Mutant Tamm-Horsfall Glycoprotein Accumulation in Endoplasmic Reticulum Induces Apoptosis Reversed by Colchicine and Sodium 4-Phenylbutyrate

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As a consequence of uromodulin gene mutations, individuals develop precocious hyperuricemia, gout, and progressive renal failure. In vitro studies suggest that pathologic accumulation of uromodulin/Tamm-Horsfall glycoprotein (THP) occurs in the endoplasmic reticulum (ER), but the pathophysiology of renal damage is unclear. It was hypothesized that programmed cell death triggered by accumulation of misfolded THP in the ER causes progressive renal disease. Stably transfected human embryonic kidney 293 cells and immortalized thick ascending limb of Henle’s loop cells with wild-type and mutated uromodulin cDNA were evaluated to test this hypothesis. Immunocytochemistry, ELISA, and deglycosylation studies indicated that accumulation of mutant THP occurred in the ER. FACS analyses showed a significant increase in early apoptosis signal in human embryonic kidney 293 and thick ascending limb of Henle’s loop cells that were transfected with mutant uromodulin constructs. Colchicine and sodium 4-phenylbutyrate treatment increased secretion of THP from the ER to the cell membrane and into the culture media and significantly improved cell viability. These findings indicate that intracellular accumulation of THP facilitates apoptosis and that this may provide the pathologic mechanism responsible for the progressive renal damage associated with uromodulin gene mutations. Colchicine and sodium 4-phenylbutyrate reverse these processes and could potentially be beneficial in ameliorating the progressive renal damage in uromodulin-associated kidney diseases.


Mutations in the uromodulin (UMOD) gene result in an autosomal dominantly transmitted syndrome characterized by precocious hyperuricemia, gout, and progressive kidney failure frequently leading to dialysis (1–3). This condition has been termed familial juvenile hyperuricemic nephropathy (FJHN), medullary cystic kidney disease type 2 (MCKD-2), and glomerulocystic kidney disease (2,4). Most UMOD mutations involve alteration of highly conserved cysteine residues that compromise processing of uromodulin/Tamm Horsfall glycoprotein (THP) (3,5). Uromodulin and THP are two large glycoproteins with an identical amino acid sequence, and both are a product of the UMOD gene. Uromodulin was isolated from the urine of pregnant women and has been found to have a small increase in Man7GlcNac2 molar percentage compared with THP, which is isolated by salt precipitation in nonpregnant individuals (6). We refer to the uromodulin gene product as THP in this article, as the antibody to uromodulin was created by using THP obtained by salt precipitation. Synthesis of THP is modified in the endoplasmic reticulum (ER), before secretion. In addition to the assembly of N-glycans, THP biosynthesis is characterized by two peculiar posttranslational steps: (1) The addition of glycosyl phosphatidylinositol (GPI)-anchor by ER transpeptidase (7), an event that ensures THP exposure at the luminal face of thick ascending limb of Henle’s loop (TALH) cells, and (2) the formation of a large number of intrachain disulfide bridges by ER protein disulfide isomerase.

Transfection experiments of the UMOD gene into Hela cells have demonstrated that intrachain disulfide bonding is important in the maturation and secretion of THP (8). Because of their effect on disulfide bonds, mutations that affect cysteine residues are often associated with defective folding of the global protein structure. Misfolded and incompletely assembled proteins, particularly as a result of cross-linking by nonnative interchain disulfide bonds (9), are prone to aggregation and retention in the ER (10). Such retention can lead to the unfolded protein response (11,12), which may activate a number of pathways, including growth arrest and programmed cell death (13).

In vitro studies of MCKD2/FJHN/glomerulocystic kidney disease–associated UMOD mutations corroborate histologic evidence from kidney biopsies of affected patients, demonstrating that intracellular accumulation of THP occurs in the ER (3–5,14). These findings are consistent with the decrease of THP in the urine of individuals with disease associated UMOD muta-
tions (4,14,15). Whereas studies indicate a pathologic accumulation of THP in the ER, the pathophysiology of renal damage is unclear.

We hypothesized that accumulation of misfolded THP in the ER leads to apoptosis. We further hypothesized that colchicine and chemical chaperones that are known to stabilize the conformation of proteins that are defective in patients with a number of inherited diseases (16) would facilitate THP excretion, reduce abnormal THP deposition, and decrease programmed cell death.

Materials and Methods

Construction of Wild-Type and Mutated UMOD Expression Vector

Wild-type (WT) UMOD cDNA were generated from a human kidney cDNA library by PCR and subcloned into pcDNA3 mammalian expression vector (Invitrogen, Carlsbad, CA). Mutant constructs were based on previously reported UMOD mutations (2). Mutant F1 that contains a 27-bp deletion that results in the in-frame deletion of amino acids 177 to 185 (g.1966_1992del; c.529_555del; p.H177_185del) and mutant F2 that contains a missense mutation that changes a conserved cysteine to tyrosine (g.1880G>A; c.443G>A; p.C148Y) were constructed by a mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer’s protocol using mutant primer sets: F1 SS, 5'-GGGGGATGGATGGC ACTATGAGTGCTCCCCGGG-3'; F1 AS, 5' CCCTCCGAGCACTCATAGTGCC ATCCATC CCCG-3'; F2 AS, 5'-GGGGGATGGATGGC ACTATGAGTGCTCCCCGGG-3'; F2 SS, 5'-CCCTCCGAGCACTCATAGTGCC ATCCATC CCCG-3'.

Selection of Stably Transfected Clones

Human embryonic kidney 293 (HEK293) cells (American Type Culture Collection, Rockville, MD) were cultured in DMEM supplemented with 10% FBS and standard antibiotics in a 5% CO2 atmosphere at 37°C. An immortalized TALH cell line derived from kidneys of New Zealand white rabbits was used (17). The TALH cell line was maintained as a monolayer culture in DMEM supplemented with 10% FBS and standard antibiotics in a 5% CO2 atmosphere at 37°C. Transfection was performed using lipofectamine reagents (Invitrogen) according to the manufacturer’s protocol. Stably transfected cells were selected by culturing in the presence of DMEM media supplemented with 10% FBS, standard antibiotics, and 0.75 mg/ml G418 in a 5% CO2 atmosphere at 37°C for 2 wk. For determining the capacity of THP expression, cells were cultured in a 24-well plate with 80% confluence, washed, and incubated with serum-free DMEM media that contained antibiotics for 24 to 72 h. Expressed THP was determined with the conditioned media by Western blot.

Cell Culture, THP Expression, and Effect of Chemical Agents

Stably transfected cells were cultured for 24 h in DMEM media that contained 10% FBS, antibiotics, and 0.375 mg/ml G418. The cells were washed (×3) with serum-free DMEM media that contained antibiotics

A. HEK293 cells

B. TALH cells

Figure 1. Expression of Tamm-Horsfall glycoprotein (THP) from human embryonic kidney 293 (HEK293; A) and thick ascending limb of Henle’s loop (TALH) cells (B) that were stably transfected with wild-type (WT) and mutant uromodulin (UMOD) genes. (a) Western blot of the conditioned media of HEK293 and TALH cell culture. After WT and two mutant UMOD constructs (F1 containing c.529_555 del and F2 containing c.443G>A) were stably transfected into HEK293 and TALH cells, the cultured cells were incubated with serum-free DMEM media for 24 h, and the conditioned media were tested for THP expression by Western blot. HEK293 and TALH cells that were transfected with expression vector pCDNA3 alone (EV) or enhanced green fluorescent protein (EGFP) were used as negative and transfection efficiency controls. (b) Secreted THP in the conditioned media was measured by ELISA. The amount of secreted THP was expressed as mean ± SD from triplicate experiments. The amount of THP in HEK293 cells was WT, 54.7 ± 1.2; F1, 16.0 ± 2.0; F2, 12.7 ± 1.2 ng/mg protein per 24 h (**P < 0.005). The amount of THP in TALH cells was WT, 14.0 ± 1.0; F1, 3.7 ± 1.2; F2, 2.7 ± 0.6 ng/mg protein per 24 h (**P < 0.005). These results were reproducible in six independent clones for each WT, F1, and F2.
and further incubated with the same media for 24 to 72 h. Secreted THP in the conditioned media was determined by Western blot and ELISA. For evaluating chemical agents, the cells were incubated in a serum-free medium supplemented with colchicine, sodium 4-phenylbutyrate (4-PBA; Calbiochem, San Diego, CA), and allopurinol (Sigma, St. Louis, MO) at concentration ranges of 0.001 to 1 mM, 1 to 10 mM, and 0.001 to 5 mM, respectively.

**Western Blot and ELISA for THP**

The conditioned media were harvested and concentrated with cold ethanol. Incubated cells were lysed in lysis buffer (20 mM Tris-HCl [pH 8.0], 1 mM PMSF, 1 mM 4-[2-aminoethyl] benzenesulfonyl fluoride, 10 mM E-64, 100 mM bestatin, 100 mM leupeptin, 100 mM aprotinin, and 5 mM EDTA). Proteins were quantified by using the Bio-Rad Proteins Assay (Bio-Rad, Hercules, CA), and equal amounts of proteins were loaded. For Western blotting, samples were separated by SDS-PAGE in reducing conditions and transferred to nitrocellulose. After blocking with 5% skim milk, membranes were incubated overnight at 4°C with sheep polyclonal primary antibody against human THP (Chemicon International, Inc., Temecula, CA; 1:2000 dilution), followed by the incubation of AP-conjugated rabbit secondary antibody against sheep (Chemicon; 1:5000 dilution) for 1 h at room temperature and visualized with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Bio-Rad). β-Actin (Abcam, Cambridge, MA) was used as an internal control for equal loading.

An ELISA was established with a precoated-antibody (rabbit anti-human THP antibody; Santa Cruz Biotechnology, Santa Cruz, CA; 0.5 μg/ml biocarbonate buffer [pH 8.4]; 1:200 dilution) bound to the solid phase, a primary antibody (sheep anti-human THP polyclonal antibody; Chemicon; 1:2000 dilution), a secondary antibody (AP-conjugated rabbit anti-sheep antibody; Chemicon; 1:5000 dilution), and an alkaline phosphatase liquid substrate system (Sigma). Urinary THP was purified by the salt-precipitation method (18).

**Deglycosylation of THP**

For differentiating THP precursor and mature forms, oligosaccharide cleavage was evaluated with two different deglycosidases, N-glycosidase F (PNGase F; New England Biolab, Beverly, MA) and endo-β-N-acetylglucosaminidase H (Endo H; New England Biolab) according to the manufacturer’s protocol.

**Immunocytochemistry**

Stably transfected WT and mutant THP-expressing HEK293 and TALH cells were grown on eight-well chamber slides, fixed with 100% cold methanol (−20°C) for 10 min at room temperature, and washed with PBS. Nonspecific antibody binding was blocked by incubating for 1 h with PBS that contained 5% normal goat serum and 1% BSA for 1 h. Cells then were incubated with sheep anti-human THP polyclonal antibody (Chemicon; 1:200 dilution) in PBS that contained 1% normal goat serum and 1% BSA and followed by incubation with FITC-conjugated donkey anti-sheep IgG antibody (Chemicon; 1:200 dilution) in PBS that contained 1% normal goat serum and 1% BSA. Immunocytochemical signals were visualized and photographed with a ×400 magnification using a fluorescence microscope (Olympus 1X71; Olympus, Melville, NY).

**FACS Analysis for Apoptosis Assay**

Apoptotic cells were measured using annexin V-FITC and propidium iodide staining (BD Bioscience, San Jose, CA) with FACS analysis. Cell sample preparation, Annexin V, and propidium iodide staining for FACS analysis were performed according to the manufacturer’s instructions. The percentage of apoptotic cells was determined by the fluorescence of individual cells measured by FACS flow cytometry (Becton Dickinson, San Jose, CA).

**Evaluation of Cell Viability**

WT and two mutant cell clones (1 × 10⁶) were inoculated in each well of a six-well plate and cultured in DMEM supplemented with 10% FBS and standard antibiotics in a 5% CO₂ atmosphere at 37°C for 24 h. The cells were incubated further in a serum-free DMEM medium in the absence and presence of 0.5 mM colchicine, 10 mM 4-PBA, and 5 mM allopurinol. After 24- to 72-h incubations, cells were detached from a plate using a cell scraper, suspended in PBS, and stained with trypan blue solution. Viable cells, which were resistant to trypan blue staining, were counted using a hemacytometer under light microscopy (Olympus 1X71). Viability was calculated with 100% being the viable cell number at 0 h incubation. Data were obtained from three individual experiments.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Retention of mutant THP in endoplasmic reticulum (ER) of HEK293 cells. (A) Detection of intracellular and extracellular THP. WT and mutant (F1 and F2) THP-expressing cells were incubated with serum-free DMEM media for 24 h, and expressed THP isoforms were determined with cell extracts or conditioned media by Western blot. The expression of β-actin is shown as a control for protein loading. Intracellular THP consisted of two forms, 75 and 86 kD, in which the latter is the same size as extracellular THP. The amount of 86-kD THP was dramatically reduced in the two mutant cells. (B) Deglycosylation of THP isoforms. The samples from cell extract and culture media were treated with N-glycosidase F (PNGase F) or endo-β-N-acetylglucosaminidase H (Endo H; New England Biolab) according to the manufacturer’s protocol.
Results

THP Excretion from HEK 293 and TALH Cells Transfected with Mutant UMOD Genes Is Significantly Reduced

The WT and the two mutant (F1 and F2) clones expressed THP with an apparent molecular weight (MW) of 86 kD, the same MW as urinary THP (Figure 1). HEK293 and TALH cells do not express endogenous THP. Negative controls and vector or enhanced green fluorescent protein (EGFP) transfected cells did not express THP. ELISA quantification of THP expressed in conditioned media indicated that mutant clones excreted significantly less THP. Compared with WT THP-expressing HEK293 cells (54.7 ± 1.2 ng/mg protein), THP expression was decreased by approximately 70% for F1 (16.0 ± 2.0 ng/mg protein) and decreased by approximately 77% for F2 (12.7 ± 1.2 ng/mg protein). Compared with WT THP-expressing TALH cells (14.0 ± 1.0 ng/mg protein), THP expression was decreased by approximately 73% for F1 (3.7 ± 1.2 ng/mg protein) and decreased by approximately 80% for F2 (2.7 ± 0.6 ng/mg protein). These results were reproducible in six independent clones for each WT, F1, and F2.

Accumulation of Mutant THP within ER

Two MW intracellular THP, 75 kD and 86 kD, were detected from WT and mutant HEK293 cells (Figure 2A). Although the ratio of 75 to 86 kD was similar in the WT cell, the amount of the 86-kD forms was reduced in both mutant cells. To characterize the oligosaccharide modifications of the two different intracellular THP, they were treated with deglycosidases PNGase F and Endo H. PNGase F hydrolyzes nearly all types of N-linked oligosaccharides of glycoproteins, from both the ER and the Golgi. In contrast, Endo H cleaves only high-mannose glycoproteins from the ER but does not cleave glycoproteins modified in the Golgi. Deglycosylation experiments showed that both the 75- and the 86-kD forms were cleaved by PNGase (Figure 2B). Whereas Endo H cleaved the 75-kD THP, the 86-kD form was resistant to Endo H digestion, indicating that the

Figure 3. Determination of apoptosis of WT (a), mutant F1 (b), and F2 (c) THP-expressing HEK293 (A) and TALH cells (B) by annexin V labeling followed by FACS analysis. WT and mutant cells (1 × 10⁶) were stained with annexin V-FITC and propidium iodide. The percentage of cells that underwent apoptosis was measured quantitatively using FACS flow cytometry. Mutant THP-expressing HEK293 cells show significantly greater early apoptotic signal (right bottom) than WT cells (3.7% [F1] versus 3.5% [F2] versus 0.8% [WT]). The percentage of end-stage apoptotic cells (right top) was also higher in the two mutant cells than in WT cells (5.3% [F1] versus 5.2% [F2] versus 2.7% [WT]). Mutant THP-expressing TALH cells also show significantly greater early apoptotic signal (right bottom) than WT cells (3.6% [F1] versus 5.1% [F2] versus 1.4% [WT]). The percentage of end-stage apoptotic cells (right top) was also higher in the mutant cells than in WT cells (17.6% [F1] versus 15.3% [F2] versus 10.5% [WT]). A similar pattern of results was seen in three independent clones for each WT, F1, and F2, respectively.
protein was a terminally glycosylated precursor in the Golgi apparatus. These results suggest that the 75-kD THP exists as a precursor in the ER and is exported into the Golgi apparatus for further oligosaccharide processing and becomes the mature 86-kD form that is excreted into the conditioned media. Similar results were obtained for TAL cells (data not shown).

**Comparison of Apoptosis between WT and Mutant THP-Expressing HEK293 and TALH Cells**

Because the accumulation of misfolded proteins in the ER has been reported to induce programmed cell death (11), we measured apoptosis by labeling THP-expressing HEK293 and TALH cells with annexin V-FITC followed by FACS analysis (Figure 3). FACS data showed more early apoptotic signal in mutant cells than in WT cells (Figure 3a), 3.7 versus 0.8% in HEK293 cells (Figure 3A), and 3.6 or 5.1 versus 1.4% in TALH cells (Figure 3B), respectively. The percentage of cells in late apoptosis was also higher in both mutant cells than in the WT cells. These data indicate that the accumulation of mutant THP in the ER is associated with significantly greater apoptosis.

**Effects of Chemical Agents on Mutant THP Release from Cells**

Chemical chaperones have been shown to rescue misfolded proteins from the ER. We examined the effect of the chemical chaperone sodium 4-PBA as well as colchicine and allopurinol on THP excretion using ELISA (Figure 4). THP secretion in both WT and mutant cells was increased by treatment of 0.001 to 1.0 mM colchicine and by 5 to 10 mM 4-PBA. At the optimal colchicine concentration of 0.5 mM, THP excretion was increased by 6.4-, 11.2-, and 9.0-fold for WT, F1, and F2 mutant THP-expressing HEK293 cells, respectively (Figure 4A). Treatment with 10 mM 4-PBA increased THP excretion by 4.1-, 3.9-, and 4.6-fold for WT, F1, and F2 mutant THP-expressing HEK293 cells, respectively (Figure 4B). At the optimal colchicine concentration of 0.5 mM, THP excretion was increased by 6.1-, 12.2-, and 10.6-fold for WT, F1, and F2 mutant THP-expressing HEK293 cells, respectively (Figure 4C). These data indicate that chemical treatments can rescue mutant THP from the ER and increase its secretion.

![Figure 4](image-url)

**Figure 4.** Effect on THP secretion by colchicine (a), sodium 4-phenylbutyrate (4-PBA; b), and allopurinol (c) in THP-expressing HEK293 (A) and TALH cells (B). WT and mutant THP-expressing HEK293 and TALH cells were cultured for 24 h and incubated further for 24 h in the serum-free DMEM media in the absence or presence of various concentrations of colchicine, 4-PBA, and allopurinol. The secreted THP in the conditioned media was measured by ELISA. THP secretion was increased by treatment of 0.001 to 1 mM colchicine or 5 to 10 mM 4-PBA for both WT and the two mutant expressing cells.
expressing TALH cells, respectively (Figure 4Ba). Treatment with 10 mM 4-PBA increased THP excretion by 3.4-, 3.8-, and 4.0-fold for WT, F1, and F2 mutant THP-expressing TALH cells, respectively (Figure 4Bb). In contrast, allopurinol tested at concentrations ranging from 0.001 to 5 mM did not affect THP secretion (Figure 4c).

**Chemical Agents Facilitate Movement of Mutant THP from Cytoplasm to Plasma Membrane**

As colchicine and 4-PBA increased the excretion of mutant THP into conditioned media, we performed additional immunologic assays to study their effect on intracellular localization of THP in HEK293 and TALH cells. In WT transfected cells, THP localized predominantly to the cell membrane (Figure 5a), whereas in HEK293 and TALH cells that were stably transfected with mutant F1 and F2, THP localization was mainly cytoplasmic (Figure 5, d and g), consistent with accumulation of mutant protein in the ER. When mutant cells were incubated with either 0.5 mM colchicine or 10 mM 4-PBA, THP signal was reduced in cytoplasm and increased at plasma membrane.

**Effects of Chemical Agents on Cell Viability**

Previous results in HEK293 and TALH cells indicated that mutant THP in the ER induces apoptosis. Treatment of these cells with colchicine and 4-PBA facilitated release of mutant THP from the ER to the plasma membrane and out of the cell. To evaluate whether treatment with chemical agents affected the rate of apoptosis in cells, we evaluated cell viability (Figure 6). Compared with WT cells, viability of the two mutant cells rapidly decreased, with the extent more severe in F2 than F1. After 72 h of incubation without chemical agent treatment, viability was reduced to 79.1, 51.2, and 25.6% for WT, F1, and F2 THP-expressing HEK293 cells, respectively (Figure 6A). After 72 h of incubation without chemical agent treatment, viability was reduced to 74.5, 48.8, and 25.6% for WT, F1, and F2 THP-expressing TALH cells, respectively (Figure 6B). In the presence of 0.5 mM colchicine or 10 mM 4-PBA, cell viability increased.

![Figure 5. Cellular localization of THP before and after treatment with chemical agents.](image-url)

WT and mutant THP-expressing HEK293 (A) and TALH cells (B) were grown and incubated for 24 h in serum-free DMEM media in the absence or presence of 0.5 mM colchicine or 10 mM 4-PBA. THP was labeled with sheep anti-THP antibody and FITC-conjugated anti-sheep IgG antibody, and cellular localization was observed under a fluorescence microscope. Without treatment with chemical agents, THP signal was most intense in the plasma membrane for WT cells (a) and in the cytoplasm for F1 (d) and F2 (g) cells. When mutant cells were incubated with 0.5 mM colchicine (F1 + colchicine [b]; F2 + colchicine [h]) or 10 mM 4-PBA (F1 + 4-PBA [c]; F2 + 4-PBA [f]), THP signal was reduced in the cytoplasm and increased at the plasma membrane. Negative control mock-HEK293 and TALH cells are shown in j.
viability was improved for WT cells and greatly improved for mutant cells. With treatment, viability in the mutant cells recovered to the level of WT cells without treatment. In contrast, 5 mM allopurinol did not change the cell viability for WT and mutant cells (Figure 6).

Effects of the chemical agents (colchicine and 4-PBA) on cell apoptosis were evaluated with FACS analysis (Table 1). FACS data demonstrated that more early apoptotic signal was shown in cells without treatment than in cells with chemical treatment. The percentage of cells in late apoptosis was also higher in cells without treatment than in cells with treatment.

**Discussion**

Uromodulin (THP) is a GPI anchor glycoprotein produced exclusively by tubule cells of the thick ascending limb (TAL) of Henle (19,20). THP exposed at the luminal face of TAL is released in urine by proteolytic cleavage of the large ectodomain of GPI-anchored domain (21). THP is the most common protein in healthy urine. A characteristic of THP biosynthesis is the very slow conversion from precursor to mature form. Because THP has a very high number of disulfide bridges (48 cysteine residues/mol), formation of a correct set of intrachain disulfide bonds seems to be the rate-limiting step for precursor export out of the ER (8,20). The majority of the >30 different disease-associated UMOD mutations identified to date affect cysteine residues, and it is likely that all lead to protein misfolding and consequently to the retention of the mutant THP in the ER (2,3,5).

An increasing number of diseases seem to be due to improper protein folding, including the ER storage diseases, resulting from the accumulation of proteins secondary to gene mutations (22–25). Identification of an apoptotic pathogenesis induced by accumulation of THP in the ER provides a therapeutic target. Treatment that disrupts intracellular THP aggregates and facilitates movement of THP from the ER to the Golgi and out of the cell may slow or prevent the characteristic tubular cell destruction. Low MW compounds can stabilize misfolded proteins, inhibiting aggregation and/or enabling the movement of these proteins to the Golgi, where they are processed for cellular secretion (26,27). The recent utility of chemical chaperones to stabilize misfolded proteins in a number of degenerative, lysosomal, and ER storage diseases spurred our interest (28–33). The utility of 4-PBA in rescuing nephrin missense mutants encouraged us to test it (30), and we observed a beneficial effect in facilitating THP transport in our transfection model (Figures 4 and 5). The increased transport of mutant THP out of the cell was accompanied by an increase in cell viability, suggesting possible clinical utility (Figure 6).

We also evaluated the effect of two medications that are commonly used to treat gout in individuals with UMOD mutations. Allopurinol is effective in controlling hyperuricemia and preventing gout in individuals with uromodulin-associated kidney diseases, but its ability to prevent renal damage is controversial (34–36). Colchicine, also used to treat gout, can affect microtubule formation, and recent reports suggest that it may delay aggresome formation by the myopathy-causing R120G αB-crystallin mutant (37). We found that allopurinol did not facilitate transport of THP in either of the mutant HEK393
and TALH transfection constructs (Figure 4), and allopurinol did not seem to improve cell viability (Figure 6). Colchicine did facilitate movement of mutant THP from the ER to the cell membrane (Figure 5) and out of the cell (Figure 4). In vitro studies also show that colchicine improved cell viability in HEK293 and TALH cells expressing mutant THP (Figure 6). Improved cell viability was associated with a decrease in apoptotic cells (Table 1). The effect of chemical treatment on post-translational modification of THP in both lysates and conditioned media was evaluated by Western blot analyses. Treatment increased the ratio of 86-/75-kD THP, consistent with an increase in matured forms of THP. These data suggest that chemical treatment reduces THP accumulation in the ER of cells, decreasing cellular apoptosis and increasing cell viability. This is the first study to demonstrate the use of colchicine as a chemical agent to prevent accumulation of ER deposits. This agent may be beneficial not only in uromodulin-associated kidney disease but also in other ER storage diseases as well.

Variability in clinical findings associated with UMOD mutations has been reported, but clear genotype-phenotype correlations have not emerged (3). Longitudinal assessment of families that segregate for UMOD mutations indicates that the disease is more severe in some families (2,6). In human families that segregate the mutations studied in this report, we have observed that the onset of renal damage occurs earlier in individuals with the F2 mutation, compared with the F1 mutation. It therefore is interesting to see that the F2 mutation is associated with a decreased rate of cell viability in the HEK293 and TALH transfection assays. The generality of this observation is unknown, but this may explain the clinical variability in renal function seen in these conditions.

Taken together, our data suggest that UMOD mutations associated with MCKD-2 and FJHN result in the failure to process THP from the ER to the Golgi and secretion out of the cell. THP retention in the ER seems to be associated with an increase in programmed cell death. Apoptosis of the TALH may account for the pathophysiology of progressive renal function seen in these diseases. Treatment of stably transfected HEK293 and TALH cells that express mutant THP with either colchicine or 4-PBA reduced the accumulation of abnormal THP in the ER, facilitating its transport to the plasma membrane and out of the cell. This effect was associated with an increase in cell viability. These findings suggest that treatment with colchicine or sodium 4-PBA may enhance the transportation of THP from the ER to the plasma membrane and secretion into the tubular lumen, reducing programmed cell death of the TALH. These chemical agents may provide therapeutic alternatives to prevent kidney damage in THP-associated kidney diseases.

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