Dysregulated apoptosis of renal tubular epithelial cells (RTEC) is an important component of the pathogenesis of several renal diseases, including HIV-associated nephropathy (HIVAN), the most common cause of chronic kidney failure in HIV-infected patients. In HIVAN, RTEC become infected by HIV-1 in a focal distribution, and HIV-1 infection has been shown to induce apoptosis in vitro. In microarray studies that used a novel renal tubular epithelial cell line from a patient with HIVAN, it was found that the ubiquitin-like protein FAT10 is one of the most upregulated genes in HIV-infected cells. Previously, FAT10 was shown to induce apoptosis in murine fibroblasts. The expression of FAT10 in HIVAN and the ability of FAT10 to induce apoptosis in human RTEC therefore were studied. This study revealed that FAT10 expression is induced after infection of RTEC by HIV-1 and that expression of FAT10 induces apoptosis in RTEC in vitro. Moreover, it was found that inhibition of endogenous FAT10 expression abrogated HIV-induced apoptosis of RTEC. Immunohistochemical studies demonstrated increased FAT10 expression in a murine model of HIVAN, in HIVAN biopsy samples, and in autosomal dominant polycystic kidney disease, another renal disease that is characterized by cystic tubular enlargement and epithelial apoptosis. These results suggest a novel role for FAT10 in epithelial apoptosis, which is an important component of the pathogenesis of many renal diseases.

ubiquitin-independent degradation of proteins via the proteasome (12). Because FAT10 has putative roles in regulating apoptosis and cell-cycle progression (13,14), we studied FAT10 expression in the HIV-1 transgenic model of HIVAN and in HIVAN biopsy specimens. Given that ADPKD is also characterized by renal tubular dilation and dysregulated apoptosis (15,16), we examined the expression of FAT10 in ADPKD. We report here that FAT10 expression is increased in renal epithelial cells in HIVAN and ADPKD, that in vitro expression of FAT10 induces apoptosis in human renal tubular epithelial cells (RTEC), and that inhibition of FAT10 expression prevents HIV-induced apoptosis in RTEC.

Materials and Methods

Tissue Specimens

The HIV-1 transgenic mouse model of HIVAN has been characterized extensively. Heterozygous transgenic mice develop proteinuria, progressive renal failure, and histologic renal disease that is identical to HIVAN (4,17,18). HIVAN biopsy tissue was collected at the time of diagnostic renal biopsy under a protocol that was approved by the Mount Sinai Institutional Review Board. ADPKD tissue sections were obtained from nephrectomy specimens that were procured by National Disease Research Interchange and Polycystic Kidney Disease Foundation. All tissues were fixed in 4% paraformaldehyde/PBS and embedded in paraffin.

Generation of the Human Proximal Tubular Cell Line

Primary human proximal tubular cells (HPT) were grown from a HIVAN biopsy specimen by mincing of a portion of a fresh cortical renal biopsy sample and plating onto collagen-coated tissue culture plates in media selective for growth of proximal tubular cells according to previously published methods (19). A subpopulation of these cells (subsequently named HPT-1) was conditionally immortalized by infection with vesicular stomatitis virus (VSV)–temperature-sensitive SV40 large T-antigen (tsTag)-IRES-Hygro, a VSV-glycoprotein (VSV-G) pseudotyped replication defective lentivirus encoding the SV40 temperature-sensitive large T antigen under the control of the immediate early cytomegalovirus (CMV) promoter (Figure 1a). For all subsequent studies, HPT-1 cells were expanded at 33°C until they reached 80% confluence and subsequently cultured at 37°C for 14 d to induce T antigen degradation and cellular differentiation.

Viral Transduction

To conditionally immortalize proximal tubular epithelial cells (PTEC), we developed a lentiviral vector encoding the tsTag allele tsA58U19 (20). tsTag was subcloned into pIRES-hyg (Clontech, Palo Alto, CA). A fragment that included the CMV promoter tsTag and hygromycin-resistance genes was subsequently subcloned into pHR/CMV-IRES2-EGFP-ΔB (gift of Dr. James C. Mulloy, Memorial Sloan Kettering Cancer Center, New York, NY), resulting in the vector pHRS-tsTag-IRES-hygro (Figure 1a).

pNL4–3:ΔG/P-EGFP, a gag/pol-deleted HIV-1 construct that contains enhanced green fluorescence protein (EGFP) in the gag open reading frame (Figure 1b) (21) and pHRS-ΔG-EGFP (EGFP control; Figure 1c), were used to generate VSV-G–pseudotyped virus for infection of HPT-1 cells as described previously (21). A multiplicity of infection (MOI) of 0.5 was used to infect HPT-1 cells because higher MOI resulted in excessive cytotoxicity at 3 and 7 d after infection.

Figure 1. Constructs used in these studies. pHR′–temperature-sensitive SV40 large T-antigen (tsTag)-IRES-hygro (a) was used to produce lentivirus to conditionally immortalize proximal tubular epithelial cells (PTEC). pNL4–3:ΔG/P-EGFP (P-EGFP; b) encodes a gag/pol-deleted HIV-1 provirus with enhanced green fluorescent protein (EGFP) cloned into the gag open reading frame. pNL4–3:ΔG/P-EGFP and pHR′-IRES-EGFP (c) were used to generate VSV-G–pseudotyped lentiviruses for infection of human proximal tubular-1 (HPT-1) cells. (d) pVirDH/E is a lentiviral vector that allows production of short hairpin RNA (shRNA) in transduced cells. (e) pV-VPW/BE-FAT10 is a bicistronic vector that expresses FAT10 and EGFP as separate proteins. (f) pcDNA4-FAT10 expresses FAT10 with N-terminal histidine and Xpress epitope tags.

Characterization of HPT-1 Cells

HPT-1 cells were grown and differentiated on glass coverslips that had been coated with type 1 rat tail collagen (BD Biosciences, San Jose, CA). Cells were fixed in 4% paraformaldehyde/PBS. Endogenous alkaline phosphatase activity was detected by incubating cells in 10 mM Tris (pH 9.0) with 0.3 mM 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium (Roche, Indianapolis, IN). For immunocytochemical detection of aquaporin-1 and cytokeratin, HPT-1 cells were permeabilized with 0.1% Triton X-100. Primary antibodies that were used to characterize HPT-1 cells included anti-aquaporin-1 (AQP11-S), anti-aquaporin-2 (AQP21-A; Alpha Diagnostics, San Antonio, TX), anti-cytokeratin (V2258; Sigma, St. Louis, MO), anti–smooth muscle myosin (MAB3568; Chemicon, Temecula, CA), and Tamm-Horsfall protein (55140; Cappel Laboratories, Westchester, PA). Primary antibodies were detected using Cy3-labeled goat anti-rabbit IgG or Cy3-labeled goat anti-mouse IgG (KPL, Gaithersburg, MD) diluted 1:100.

Analysis of FAT10 RNA Expression in HPT-1 Cells and Kidneys from HIV-1–Transgenic Mice

Total RNA was harvested from HPT-1 cells after infection with VSV-NL4–3:ΔG/P-EGFP or VSV-HR′-IRES-EGFP and from kidneys

Materials and Methods

Tissue Specimens

The HIV-1 transgenic mouse model of HIVAN has been characterized extensively. Heterozygous transgenic mice develop proteinuria, progressive renal failure, and histologic renal disease that is identical to HIVAN (4,17,18). HIVAN biopsy tissue was collected at the time of diagnostic renal biopsy under a protocol that was approved by the Mount Sinai Institutional Review Board. ADPKD tissue sections were obtained from nephrectomy specimens that were procured by National Disease Research Interchange and Polycystic Kidney Disease Foundation. All tissues were fixed in 4% paraformaldehyde/PBS and embedded in paraffin.
from adult transgenic mice with severe histologic renal disease and proteinuria, transgenic mice without significant histologic renal disease, and normal mice. Ten micrograms of RNA from each sample was resolved on 1.2% agarose/formaldehyde gel and transferred to a 0.45-μm Biodyne membrane (Pall Corp., East Hills, NY).

For analysis of FAT10 expression in HPT-1 cells, a portion of the second exon of human FAT10 was amplified by PCR using cDNA from VSV-NL4–3 and primers 5'-GATTCCTAACGAGCGAGAAA-3' (sense) and 5'-CAATCCAAACCTACTCTCATG-3' (antisense) with the thermocycler parameters 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C for 40 cycles. For analysis of FAT10 expression in murine kidneys, the murine FAT10 coding sequence was amplified using cDNA from HIV-transgenic kidneys using primers 5'-CGGGATCCATGGCTTCTGTCCGCACCTGTG-3' (sense) and 5'-GAATTCGCTTCCTGCCTCTGTGTGTTGATATCCGCACACAGAGGCAGGAAGCATTTTTTGG-3' (antisense) and the following thermocycler parameters: 15 min at 95°C for one cycle; 30 s at 94°C, 1 min at 58°C, and 1 min at 72°C for four cycles; and 30 s at 94°C, 1 min at 64°C, 1 min at 72°C for 31 cycles.

PCR products were purified using Qiiaquick columns (Qiagen) and labeled with dCTP32 (Perkin-Elmer, Wellesley, MA) using Ready to Go dCPT Labeling Beads (Amersham Biosciences, Piscataway, NJ) according to the manufacturer’s protocol. Glyceraldehyde-3-phosphate dehydrogenase and ribosomal RNA bands were analyzed to ensure equal loading of RNA.

Generation of FAT10 Expression Vectors

The FAT10 coding sequence was amplified from plasmid MGC-21200 (ATCC) using primers 5'-CGGGATCCATGGCTTCTGTCCGCACCTGTG-3' (sense) and 5'-GAATTCGCTTCCTGCCTCTGTGTGTTGATATCCGCACACAGAGGCAGGAAGCATTTTTTGG-3' (antisense; EcoRI adapter in italics) and the following thermocycler parameters: 15 min at 95°C for one cycle; 30 s at 94°C, 1 min at 58°C, and 1 min at 72°C for four cycles; and 30 s at 94°C, 1 min at 64°C, and 1 min at 72°C for 31 cycles.

The PCR product was cloned into VVPW/BE (see below) and pcDNA4/HisMaxC (Invitrogen) using HotStar Master Mix kit (Qiagen, Valencia, CA) and the primers 5'-CGGGATCCATGGCTTCTGTCCGCACCTGTG-3' (sense) and 5'-GAATTCGCTTCCTGCCTCTGTGTGTTGATATCCGCACACAGAGGCAGGAAGCATTTTTTGG-3' (antisense) and the following thermocycler parameters: 15 min at 95°C for one cycle; 30 s at 94°C, 1 min at 58°C, and 1 min at 72°C for four cycles; and 30 s at 94°C, 1 min at 64°C, 1 min at 72°C for 31 cycles.

Generation of Short Hairpin RNA Vectors

We constructed two short hairpin RNA (shRNA) vectors to knock down expression of FAT10. One vector (pVIRHD/E-FAT10.1) is targeted against the first exon of FAT10, and the other (pVIRHD/E-FAT10.2) is targeted against the second exon. The VIRHD/E/siLuc lentivector expressing the anti-luciferase siRNA was used as negative control. Each vector was constructed by annealing complementary 61-mer oligonucleotides that contained the 19 nucleotide sequence that corresponded to the target FAT10 sequence in the sense and antisense orientation separated by a nine-nucleotide spacer region, allowing generation of a shRNA. Oligonucleotide sequences are provided in Table 1. Annealed oligonucleotides were inserted under the control of the human β1 promoter in the self-inactivating pVIRHD/E lentiviral vector (Figure 1f) derived from VVPV/EGFP (22). The resulting lentiviral vectors, which also express the reporter EGFP from the constitutive murine phosphoglycerate kinase promoter, were packaged into VSV-G-pseudotyped virions using the methods described above in the Viral Transduction section (Gusella et al., unpublished observations, 2005).

Generation of Anti-FAT10 Antiserum

Rabbit polyclonal antiserum against FAT10 was raised against the peptide APNASLCVHVHRSE, which spans the splice junction between the first and second exons of FAT10. This peptide was conjugated to keyhole limpet hemocyanin before inoculation. Peptide synthesis, inoculations, and harvesting of antiserum were performed by Open Biosystems (Huntsville, AL). Antiserum that was used in these studies was collected 70 d after inoculation.

Immunohistochemistry

Paraformaldehyde-fixed paraffin-embedded tissue was stained for FAT10 using FAT10 antiserum at a 1:100 dilution followed by incubation with biotinylated rabbit anti-goat IgG at a 1:200 dilution. The Vector ABC Elite Kit and aminoethylcarbazole (Vector Labs, Burlingame, CA) were used according to a previously published protocol (8). For ensuring specificity of the antiserum, staining controls included using preimmune serum from the same rabbit that was used to generate the antiserum and incubating the antiserum with 10 μg/ml of the immunizing peptide for 60 min before use in immunohistochemistry.

Table 1. Oligonucleotides used for generating shRNA vectors

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<th>Vector</th>
<th>Sense Oligonucleotide</th>
<th>Antisense Oligonucleotide</th>
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<tr>
<td>pVIRHD/E-FAT10.1</td>
<td>5'-GATTCCTGCTTCCTGCCCTCTGTGTGTTGATATCCGCACACAAGAGGAGGGAAGATTTTTTTGG-3'</td>
<td>5'-AATTCAAAAAACATGCTTCTGCTGCTGTGTTGATATCCGCACACAACAGAGGGAAGGGAAGCATTTTTTTGG-3'</td>
</tr>
<tr>
<td>pVIRHD/E-FAT10.2</td>
<td>5'-GATCCGGAACATGTCGCCCTGAAGATGTTGATATCCGCACACACAGAGGGAAGATTTTTTTGG-3'</td>
<td>5'-AATTCAAAAAACATGCTTCTGCTGCTGTGTTGATATCCGCACACAACAGAGGGAAGGGAAGCATTTTTTTGG-3'</td>
</tr>
<tr>
<td>pVIRHD/E/siLuc</td>
<td>5'-GATCCGGAACATGTCGCCCTGAAGATGTTGATATCCGCACACACAGAGGGAAGATTTTTTTGG-3'</td>
<td>5'-AATTCAAAAAACATGCTTCTGCTGCTGTGTTGATATCCGCACACAACAGAGGGAAGGGAAGCATTTTTTTGG-3'</td>
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<td>5'-GATCCGGAACATGTCGCCCTGAAGATGTTGATATCCGCACACACAGAGGGAAGATTTTTTTGG-3'</td>
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aShRNA, short hairpin RNA.
**FAT10 shRNA Inhibition Studies**

Separate populations of HPT-1 cells were transduced with VSV-pseudotyped VIRHD/e-FAT10.1 and VIRHD/E-FAT10.2 at an MOI of 10. After expansion and differentiation, 10⁷ cells were transfected with pcDNA4-FAT10 (Figure 1e) using Lipofectamine 2000 (Invitrogen). Lysate from pcDNA4-FAT10–transfected HPT-1 cells was used for detection of Xpress-tagged FAT10 by Western blotting using Anti-Xpress-HRP (Invitrogen) diluted 1:2000.

**Flow Cytometry**

Differentially HPT-1 cells were transfected with VVPW/BE-FAT10 or VVPW/BE (control) using Lipofectamine 2000 (Invitrogen) at a DNA:lipid ratio of 1:3 according to the manufacturer’s protocol. Twenty-four hours after transfection, 1.2 × 10⁶ HPT-1 cells were stained using the Vybrant apoptosis #6 kit (Invitrogen) according to the manufacturer’s protocol. Cells then were analyzed by flow cytometry at the Mount Sinai Flow Cytometry Shared Research Facility using a FACSVantage flow cytometer (BD Biosciences). Transfected cells were identified by the presence of EGFP fluorescence. Apoptotic cells were identified by the presence of annexin V staining in the absence of propidium staining, and necrotic cells were identified by the presence of propidium staining. χ² testing was used to compare the number of apoptotic and apoptotic plus necrotic cells that were transfected with VVPW/BE-FAT10 to cells that were transfected with VVPW/BE. A two-sided P < 0.05 was used to define statistical significance.

To study the effect of FAT10 inhibition on HIV-induced apoptosis, we infected separate populations of HPT-1 cells that had been stably transduced with VIRHD/e-FAT10.1, VIRHD/E-FAT10.2, or VIRHD/E/siLuc with VSV-NL4–3:ΔG/P-EGFP as described in the section Viral Transduction. Seven days after infection, we analyzed apoptosis in each population as described above.

**Results**

**Development of a Tubular Epithelial Cell Line HPT-1 from a Patient with HIVAN**

Host genetic factors strongly influence susceptibility to HIVAN. Therefore, to maximize the relevance of our studies to HIVAN pathogenesis, we developed a PTEC line from a patient with HIVAN for use in these studies. We chose PTEC because they are the predominant epithelial cell type in the kidney, we have shown them to be infected by HIV-1, and they are important in disease progression in HIVAN (8).

Primary PTEC that were grown from a HIVAN biopsy were conditionally immortalized by stable transduction with a lentivirus encoding the SV40 temperature-sensitive large T antigen under the control of the CMV promoter (Figure 1a). The resulting cell population (subsequently referred to as HPT-1) grows indefinitely under permissive conditions (33°C), but at 37°C, T antigen degrades (Figure 2a) and the cells induce apoptosis maximally. HPT-1 cells express PTEC markers such as cytokeratin, alkaline phosphatase, and aquaporin-1 (Figure 2b, c). HPT-1 cells do not express nonspecific proteins such as vimentin or smooth muscle myosin, Tamm Horsfall protein, or aquaporin-2 (markers for thick ascending limb of Henle and collecting duct, respectively [data not shown]). HPT-1 cells are not latently infected by HIV-1 as determined by PCR analysis (data not shown).

**FAT10 mRNA Expression after In Vitro HIV-1 Transduction of Human Proximal Tubular Cells**

In preliminary studies using oligonucleotide microarrays, we found that FAT10 was one of the most robustly upregulated genes in HIV-infected HPT-1 cells (24). To determine the specificity of the increased expression of FAT10, we performed Northern blotting using RNA from HPT-1 cells that were infected with VSV-NL4–3:ΔG/P-EGFP (Figure 1b), a gag/pol-deleted virus that was derived from the same proviral construct that was used to create the HIV-1–transgenic HIVAN model (17,21). VSV-HR′-IRES-EGFP, a lentivirus that expresses EGFP (Figure 1c), was used as a control. Because infection of RTEC in vitro by HIV-1 is inefficient (6,25), both viruses were pseudotyped with VSV-G to maximize the efficiency of infection. FAT10 expression was markedly increased in HPT-1 cells that were infected with VSV-pNL4–3:ΔG/P-EGFP at 3 and 7 d after infection but not at 12 and 24 h after infection (Figure 3a). FAT10 was not expressed in control-infected cells at any time point (Figure 3a) or in noninfected cells (data not shown).

**FAT10 RNA Expression in Kidneys from HIV-1–Transgenic Mice**

We next studied FAT10 expression in kidneys from the HIV-1–transgenic model of HIVAN. These mice develop proteinuria, renal failure, and histologic renal disease that is identical to HIVAN (18). Kidneys were obtained from transgenic mice with severe proteinuria and histologic disease, transgenic mice without overt proteinuria or histologic disease, and normal adult mice. FAT10 expression was markedly increased in kidneys from HIV-transgenic mice with severe renal disease, with lesser FAT10 levels detectable in kidneys from HIV-transgenic mice without overt renal disease (Figure 3b). FAT10 expression was minimally detectable in kidneys from normal adult mice (Figure 3b).
FAT10 Expression Induces Apoptosis in HPT-1 Cells

Because FAT10 expression and epithelial apoptosis are increased in HIVAN, we studied whether FAT10 is capable of inducing apoptosis in HPT-1 cells. HPT-1 cells were transfected with a plasmid encoding FAT10 and EGFP in a dicistronic vector (pVVPW/BE-FAT10; Figure 1d), allowing identification of transfected cells by EGFP fluorescence. Twenty-four hours after transfection with pVVPW/BE-FAT10, 18.1% of EGFP-expressing cells were apoptotic as determined by annexin V staining (Figure 4, a and c), whereas only 1.9% of HPT-1 cells that were transfected with the control vector (VVPW/BE) were apoptotic (Figure 4, b and c). Moreover, 47.3% of pVVPW/BE-FAT10–transfected cells were either apoptotic or necrotic (positive for propidium and/or annexin V staining), but only 9.3% of vector-transfected cells were apoptotic or necrotic (Figure 4c). Levels of apoptosis and apoptosis plus necrosis were significantly greater in the cells that expressed FAT10 ($P < 0.0001$).

Inhibition of FAT10 Prevents HIV-Induced Apoptosis of RTEC

To determine whether FAT10 has a direct causal role in HIV-induced apoptosis of RTEC, we studied whether specific inhibition of endogenous FAT10 using shRNA vectors could prevent apoptosis of RTEC after infection with HIV-1. We generated two lentiviral vectors, VIRHD/e-FAT10.1 and VIRHD/E-FAT10.2, to stably transduce HPT-1 cells with shRNA directed against two separate regions of the FAT10 transcript. To determine the efficacy of these vectors in suppressing FAT10 protein expression, we stably transduced HPT-1 cells with the shRNA vectors and subsequently transfected them with the FAT10 expression vector pcDNA4-FAT10. Both shRNA vectors effectively inhibited expression of FAT10 protein (Figure 5a).

We then tested whether preventing upregulation of endogenous FAT10 could prevent apoptosis in HPT-1 cells after infec-

Figure 3. FAT10 mRNA expression is increased in HIV-infected HPT-1 cells and in kidneys from HIV-transgenic mice. (a) Infection with gag/pol-deleted HIV-1 induced expression of FAT10 at 3 and 7 d after infection. Expression of FAT10 mRNA was markedly increased in severely diseased kidneys from HIV-transgenic mice. (b) Lesser expression of FAT10 was present in kidneys from transgenic mice without overt nephropathy, and there was minimal expression in kidneys from normal adult mice.

Figure 4. FAT10 expression induces apoptosis in HPT-1 cells. Twenty-four hours after transfection with VVPW/BE-FAT10, EGFP-expressing HPT-1 cells were examined by flow cytometry. A total of 18.1% of cells that were transfected with pVVPW/BE-FAT10 were apoptotic as determined by annexin V staining (a and c), whereas only 1.8% of vector-transfected cells became apoptotic (b and c; $P < 0.0001$). A total of 47.3% of HPT-1 cells that were transfected with FAT10 were either apoptotic (annexin V positive, propidium negative) or necrotic (positive for propidium) in contrast to vector-transfected cells (9.3%; c; $P < 0.0001$).
tion by HIV-1. We infected HPT-1 cells that had been stably transduced with VIRHD/E-FAT10.1, VIRHD/E-FAT10.2, or VIRHD/E/siLuc with VSV-NL4–3/H9004G/P-EGFP. Cells that expressed either anti-FAT10 shRNA construct demonstrated significantly lower levels of apoptosis as compared with cells that expressed the shRNA control vector VIRHD/E/siLuc (P < 0.0001; Figure 5b).

**FAT10 Protein Expression Is Increased in Kidneys from HIV-1–Transgenic Mice**

We used FAT10 antiserum to perform immunohistochemical analysis of FAT10 expression and localization in kidneys from HIV-1–transgenic mice and in normal controls. FAT10 staining was focal with expression in RTEC in some nephrons, (Figure 6a), sloughing (often apoptotic) epithelial cells (Figure 6b) and cells just beneath the epithelial layer (Figure 6c). Focal expression of FAT 10 also was present in glomeruli (Figure 6d) and in vascular smooth muscle cells (Figure 6e). In kidneys from normal adult mice, FAT10 was not detected in glomeruli, tubules, or interstitium (Figure 6f) but was detected in vascular smooth muscle cells (data not shown). Preimmune serum controls were negative (Figure 6g), and preincubation of the antiserum with the peptide that was used to generate the antiserum abolished all staining (Figure 6h). Immunostaining with other polyclonal antibodies to FAT10 donated by Dr. C. Lee [26] and Dr. S. Weissman [13]) resulted in similar staining (data not shown).

**FAT10 Expression in HIVAN and ADPKD**

To determine whether FAT10 expression is increased in patients with HIVAN, we performed immunohistochemistry using HIVAN biopsy specimens. FAT10 was expressed in areas of periglomerular fibrosis surrounding Bowman’s capsule (Figure 7a, top left), in tubular epithelial cells (Figure 7a, middle left), and in arterial smooth muscle cells (Figure 7a, bottom left). Preimmune serum controls using serial sections were negative (Figure 7a, right).

Because ADPKD, like HIVAN, is characterized by tubular cystic dilation and dysregulated RTEC apoptosis, we performed immunohistochemistry to investigate whether FAT10 is
expressed in kidneys from patients with ADPKD. FAT10 was strongly expressed in RTEC that lined cysts (Figure 7b), particularly in areas where the epithelium had become hyperplastic (Figure 7b, bottom left, arrowheads). Preimmune controls were negative (Figure 7b, right), and FAT10 expression was not detected in normal adult human kidneys (Figure 7c).

Discussion

HIVAN and ADPKD are important causes of renal disease. HIVAN is the third-leading cause of ESRD in black individuals who are aged 20 to 64 yr and is the most common cause of chronic kidney failure in HIV-1–infected patients (1). ADPKD is the most common lethal inherited disease in humans, occurring in 1 in 800 live births and affecting 4 to 6 million people worldwide (2).

HIVAN is caused by expression of HIV-1 genes in infected renal epithelial cells (4,5,27,28). Host genetic factors are important in modulating the predisposition to developing HIVAN in patients and in HIV-1–transgenic mice (9–11,29). Most previous studies of the response of renal epithelial cells to HIV-1 infection have used cell lines from rodents or hosts that are not susceptible to developing HIVAN (white individuals). In these studies, we used RTEC from a patient with HIVAN to maximize the likelihood that the in vitro cellular response to infection will be relevant to HIVAN pathogenesis.

UBL have important roles in the pathogenesis of several diseases, including malignancy, neurodegenerative diseases, immune disorders, and infectious diseases (30). FAT10 is a 165–amino acid UBL that contains two ubiquitin-like domains, each of which is approximately one third identical to ubiquitin (14). FAT10 first was cloned by Fan et al. (31), who discovered the gene by cDNA hybridization selection while analyzing the human HLA-F locus for novel genes. FAT10 initially was reported to be expressed in mature B lymphocytes and dendritic cells, suggesting a role for the gene in antigen processing/presentation and immune response (32). Other investigators have found, however, that a variety of transformed cell lines express FAT10 when they are exposed to TNF-α and IFN-γ but not IFN-α (14). FAT10 first was cloned by Fan et al. (31), who discovered the gene by cDNA hybridization selection while analyzing the human HLA-F locus for novel genes. FAT10 initially was reported to be expressed in mature B lymphocytes and dendritic cells, suggesting a role for the gene in antigen processing/presentation and immune response (32). Other investigators have found, however, that a variety of transformed cell lines express FAT10 when they are exposed to TNF-α and IFN-γ but not IFN-α (14). This is in contrast to the UBL ISG-15, which is induced by IFN-α but not IFN-γ (33). Although both the cytokine-mediated induction of FAT10 in vitro and its genomic localization suggest that FAT10 may have a role in immunity, the in vivo role of FAT10 remains obscure.

The first evidence that FAT10 is a proapoptotic molecule came from Raasi et al., who reported that attempts to produce cell lines that would constitutively express FAT10 protein were unsuccessful as a result of increased cell death (34). The same group later found that inducible expression of FAT10 in mouse fibroblasts results in caspase-dependent apoptosis that is abrogated by deletion of the c-terminal diglycine motif of FAT10 (14). Because this motif is necessary for covalent attachment of ubiquitin and UBL to target proteins, this study suggested that
FAT10 must become covalently attached to as-yet-unidentified protein(s) to induce apoptosis. Further supporting a role for FAT10 in apoptosis, Coyle et al. (35) reported that induction of apoptosis in hepatoma cells by exposure to TGF-β results in strong upregulation of FAT10. Because TGF-β is upregulated in renal specimens from patients with HIVAN (36) and has been implicated as a mediator in the pathogenesis of many forms of progressive renal failure, FAT10 may be involved in TGF-β–induced apoptosis.

FAT10 conjugation to proteins was shown recently to target proteins for ubiquitin-independent proteasomal degradation (12). Although the proteins to which FAT10 becomes covalently conjugated remain unknown, FAT10 has been shown to interact noncovalently with two proteins, MAD2 (13) and NEDD8 ultimate buster 1L (NUB1L) (37). MAD2 is a mitotic spindle assembly checkpoint protein whose inhibition can lead to genomic instability and subsequent apoptosis (38). NUB1L has been reported to bind noncovalently to another UBL, NEDD8. MAD2 (13) and NEDD8 (12). Although the proteins to which FAT10 becomes covalently conjugated remain unknown, FAT10 and NEDD8 may result from covalent and/or noncovalent interactions of FAT10 with several cellular proteins.

Ubiquitin and UBL are critical co-factors in the HIV-1 life cycle. HIV-1 Tat, for example, must be ubiquitylated to maximally transactivate transcription from the HIV-1 LTR promoter (42), and the Gag late protein p6 must be ubiquitylated to allow budding of HIV-1 virions from the cell membrane (43). P6 also was shown recently to be capable of modification by the UBL SUMO-1 (44). Other HIV-1 proteins, such as Vif and Vpu, induce ubiquitylation and subsequent degradation of host proteins (45–47). Whether HIV-1 proteins are targets of FAT10 conjugation or induce FAT10 conjugation to host proteins is currently under investigation.

Conclusion

These studies demonstrate that HIV-1 expression in RTEC induces expression of FAT10 and that FAT10 expression induces apoptosis in RTEC. Moreover, inhibition of FAT10 expression results in suppression of HIV-induced apoptosis of RTEC. Because FAT10 expression is increased in HIVAN and ADPKD, it may have an important role in the dysregulation of apoptosis that occurs in the renal epithelium in these diseases.

Acknowledgments

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References


