Lysosomal Cystine Storage Augments Apoptosis in Cultured Human Fibroblasts and Renal Tubular Epithelial Cells

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Abstract. Nephopathic cystinosis is a lethal disorder of lysosomal cystine storage due to defective lysosomal cystine transport. How lysosomal cystine causes this multisystemic disorder culminating in end-stage renal disease is not known, because the cystine is isolated from cellular metabolism by the lysosomal membrane. It is here reported that in both normal and nephopathic cystinotic fibroblasts and cultured renal proximal tubule epithelial cells, increased lysosomal cystine causes an increased rate of apoptosis. In nephopathic cystinotic fibroblasts, the rate of apoptosis is 14.8% after exposure to TNF-α versus 7.8% in control normal fibroblasts. Anti-Fas antibodies and UV exposure induced apoptosis in 18.1% and 17.4% of nephopathic cystinotic fibroblasts, respectively, versus 5.2% and 7.1% in normal fibroblasts when analyzed by CaspACE (P < 0.05). Similar results were found when the cells were analyzed by TdT-mediated dUTP nick end labeling (TUNEL). When the cystine content of normal fibroblasts is increased by exposure to cystine dimethylester (CDME), the apoptotic rate is increased to the rate seen in nephopathic cystinotic cells.

Decreasing the cystinotic cells’ cystine content by use of cysteamine results in normalization of the apoptotic rate. Renal proximal tubule epithelial (RPTE) cells are much more sensitive to CDME than fibroblasts, reaching 43.8% apoptosis 6 h after exposure to CDME alone, compared with 38.2% when exposed to TNF-α alone. Serum withdrawal causes an apoptotic rate of 8.7% in nephopathic cystinotic fibroblasts, compared with 6.1% in normal fibroblasts. That rate increases to 37.3% in normal fibroblasts after CDME exposure. Fibroblasts from two cystinotic variants, benign ocular and intermediate cystinosis, do not display increased apoptosis with increased lysosomal cystine. It is concluded that enhanced apoptosis resulting from lysosomal cystine storage may lead to inappropriate cell death and decreased cell numbers in many tissues and hence contribute to the nephopathic cystinotic phenotype. The variant forms may represent co-segregation or linkage of rare alleles that confer resistance to apoptosis, moderating the cell loss and causing the milder disease expression.

Nephopathic cystinosis results from failure of expression of CTNS, located at 17p13, which codes for cystinosin, a 367-amino acid peptide that functions to transport cystine from lysosomes. It is the only lysosomal amino acid transporter yet cloned. The cDNA has 12 exons and is 2.6 kb in length (1). Nephopathic cystinosis has as its major pathophysiologic effects the progressive loss of renal function beginning with the renal Fanconi syndrome at less than 1 yr of age, followed by the onset of glomerular failure after approximately age 7 yr, and reaching ESRD by 10 yr of age. The children are stunted, rarely achieving a height greater than the 50th percentile for a 3-yr-old in the untreated state, and they develop hypothyroidism between the ages of 8 and 10 yr. Renal rickets may also occur secondary to phosphaturia. They display a pathognomonic salt and pepper retinopathy and corneal crystals, which lead to photophobia, corneal ulcerations, and severe debility due to pain as well as impaired vision. Diabetes, esophageal dysmotility, and myopathy may be late complications (2).

The proximate cause of cell death in the cystinotic phenotype is not known, nor is it readily inferred, because the cystine is isolated within lysosomes. The only apparent mode of egress for cystine from lysosomes lacking a functional cystine transporter is exocytosis. After exocytosis, the lysosomal cystine would be deposited at the external face of the plasma membrane, where the amino acid transporter (X̄ gc) is available to transport cystine directly back into the cytosol. There it would encounter a normal cellular concentration of GSH (5 mM) and be reduced to cysteine, with the concomitant oxidation of GSH to GSSG. NADPH-GSSG reductase functions to reduce GSSG back to GSH, effectively completing the transformation of lysosomal cystine to cystolic cystine (3). The cysteine so generated is then available for protein and GSH synthesis (4). Nothing in this sequence suggests a disadvantage for cystinotic cells nor offers an explanation for the lethality of this phenotype.

Recent studies on apoptosis have implicated lysosomes as participants in the critically important process of programmed cell death (5). Lysosomal participation in apoptosis has been documented in a number of studies, but the extent of involvement is still being determined (6,7). It is clear that permeabilization of the lysosomal membrane occurs in this process and
that cathepsins B and D are released. Movement of cathepsins
from granular to cytosolic locations during apoptosis has been
found in fibroblasts undergoing an increase in oxidative stress.
Induction of apoptosis in macrophage-like cells occurs upon
exposure to a lysosomotopic detergent, which causes graded
lysosomal leakage (8). Similar effects have been seen after
photo-oxidation of lysosomes, leading to increased membrane
permeability and resulting in TdT-mediated dUTP nick end
labeling (TUNEL) positivity of the nucleus, an accepted mea-
sure of apoptosis (9). We here report that lysosomal cystine
loading alone causes increased apoptosis in cultured RPTE
cells and also causes increased apoptosis in cystinotic and
normal fibroblasts after standard apoptotic stimuli. Two fibro-
blast lines derived from variant forms of cystinosis, interme-
diate, in which renal death occurs in the teens or twenties, and
ocular, in which no renal involvement occurs (2), do not show
an increased apoptotic response, even though the lysosomal
cystine content is in the same range as in the nephropathic
cells.

Table 1. Genotype and cystine content of cell lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Phenotype</th>
<th>Genotype</th>
<th>Cystine Content (nmol cystine/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM00008</td>
<td>Nephropathic</td>
<td>46XX, 65-kb del</td>
<td>7.5</td>
</tr>
<tr>
<td>GM00760</td>
<td>Nephropathic</td>
<td>46XY, 753 G→A, premature stop</td>
<td>0.78</td>
</tr>
<tr>
<td>GM00046</td>
<td>Nephropathic</td>
<td>46XY, 5-bp del, frameshift</td>
<td>1.51</td>
</tr>
<tr>
<td>GM08761</td>
<td>Ocular</td>
<td>46XX, not determined</td>
<td>6.29</td>
</tr>
<tr>
<td>GM00379</td>
<td>Intermediate</td>
<td>IVS11+2 T→C</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>753 G→A (W138X)</td>
<td></td>
</tr>
<tr>
<td>GM00010</td>
<td>Normal</td>
<td>46XY, apparently normal</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>GM05399</td>
<td>Normal</td>
<td>46XY, apparently normal</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RPTE</td>
<td>Normal</td>
<td>46XY, apparently normal</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*The cystine content was measured by a cystine binding protein assay as described in Materials and Methods. RPTE, renal proximal
tubule epithelial cells.

Table 2. The apoptosis rate in cystinotic nephropathic, variant, and normal fibroblasts

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Phenotype</th>
<th>% Apoptosis</th>
<th>TNF-α</th>
<th>Anti-Fas</th>
<th>UV</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM008</td>
<td>Nephropathic</td>
<td>14.9±0.6</td>
<td>17.7±2.9</td>
<td>12.8±4.8</td>
<td>2.2±0.2</td>
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<tr>
<td>GM760</td>
<td>Nephropathic</td>
<td>13.5±4.3</td>
<td>14.4±2.7</td>
<td>26.3±5.4</td>
<td>3.1±0.7</td>
<td></td>
</tr>
<tr>
<td>GM046</td>
<td>Nephropathic</td>
<td>16.1±4.1</td>
<td>22.3±2.9</td>
<td>13.1±2.0</td>
<td>2.2±0.6</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>14.8</td>
<td>18.1</td>
<td>17.4</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>GM08761</td>
<td>Ocular</td>
<td>8.2±0.98</td>
<td>5.9±1.9</td>
<td>6.4±2.0</td>
<td>2.3±0.3</td>
<td></td>
</tr>
<tr>
<td>GM00379</td>
<td>Intermediate</td>
<td>11.4±2.0</td>
<td>7.7±1.0</td>
<td>8.4±0.94</td>
<td>3.1±0.2</td>
<td></td>
</tr>
<tr>
<td>GM010</td>
<td>Normal</td>
<td>9.2±1.9</td>
<td>6.5±2.3</td>
<td>7.0±0.6</td>
<td>2.8±0.2</td>
<td></td>
</tr>
<tr>
<td>GM05399</td>
<td>Normal</td>
<td>6.3±1.9</td>
<td>4.9±0.8</td>
<td>7.1±0.5</td>
<td>1.9±0.1</td>
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</tr>
<tr>
<td>Average</td>
<td></td>
<td>7.8</td>
<td>5.2</td>
<td>7.1</td>
<td>2.4</td>
<td></td>
</tr>
</tbody>
</table>

*Cells were treated with apoptosis inducers as indicated, stained with CaspACE, and analyzed by fluorescence microscopy. A minimum
of 250 cells were scored per condition, and this was done in triplicate, thus at least 750 cells were scored per condition. T-statistics: P <
0.001 for average nephropathic versus average normal; P < 0.05 for ocular versus nephropathic; P > 0.05 for ocular versus normal,
intermediate versus nephropathic, and intermediate versus normal.

Materials and Methods

Cell Culture
Normal and cystinotic fibroblasts were purchased from The Coriell
Mutant Cell Repository, and cultured in Coon modification of Ham
F12 medium, supplemented with 10% fetal calf serum (FCS). Renal
proximal tubule epithelial (RPTE) cells were purchased from Bio-
whittaker, cultured in renal epithelial basal medium supplemented
with one Singlequots kit per 500 ml to make renal epithelial growth
media (REGM, Biowhittaker). Fibroblasts and RPTE were maintained
in a 5% carbon dioxide, 95% air, humidified incubator at 37°C (4).

Induction of apoptosis and assays for its detection were performed
using commercially available reagents. Normal and cystinotic fibro-
blasts were matched for passage number (±3 passages) and cell
density, and then exposed to one of three apoptotic triggers: TNF-α (2
ng/ml) with actinomycin D (2.5 μg/mL) for 16 h; anti-Fas antibody
(500 ng/ml) with actinomycin D (2.5 μg/mL) for 16 h; or UVB light
(60 mJ) (10–13). After exposure, the cells were maintained in Coon
modification of Ham F12 medium for 16 h before analysis. The cells
were then assayed for apoptosis. Serum withdrawal was also used as
an apoptotic stimulus (14–17), in which case the cells were incubated
activated caspase(s). Cells were incubated in FITC-VAD-Fmk, the able form of the pan-caspase inhibitor zVAD-Fmk, which binds to employed. CaspACE (Promega) is an FITC-conjugated cell-perme-

as described. Three commercially available apoptosis assays were accomplished by exposure to 0.1 mM or 0.25 CDME in normal medium to inhibit cystine re-accumulation.

Normal fibroblast lysosomes were loaded with cystine by the addition of 0.5 mM cysteamine-HCl (4) (Sigma) in Ham F12 medium lacking cystine (Life Technologies), with 10% FCS, for 1 h followed by exposure to the apoptotic triggers in cystine-free medium to prevent lysosomal cystine loss, and then analyzed for apoptosis as described. Lysosomal cystine loading of RPTE cells was accomplished by exposure to 0.1 mM or 0.25 CDME in normal REGM for 1 h before treatment with or without apoptotic triggers.

Cystine Binding Protein Assay

Intracellular lysosomal free cystine was determined using a cystine binding protein (CBP) assay as described (19). CBP was procured from Riverside Scientific. The assay has a sensitivity of 0.1 μM.

Total cell protein was determined by a modification of the Lowry method (20). Statistical analyses were performed using paired t test for means with SSPS for Windows. Results are mean ± 1.0 SD; error bars are ± 1.0 SD (Figure 5).
Immunhistochemistry of Normal and Cystinotic Fibroblasts

Cells were stained with Lysotracker Red (Molecular Probes) for 5 min at room temperature, washed twice in PBS, and then fixed in 4% formalin (1 h). Slides were immersed in blocking buffer (PBS, pH 7.2, 0.5% Carnation dry milk, 0.1% Triton X-100) for 20 min at room temperature, washed twice in PBS, and then incubated with primary antibody (anti-cathepsin B, Santa Cruz Biotechnologies) diluted 1:100 for 2 h at room temperature, followed by incubation with secondary antibody (FITC-conjugated rabbit anti-goat, Santa Cruz Biotechnologies), at 4°C overnight. Slides were washed twice with PBS and then sealed with coverslips and viewed using a Leica DMRX deconvoluting microscope.

Results

The cell lines studied, their genotypes, and nominal cystine content are listed in Table 1. The mutations in cell lines GM00008, GM00760, and GM00046 cause typically severe nephropathic cystinosis with ESRD by 10 yr of age. The cystine content shown in the cystinotic lines in Table 1 varies between 0.8 and 15.7 nmol/mg protein, which is that typically seen in cultured cystinotic fibroblasts (2).

Apoptosis induced in three nephropathic cystinotic, two normal, one intermediate cystinotic, and one ocular cystinotic fibroblast line by three separate inducers is shown in Tables 2 and 3. In Table 2, apoptosis in these lines was assessed by CaspACE. The cystinotic cells on average display about 2 to 3 times the apoptotic rate for the three apoptotic triggers compared with the normal cell lines. The cystinosis variant lines do not show increased apoptosis under these conditions. The differences are statistically significant at $P < 0.05$ between the averages for cystinotic and normal lines and between nephropathic and ocular cystinotic lines. There is no significant difference in the rate of apoptosis between intermediate and nephropathic lines, ocular cells and normal cells, or intermediate versus normal cells (Table 2). Similar results were obtained when the cells were analyzed by TUNEL, except that
line GM00008 did not differ significantly from the normal lines’ response after anti-Fas or UV exposure, nor did line GM00046 after TNF-α exposure (Table 3). Again, the variant lines did not show augmented apoptosis compared with the normal fibroblast lines.

Modulation of the apoptotic response by altering the lysosomal cystine content of normal or nephropathic cystinotic fibroblasts is shown in Figure 1. The bar graphs show the apoptosis rates induced by exposure of two cystinotic and two normal cell line cells to TNF-α or UV radiation before and after correction of the cystine content of nephropathic fibroblasts to normal levels with MEA and before and after increasing the cystine content of normal fibroblasts to cystinotic levels by pre-incubation with CDME. The mean rate of apoptosis for the cystinotic cells before cystine depletion was 15.6 ± 2.7%; after cystine depletion by exposure to MEA, it fell to 6.1 ± 2.8%. This difference is significant at P < 0.001. The average control apoptotic rate for the normal fibroblast lines was 7.2 ± 1.3%, which rose to 18.7 ± 5.4% after exposure to CDME (P < .001). The effect holds whether induction was by TNF-α or UV light. Representative fluorescence micrographs displaying this effect after UV exposure are shown in Figure 2. The high rate of apoptosis in cystinotic fibroblasts with initial cystine content of 4.0 nmol/mg protein is shown in Figure 2A. This fell after treatment with cysteamine, which lowered the cystine content to < 0.1 nmol/mg protein (Figure 2B). The normal rate of apoptosis in normal fibroblasts (cystine content <0.1 nmol/mg protein) is shown in Figure 2C, and this increases to 37.3 ± 3.9%.

Table 4. Apoptosis in normal and nephropathic cystinotic fibroblasts exposed to serum withdrawal

<table>
<thead>
<tr>
<th>Condition</th>
<th>%Apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM00008</td>
<td>2.8±0.4</td>
</tr>
<tr>
<td>GM05399</td>
<td>3.1±0.5</td>
</tr>
<tr>
<td>Untreated controls</td>
<td></td>
</tr>
<tr>
<td>Serum withdrawal</td>
<td>8.7±1.0</td>
</tr>
<tr>
<td>Serum withdrawal and MEA</td>
<td>7.2±0.4</td>
</tr>
<tr>
<td>Serum withdrawal and CDME</td>
<td>–</td>
</tr>
<tr>
<td>GM00008</td>
<td>6.1±0.3</td>
</tr>
<tr>
<td>GM05399</td>
<td>37.3±3.9</td>
</tr>
<tr>
<td>Serum withdrawal and CDME</td>
<td></td>
</tr>
</tbody>
</table>

* The cystine content was decreased in cystinotic cells from 2.3 to 0.5 nmols cystine/10⁶ cells by pretreatment with MEA before incubation in cystine-free and serum-free Ham F₁₂ medium. The cystine content in normal was increased from 0.4 to 1.3 nmols cystine/10⁶ cells by treatment with CDME before incubation in serum-free Coon F₁₂ (see Materials and Methods). Cells were stained with CaspACE and analyzed as previously described. P = 0.068 for 008 serum withdrawal versus 05399 serum withdrawal; P = 0.172 for 008 serum withdrawal versus 05399 serum withdrawal plus MEA; and P = 0.006 for 05399 serum withdrawal versus 05399 serum withdrawal plus CDME.

Figure 3. The effect of CDME or MEA on the morphology of normal or nephropathic cystinotic fibroblasts. Fibroblasts were cultured under the usual conditions and exposed to TNF-α for 16 h as described in Materials and Methods. Panels a through f are nephropathic cystinotic fibroblasts. Panels b and e show cells after TNF-α exposure alone; panels c and f show the effect of CDME in normal cells (c) or MEA in cystinotic cells (f). Note typical apoptotic morphology in panels b, c, and e, with more apoptotic cells in e (cystinotic) than b (normal). Enhanced apoptosis is seen in normal cells to which CDME is added (c). More normal morphology is seen in nephropathic cystinotic cells after MEA treatment (f). Photographs via phase microscopy.

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Figure 4. Loss of colocalization of cathepsin B in normal and nephropathic cystinotic fibroblasts after TNF-α exposure. Fibroblasts were maintained under normal culture conditions, exposed to TNF-α, loaded with Lysotracker red, fixed, and stained for cathespin B using an anti-cathepsin B antibody as described in Materials and Methods. Panels a through f are normal fibroblasts; a through c are control cells; d through f are treated with TNF-α. Panels g through l are cystinotic fibroblasts; g through i are control; j through l are treated with TNF-α. Note loss of granularity and decreased colocalization in c versus f and i versus l. Photographs via a deconvoluting microscope.
The RPTE cystine content after exposure to 0.25 mM CDME for fibroblasts) followed by lysis and release from the culture dish. The time course was accelerated, with these cells attaining a maximum rate of apoptosis within 6 h, (as opposed to 17 h in cultured cells (31–33), and it could be that exposure to CDME alone alters the redox potential sufficiently to trigger the apoptotic response (24). In contrast, extracellular cystine deprivation is pro-apoptotic (25–27), and cells can be rescued from apoptosis by extracellular cystine supplementation via the ectomembrane enzyme, glutathione tranpeptidase, which is localized to the plasma membrane and which functions in the synthesis of reduced glutathione (28). Accessibility of extracellular cystine to this plasma membrane complex leads to enhanced GSH synthesis, which may not be the case in cystine originating from lysosomes. Apoptosis is known to play a role in the renal tubular dysfunction seen in transplantation rejection phenomenon and also in certain forms of retinopathy. The renal tubule and retina are highly sensitive to apoptosis (29,30). These two tissues are the first to be affected in nephropathic cystinosis (2), and it is feasible that the order of tissues involved in the disease reflects the intrinsic sensitivity of each to apoptosis. CDME is known to disrupt renal tubule cell function in animal models and cultured cells (31–33), and it could be that exposure to CDME alone alters the redox potential sufficiently to trigger the apoptotic response (Figures 5 and 6). CDME is known to traverse both plasma and lysosomal membranes, but it is only concentrated within the lysosomes, where resident hydrolases remove the methylester producers, generating free cysteine. The acidic environment produces a net positive charge, inhibiting exit of cysteine via the lysosomal cystine transporter cystinostin (18,21). We speculate that under normal circumstances in normal cells, the influx of cysteine into lysosomes via proteolysis of disulfide-containing proteins (22,23) proceeds at a rate less than the normal egress rate of cysteine via cystinosis. This would yield a small and relatively constant rate of release of cystine into the cytosol, which could be readily reduced to cysteine by glutathione. In cystinotic fibroblasts, which lack functional cystinosin, cystine accumulates to very high levels (2). Apoptotic stimuli lead to lysosomal permeabilization and translocation of cathepsins B and D into the cytosol (5–9) (Figure 4); it is therefore possible that rapid release of cystine into the cytosol occurs during this permeabilization phase and results in a locally altered redox potential, and/or reaction with critical protein thiol, that may sensitize the cells to, or trigger, the apoptotic response (24). In contrast, extracellular cystine deprivation is pro-apoptotic (25–27), and cells can be rescued from apoptosis by extracellular cystine supplementation via the ectomembrane enzyme, glutathione tranpeptidase, which is localized to the plasma membrane and which functions in the synthesis of reduced glutathione (28). Accessibility of extracellular cystine to this plasma membrane complex leads to enhanced GSH synthesis, which may not be the case in cystine originating from lysosomes. Apoptosis is known to play a role in the renal tubular dysfunction seen in transplantation rejection phenomenon and also in certain forms of retinopathy. The renal tubule and retina are highly sensitive to apoptosis (29,30). These two tissues are the first to be affected in nephropathic cystinosis (2), and it is feasible that the order of tissues involved in the disease reflects the intrinsic sensitivity of each to apoptosis. CDME is known to disrupt renal tubule cell function in animal models and cultured cells (31–33), and it could be that exposure to CDME alone alters the redox potential sufficiently to trigger the apoptotic response (Figures 5 and 6). CDME also causes inhibition of Na⁺/H⁺-dependent transporters (33). Free cystine has been known to be nephrotoxic in experimental animals since 1925 (34); however, the mechanism remains to be determined. Cystinosis patients display a typical swan neck deformity of
the proximal tubule (35,36), concomitant with development of the Fanconi syndrome and consistent with hypocellularity of that structure.

Enhanced sensitivity to apoptosis due to increased lysosomal cystine loading offers another insight into the interplay of lysosomes and apoptosis as well a new perspective in understanding the pathophysiology of cystinosis. Increased lysosomal cystine in fibroblasts or RPTE cells either as the result of cystinosin deficiency, or induced by incubating normal cells in cystine dimethylester, causes increased apoptosis. This finding is important because it excludes other effects of defective CTNS expression and alternative effects of CDME exposure, because the only common element in the two conditions is elevated lysosomal cystine. Enhanced sensitivity to apoptosis is observed after triggers that stimulate both the intrinsic apoptotic pathway (UV light) and the extrinsic pathway (TNF-α, anti-Fas antibody, or serum withdrawal). The intrinsic and extrinsic pathways converge at the BID/Bcl2 locus and then ultimately activate caspases (37). It is somewhat counterintuitive that lysosomal cystine release increases apoptosis, because the executioner caspases are thiol proteases, which should be inhibited by the disulfide cystine.

Inappropriate apoptotic cell death may occur in cystinotic tissues after extrinsic or intrinsic apoptotic stimuli are presented, which would fail to be executed in normal tissues due to damping of the apoptotic cascade. This aberrant sensitivity could account for the cystinotic phenotype by causing inappropriate cell death throughout embryogenesis and the life of the individual, leading to a hypocellular state in many tissues. The mechanisms causing this augmented rate of cell death remain to be determined. It is possible that the cells may proceed to necrosis in some cases under these circumstances, as suggested by the increase in PI positivity seen in RPTE cells treated with both TNF-α (38) and CDME.

Patients with variant forms of cystinosis are either heterozygous for CTNS mutations, which, in the homozygous state,
cause the nephropathic form of the disease, or they are homozygous for predicted milder mutations (39,40). The leukocyte cystine content overlaps among the various clinical forms of cystinosis, ranging from 1.3 to 11.6 nmol/mg protein in nephropathic, 1.7 to 2.5 in the intermediate form, and between 0.5 and 1.8 in the ocular form. A similar overlap is shown in cultured fibroblasts in Table 1. Distinction among the varieties is based on the age at onset of symptoms and clinical severity (2.39–41).

Two variant cystinotic cell lines did not show enhanced rates of apoptosis despite increased lysosomal cystine (Tables 2 and 3). The combination of a severe nonsense mutation and a splice-site mutation allows for some residual cystine transport activity and, because the splice site mutation in cell line GM00379 causes truncation of cystinosin just distal to the second lysosomal recognition site, permits some lysosomal localization of cystinosin and cystine transport activity (39,42). We speculate, however, that these phenotypic cystinosis variants are not due to residual cystinosin activity, as the lysosomal cystine content overlaps that of the nephropathic form; rather, they are due to linkage or to co-segregation of a gene or genes, which confers relative resistance to apoptosis. The cystinosis mutation itself is rare; if a rare allele is also responsible for resistance to apoptosis, then the extreme scarcity of these variants is explained, as is the milder phenotype. The phenotype could also be caused by diminished expression of a pro-apoptotic gene such as APC, as has recently been described in familial adenomatous polyposis (43). This hypothesis raises the possibility of increased neoplastic transformation in these patients, however none has yet been recognized.

We conclude that nephropathic cystinotic fibroblasts enter the pathway for programmed cell death more readily than do normal fibroblasts and that normal fibroblasts and RPTE cells display augmented entry into that pathway after their lysosomal cystine content is artificially increased. Study of the pathway(s) by which cellular perturbations result in modulation of the apoptotic cascade may lead to a clearer understanding of the regulation of apoptosis, and a better understanding of the development of the cystinotic phenotype.

Acknowledgments

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References


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2001, pp 308–319

592, 1992

522, 1996

244, 1993


