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The role of metallothionein IIa in defending lens epithelial cells against cadmium and TBHP induced oxidative stress

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Abstract

Purpose—Heavy metals and other forms of oxidative stress have been implicated as key factors in the formation of age-related cataract in humans. Metallothioneins are a group of proteins known to play important roles in defending cells against the cytotoxic effects of heavy metals. However, little is known about their involvement in defending against other forms of oxidative stress. Here, we examined the ability of metallothionein IIa (MTIIa) to protect human lens epithelial cells against cadmium and tertiary butyl hydroperoxide (TBHP)-induced oxidative stress.

Methods—MTIIa over-expressing human lens epithelial cells (SRA01/04) were created by retroviral mediated gene transfer. Normal and MTIIa over-expressing cells were exposed to various concentrations of cadmium and TBHP and subsequently monitored for cell death, changes in cell phenotype and differences in growth rate. In addition, expression levels of three other important antioxidant genes, heme oxygenase-1, thioredoxin reductase-1, and manganese superoxide dismutase were monitored by real-time RT-PCR following exposure to TBHP.

Results—Analysis of the over expressing cell lines revealed an approximate 3–4 fold increase in MTIIa expression relative to control cells, resulting in as much as 20% protection against cadmium-induced oxidative stress ($p<0.001$). The MTIIa over expressing cells were also significantly more resistant to TBHP treatment while control cells exhibited significant shrinking and rounding-up following 3–6 h TBHP treatment, no changes were observed in TBHP-treated over expressing cells. When control cells were treated for 3 h or overnight with TBHP, 40–45% cell death occurred by day three. However, no cell death was observed at this time for the treated MTIIa over-expressing cell line. In addition, TBHP induced the expression of MTIIa, heme oxygenase-1, thioredoxin reductase-1, and MnSOD in both normal and MTIIa over-expressed cell lines. Interestingly the latter three genes were induced at 2-3 fold higher levels in TBHP-treated MTIIa over-expressing cells, compared to treated control cells ($p=0.001$, $p=0.02$, and $p=0.01$, respectively).

Conclusions—These data indicate that over-expression of MTIIa in human lens epithelial cells results in protection against cadmium and TBHP-induced oxidative stress. In addition, the results suggest that MTIIa, and/or its ability to chelate metals, may play a role in regulating expression of other important antioxidant genes in response to oxidative stress.

Heavy metals and various forms of oxidative stress are known to be associated with cell death and cataract. Human exposure to heavy metals such as cadmium arise from widespread sources

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including cigarette smoke, air pollution, leaching of landfills, industrial waste, emissions from fossil fuels, fertilizers, and corrosion of plumbing [1,2]. Cadmium has been shown to be present in most human ocular tissues [3], and has a biological half-life in humans of up to 30 years [4]. Large amounts of the heavy metal have been detected in lenses of chronic smokers [5] who also exhibit early cataract formation [6]. Increased cadmium levels have also been reported in cataracts compared to clear human lenses [5].

In addition to heavy metals, tertiary butyl hydroperoxide (TBHP) is another form of oxidative stress that results in lipid oxidation, is known to deplete intracellular pools of calcium [7], as well as glutathione [8,9], and produces cell death [10–12]. TBHP increases levels of protein/thiol mixed disulfides in cultured lenses [13], causes cross-linking of lens aminophospholipids [14], and adversely affects membrane permeability in rabbit lenses, likely as a result of glutathione oxidation [15].

Biological systems have evolved numerous ways to defend against cytotoxic effects of heavy metals and other forms of oxidative stress. One major group of proteins believed to regulate and protect cells against metal toxicity is the metallothioneins (MTs), 6–7 kDa polypeptides that bind a wide spectrum of metals and are rapidly induced by metals and other agents in numerous tissues [16]. In addition to metals, MTs are also induced by steroids in rat fibroblasts [17] and primary human skin fibroblasts [18], carcinogens in mice [19], chemicals that induce oxidative stress in rodent cells [20], and X-irradiation and UV-induced DNA damage in multiple cell types [21].

Humans possess 16 known isoforms of MTs, grouped into four classes: MTs I, II, III, and IV. We have previously shown that the human lens expresses MT classes I and II including MT isoforms Ia, Ig, If, Ih, Ie, and IIa [22]. Only one isoform, MTIIa, is specific for the lens epithelium whereas MTI isoforms are expressed at lower levels in both the lens epithelium and lens fibers [22]. In addition, MTIIa exhibits increased expression in age-related cataract relative to clear human lenses [23], suggesting a possible role for MTIIa in lens protection and/or prior exposure of the lens to toxic metals.

A number of studies have demonstrated a direct role for MTs in protecting multiple cell types against a wide range of insults associated with metal exposure, oxidative stress, and cataract. Over-expression of MT in a human trophoblastic cell line was shown to protect against cadmium-induced apoptosis [24]. MT I- and MT II-null mice were more sensitive to metal exposure and oxidative stress than wild type mice [25–29], and over-expression of MTIa in a human retinal pigment epithelial cell line provided direct protection against cadmium exposure, heme- and iron-induced oxidation and UV light-induced apoptosis [30]. However, the possible role of MTIIa in the protection of human lens epithelial cells (HLECs) against these oxidative agents has not been examined.

Based on the known association between toxic metals, oxidative stress and lens cataract, and the detection of increased MTIIa expression in age-related cataract relative to clear lenses [23], we sought to further define the role of MTIIa in the protection of HLECs against cadmium and TBHP-induced oxidative stress. Our results provide evidence that over-expression of MTIIa in HLECs results in significant protection against cadmium and TBHP-induced cell damage and death. Both MTIIa over-expressing cells and control cells exhibited increased expression of other important antioxidant enzymes in response to TBHP treatment. However, these enzymes were approximately 2–3 times more highly expressed in treated MTIIa over-expressing cells relative to treated controls. Taken together, these data indicate an important role for MTIIa in protection of the lens against various forms of oxidative stress.

METHODS

Creation of SRA01/04-MTIIa over-expressing cells

Human lens epithelial cells (SRA01/04; HLECs) and 293-FT kidney cells were grown and cultured in Dulbecco's modified Eagle's medium (Invitrogen, Gaithersburg, MD) supplemented with 15% fetal bovine serum (Invitrogen), gentamicin (50 units/ml; Invitrogen), penicillin-streptomycin antibiotic mix (50 units/ml; Invitrogen) and Fungizone (5 μl/ml; Invitrogen) at 36.5 °C in the presence of 5% CO_2. Over-expressing SRA01/04-MTIIa cell lines were created using the ViraPower™ Lentiviral Expression System (Invitrogen) as described by the manufacturer. Briefly, primers were designed to amplify full length MTIIa transcripts and the resulting product was cloned into the expression vector, sequenced to ensure product authenticity and orientation, and subsequently used for transfecting 293-FT kidney cells for creation of the viral construct. Virus particles were harvested from the 293-FT cells and various titers were used to infect SRA01/04 HLECs. MTIIa over-expressing cells were selected for using blasticidin-containing media (6 μg/ml; Invitrogen). Multiple HLEC colonies were selected and tested for MTIIa over-expression.

Confirmation of MTIIa over-expression in SRA01/04 cells

Gene-specific primers were designed for MTIIa using the BLAST program and GenBank data base (National Library of Medicine, Bethesda, MD). Total RNA was isolated from control and MTIIa-expressing HLECs and semi-quantitative RT-PCR was performed with 100 ng of RNA using the OneStep RT-PCR system according to the manufacturer's protocol (Invitrogen) to confirm MTIIa over-expression. Products were separated by gel electrophoresis on 1.5% agarose gels and visualized by ethidium bromide staining. The cell line exhibiting the highest level of MTIIa over-expression was chosen for use in the following studies.

Cadmium and TBHP cell treatments

Control and over-expressing HLECs were plated in 96 well plates at a density of 10,000 cells per well and cultured in DMEM containing 15% serum. Both cell lines were exposed to 0, 10, 20, 40, 80, 160, 320, 640, 1280, and 2500 μM cadmium for 24 h and cell viability was monitored by MTS assays. For TBHP treatments, approximately $5x10^5$ control and over-expressing HLECs were plated in 35 mm plates and cultured in DMEM as described above for 20 h. The cell medium was then replaced with serum free medium for 30 min and the cells were subsequently challenged with serum free medium containing 0.5 mM TBHP for 3 h, 6 h, and overnight. Morphological studies were conducted using phase contrast light microscopy immediately following TBHP treatment. For cell growth studies, HLECs were exposed to 0.5 mM TBHP for either 3 h or overnight. Immediately following TBHP treatment the medium was changed to serum containing DMEM which was changed every other day. Cell counts were conducted using a Coulter cell counter on days 1, 3, 5, 7, 9, and 11 following TBHP treatment. All experiments were conducted in triplicate.

Cell viability assays using MTS reagent

Cell viability assays were conducted using eight replicates and a CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay (Promega, Madison, WI). This kit contains the tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (MTS) and was used to monitor cell viability following the manufacturer's protocols. MTS color change was analyzed using an ELX-800 universal plate reader (Bio-Tek Instruments, Winooski, VT) set at an absorbance reading of 492 nm.

Real-time RT-PCR analysis

Total RNA was isolated from control and MTIIa over-expressing cells at 0, 3, 6, and 16 h post-TBHP treatment (0.5 mM for 3 h) using Trizol reagent (Invitrogen). RNA was resuspended in RNAse-free water, digested with DNAse 1 (Invitrogen) and purified using Qiagen columns (Qiagen, Hideer, Germany). Real-time RT-PCR was performed as described previously [31] using the Quantitect™ SYBR Green PCR kit (Qiagen) with the Icycler IQ™ real-time detection system (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's protocol. Total RNA (12.5 ng) from control and MTIIa over-expressing cell lines, with and without 0.5 mM TBHP treatment, was first incubated at 50 °C for 50 min followed by a 15 min 95 °C step. Amplification of cDNA was performed using a total of 40 cycles with each cycle containing a 30 s incubation at 95 °C, followed by an annealing step of 56 °C for 30 s and an extension step of 72 °C for 30 s. The primer sequences for MTIIa were: forward primer AAG TCC CAG CGA ACC CGC GT and reverse primer CAG CAG CTG CAC TTG TCC GAC GC. The primer sets used for heme oxygenase-1 (HO-1), thioredoxin reduc-tase-1 (TrxR1), and manganese superoxide dismutase (MnSOD) have been described previously [31]. Melting curves revealed a single peak for each primer set indicating that no primer-dimer formation occurred. β-Actin was used as an internal control for each reaction to ensure equal loading of RNA. Amplification plots, representing three replicates, were created and the threshold cycle was used to calculate the fold change of each gene.

RESULTS

MTIIa over-expressing HLECs were created using a lentiviral expression system as described above. MTIIa HLECs exhibited an approximate 3–4 fold higher level of MTIIa expression compared to the parent cell line (Figure 1A). In order to determine if over-expression of the MTIIa gene was capable of protecting lens cells against cadmium toxicity, control and over-expressing cells were exposed to various levels of cadmium chloride for 24 h. MTS assays indicated that concentrations of cadmium greater than 160 μM resulted in significant HLEC death for both control and MTIIa over-expressing cell lines (Figure 1B). However, significant increases in cell viability ($p<0.001$) of approximately 20% were detected for cadmium-treated MTIIa over-expressing cells relative to treated control cells at 40 and 80 μM cadmium levels, indicating that over-expression of MTIIa is capable of protecting lens epithelial cells against the cytotoxic effects of cadmium at these concentrations (Figure 1B).

In order to determine if MTIIa plays a role in the detoxification of other forms of oxidative stress, control and over-expressing cell lines were exposed to 0.5 mM TBHP for various time periods. Changes in cell phenotype and morphology were monitored using phase contrast light microscopy. Following 3 h of TBHP treatment, distinct morphological changes were seen in the treated control cells (condensed nuclei; Figure 2D), but not in the treated MTIIa over-expressing cells (Figure 2J). Following 6 h of TBHP exposure, the control cells began to round-up, indicating that they were starting to detach from the plate (Figure 2E). MTIIa over-expressing cells treated in the same manner exhibited no difference in cell phenotype (Figure 2K). Even more dramatic differences were observed following overnight exposure of these cells to TBHP. Significant cell shrinking, an increase in the number of condensed nuclei, the formation of threadlike structures, and an increase in cell death were observed in control cells treated overnight with TBHP (Figure 2F). However, none of these differences were observed in the TBHP-treated over-expressing cells (Figure 2L), indicating that MTIIa is likely to play a role in protecting HLECs against TBHP stress.

Since it was apparent that an increase in cell death was occurring in control cells following exposure to TBHP, we monitored cell growth rates for both control and over-expressing cells on days 1, 3, 5, 7, 9, and 11 following 3 h or overnight exposures to 0.5 mM TBHP (overnight exposure monitored up to nine days only). At time 0 after the 3 h TBHP-treatment, 20% of

treated control cells had died, compared to no cell death for the treated over-expressing cells (Figure 3A; $p<0.001$). At time 0 following the overnight treatment with TBHP, cell death was 32% for control cells compared to 20% for over-expressing cells (Figure 3B; $p<0.001$). exposures to TBHP (3 h and overnight) had similar effects on both control and over-expressing cells (Figure 3). Forty to 45% cell death occurred by day three following 3 h or overnight exposures of control cells to TBHP while no cell death was observed at this time in the treated MTIIa over-expressing cell line (Figure 3). TBHP-treated control cells did not exhibit an increase in growth for any of the times tested (Figure 3). However, treated MTIIa over-expressing cells continued to grow after day three with a population doubling occurring by day nine (Figure 3). The growth curve for MTIIa over-expressing cells exposed to TBHP closely resembled the growth curve for untreated control cells indicating that over-expression of MTIIa is capable of conferring resistance to TBHP-induced cell damage. Interestingly, the growth rate of untreated MTIIa expressing cells was much higher than untreated control cells (Figure 3).

We showed in a previous report that oxidative challenge of HLECs resulted in an increased expression of three anti-oxidant genes, HO-1, TrxR1 and MnSOD [31]. Thus, it was of interest to examine the effects of TBHP treatment on the expression profiles of these antioxidant genes in both treated control and MTIIa over-expressing cells using real-time RT-PCR. In addition, we examined the expression levels of MTIIa in both of these cell lines to determine if MTIIa could be induced by TBHP. The expression levels were examined at 0, 3, 6, and 16 h of normal culture following a 3 h treatment of both control and over-expressing cells with 0.5 mM TBHP (MTIIa was investigated only at 0, 3, and 6 h; Figure 4). This analysis indicated that the expression of these four genes was increased in both cell lines following TBHP treatment, relative to untreated cells (Figure 4), suggesting that all four genes are responsive to TBHP. A maximal increase in expression of all four genes occurred in both cell lines at 6 h after the 3 h TBHP-treatment (Figure 4). The expression of HO-1 and TrxR1 returned to near baseline levels by 16 h post-TBHP treatment (Figure 4B,C), while that for MnSOD remained high in the MTIIa over-expressing cell line (Figure 4D). TBHP-induced expression of HO-1, TrxR1 and MnSOD was 175 fold, 12 fold, and eight fold higher, respectively, in MTIIa over-expressing cells, compared to untreated over-expressing cells at the 6 h time period (Figure 4B-D). Interestingly, the expression of these three genes at 6 h was substantially higher (2–4 fold) in the over-expressing cell line treated with TBHP relative to control cells treated in the same fashion (Figure 4B-D; HO-1: $p=0.001$; TrxR1: $p=0.02$; MnSOD: $p=0.01$).

DISCUSSION

These data demonstrate that over-expression of MTIIa, the most highly expressed metallothionein in the lens [22], is capable of defending HLECs against the cytotoxic effects of cadmium- and TBHP-induced oxidative stress. MTIIa provided significant protection against 40 and 80 μM levels of cadmium (Figure 1B), which are approximately the levels found to be present in cataracts of smokers [5]. Dramatic differences in lens epithelial cell morphology were detected in control cells exposed to TBHP, while little to no change was observed in treated MTIIa over-expressing cells (Figure 2). In addition, exposure of control cells to TBHP resulted in significantly more cell death relative to the treated over-expressing cell line (Figure 3). MTIIa, HO-1, TrxR1, and MnSOD were all induced in normal lens epithelial cells exposed to TBHP, but interestingly, the latter three genes were induced at significantly higher levels in MTIIa over-expressing cells treated in the same manner (Figure 4). The robust expression of other antioxidant genes in TBHP-treated MTIIa over-expressing cells may in part explain their increased resistance to TBHP. Taken together, these data demonstrate that MTIIa plays an important role in defending the lens against the toxic effects of cadmium and TBHP exposure.

It is well established that oxidative stress, resulting from heavy metal exposure and UV-light, contributes to the development of cataract in humans. The lens has evolved numerous ways to defend itself against these stresses with one major group of proteins, the MTs, playing an important role. Numerous studies have demonstrated that MTs are involved in the detoxification of many different metals in a variety of cell lines. Here, we have confirmed these results in HLECs by demonstrating that MTIIa over-expression protects against the cytotoxic effects of oxidative stress induced by 40 and 80 μM levels of cadmium. However, few data exist in the literature concerning the role of MTs in defending lens epithelial cells against other forms of oxidative stress.

Previous studies have shown that the mouse MT1 gene [32] and trout MTA gene [33] are induced upon exposure of cells to hydrogen peroxide. It has also been shown that the trout MTB gene can be induced by both hydrogen peroxide and TBHP [34], over-expression of MT can protect NIH 3T3 cells against TBHP toxicity [35], astrocytes isolated from MT-null mice are more sensitive to TBHP exposure [35] and MTIII can defend against hydrogen peroxide-induced DNA damage [36]. A recent study has shown that expression of MTIIa is significantly increased in TBHP-treated HeLa cells [37]. However, the role of MTs in defending human cells, and the lens, against the toxic effects of oxidative stresses other than heavy metals has not been thoroughly investigated. Here, we have provided evidence that over-expression of MTIIa is capable of protecting lens epithelial cells against the toxic effects of TBHP exposure. Normal HLECs exposed to TBHP exhibited significant cell shrinking, an increase in the number of condensed nuclei and an increase in cell death when compared to MTIIa over-expressing cells (Figure 2). Indeed, TBHP treatment of control cells resulted in 40–45% cell death, with no cell death observed in MTIIa over-expressing cells treated in the same manner (Figure 3).

Interestingly, MTIIa over-expressing cells exhibited a dramatic increase in proliferation relative to control cells even in the absence of exogenous stress (Figure 3). MTs are known to influence both cell proliferation and death [38,39] and seem to be expressed at higher levels in fetal tissues than in adult tissues [40]. It has been reported that MT expression is 2–3 fold higher in proliferating compartments of colon cancer cells relative to growth inhibited cells [41] and that the level of MTIIa expression in breast cancer tissue correlates with increased proliferation [42]. Over-expression of MTIIa in MCF-7 cells results in a two fold increase in the rate of cell growth [43]. Although the mechanisms by which MTs are able to exert their effects on cell proliferation are still unknown, the present data, none the less, support the increasing evidence that these proteins may play an important role in the growth rate of multiple cell types, including lens epithelial cells.

Since our data indicated that over-expression of MTIIa results in significant protection against TBHP toxicity, and since we had previously shown that three important antioxidant genes were induced in HLECs following oxidative challenge [31], it was of interest to determine if these genes were involved in the observed protection against TBHP. Here, we demonstrated that HO-1, TrxR1, and MnSOD exhibit increased expression levels following TBHP exposure in both control and MTIIa over-expressing cell lines (Figure 4B–D). It is well known that these genes, and many other antioxidant related genes, are induced by the presence of many forms of oxidative stress. However, these three genes were found to be induced at significantly higher levels in our MTIIa over-expressing cell line relative to control cells following TBHP exposure. Whether this finding may be linked with the elevated proliferation rate of the MTIIa over-expressing cells, as discussed above, is not known.

It is known that the metal-responsive transcription factor-1 gene, an essential zinc finger transcription factor that induces the expression of many genes that respond to the presence of heavy metals, requires elevated zinc concentrations for efficient DNA binding. Interestingly,

in a MTF-1 dependent transcriptional system, it has been shown that the presence of zinc-saturated MT is necessary to obtain transcriptional activation of MTF-1 responsive promoters by cadmium, copper, or hydrogen peroxide [44]. This phenomenon is explained by the fact that cadmium or copper have a higher affinity for MT than zinc, and the presence of these metals results in the release of zinc from MT proteins. Oxidation of MTs by hydrogen peroxide also results in the release of zinc which in turn becomes available for the activation of MTF-1 [44]. HO-1 [45] and MnSOD [46,47] are known to be induced by heavy metals, and this induction could be through MTF-1. It is possible that, in our system, over-expression of MT results in the sequestration of more zinc from the cell culture media. Treatment of these cells with TBHP may result in oxidation of MT which increases the availability of zinc leading to the induction of these two genes. Although expression of TrxR1 has not been linked to MTF-1, it is possible that its increased induction in MTIIa over-expressing cells treated with TBHP is also the result of a similar mechanism.

In summary, the present data demonstrate that MTIIa is capable of protecting HLECs against cytotoxic effects that include cadmium exposure. Although MTs are almost exclusively thought of as proteins that regulate and defend cells from damage induced by heavy metal exposure, we have shown that MTIIa is also capable of defending lens epithelial cells against TBHP-induced cell damage. TBHP exposure of normal HLECs resulted in significant cell shrinking, an increase in the number of condensed nuclei and a 40–45% increase in cell death when compared to MTIIa over-expressing cells treated with the hydroperoxide. In addition, TBHP exposure resulted in upregulation of four other important antioxidant proteins, HO-1, TrxR1, MnSOD, and MTIIa. However, this upregulation was greatly amplified in the MTIIa over-expressing cell line (with the exception of MTIIa), indicating that MTIIa is likely to be involved in regulating expression of these genes to some degree. Overall, we have demonstrated a direct, and potentially an indirect, mechanism by which MTIIa is capable of defending lens epithelial cells against toxic effects of both cadmium and TBHP exposure.

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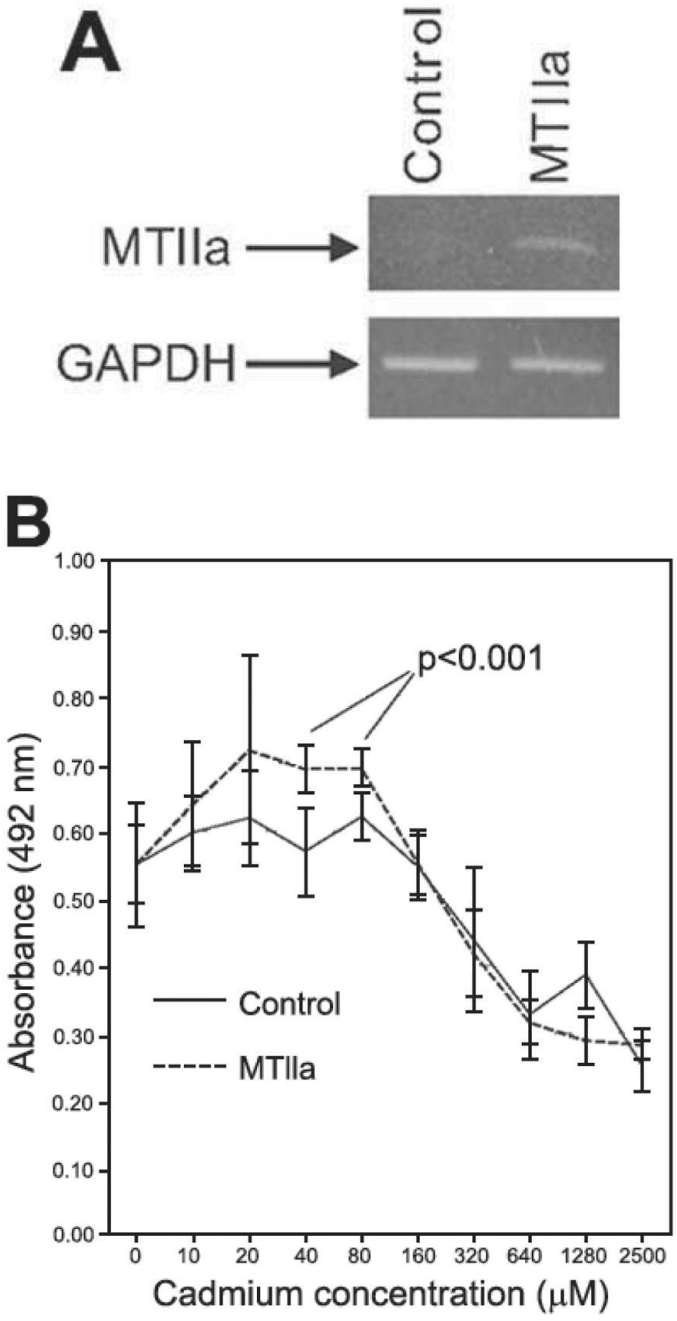

Figure 1.

Metallothionein IIa over-expression protects human lens epithelial cells against cytotoxic effects of cadmium exposure. **A**: Semi-quantitative RT-PCR confirmation of MTIIa over-expression in retroviral infected HLECs (MTIIa) relative to uninfected control cells (control). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) shows equal loading of RNA between the two cell lines. The results shown are representative of three experiments. Note the 3–4 fold increase in MTIIa gene expression in the MTIIa cells compared to controls. **B**: Representative graph depicting increased resistance to cadmium treatment of the MTIIa over-expressing cell line (dashed line) relative to control cells (solid line). Cadmium treatments were conducted for 24 h. The absorbance readings (reflecting cell viability, with larger values indicating increased

numbers of live cells) and cadmium concentrations are indicated. Each error bar represents the standard deviation of eight separate cell-viability assays. Differences at 40 and 80 μM cadmium are significant at $p<0.001$.

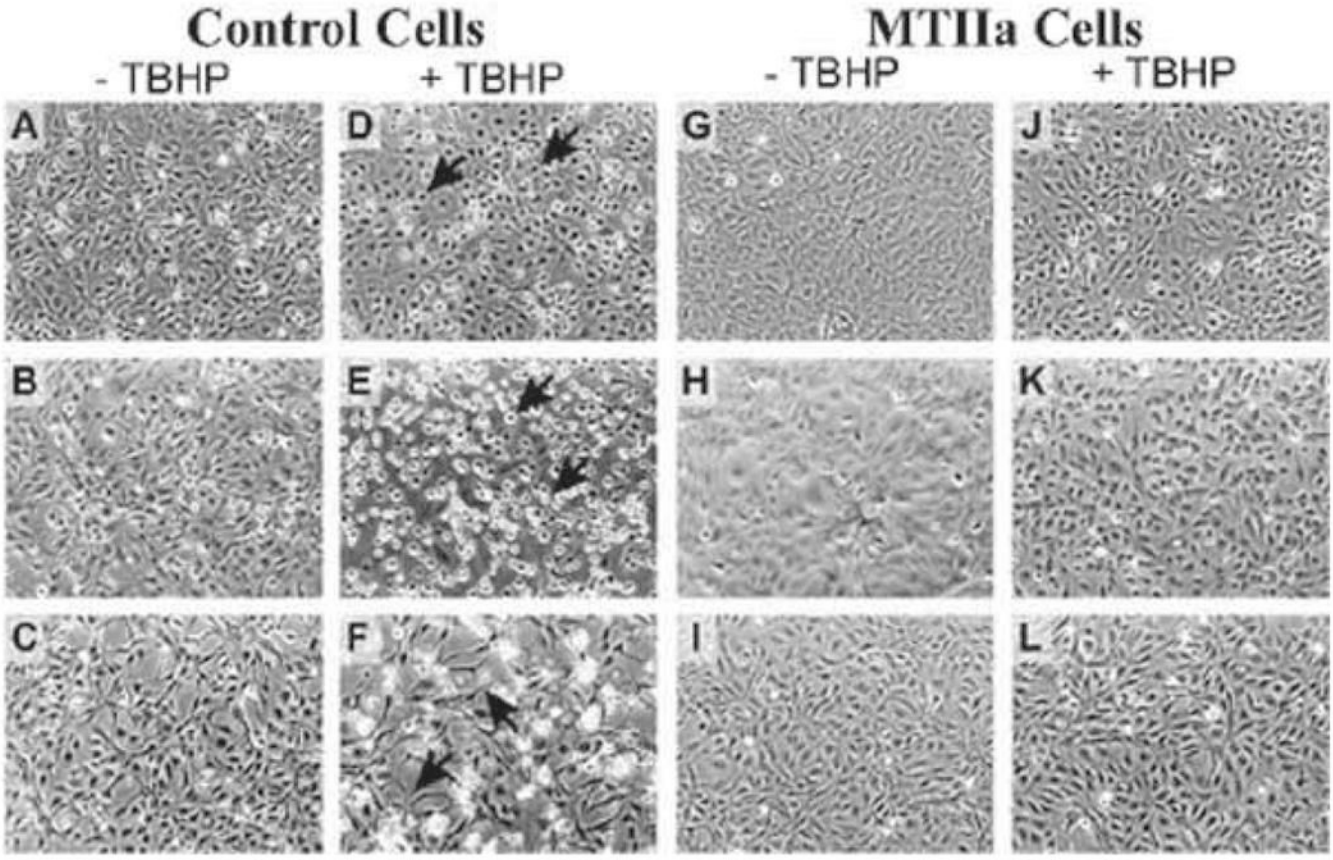

Figure 2.

Metallothionein IIa over-expression in human lens epithelial cells confers resistance to TBHP exposure. The top row shows cells after 3 h exposure, the middle row shows cells after 6 h exposure, and the bottom row shows cells after overnight exposure. **A**–**C**: Light micrograph images of control cells not exposed to TBHP. **D**–**F**: Light micrograph images of control cells following 0.5 mM TBHP treatment for indicated times. Arrows identify condensed nuclei (**D**), rounded-up cells (**E**), and threadlike structures (**F**). **G**–**I**: Light micrograph images of MTIIa over-expressing cells not exposed to TBHP. **J**–**L**: Light micrograph images of MTIIa over-expressing cells following 0.5 mM TBHP treatment for indicated times. All results are representative of those obtained for three experiments.

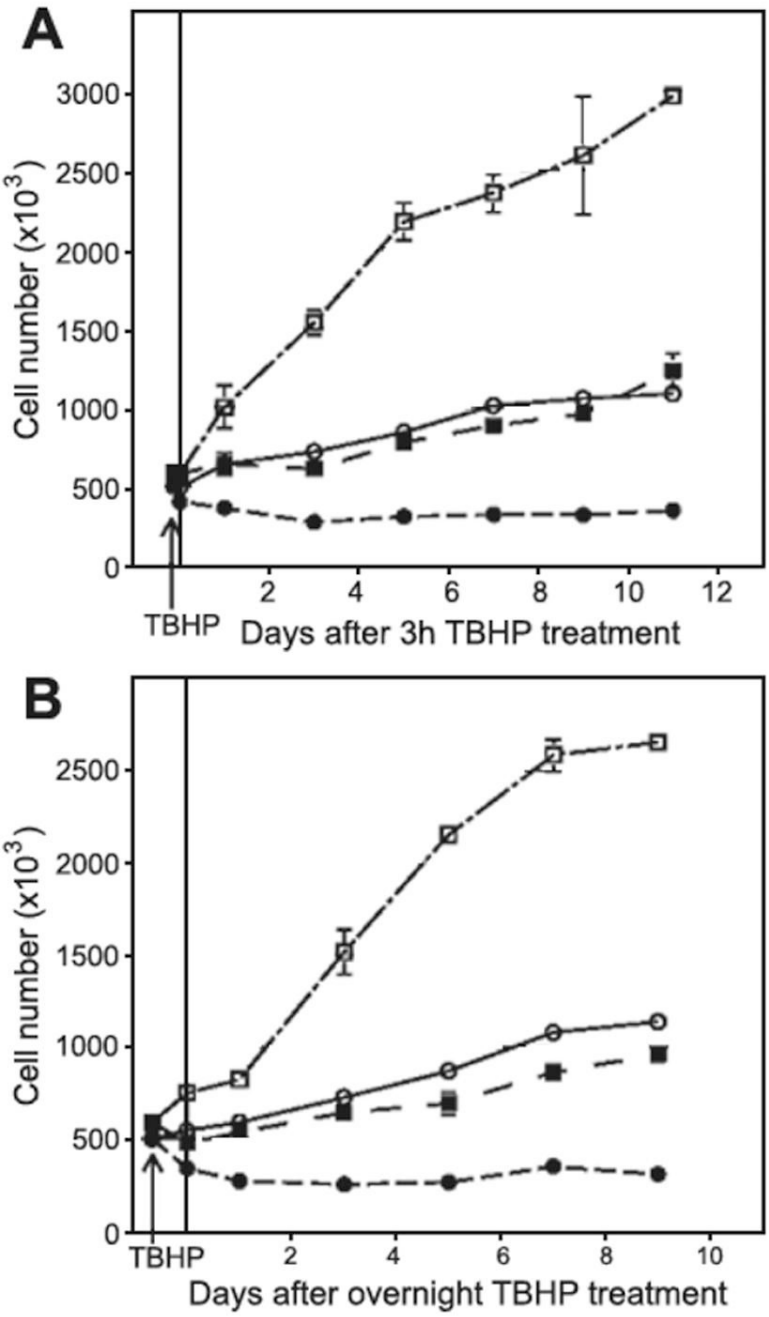

Figure 3.

Growth of control and MTIIa over-expressing human lens epithelial cells on days 1, 3, 5, 7, 9, and 11 following 0.5 mM TBHP treatment for indicated times. **A** shows the number of control and MTIIa over-expressing cells with and without a 3 h 0.5 mM TBHP treatment on indicated days after exposure. **B** shows the number of control and MTIIa over-expressing cells with and without an overnight 0.5 mM TBHP treatment on indicated days after exposure. In both **A** and **B**, the open circles represent control cells minus TBHP, the filled circles represent control cells plus TBHP, the open squares represent MTIIa over-expressing cells minus TBHP, and the filled squares represent MTIIA over-expressing cells plus TBHP. The error bars represent

standard deviations of three separate assays (the absence of bars indicates a very small standard deviation).

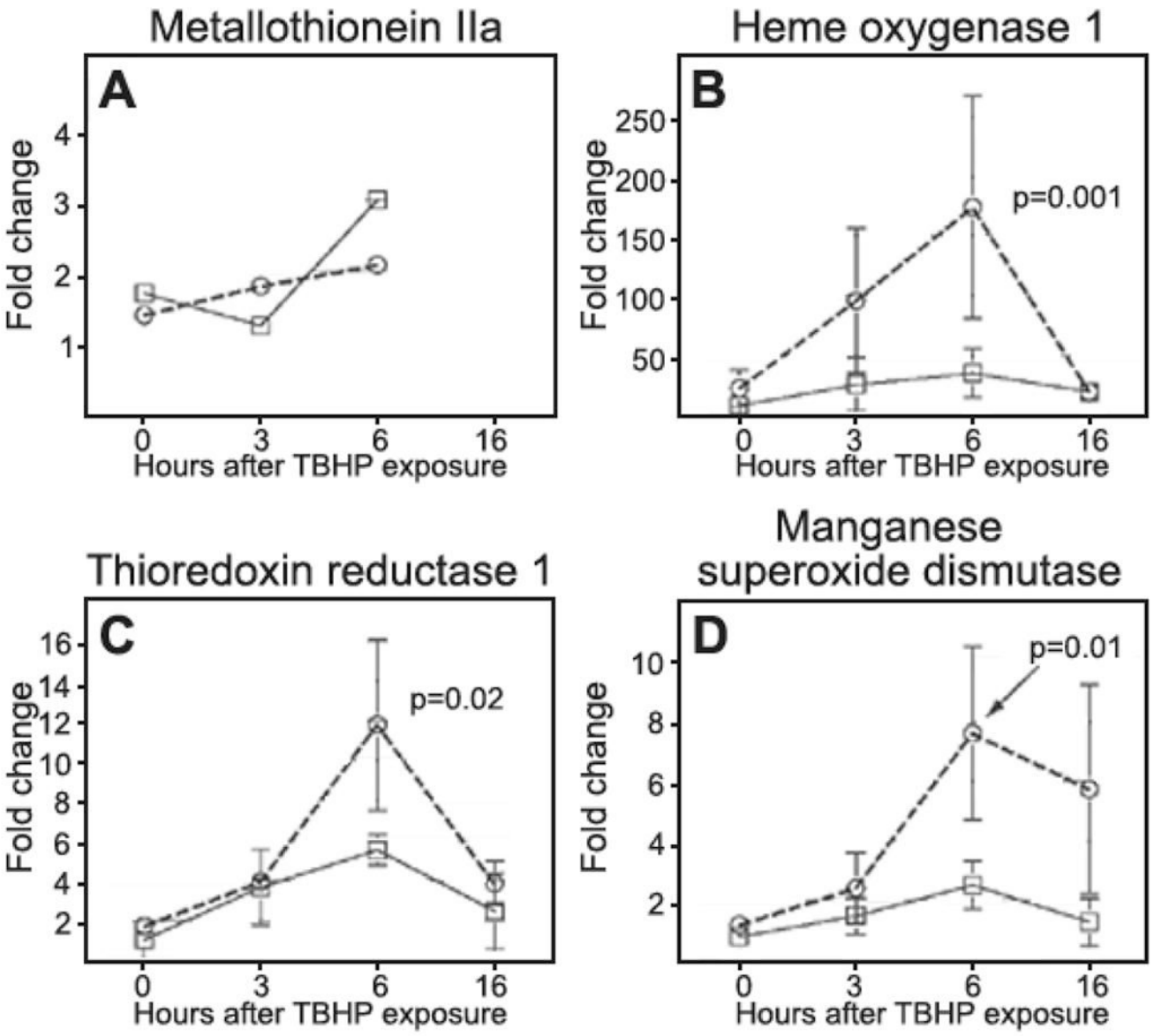

Figure 4.

Expression of MTIIa, HO-1, TrxR1, and MnSOD in cells after treatment with TBHP. Real-time RT-PCR analysis of MTIIa (**A**), heme oxygenase-1 (**B**), thioredoxin reductase-1 (**C**), and manganese superoxide dismutase (**D**) in control (solid lines) and MTIIa over-expressing (dashed lines) human lens epithelial cells. Both cell lines were first treated with 0.5 mM TBHP for 3 h and then cultured in normal medium for the indicated times. Results are expressed as calculated fold changes for TBHP-treated control and MTIIa over-expressed cells, compared to the same cells not treated with TBHP. β-Actin was used as an internal control for each reaction. The error bars represent standard deviations for n=3 (**B**–**D**).