathologists (X.L. and L.D.) independently evaluated the staining. A consensual score was established on a double-headed light microscope.

Positive immunostaining was classified as diffuse when more than 50% of the cells were involved or focal when fewer than 50% were involved.

In Situ Hybridization

The protocol used for in situ hybridization was described in detail by Audié et al. The probes used were oligonucleotides encompassing the tandem repeat sequences of MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC6, and MUC7 (Eurogentec, Liège, Belgium). They were 3’-sulfur 35–labeled by terminal deoxynucleotidyl transferase (Amersham, Les Ullis, France). Silane-covered slides were deparaffinized, rehydrated, and incubated with 2 µg/mL of Proteinase K solution in a 0.1-mol/L concentration of tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.0, and a 50-mmol/L concentration of EDTA, pH 8.0, at 37°C for 15 minutes. After fixation in 4% paraformaldehyde solution in phosphate-buffered saline, pH 7.4, for 15 minutes, the slides were treated in 0.25% acetic anhydride and a 0.1-mol/L triethanolamine solution, pH 8.0, for 10 minutes and prehybridized in 4× sodium saline phosphate EDTA buffer (SSPE) containing 1× Denhardt buffer for 45 minutes. Hybridization was performed for 14 to 16 hours at 42°C in a 4× SSPE solution containing a 1.2-mol/L concentration of sodium phosphate buffer, pH 7.2, 0.1% N-lauroylsarcosine, 1× Denhardt buffer, 3 mg/mL of yeast transfer RNA, a 20-mmol/L concentration of dithiothreitol, and 50% formamide containing 0.75 × 10^6 disintegrations per minute per micro-liter of 35S-labeled oligonucleotide in moist chambers.

Posthybridization washes were performed successively in SSPE solutions of decreasing concentrations, and after a final wash in 0.1× SSPE at 42°C for 30 minutes, slides were dehydrated, dried, and dipped in LM-1 emulsion (Amersham). The sections were placed in a 4°C desiccation chamber for 3 weeks. They were developed and counterstained with methyl green pyronin (Sigma Chemical, St Louis, MO).

The controls consisted of competition studies with excess of unlabeled relevant and irrelevant oligonucleotides and examination of nonepithelial structures on the slides (vessels, fat) as negative controls. Adapted positive control tissues also were used.

Two pathologists (X.L. and L.D.) independently evaluated the results. Semiquantitative scoring was performed on a double-headed light microscope, according to the intensity of labeling as follows: −, absent; +, weak (visible at magnification ×200); ++, moderate (visible at ×100); ++++, intense (visible at ×25).

Results

Normal Kidney

Results for MUC1, MUC3, and MUC6 are summarized in Table 1.

By in situ hybridization, as early as 7.5 weeks of gestation, the probe for MUC1 gave a very weak and focal signal on the metanephric blastema Image 1. At 9.5 weeks, MUC1 was expressed in metanephric blastema, in the
ureteric buds, and in renal differentiated tubules weakly by in situ hybridization and more intensely by immunohistochemical analysis Image 2. No staining was seen in the proximal convoluted tubules and glomeruli. The immunostaining was membranous with an apical polarity in the renal tubules. The distribution of the immunostaining remained constant during fetal life. MUC1 also was expressed diffusely at 6 months of life, always with an apical pattern.

MUC3 by in situ hybridization was found to be expressed from 8 weeks in immature tubules with a moderate signal Image 3 and very weakly in metanephric blastema. After 13 weeks, MUC3 was present in the proximal renal convoluted tubules with moderate staining Image 4. The signal was detected until 6 months of life, always in the proximal convoluted tubules with a weak and focal intensity.

For MUC6, as early as 9.5 weeks, a weak signal was seen in the nephrogenic zone in the tips of ureteric buds by in situ hybridization and was more intense by immunohistochemical analysis Image 5. After this time, MUC6 was expressed constantly at the ends of the ureteric buds in

<table>
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<tr>
<th>Table 1</th>
<th>MUC1, MUC3, and MUC6 Expression in Normal Fetal Kidney*</th>
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<tr>
<td></td>
<td>MUC1</td>
</tr>
<tr>
<td></td>
<td>Gestational Period</td>
</tr>
<tr>
<td>7.5 wk</td>
<td>+ (MB)</td>
</tr>
<tr>
<td>8 wk</td>
<td>+ (UB)</td>
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<tr>
<td>9.5 wk</td>
<td>+ (UB)</td>
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<tr>
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<tr>
<td>11 wk</td>
<td>+ (UB, DT)</td>
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<tr>
<td>13 wk</td>
<td>+ (UB, DT)</td>
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<tr>
<td>18 wk</td>
<td>+ (DT, CD)</td>
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<tr>
<td>24 wk</td>
<td>+ (DT, CD)</td>
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<tr>
<td>30 wk</td>
<td>ND</td>
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<tr>
<td>39 wk</td>
<td>+ (DT, CD)</td>
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<td>6 mo</td>
<td>+ (DT, CD)</td>
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CD, collecting ducts; DT, distal tubule; IT, immature tubule; MB, metanephric blastema; ND, not done; PT, proximal renal tubule; UB, ureteric bud.

*For in situ hybridization, the intensity of labeling was scored as follows: –, absent; +, weak (visible at magnification ×200); ++, moderate (visible at ×100); ++++, intense (visible at ×25). For immunohistochemical analysis, positive immunostaining was classified as diffuse when more than 50% of the cells were involved or focal when fewer than 50% were involved.
nephrogenic zones. From 18 weeks, by in situ hybridization and immunohistochemical analysis, MUC6 also was expressed in the collecting ducts until 6 months of life but more weakly and focally after 30 weeks of gestation. MUC2, MUC4, MUC5AC, MUC5B, and MUC7 were not detected on renal structures.

**Malformed Kidney**

By immunohistochemical analysis, we observed that MUC1 was expressed diffusely in the epithelial cells covering all the cysts of ARPKD. It also was expressed in all renal dysplasias in the cysts and primitive ducts. By in situ hybridization, MUC3 was not detected in malformed cysts (ARPKD and dysplasia) but sometimes was present focally in residual proximal normal tubules between the cysts in renal dysplasia. No expression of MUC6 by in situ hybridization or immunohistochemical analysis was observed in malformed cysts of ARPKD or dysplasia. No expression of MUC2, MUC4, MUC5AC, MUC5B, or MUC7 was seen.
Discussion

Differential expression of human mucin genes in epithelial organs has been reported in the lung and digestive tract during development in mature as in immature cells. In the kidney, to date, no extensive study was available concerning the expression of mucin genes during renal formation.

Chambers et al reported high-level expression of MUC1 from 12.5 weeks until term in the collecting ducts of the kidney. In the present study, we observed expression of MUC1 in metanephric blastema and immature tubules as early as 7.5 weeks of gestation. In differentiated tubules, MUC1 is expressed strongly and diffusely in distal renal tubules and collecting ducts with an apical staining evident in immunohistochemical analysis. MUC1 expression remains constant and high throughout embryonic and fetal life. So, MUC1 may be suspected to be implicated in all stages of renal morphogenesis. Indeed, Hudson et al demonstrated that MUC1 transfection in Madin-Darby canine kidney cells induced polarization of tubular cells and cell structures mimicking tubulogenesis. MUC1 is a large glycoprotein, which by its intracytoplasmic tail interacts directly with β-catenin. However, β-catenin is a key protein for the formation of cell-cell junctions by interaction with E-cadherin. In adult kidneys, Leroy et al observed that MUC1 is expressed in normal distal convoluted tubules and in collecting ducts.

In the case of MUC3, we showed that MUC3 messenger RNA was detected in metanephric blastema and immature tubules at 8 weeks of gestation, but always with a weak signal. After 8 weeks, MUC3 expression was found in proximal convoluted renal tubules, but always at a very low level. In adult kidney, Leroy et al by in situ hybridization, demonstrated weak and focal expression of MUC3 restricted to the proximal convoluted tubules, which suggests a possible role for MUC3 for renal tubular differentiation. MUC3 is a membrane-bound mucin and is a member of a cluster of mucin genes located on 7q22 (MUC3, MUC11, MUC12, MUC17). But this family of genes is not well characterized. Two putative genes for MUC3 were proposed (MUC3A, MUC3B) with strong homologies (98%) but incompletely sequenced and characterized for their respective expression patterns; these findings need to be confirmed. Because of such similarity, the probe that we used for in situ hybridization would recognize transcripts from both genes.

Two epidermal growth factor (EGF)-like domains characterize MUC3 in the 3’ terminus extremity. Functions of these domains remain unknown, but such motifs are present in growth factors. A hypothesis is that MUC3 and MUC4 may be representing a reserve fund of EGF. Interestingly, EGF and transforming growth factor (TGF)-α have been implicated in renal tubular development by mediating epithelial branching and motility. Moreover antibodies raised against EGF receptors block the development of renal structures from the ureteric bud. EGF-like growth factors such as heparin-binding epidermal growth factor–like growth factor also are suspected to mediate epithelial-mesenchymal renal interactions.

In immunohistochemical analysis, diffuse apical staining is shown with MUC1 antibody in the cysts of autosomal recessive polycystic kidney disease (MUC1 antibody, ×400).

Immunohistochemical analysis reveals no expression of MUC6 in autosomal recessive polycystic kidney disease (MUC6, ×200).
Reid and Harris\(^1\) described MUC6 expression in fetal kidney. These authors studied 6 fetuses aged 13 to 23 weeks of gestation. They observed that MUC6 was expressed at 13 weeks’ gestation and was located in the ureteric bud epithelium and in some collecting ducts. At 23 weeks, the expression was similar, with an increase level of messenger RNA.\(^1\) In our series, we were able to detect MUC6 as early as 9.5 weeks of gestation in ureteric buds and in collecting ducts. We observed that the level of MUC6 expression was heterogeneous throughout renal maturation, with constant expression in ureteric buds and collecting ducts. MUC6 also was expressed weakly at 6 months of life. In the study by Reid and Harris,\(^1\) no expression of MUC6 by in situ hybridization or immunohistochemical analysis was seen in adult kidney. Leroy et al\(^8\) showed that MUC6 was detectable in adult kidney by reverse transcriptase–polymerase chain reaction, but no signal could be detected by in situ hybridization or immunohistochemical analysis, possibly reflecting a very low level of expression. MUC6 seems to be an important MUC gene during renal development because its expression is associated with events of tubulogenesis and branching and functional maturation of the kidney, and expression clearly decreases in adult mature kidney. MUC6 belongs to the cluster of mucin genes that is mapped on locus 11p15.5, encoding for mucins implicated in the biosynthesis of mucus gel. TGF-\(\beta\)-like domains are found in the structure of MUC6 apomucin.\(^20\) TGF-\(\beta\) also is expressed at the tips of the ureteric buds and is supposed to inhibit nephron formation and branching morphogenesis. So, TGF may participate in the balance of regulation between stimulation and inhibition of the growth of the ureteric bud.\(^21\) Thus, MUC6 might have a role in the mechanisms of induction and inhibition. In the lung, another organ known for the importance of branching and elongation events, another gene belonging to this cluster, MUC5AC, is seen at the tips of buds during organogenesis.\(^5\)

ARPKD is a genetic disease that leads to polycystic kidneys with renal failure. In ARPKD, cysts are developed from renal collecting tubules. Cyst formation is associated with abnormal differentiation and alterations of the basement membrane.\(^22\) Renal dysplasia is a disorder of metanephric differentiation associated with abnormal mesenchymal-epithelial transformations. It is characterized by architectural distortion, abnormal metanephric elements, and cysts of various sizes connected to dysplastic tubules within an undifferentiated tissue. Dysplastic tubules may represent abnormal collecting ducts.\(^23\)

In these renal malformations, we observed that only MUC1 expression was retained in the epithelium of cysts. MUC1, the earliest MUC gene to be expressed during renal morphogenesis, seems to be expressed independently of these malformative processes. In contrast, expression of MUC6, the latest gene to be activated, was lost in these malformations, while it is expressed in the normal collecting ducts. Therefore, these findings may suggest a possible dysregulation of MUC6 during nephrogenesis.

The present results demonstrate the expression of human mucin genes (MUC1, MUC3, and MUC6) during all the stages of the formation of fetal kidney, suggesting a role in the renal maturation and differentiation processes. They also clearly suggest a role for 11p15 MUC genes during specific stages of morphogenesis of epithelial organs, even when these organs need no protection by a secreted mucus film.

References


