Role of the Proteasome in TGF-β Signaling in Lens Epithelial Cells

Matthew R. Hosler,1,2 Shuh-Tuan Wang-Su,1 and B. J. Wagner1,2,3

PURPOSE. The durability of the ubiquitin proteasome pathway in the mammalian lens makes this enzyme system a potential contributor to cataracts and posterior capsular opacification (PCO). The present study addresses proteasome involvement in TGF-β-induced, cataract-associated gene activation in human lens cells.

METHODS. HLE B-3 cells were treated with TGF-β, in combination with the proteasome inhibitors MG-132 or lactacystin. TGF-β target gene expression was measured by semiquantitative RT-PCR. Annexin-FITC staining and flow cytometry were used to assess apoptosis levels. Western blot analyses were performed with anti-SnoN and anti-Smad2 antibodies.

RESULTS. TGF-β induced the expression of α-smooth muscle actin, fibronectin, and TGF-β-inducible gene mRNA in HLE B-3 cells and primary cultured human lens cells from donor tissues. TGF-β also induced a time-dependent decrease in the level of the Smad repressor SnoN. γ-Glutamyl-cysteine synthetase (γ-GCS) mRNA levels decreased in the presence of TGF-β. Proteasome inhibitor cotreatment blocked the induction of α-SMA mRNA, the loss of SnoN protein, the decrease in γ-GCS mRNA, and TGF-β-induced apoptosis.

CONCLUSIONS. The HLE B-3 cell line and primary cultured human lens cells respond similarly to TGF-β treatments by activating cataract-related gene expression. This response in both of these model systems is blocked by inhibiting the proteasome. This suggests that the proteasome can mediate cataract and PCO-associated changes and therefore is a novel target of medical therapy. (Invest Ophthalmol Vis Sci. 2006;47: 2045–2052) DOI:10.1167/iovs.05-0650

The ubiquitin proteasome pathway (reviewed in Refs. 1–4) involves a series of enzymes that ubiquitinate and degrade target substrate proteins. The ATP-dependent 26S proteasome degrades ubiquitin-tagged substrates. This pathway is necessary to remove key proteins coordinately, as part of normal cell signal transduction. Examples include cyclin-dependent kinase inhibitors,5 the MAP kinase inhibitor ICER,6 and TGF-β–dependent Smad inhibitors.7 Interference with the ubiquitin proteasome pathway at several levels is reported to modulate these signals and suggests the role played by the proteasome in these processes. The most common way to block this pathway is using small peptides or peptide analogues that bind and inhibit the activity of the 20S core protease, thereby blocking the whole pathway. MG132 and lactacystin are examples of this type of inhibitor. This current work investigates the role of the proteasome in TGF-β induction of lens disease.

Posterior capsular opacification (PCO) is a serious complication of cataract surgery.8–11 TGF-β has been linked with PCO formation in clinical and experimental studies.12 One effect of TGF-β is induction of lens cells to undergo an epithelial mesenchymal transition (EMT) during PCO formation.13,14 This process involves the activation of several EMT-associated target genes including α-smooth muscle actin (α-SMA), fibronectin, and TGF-β-inducible gene (β-ig).15 Reports suggest that blocking lens cell growth and transdifferentiation after cataract extraction surgery can prevent PCO formation.15,16,17 Several strategies are reported, and one of the most promising appears to be blocking TGF-β signaling.13,18 Treatments described in the literature are potentially associated with numerous obstacles, and none have found their way into clinical use.19–22 Therefore, compounds must be sought that can block TGF-β and related lens processes, while being closer to use in human subjects for this purpose. The proteasome has been reported to modulate cell signaling proteins, including those associated with TGF-β.23 In this way the proteasome can contribute to TGF-β-mediated lens disease, making proteasome inhibitors novel agents that could prevent PCO.

TGF-β induces expression of numerous groups of proteins that direct downstream gene regulation. These induced factors include groups of transcriptional activators and groups of transcriptional repressors.24,25 Though the TGF-β/R-Smad pathway itself typically activates promoters, its target genes can increase or decrease expression of farther downstream target genes. An example of a Smad target that acts as a transcriptional repressor is the protein Fra-1. TGF-β/R-Smads are reported to increase Fra-1 levels leading to transcriptional repression at the AP-1 promoter.24,26 Through Fra-1, TGF-β decreases expression of genes such as γ-glutamylcysteine synthase (γ-GCS). Effective treatments that block TGF-β/R-Smad signaling would also reverse this type of repression.

Apoptosis has been described in lens cell models as a response to TGF-β, and occurs in association with PCO induction. It has been reported in human anterior subcapsular cataract (ASC),27,28 ASC animal models,29,30 human PCO,12,51 and animal models of PCO.17,22 Details regarding the exact role of, or requirement for, apoptosis in TGF-β-induced PCO are not clear. However, it is a distinct occurrence related to these types of human cataract and represents an independent consequence of TGF-β lens signaling that could be blocked when trying to prevent PCO.

The TGF-β receptor, downstream activator Smads, and Smad inhibitors have been identified as targets of the ubiquitin proteasome pathway.35–35 It is reported that cotreatment with a proteasome inhibitor can cause accumulation of Smad inhibitors and block TGF-β signaling.23 A well-studied group of such
Smad inhibitors are the Ski family of proteins of which SnøN is a member. Proteasome inhibitor treatment induces accumulation of various TGF-β signal proteins; however, it appears that they predominantly cause accumulation of the Smad inhibitor proteins. Proteasome inhibition, therefore, is expected to block TGF-β actions such as those leading to ASC and PCO.

Because the proteasome appears resistant to aging and oxidation in the lens,36–38 and Smad inhibitors are critical proteasome substrates, we hypothesize that the proteasome is required for TGF-β signaling in the lens. The present study models TGF-β-induced EMT gene activation and gene repression in lens cells. We report the ability of proteasome inhibitors to block positive TGF-β-induced changes that occur during PCO formation and to reverse TGF-β induced gene repression. The proteasome is a relatively new target for FDA-approved treatment of several diseases. Our findings coupled with the clinical progress of this drug class could greatly facilitate testing of proteasome inhibitors for the purpose of preventing PCO.

Materials and Methods

Reagents

TGF-β2, MG132, lactacystin, Eagle's MEM, gentamicin, and glutathione reagents, were all purchased from Sigma-Aldrich (St. Louis, MO). RT-PCR reagents were purchased from Roche Applied Sciences (Indianapolis, IN). Accutase cell separation enzyme was obtained from Innovative Cell Technologies Inc. (San Diego, CA). Fetal bovine serum and glutamine were purchased from Gemini Bio-products (Calabasas, CA). Annexin stain was purchased from Caltag (Burlingame, CA). SnoN antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Smad2 antibody was purchased from Upstate Cell Signaling Solutions (Waltham, MA).

Cell Culture and Treatment

The human lens epithelial cell line, HLE B-3, was generously provided by Usha Andley (Washington University, St. Louis, MO).9 The cells were cultured in Eagle’s MEM, with 20% fetal bovine serum, glutamine (15 mg/mL), and gentamicin (50 μg/mL). Cells were treated and harvested between 80% and 90% confluence for all mRNA and protein assays. All epithelia were cut into quarters, and each quarter portion passaged once into five wells each. All procedures complied with the Declaration of Helsinki and were approved by the Institutional Review Board of the New Jersey Medical School, University of Medicine and Dentistry of New Jersey (UMDNJ).

Reverse Transcriptase–Polymerase Chain Reaction

Semiquantitative reverse transcription–polymerase chain reaction (RT-PCR) was used to measure target mRNA levels relative to β-actin.53 HLE B-3 cells were treated overnight with 1 ng/mL TGF-β. Total RNA was isolated from cells (RNAzol; Tel-Test, Friendswood, TX) and reverse transcribed at 42°C for 60 minutes, followed by heat denaturation at 95°C for 5 minutes and cooling to 4°C. The reaction mixture (40 μL) included 1 μg of total RNA, 2.5 μL oligo (dT)16–40 U Rnase inhibitor, and 100 U MuLV reverse transcriptase (Applied Biosystems, Inc. [ABI], Foster City, CA). The PCR mixture (50 μL) included 5 μL from the reverse transcription reaction, 0.3 μM each sense and antisense primer, and 1.25 U DNA polymerase (AmpliTaq; ABI). The PCR was performed with an initial denaturation for 2 minutes at 95°C, followed by 25 or 30 amplification cycles, each comprising 1 minute at 94°C, 1 minute at 60°C (56°C for γ-GCS), and 1 minute at 72°C, followed by 7 minutes at 72°C and cooling to 4°C. The PCR products were separated by 2% agarose gel electrophoresis and visualized with ethidium bromide. The bands were quantitated by densitometry and normalized with β-actin. Each primer set was tested over a range of 15 cycles to assure that the selected number of cycles was in the linear range. Product formation for indicated genes was linear at either 25 or 30 PCR cycles. The primer sequences specific for the genes examined and predicted product sizes are shown in Table 1. All primers were synthesized by the New Jersey Medical School Molecular Resource Facility (Newark, NJ).

Western Blot

SDS sample buffer (500 μL) was applied directly to 25-cm² flasks to lyse cells.46 An additional 400-μL sample buffer was added to rinse any residual cell lysate from flasks, and added to the initial sample. Samples were incubated for 10 minutes at 95°C, followed by three 40-second bursts at microtip setting number 5 in a sonicator (model XL2020; Misonix, Farmingdale, NY). Protein concentration was measured by the Bradford protein assay (Bio-Rad, Hercules, CA), and all samples

Table 1. Primer Sequences for RT-PCR Analysis

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Primer Sequence (5'→3')</th>
<th>Reference</th>
<th>Expected Product Size (bp)</th>
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<tr>
<td>β-Actin</td>
<td>Forward: CTTGCGGCGGCGCATGACCCCA</td>
<td>44</td>
<td>243</td>
</tr>
<tr>
<td></td>
<td>Reverse: TCGGCTTAGGGTGGCGGCTGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β-inducible gene (β4γ)</td>
<td>Forward: CGCTACCAACCAACACATGCA</td>
<td>45</td>
<td>660</td>
</tr>
<tr>
<td></td>
<td>Reverse: GGTTGCGCAGCTGTCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-SMA</td>
<td>Forward: CGCTGAGGGGCTGAGCC</td>
<td>15</td>
<td>516</td>
</tr>
<tr>
<td></td>
<td>Reverse: TCGGAGTGGACCCAGATTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Forward: GCAGCTTGATGATGATTG</td>
<td>15</td>
<td>421</td>
</tr>
<tr>
<td></td>
<td>Reverse: ATCGACCCGATGATTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ-CGS (heavy subunit)</td>
<td>Forward: GTGACGACGACGACG</td>
<td>24</td>
<td>531</td>
</tr>
<tr>
<td></td>
<td>Reverse: TGAGACGACGACGACGAG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
were diluted to equal protein concentrations. On a 10% SDS polyacrylamide minigel 25 μg protein was loaded per lane. Gels were electro-botted for 1 hour, 15 minutes at 100 V onto nitrocellulose membranes. Membranes were blocked for 1 hour at room temperature in 5% dry milk. A 1:200 dilution of SnoN primary antibody in the same dry milk solution was applied at room temperature for 1 hour. A 1:2000 dilution of the secondary antibody in the blocking solution was then applied to the blots for 1 hour at room temperature. Membranes were rinsed and visualized by enhanced chemiluminescence with the HRP kit (PerkinElmer, Wellesley, MA). Chemiluminescence reactions were visualized with a series of five 2-minute captures on a gel documentation system (Syngene, Cambridge, UK), and the optimum exposure selected from the five captures. Band intensity was then quantified (GeneTools software package; Syngene).

**Annexin Staining**

HLE B-3 cells were grown in six-well plates for Annexin staining. At the end of the treatment period, cells were removed from wells using the enzyme treatment (Accutase) at 37°C for 5 minutes. This enzyme reaction was stopped by addition of equal volume of growth media containing 20% fetal bovine serum. Cells were rinsed in binding buffer (Caltag) and resuspended at a concentration of 1 million cells per milliliter. Cell sample (100 μL) was mixed with 10 μL annexin V-FITC and 10 μL of propidium iodide solution, as adapted from the manufacturer’s instructions. Cells were incubated on ice and in the dark for 10 minutes and immediately analyzed by flow cytometry (FACS Calibur System; BD Biosciences, Franklin Lakes, NJ).

**Statistical Analysis**

A statistical software package, (JMP, ver. 4; SAS Institute Inc, Cary, NC) was used to perform the analyses. The statistical significance of pair-wise comparisons was assessed by two-tailed Student’s t-test. As indicated for analysis of Western blot quantitation, the significance of protein levels across treatment conditions was assessed by one-way analysis of variance (ANOVA). P < 0.05 was considered to be statistically significant.

**RESULTS**

**TGF-β and Expression of EMT-Associated Genes Used as Markers for PCO**

Semiquantitative RT-PCR was used to measure mRNA expression for three genes: α-SMA, fibronectin, and β-ig. mRNAs were prepared from HLE B-3 cells after 16 hours of treatment with 1 ng/mL TGF-β. As seen in Figure 1, expression of each of these cataract and EMT-associated genes was increased more than twofold in the presence of 1 ng/mL TGF-β. α-SMA expression was increased the most, followed by fibronectin and then β-ig.

**Proteasome Inhibitors and TGF-β Transcriptional Activation**

HLE B-3 cells treated with 1 ng/mL TGF-β, 2.5 μM MG132, or a combination of both were harvested for mRNA after 16 hours incubation. RT-PCR was performed using primers against the α-SMA gene. Figure 2A illustrates that the TGF-β-induced increase in α-SMA mRNA levels was completely inhib-
lit by the full dose of MG132. The 1.25-μM dose of MG132 resulted in a partial blockage of induction of α-SMA.

A similar experiment using lactacystin as the proteasome inhibitor showed similar results; however, a higher concentration of this less potent inhibitor was needed. HLE B-3 cells were treated overnight with 1 ng/mL TGF-β, 10.0 μM lactacystin alone, or a combination of both. Cells were harvested for mRNA (RNAzol reagent; Tel-Test). RT-PCR was performed using primers against the α-SMA gene. The TGF-β treatment induced α-SMA mRNA, and 10 μM lactacystin completely inhibited this induction seen in Figure 2B. Lactacystin (5 μM) applied together with TGF-β did not prevent induction of α-SMA mRNA.

Primary cultures of explanted human donor lens epithelium treated with 1 ng/mL TGF-β, 2.5 μM MG132 or the combination of both showed similar effects of TGF-β activation which was blocked by proteasome inhibition (Fig. 2C). The increase in α-SMA mRNA was notably less in the primary cultures, but the inhibition by MG132 was similar to that in the HLE B-3 cell line.

**Proteasome Inhibitors and TGF-β Transcriptional Repression**

Figure 3A illustrates the negative regulation of the γ-GCS gene by TGF-β treatment. HLE B-3 cells were treated with 1 ng/mL TGF-β as just describe. TGF-β caused up to an 80% reduction in the expression of the γ-GCS gene. Cotreatment with 2.5 μM MG132 caused a reversal of the observed TGF-β effect.

Figure 3B illustrates a similar cotreatment of HLE B-3 cells with 1 ng/mL TGF-β in combination with the proteasome inhibitor, lactacystin. Cotreatment with 10 μM lactacystin blocks the TGF-β reduction in γ-GCS gene expression, similar to MG132.

**TGF-β, MG132, and Apoptosis in HLE B-3 Cells**

HLE B-3 cells were treated in culture with 10 ng/mL TGF-β and 2.5 μM MG132 to study the effects on apoptosis after 24 hours of incubation. Cells were separated and suspended, and then dual stained with annexin V-FITC and propidium iodide. For each sample, 10,000 cells were counted, and the proportion of apoptotic cells expressed as a percentage of all cells counted.

Figure 4 illustrates that 10 ng/mL TGF-β induced a statistically significant level of apoptosis. In the samples treated with this TGF-β dose combined with MG132, the apoptosis induction was blocked. Apoptosis levels in cells treated with MG132 alone were similar to the control.

**Effect of Proteasome Inhibition on SnoN and Smad2**

HLE B-3 cells were treated for the indicated lengths of time with 1 ng/mL TGF-β alone, 2.5 μM MG132 alone, or a combination of the two. Western blot quantification of SnoN protein levels is shown in Figure 5A. TGF-β induced a decrease in SnoN levels at 1, 4, and 16 hours. MG132 alone induced accumulation of SnoN at all time points, and the combination also showed SnoN accumulation at all time points. As emphasized by asterisks in Figure 5A, one-way ANOVA indicated that the changes seen across the three treatment conditions were significant at 4 and 16 hours.

Similar TGF-β treatment and Western blot for SnoN protein using lactacystin as the proteasome inhibitor is shown in Figure 5B. TGF-β induced a decrease in SnoN levels at 1, 4, and 16 hours. Consistently, the TGF-β-induced decrease appeared to be less pronounced at the 2-hour time point. Like MG132, lactacystin alone induced accumulation of SnoN at all time points. The asterisks in Figure 5B indicate the results of the one-way ANOVA, which indicated that the changes seen across the three treatment conditions were significant at 1 and 16 hours.
Smad2 immunoblots and graph of densitometric scans are shown in Figure 6. TGF-β and MG132 treatments, protein preparation, and Western blot analysis were performed similarly to those for SnoN. TGF-β induced an increase in Smad2 levels primarily at 1 hour, and only slightly at the other three time points. MG132 alone caused accumulation of Smad2 to a degree similar to that caused by TGF-β alone. The combination of TGF-β and MG132, however, did not have an additive effect on Smad2 levels. In contrast to SnoN, no statistically significant changes in Smad2 levels across treatment conditions were found by ANOVA.

FIGURE 5. Western blot analysis showing levels of SnoN protein after treatment with a combination of TGF-β plus proteasome inhibitor. Cells were harvested for protein studies after 1, 2, 4, or 16 hours. (A) SnoN Western blot analysis, and densitometric quantitation of SnoN bands. HLE B-3 cells were treated with 1 ng/mL TGF-β, 2.5 μM MG132, or 1 ng/mL TGF-β plus 2.5 μM MG132. (B) Densitometry quantitation of SnoN Western blot analysis with lactacystin. HLE B-3 cells were treated with 1 ng/mL TGF-β, 10 μM lactacystin, or 1 ng/mL TGF-β plus 10 μM lactacystin. Data are the mean ± SEM of triplicate independently treated samples from one of two experiments that yielded similar results (n = 3). *Statistically significant changes in SnoN protein levels across treatment conditions (P < 0.05; ANOVA).

Effect of Proteasome Inhibition on HLE B-3 Cell Morphology

Phase-contrast microscopic imaging of HLE B-3 cells is shown in Figure 7. Figures 7A and 7B show that TGF-β induced significant elongation of the B-3 cells. Figure 7C shows that proteasome inhibition alone had no effect on cellular morphology. Figure 7D shows that proteasome inhibition prevented the TGF-β induction of the morphology change seen in 7B. The cultures were followed after treatment for 24 and 48 hours. The changes as shown persisted through 24 hours. However, excess cell death was apparent with 10 ng/mL TGF-β alone starting at 36 hours, making the later time point difficult to evaluate.

DISCUSSION

The Proteasome’s Role in TGF-β Induction of Cataract and PCO-Related Genes

The EMT process has been linked to anterior subcapsular cataract (ASC), and posterior capsular opacification (PCO). mRNA levels of three of the most commonly studied genes used as markers for EMT were induced by TGF-β in our human lens epithelial cell line and human lens epithelial cell primary culture model. The induction of the α-SMA marker gene was blocked by inhibiting the proteasome (Fig. 2). One explanation for this is that the proteasome inhibitor treatment is inducing accumulation of a Smad inhibitor protein. TGF-β
decreases the level of the Smad repressor protein SnoN and proteasome inhibitor treatment causes accumulation of this repressor even in the presence of TGF-β (Fig. 5).

The Ski/SnoN family of oncoproteins are classified as such because of their inhibitory effects on the TGF-β pathway and their association with the transformed phenotype. Because the HLE B-3 cell line is transformed, it may express elevated levels of SnoN that are related to the transformation process. We confirmed the physiological relevance of our results by showing that proteasome inhibition also blocked TGF-β-induced gene expression in primary cultured human lens epithelial cells, as shown in Figure 2C. The primary cultured cells also increased α-SMA mRNA levels in response to TGF-β, and MG132 blocked this induction. This suggests that the TGF-β signaling axis, including SnoN repression, is present and functioning in the untransformed human lens cells. The results of these experiments are consistent with related nontransformed tissue studies showing that lenses from Smad3 knockout mice fail to undergo EMT normally in response to TGF-β.77

The primary cultured lens cells exhibited significant levels of α-SMA expression without treatment, and the degree of stimulation by TGF-β was less than in the B-3 cell line. We speculate that this could be due to relatively high background levels of α-SMA in all the samples, as they undergo a wound-healing response secondary to the culture procedure. Injury-induced gene activation has been described in several different lens models. Saika et al.49 modeled lens injury and healing in mice by surgically injuring the lenses in situ and observing the effects. Healing lenses were found to express elevated levels of α-SMA and lumican. Immunohistochemistry of human postoperative lens samples has shown nuclear translocation of Smads 3 and 4 in lens epithelial cells adjacent to surgical lens epithelial incisions.50 These findings are consistent with reports of elevated TGF-β-associated elements in cultured lenses, where the stimulation was attributed to lens adherence to culture vessels.15,51

**The Role of the Proteasome in TGF-β Induction of Apoptosis**

Based on reports relating PCO to apoptosis,29,52–55 we examined TGF-β and proteasome inhibition of apoptosis in HLE B-3 cells. The exact role and importance of apoptosis in PCO is not fully clear. However, TGF-β induces apoptosis in PCO models and human PCO disease. Figure 4 shows that TGF-β induces significant levels of apoptosis in the HLE B-3 cells and that, at the concentration used, MG132 cotreatment blocks this induction. Although blocking apoptosis is not itself a likely goal for PCO treatment, this inhibition further confirms the proteasome’s role in TGF-β function, and its potential for contributing to PCO.59 This may also illustrate a link between TGF-β, the proteasome and other cataract subtypes associated with apoptosis.56–59 The association of apoptosis and cataract is still an area of active investigation.29,53,54,60–62

**TGF-β Utilization of the Proteasome during Smad Activation**

Several Smad-associated signal proteins downstream of the TGF-β receptor are proteasome substrates, an important reported target being the Smad repressor, SnoN.7,63,64 The proteasome inhibitor-induced accumulation of SnoN seen in our experiments is consistent with the proteasome’s being necessary for normal TGF-β signal progression.

The current work included the observation that TGF-β induced a decrease in SnoN protein levels, except at the 2-hour time point. This observation agrees with the study by Stroschein et al.65 which showed a rapid decrease in SnoN mRNA and protein levels followed immediately by an increase that peaked at 2 hours after TGF-β ligand stimulation. They also found increased levels of SnoN bound to Smad4, peaking at 2 hours. This phenomenon is explained by the fact that there are TGF-β response elements in the SnoN promoter. They postulate that TGF-β activates SnoN gene expression and levels, and that SnoN provides negative feedback on TGF-β to facilitate precise regulation of that pathway.

Other TGF-β receptor-associated proteins are proteasome substrates (reviewed in Ref. 66), including Smad2.67–69 Considering the potential for Smad2 accumulation to oppose the accumulation of SnoN, we examined Smad2 levels under the

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**Figure 7.** Phase-contrast microscope images of HLE B-3 cells treated overnight with (A) vehicle, (B) 10 ng/ml TGF-β, (C) 2.5 μM MG132, or (D) 10 ng/ml TGF-β plus 2.5 μM MG132. Each image shown is representative of four independently treated samples that yielded similar results (n = 4).
same TGF-β and proteasome inhibitor treatment conditions. Figure 6 shows that MG132 alone had less of an effect on the accumulation of Smad2, and the accumulation was transient. Smad2 accumulation was seen only with MG132 treatment at 1 hour, after which Smad2 levels were similar to control levels. Our proteasome inhibitor treatment therefore appears to affect the repressor SnoN preferentially, with higher potency and over a longer period, explaining its inhibitory effects on TGF-β-induced gene expression.

The Role of TGF-β and the Proteasome in PCO

Blocking PCO has been attempted with several medicines. Some of these compounds induce apoptosis as part of their mechanism of action. Thus, the inhibition of TGF-β induced apoptosis by proteasome inhibitors that we show in Figure 4 might seem to be counterproductive as a treatment for PCO. However, PCO is also reported to involve the antiapoptotic growth factor FGFR in addition to TGF-β. Proteasome inhibitors are seen to block the action of this and similar cytokines. Depending on the balance of effector molecules (e.g., p53, Bax, JNK, bcl2, NFκB and myc) prevailing in the cell at a given time and the concentration of the proteasome inhibitor used, the direction of the cellular response will change when the proteasome is inhibited. For example, cancer cells proliferating due to abnormally high degradation of p53 are killed by inhibiting the proteasome to restore p53 levels, whereas normal cells are relatively unaffected. We therefore predict that proteasome inhibition will be effective at blocking PCO, based on its action against several PCO-related growth factors.

The pathogenesis of PCO is believed to include the proliferation and morphologic transformation of the lens epithelial cells. Specifically, lens epithelial cells have been found to become elongated, and to remodel their extracellular matrix proteins. The HLE B-3 cell line is one of several lens models shown to undergo morphologic changes induced by TGF-β. We used the B-3 cell line and previously optimized conditions to determine whether proteasome inhibition would modulate this 10 ng/mL TGF-β-induced morphologic change. We show in Figure 7 that inhibition of the proteasome blocks TGF-β induction of cellular elongation. This further suggests that proteasome inhibition will be effective at blocking the molecular pathogenesis of PCO.

The interaction we see between TGF-β and proteasome inhibitors in this study makes this class of drugs an attractive possibility in the search for medical prevention of PCO. A member of this class was recently approved by the FDA for the treatment of multiple myeloma and is in numerous clinical trials for treatment of several tumor types (reviewed in 75). This recent clinical progress can greatly facilitate novel applications of these drugs in such areas as the prevention of PCO.

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