Ribonuclease 7 is a potent antimicrobial peptide within the human urinary tract

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Although the urinary tract is constantly challenged by microbial invasion, it remains free from colonization. Although little is known about how the urinary tract maintains sterility, the presence of antimicrobial peptides (AMPs) in the urine suggests that they may play a role in its protection from infection. Ribonuclease 7 (RNase 7) is a potent AMP that was first identified in the skin. Here, we characterize the expression and relevance of RNase 7 in the human kidney and urinary tract. Using RNA isolated from healthy human tissue, we performed quantitative real-time PCR and found basal *RNASE7* expression in kidney and bladder tissue. Immunohistochemical and immunofluorescent analysis localized RNase 7 to the urothelium of the bladder, ureter, and the intercalated cells of the collecting tubules. In control urine samples from healthy individuals, the concentration of RNase 7 was found to be in the low micromolar range; very abundant for an AMP. Antibacterial neutralization assays showed that urinary RNase 7 has potent antimicrobial properties against Gram-negative and Gram-positive uropathogenic bacteria. Thus, RNase 7 is expressed in the human kidney and urinary tract and it may have an important antimicrobial role in maintaining tract sterility.

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The urinary tract, except for the urethral meatus, is usually sterile despite its proximity with fecal flora. The precise mechanism by which the urinary tract maintains sterility is not well understood.[1] Recently, antimicrobial peptides (AMPs) have been shown to have an important role in innate immunity.[2] AMPs are a ubiquitous component of innate immunity produced by epithelial cells or hematopoietic cells. AMPs are mainly cationic proteins that possess antimicrobial activity against bacteria, enveloped viruses, fungi, and some protozoa. They may be constitutively expressed and/or induced by invading pathogens.[3–5] Although many AMPs have been described in other organ systems, only a few AMPs have been studied in the human urinary tract.[5] In this study, we describe the expression and function of ribonuclease 7 (RNase 7), a novel AMP in human kidney tissue and the urinary tract.

Harder and Schröder[6] first identified RNase 7 as an abundant protein in the human epidermis while examining protein extracts of normal skin for antimicrobial activity. Subsequent studies demonstrated that *RNASE 7* is expressed in other organs, including the liver, gastrointestinal tract, heart, skeletal muscle, and respiratory tract. *RNASE 7* expression was noted in the kidney, although the extent of its expression and precise location were not characterized.[6,7] Although the mechanisms for the antimicrobial properties of RNase 7 are not completely understood, its bactericidal activity has been linked to its capacity to permeate and disrupt the bacterial membrane, independent of its ribonuclease activity.[4,7–12] Consequently, RNase 7 has potent antimicrobial activity against Gram-negative bacteria, Gram-positive positive bacteria, and yeast.[6,13–16] It has been stated that, on a per molar base, RNase 7 is one of the most potent human AMPs that has been described, with antibiotic concentrations ranging in the low micromolar range.[4]

RESULTS

Human kidney and bladder tissue express high levels of *RNASE7* mRNA

In the bladder, mean *RNASE7* expression was 117,530 ± 1880 transcripts per 10 ng RNA. *RNASE7* expression was significantly greater in the bladder than within the kidney ($P = 0.0003$). In the kidney, *RNASE7* expression was analyzed separately in the cortex, medulla, and pelvis. All tissue sections tested expressed *RNASE7* (Figure 1). *RNASE7* expression was greatest in the renal pelvis compared with other parts of the kidney, with a mean *RNASE7* expression of 1395 ± 65 transcripts per 10 ng RNA. *RNASE7* expression was significantly greater in the renal pelvis than the renal cortex and medulla ($P = 0.0015$).

RNase 7 is expressed throughout the human kidney and urinary tract

To investigate the distribution of RNase 7 in healthy human kidney, ureter, and bladder tissue, we performed immunohistochemistry (IHC) and immunofluorescence using antibodies directed against RNase 7. RNase 7 immunoreactivity was present throughout the urothelium of the ureter and bladder of all investigated specimens ($n = 4$; Figure 2). RNase 7 was also expressed in the renal cortex and renal medulla of all specimens (Figure 3). RNase 7 showed cell-specific expression in the cortical and medullary collecting tubules. The glomeruli, proximal tubules, and loops of Henle did not show RNase 7 staining. Negative controls showed no RNase 7 immunoreactivity (Figures 2 and 3).

RNase 7 is expressed in a specific subset of cells in the human collecting tubule

Expression of RNase 7 in individual cell types of the collecting tubule was performed using markers for principal cells (aquaporin-2), α-intercalated cells (anion exchanger-1

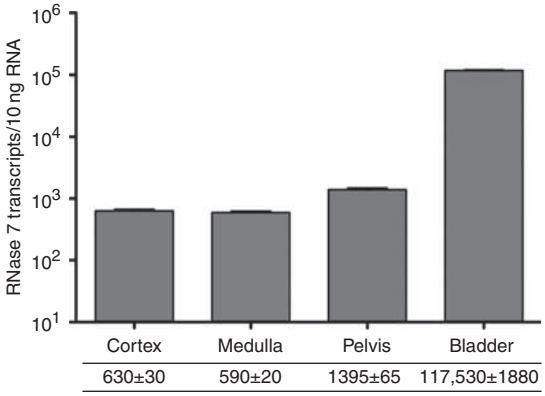

Figure 1 | Abundant expression of ribonuclease 7 (*RNASE 7*) in human kidney and bladder. Transcript levels quantified by real-time PCR in the kidney and bladder. Shown are the results of three independent samples. On the line below, the mean transcript levels are shown with the s.e.m. *RNASE 7* expression was significantly greater in the bladder than the kidney (cortex, medulla, and pelvis; $P = 0.003$). *RNASE 7* expression was significantly greater in the renal pelvis than the cortex and medulla ($P = 0.0015$).

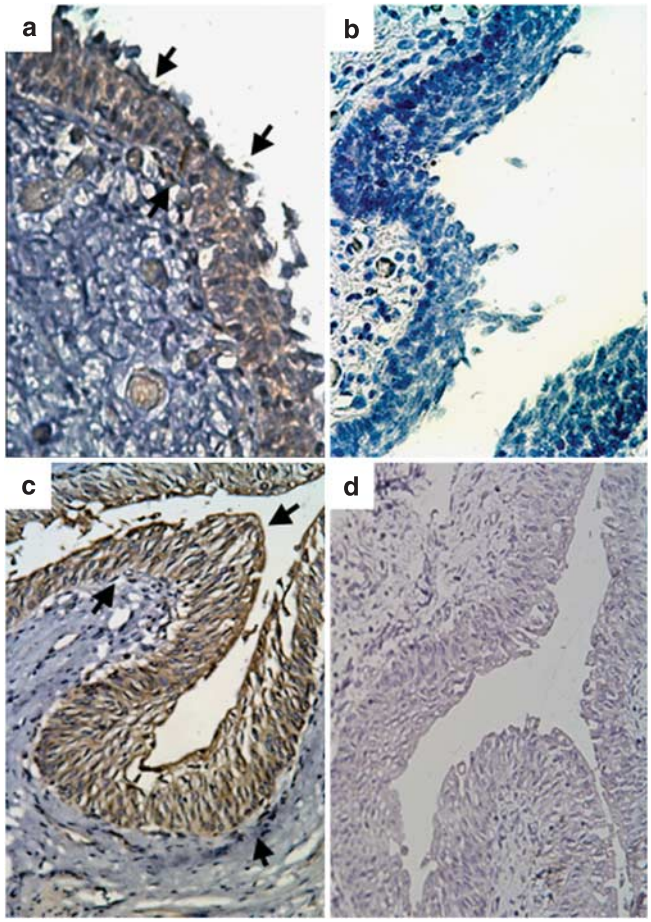

Figure 2 | Ribonuclease 7 (RNase 7) is expressed throughout the urothelium of the ureter and bladder. Immunohistochemistry (IHC) demonstrates RNase 7 expression (brown/arrows) throughout the urothelium of the (**a**) human bladder and (**c**) ureter. Negative control (**b**) bladder and (**d**) ureter. Original magnification × 40.

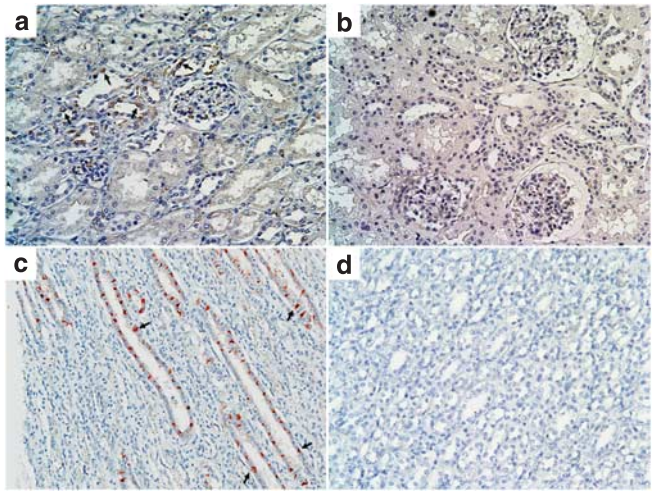

Figure 3 | Ribonuclease 7 (RNase7) shows cell-specific expression throughout the human kidney. Immunohistochemistry demonstrates isolated cell-specific expression (arrows) in the collecting tubule of (**a**) human kidney cortex and (**c**) medulla. Negative control (**b**) cortex and (**d**) medulla. Original magnification × 20.

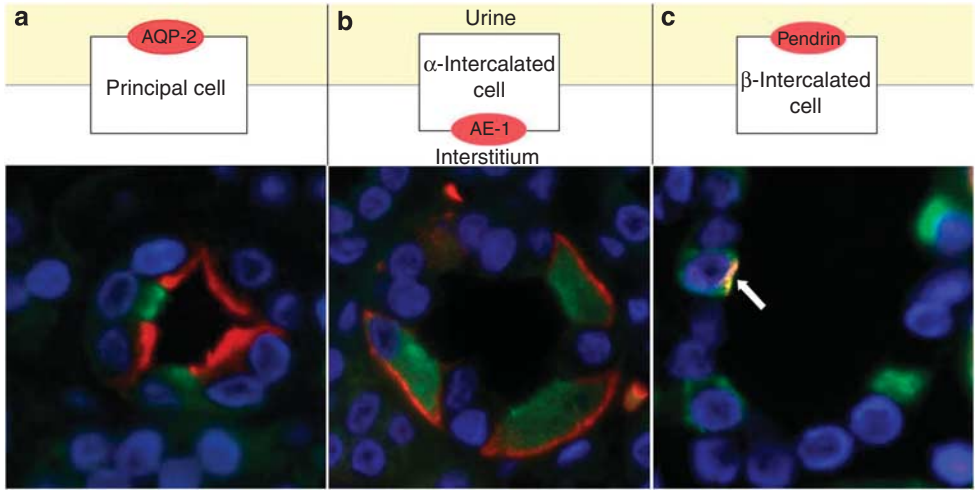

Figure 4 | Cell-specific expression of ribonuclease 7 (RNase 7) in the renal collecting tubule. Human kidney was labeled for RNase 7 (green), nuclei (blue), and cell type markers (red). Cell type markers consisted of aquaporin-2 (AQP-2) for principal cells, anion exchanger-1 (AE-1) for α-intercalated cells, and pendrin for β-intercalated cells. Isolated cells positive for RNase7 were identified in the collecting tubule. (**a**) Principal cells (apical red staining) were negative for RNase 7 (green). (**b**) RNase 7 (green) was expressed by α-intercalated cells (red basolateral AE-1 staining). (**c**) RNase 7 (green) and pendrin (red) colocalized in β-intercalated cells to demonstrate apical yellow staining (arrow). Original magnification × 100.

(AE-1)), and β-intercalated cells (pendrin) (Figure 4 and Supplementary Figure S1 online). The α- and β-intercalated cells expressed RNase 7, but principal cells did not. Occasionally, pendrin-positive and AE-1-positive cells did not demonstrate RNase 7 expression. Negative controls showed no RNase 7 expression.

RNase 7 exists in human urine in measurable titers

Immunoblot analysis of cationic RNase 7 protein extracted from urine specimens identified an immunoreactive peptide that migrated to 14.5 kDa (data not shown).[6] Enzyme-linked immunosorbent assay (ELISA) demonstrated that RNase 7, normalized to urine creatinine, was present in all urine samples with concentrations ranging from 5.60 to 20.0 ± 0.92 μg/mg Cr (235–3467.2 μg/l), which corresponds to 0.17–0.3 μmol/l (Figure 5).

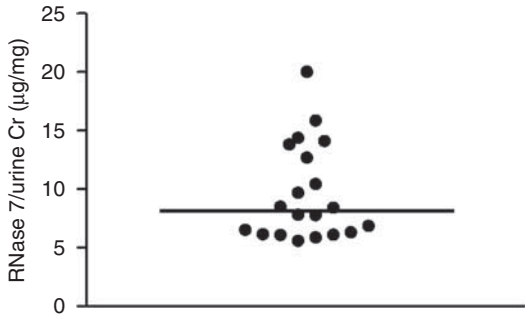

Figure 5 | Ribonuclease 7 (RNase 7) is present in urine. Urinary RNase 7 expression standardized to urine creatinine. Shown are the enzyme-linked immunosorbent assay (ELISA) results from 20 healthy controls.

RNase 7 displays antibacterial properties in human urine

To determine if RNase 7 has antibacterial effects in human urine, we inoculated urine samples from healthy individuals with either uropathogenic *Escherichia coli*, *Pseudomonas*, *Enterococcus*, *Klebsiella*, or *Proteus mirabilis* (Figure 6 and Supplementary Figure S2 online). Next, we analyzed whether the addition of antibodies directed against RNase 7 would neutralize the antimicrobial activity of RNase 7 and lead to enhanced bacterial growth. RNase 7 antibodies inhibited the action of RNase 7 as bacterial growth of Gram-negative bacteria and *Proteus* significantly increased (Figure 6 and Supplementary Figure S2 online). The presence of equivalent concentrations of irrelevant antibodies (derived from goat pre-immune serum) had no effect on bacterial growth.

Because the pH of human urine is highly variable, we used this approach to determine the effective pH range for the antimicrobial effects of RNase 7 in urine. Urine samples from healthy individuals were buffered to pH 5.0, 7.0, or 9.0. Bacterial growth was monitored (optical density (OD) 600 nm) in the presence or absence of antibodies directed against RNase 7. We observed significant bacterial growth at all pH values tested, although growth was impeded in alkaline conditions (Figure 6 and Supplementary Figure S2 online). Enterococcus demonstrated very little growth under all conditions (Supplementary Figure S2 online). These results are consistent with previous reports on the influence of pH on bacterial growth.[17]

The application of antibodies directed against RNase 7 resulted in increased bacterial growth under most urinary pH values tested. However, this phenomenon was not uniform under all conditions (Figure 6 and Supplementary Figure S2 online).

DISCUSSION

Previous studies have demonstrated that RNase 7 is an important AMP in skin, hair follicles, and the oral cavity.[6,16,18,19] In this study, we demonstrate that RNase 7 is

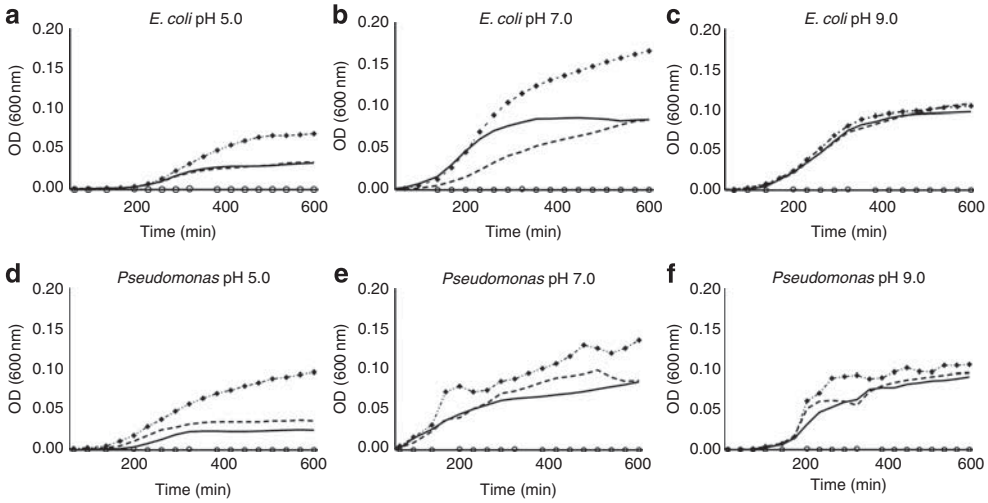

Figure 6 | Ribonuclease 7 (RNase 7) contributes to impaired growth of *Escherichia coli* and *Pseudomonas* in human urine. The antibacterial properties of RNase 7 were measured as changes in the turbidity of cultured human urine using the absorbance at 600 nm (OD_{600}). Human urine samples were inoculated at various urinary pH conditions with (**a**–**c**) *E. coli* or (**d**–**f**) *Pseudomonas* as shown by the solid black line. The open circles represent noninoculated urine samples. Addition of irrelevant antibody to inoculated urine samples (dashed line) did not affect bacterial growth. Blocking of RNase 7 activity, with the addition of RNase 7 antibody (dashed line with diamonds), resulted in increased growth of all bacterial isolates.

a novel AMP expressed in the human urinary tract. Our results demonstrate that RNase 7 is constitutively expressed in the mature human kidney, ureter, and bladder. Using quantitative real-time PCR and ELISA, we demonstrate that RNase 7 activity is greatest in the bladder and renal medulla. Specifically, immunohistochemical labeling demonstrates that RNase 7 is expressed throughout the urothelium of the lower urinary tract and the intercalated cells of the collecting tubules. These results suggest that intercalated cells have a novel role in innate immunity. Finally, we identify high concentrations of RNase 7 in the urine and demonstrate that RNase 7 has urinary antimicrobial activity against a variety of uropathogenic bacteria.

Our quantitative real-time PCR results demonstrate that *RNASE7* is expressed at high levels throughout the urinary tract. The basal uroepithelial expression of *RNASE7* is greater than the expression of previously described urinary tract AMPs like cathelicidin, human β-defensin 1 (hBD-1), and hBD-2.[20–23] Furthermore, our results indicate that renal *RNASE7* expression is comparable with *RNASE7* expression in keratinocytes.[16] When comparing *RNASE7* expression in the bladder with *RNASE7* expression in the skin, our results demonstrate that the bladder expresses nearly 100 times more *RNASE7* than primary keratinocytes.[16] High expression levels may be required in the bladder to produce bactericidal titers of RNase 7 because it is constantly excreted into and/or diluted by urine.

Our quantitative real-time PCR results also demonstrate that *RNASE7* expression increased from the upper urinary tract to the lower urinary tract—following the flow of the urinary stream. Similarly, immunostaining demonstrated that RNase 7 expression is more homogeneous throughout the lower urinary tract. IHC demonstrated cell-specific RNase 7 expression in a minority of isolated cells in the cortical and medullary collecting tubules before becoming more uniform throughout the uroepithelium of the ureter and bladder. RNase 7 was not expressed in the glomeruli, proximal tubules, loops of Henle, or interstitium. This expression pattern differs from the expression of other AMPs that have been described in the kidney. For example, hBD-1, hBD-2, and cathelicidin do not show cell-specific expression, and they have limited or no intracellular expression.[20–22,24,25]

Because RNase 7 protein expression is limited to a minority of cells in the renal cortex and medulla, only a small percentage of cells account for the relatively high *RNASE7* expression levels, especially when compared with more homogenous epithelial organs like the epidermis. Overall, both real-time PCR and IHC indicate that RNase 7 expression is present in locations where microbial exposure occurs most frequently. A similar expression pattern has been described in the epidermis and in hair follicles. In the skin, RNase 7 expression is greatest in the uppermost epidermal layers, where microbial insult most likely occurs. Likewise, in hair follicles RNase 7 expression is greatest in the outer root sheath.[14,19]

Our results indicate that α- and β-intercalated cells constitutively express RNase 7. Historically, intercalated cells have been shown to play important roles in the regulation of acid/base homeostasis. Intercalated cells account for one-third of the cells within the cortical and medullary collecting tubules.[26–28] Given their physiological position in the collecting tubule, intercalated cells are ideally positioned to defend the kidney from ascending urinary tract infections (UTIs) as they are one of the initial cell types encountered by ascending microbes before they infiltrate the renal parenchyma. The identification of RNase 7 in both subtypes of

intercalated cells defines a new role for these cells, indicating that they are critical for the production and secretion of AMPs into the urine.

Our ELISA results indicate that RNase 7 is secreted into the urine. Given the size of RNase 7 (14.5 kDa), it is possible that some urinary RNase 7 peptides originate, at least in part, from plasma filtrate. However, there is little evidence suggesting that RNase 7 persists in the plasma.[7] Additionally, to persist in the urine, RNase 7 would need to escape the efficient peptide absorption mechanisms in the proximal tubule.[29,30] Finally, the urine samples underwent centrifugation before processes, removing cellular sources of RNase 7. This finding suggests that the predominant source of urinary RNase 7 most likely originates from local production by intercalated cells of the distal nephron and the urothelium of the bladder and ureters.

In our study of 20 healthy individuals, constitutive urinary RNase 7 protein concentration was between 5.6 and 20.0 μg/mg Cr (0.17–0.3 μmol/l). When comparing urinary RNase 7 concentrations with the concentrations of other urinary AMPs (that is, cathelicidin, hBD-1, and hBD-2), RNase 7 concentrations are much greater. hBD-2 is not constitutively secreted in the urine, whereas median urinary concentrations of cathelicidin and hBD-1 are 1.6×10^{-5} and 2.5×10^{-5} μmol/l, respectively.[20,21,23,25] Constitutive RNase 7 expression at these concentrations shows potent antimicrobial activity against several pathogenic microbes, including *Pseudomonas aeruginosa, Staphylococcus aureus*, and vancomycin-resistant *Enterococcus faecium*.[6,15,31,32]

As observed in other systems, RNase 7 demonstrates antimicrobial activity in the urine. When the antimicrobial activity of RNase 7 was inhibited with antibodies directed against RNase 7, bacterial growth of *E. coli*, *Pseudomonas*, *Klebsiella*, and *Proteus* significantly increased. This effect was observed at all urinary pH values, but was less pronounced under alkaline urine conditions. This phenomenon may be secondary to decreased activity of RNase 7 at a higher pH, or it may reflect differential expression of microbial proteins that are important for RNase 7 antimicrobial activity. The effect of urine pH on RNase 7 activity and bacterial gene expression are currently under investigation. Overall, these data suggest that RNase 7 is involved in maintaining urine sterility, which is consistent with the proposed role of RNase 7 in innate immunity and antimicrobial defense.[4,13,16]

In conclusion, this is the first study to identify and evaluate RNase 7 in the human kidney and urinary tract. Our results suggest that RNase 7 is an epithelial-derived AMP that plays an important role in the innate immunity of the human uroepithelium. We demonstrate that RNase 7 contributes to host defense against Gram-negative and Gram-positive bacteria. Because RNase 7 production occurs in intercalated cells of the distal nephron, we believe these cells are important in the production of AMPs. Further evaluation of human epithelial AMPs, like RNase 7, may lead to the development of new treatment strategies for antibiotic-resistant UTIs.

MATERIALS AND METHODS

Study approval

The Nationwide Children's Hospital (NCH) institutional review board approved this study (IRB-07-00383). Uropathogen bacterial isolates were obtained from patients seeking treatment for UTIs at NCH as approved by the institutional review board (IRB-06-00603).

Human tissue and urine samples

Human kidney and ureter tissue was obtained through the NCH Department of Pathology. Tissue samples were obtained from pediatric patients undergoing nephrectomy for Wilm's tumor. Tissue samples were free of microscopic signs of disease or inflammation. The tissue was preserved as neutral formalin-fixed paraffin-embedded sections and dissected into cortex, medulla, or renal pelvis before storage. Human bladder uroepithelium, snap frozen in liquid nitrogen, was obtained from children undergoing ureteral re-implantation for reasons other than recurrent infection. Three bladder tissue specimens were obtained.

Urines samples ($n = 20$) were obtained from healthy volunteers with no history of UTI. The urine samples were centrifuged to remove urine sediment and protease inhibitor cocktail was added (Thermo Scientific, Rockford, IL).

Ribonucleic acid isolation and reverse transcription

Total RNA was isolated from frozen tissue using the Promega Total RNA Isolation System (Promega, Madison, WI). For cDNA synthesis, 4–8 μg of total RNA was reverse transcribed with Superscript III reverse transcriptase using an oligo-$(dT)_{12-18}$ primer according to the supplier's protocol (Invitrogen, Carlsbad, CA). A single cDNA preparation from each specimen was used for the assay of all antimicrobial products tested.

Cloning of gene-specific plasmids for standard curves

The cDNAs encoding *RNase7* and GAPDH (glyceraldehyde 3-phosphate dehydrogenase) were cloned into a 4-Topo plasmid vector (Invitrogen) according to the manufacturer's instructions. Plasmids were sequenced to confirm that the correct constructs were obtained. Serial dilutions of gene-specific plasmids were quantitated (by both spectrophotometric absorbance at 260 nm and ethidium bromide staining agarose gel electrophoresis with DNA standards) and then used in real-time PCR to generate standard curves for each reaction.

Real-time PCR

Total cellular RNA was extracted from the collected specimens using SV Total RNA Isolation System (Promega) according to the manufacturer's protocol. Real-time PCR was performed using single-stranded cDNA from human kidney and bladder tissue with specific oligonucleotide primer pairs using the 7500 Real-Time PCR System (Applied Biosystems, Carlsbad, CA) equipped with a fluorescence detection monitor. PCR intron spanning primers were selected using previously published standards and sequences were confirmed using DNAstar Laser Gene SeqBuilder (RNase 7 forward primer: 5′-GGAGTCACAGCACGAAGACCA-3′ and RNase 7 reverse primer 5′-CATGGCTGAGTTGCATGCTTGA-3′).[16]

Briefly, cDNA corresponding to 10 ng RNA served as a template in a 25 μl reaction containing 75 nmol/l of each primer, and 1 × Light-Cycler-Fast Start DNA Master SYBR Green mix. The PCR conditions were: initial denaturation at 95 °C for 10 min, followed by 40 cycles with each cycle consisting of denaturation at 94 °C for

30 s, annealing at 62 °C for 30 s, and extension at 72 °C for 30 s. The cycle-to-cycle fluorescence emission was monitored at 530 nm and analyzed using 7500 Software V2.0.3 (Applied Biosystems). Gene-specific plasmid standards were included with every set of reactions. Absolute transcript levels are shown per 10 ng total RNA.

RNase 7 antibodies

Antibodies against RNase 7 were commercially purchased (Abcam, Cambridge, MA; Novus Biologicals, Littleton, CO; and Sigma-Aldrich, St Louis, MO). According to the manufacturer, the Novus and Sigma antibodies do not crossreact with other proteins. The Abcam antibody does have some crossreactivity between RNase 7 and RNase 8. However, previous studies indicate that human RNase 8 expression is limited to the placenta and absent in the kidney.[33]

ELISA

Flat-bottomed, 96-well plates (Maxisorb, Nunc, Rochester, NY) were coated overnight at 4 °C with polyclonal antibody to RNase7 (3 μg/ml; Abcam). After blocking with synthetic blocking buffer (Kem-En-Tec Diagnostics, Taastrup, Denmark) for 2 h at room temperature, standards and samples were added to the wells and incubated for 2 h at room temperature. Serial dilutions of recombinant RNase 7 protein served as the standards (Novus Biologicals). Following incubation with a different biotinylated (Lightning-Link Biotin Antibody Labeling Kit, Novus Biologicals) polyclonal antibody to RNase7 (2 μg/ml; Novus Biologicals) for 2 h at room temperature, streptavidin-horseradish peroxidase (Biolegend, San Diego, CA) was added for 30 min. After incubation with TMB substrate solution for 15 min (Kem-En-Tec Diagnostics), the reaction was terminated with STOP solution (Cell Signaling Technology, Danvers, MA) and read at a wavelength of 450 nm and with 570-nm background subtraction. The detection limit of the ELISA was 0.1 ng/ml.

Results from the ELISA assay were divided by urine creatinine to establish standardized urine RNase 7-to-creatinine ratios (μg/mg) to account for urine dilution. Urine creatinine concentrations were determined using the Oxford Biomedical Research creatinine microplate assay (Rochester Hills, MI).

Immunoblot analysis

Urinary proteins were extracted from human urine samples using the Proteospin Urine Protein Concentration Micro Kit according to the manufacturer's instructions (Norgen Biotek Corporation, Thorold, ON, Canada). The urine protein samples were mixed with Laemmli sample buffer and incubated at 95 °C for 5 min. The samples were loaded onto 18% sodium dodecyl sulfate gradient gels and subjected to electrophoresis (applied constant voltage of 100 V). After the peptide/protein separation, the material in the gel was transferred to nitrocellulose by application of 100 V for 90 min. The membranes were blocked in 2% fat-free milk and incubated with the rabbit polyclonal RNase 7 antibody (Abcam) in phosphate-buffered saline with 2% fat-free milk overnight at 4 °C. This was followed by incubation with the secondary antibody, a monkey horseradish peroxidase-conjugated anti-rabbit IgG diluted 1:8000 in phosphate-buffered saline with 5% fat-free milk, for 1 h at room temperature. The proteins and peptides were visualized using an ECL detection system and chemiluminescence film according to the manufacturer's instructions (BioExpress, Kaysville, UT).

Immunohistochemistry

IHC was performed on human kidney specimens to evaluate RNase 7 expression at the cellular level. Following deparaffinization and rehydration, antigen retrieval was performed in a pressure cooker for 20 min using 0.01 mol/l citrate buffer (pH 6.0). This step was followed by a biotin block and a serum-free protein block (Superblock, ScyTek Laboratories, Logan, UT). The slides were incubated overnight at 4 °C with polyclonal rabbit RNase 7 antibody (Sigma-Aldrich) diluted 1:50 in phosphate-buffered saline containing 3% fetal bovine serum followed by anti-polyvalent biotinylated antibody (anti-mouse, rat, rabbit, guinea pig) and UltraTek Streptavidin/HRP (ScyTek Laboratories).

Sections were developed using 0.1% diaminobenzidine tetrachloride (Arcos Organics, Geel, Belgium) with 0.02% hydrogen peroxide and counterstained with hematoxylin. Negative controls sections were incubated with nonimmune serum in place of RNase7 antibody.

Immunofluorescence

Double-labeled immunofluorescence was performed to help localize RNase 7 expression in the kidney. Sections were double labeled for principal cells with goat polyclonal anti-human aquaporin-2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

Sections were double labeled for intercalated cells with mouse IgG_1 monoclonal anti-human AE-1 antibody (gift from M Jennings) or mouse IgG_1 monoclonal anti-human pendrin antibody (Medical and Biological Laboratories, Naka-ku Nagoya, Japan). The α-intercalated cells show basolateral AE-1 expression and β-intercalated cells show apical pendrin expression.[28] Rhodamine donkey polyclonal anti-goat (Jackson ImmunoResearch Laboratories, West Grove, PA), rhodamine goat anti-mouse (Jackson ImmunoResearch Laboratories), and fluorescein isothiocyanate donkey polyclonal anti-rabbit (Santa Cruz) served as the secondary antibodies.

All sections were prepared as outlined above. They were incubated with a mixture of antisera against RNase 7 (1:50; Sigma-Aldrich) and antisera against AE-1 (1:50), pendrin (1:50), or aquaporin-2 (1:200) at room temperature for 90 min. The secondary antibody was applied for 1 h at room temperature and the sections were mounted using mounting media with 4,6-diamidino-2-phenylindole. Nonimmune serum was used as a negative control.

The slides were examined with a Leica DM4000B microscope (Wetzlar, Germany) and digitally photographed using the ×100 objective and Spot RT camera/software (Diagnostic Instruments, Sterling Heights, MI). The final images were processed with Adobe Photoshop software (Adobe Systems, San Jose, CA).

Antimicrobial neutralization assay

RNase 7 antimicrobial activity against uropathogenic *E. coli* (UTI-89), *P. aeruginosa* (PEDUTI-61), *Enterococcus* (PEDUTI-39), *Klebsiella* (PEDUTI-65), and *Proteus mirabilis* (PEDUTI-44) was evaluated in human urine. These bacterial strains were isolated from positive urine cultures of patients at NCH. In brief, the bacteria were cultured at 37 °C overnight in Luria-Bertani broth to saturation. Bacteria (1 μl) was added to 100 μl a healthy individual in a 96-well flat bottom plate (Thermo Scientific, Nunc, Worcester, MA). To each well, 10 μg of anti-RNase 7 antibody (Novus) or equivalent concentrations of irrelevant antibody (derived from preimmune goat serum) were added.[16]

Bacterial growth was monitored using a Synergy HT multi-mode microplate reader (BioTek Instruments, Winooski, VT) at a final volume of 101 μl. The turbidity of the culture was measured and recorded at $t = 0$ and every 10 min thereafter for 10 h using the absorbance at 600 nm (OD_{600}). Sterility of urine was validated by incubation in the absence of bacterial inoculation. The assays were

performed at a urinary pH of 5.0, 7.0, and 9.0. The urinary pH was adjusted by the titration of 0.1 N hydrochloric acid or sodium hydroxide.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Figure S1. Cell-specific expression of RNase 7 in the renal collecting tubule.

Figure S2. RNase 7 contributes to impaired bacterial growth in human urine.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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