Lens Epithelium-Derived Growth Factor: Increased Survival and Decreased DNA Breakage of Human RPE Cells Induced by Oxidative Stress

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PURPOSE. Lens epithelium-derived growth factor (LEDGF) has been shown to be a growth and survival factor and to be present in a wide variety of cell types. The purpose of this study was to determine whether LEDGF enhances survival of human retinal pigment epithelial (RPE) cells when challenged by oxidative stress or by ultraviolet (UVB) irradiation in a culture system.

METHODS. Primary RPE cells were cultured in standard Dulbecco’s modified Eagle’s medium (DMEM) containing 15% fetal bovine serum. Protein blot analysis with antibodies to LEDGF was used to detect LEDGF in RPE cells. Initially, RPE cells were cultured in the standard medium for 1 day to allow attachment to the culture plates and then cultured in serum-free DMEM, with and without LEDGF. The trypan blue exclusion method was used to test RPE cell viability. Single-cell electrophoresis was used to evaluate single strand breaks of genomic DNA after exposure to H2O2 or irradiation by UVB.

RESULTS. LEDGF was present in RPE cells, predominantly in the nucleus. RPE cells grew for 1 week and survived for 3 weeks in the presence of LEDGF. In the absence of LEDGF, they increased in number for the first week and gradually died in the following 2 weeks. LEDGF protected RPE cells against H2O2 exposure and UVB irradiation. DNA damage induced by H2O2 exposure or UVB irradiation was lower in the presence than in the absence of LEDGF. The expression of heat shock protein (Hsp)27 was elevated by LEDGF.

CONCLUSIONS. LEDGF enhanced survival of RPE cells in culture when challenged by oxidative stress and UVB irradiation. LEDGF protected DNA from single-strand breakage and up-regulated the expression of Hsp27. These results suggest that LEDGF may be a potential agent for protecting RPE cells under various stress conditions. (Invest Ophthalmol Vis Sci. 2001;42: 2935–2941)

The retinal pigment epithelium (RPE) is a single layer of cells situated between the photoreceptors of the neural retina and the choriocapillaris and choroid.1–2 RPE cells have many crucial functions such as transportation of ions, fluids, amino acids, glucose, and other organic molecules; protection of the retina from choroidal flow; phagocytosis of photoreceptor outer segments; synthesis of acid mucopolysaccharides; and resynthesis of photopigments and vitamin A metabolism.1 Because of their anatomic location, RPE cells are exposed to high levels of oxygen radicals produced by phototransduction and the diffusion from choroidal circulation.4 The RPE also accumulates lipofuscin granules, which are products of endogenous oxidative processes. Lipofuscin granules have been implicated in cellular aging.8

Ultrafilter (UV) radiation can damage ocular tissues and have phototoxic effects on the retina.9 Such radiation, including UVA and UVB, can be transmitted to the retina in children and young adults10 and to aphakic and pseudophakic eyes after surgery. Damage to the RPE cell and its DNA have been suggested to be manifestations of response to UV radiation.11,12 Damage caused by UV radiation may also be associated with age-related diseases.

Several studies have demonstrated that basic fibroblast growth factor (bFGF),13,14 platelet-derived growth factor (PDGF),15,16 connective tissue growth factor (CTGF),17 insulin-like growth factor (IGF),18 and pigment epithelium-derived factor (PEDF)19,20 may influence the development, maintenance, and restoration of RPE cells. We have recently isolated a novel growth and survival factor, lens epithelium-derived growth factor (LEDGF), from a human lens epithelial cell (LEC) cDNA library with autoantibodies from patients with age-related cataracts.21,22 LEDGF is one of a family of homologous proteins including hepatoma-derived growth factor (HDGF)23 and HDGF-related protein (HRP-1 and -2).24 LEDGF is found in the nucleus of most cell types and enhances the growth and survival of many such cells.22

In earlier studies, we reported that LECs, mouse keratinocytes, monkey kidney cos7 cells, and human fibroblasts could be cultured successfully in a serum-free environment if LEDGF was present. But in the absence of LEDGF, most cells died after 2 to 7 days in culture.22 We showed that antibodies (Abs) to LEDGF kill LECs and retinal photoreceptor cells.21,25,26 When LEDGF is present in the serum, it enhances resistance and prolongs survival of LECs against heat and oxidative stress.22 We suggested that the mechanism for the protective effects of LEDGF may be that it stimulates the upregulation of heat shock protein (Hsp).

To determine whether LEDGF plays a similar role in RPE cells—that is, protection of RPE cells against serum deprivation, H2O2 stress, and UVB irradiation—we cultured RPE cells with and without LEDGF and challenged them with these stress conditions. We found that LEDGF enhanced survival of RPE cells and suggest that one of the survival mechanisms of RPE cells is the upregulation of Hsp27 induced by LEDGF.
MATERIALS AND METHODS

Cell Cultures

Human RPE cells were obtained from eye bank donor eyes. The eye was cut across the posterior pole, and the vitreous and neural retina were removed. The remaining eyecup was washed with phosphate-buffered saline (PBS; Gibco, Grand Island, NY), and 0.025% trypsin-EDTA (Gibco) was added to the eyecup and incubated at 37°C in a humidified chamber. The cells were then gently scraped and seeded in Dulbecco's modified Eagle's medium (DMEM; Gibco) containing 15% fetal bovine serum (FBS; Gibco) in a 100 × 20-mm culture dish. These primary cultures of RPE cells were grown in DMEM containing 15% FBS in a 5% CO₂ environment at 37°C.27

After checking for cell attachment, the medium was replaced with the same medium every other day. The human RPE cells used for the experiments were from passage numbers 2, 3, and 4, and each experiment on the cells was performed in quadruplicate.

Cell Proliferation and Viability Assay

Cell proliferation and viability were assessed by cell counting with trypan blue (Gibco) staining. After treatment, detached and floating cells were removed by washing with PBS. Attached cells were dissociated with 0.025% trypsin-EDTA solution and suspended in PBS. To determine the number of live cells, cells were stained with 0.4% trypan blue, and the unstained live cells and stained dead cells were counted with a hemocytometer.

Immunocytochemistry

Human RPE cells that had been cultured on a coverslip in a culture plate were fixed with cold methanol. After washing with PBS, they were incubated with hydrogen peroxide (Peroxidase Blocking Reagent; Dako, Carpinteria, CA) to block endogenous peroxidase activity. Then cells were blocked with 10% goat serum for 30 minutes at room temperature. Blocked cells were rinsed and washed in PBS and incubated with an Ab to LEDGF (1:500 dilution). The cells were incubated in goat biotinylated anti-rabbit IgG (LSAB2 System; Dako) as a secondary Ab. After washing with PBS, the membrane was incubated in streptavidin conjugated with horseradish peroxidase. The color was developed with streptavidin and biotin chromogen (Liquid DAB+ Substrate-Chromogen System; Dako).

Protein Blot Analysis

Proteins were dissolved in 2% sodium dodecyl sulfate (SDS) sample buffer and separated on 12% SDS-polycrylamide gel by electrophoresis (SDS-PAGE). The separated proteins were blotted onto a nitrocellulose membrane (Trans-Blot Transfer Medium; Bio-Rad, Hercules, CA). The transferred nitrocellulose membrane was incubated in 5% nonfat dry milk (Blocking Grade Blocker; Bio-Rad) in Tris-buffered saline (TBS; Bio-Rad) overnight at 4°C and then incubated with an Ab to LEDGF (1:5000 dilution) and with Ab to LEDGF neutralized with purified GST-LEDGF as a control (at 1:5000 dilution) overnight at 4°C. The sample slides were placed into electrophoresis buffer (1 mM EDTA, 2936 Matsui et al. JOVS, November 2001, Vol. 42, No. 12

DNA Single-Cell Strand Breakage Assay

DNA strand breaks were assessed by exposure to 50 μM H₂O₂ for 20 minutes or to 0.05 or 0.09 J/cm² UVB irradiation. Single-cell gel electrophoresis described by Singh et al.28 was modified as follows: The cells treated with H₂O₂ or UVB were harvested and mixed with 0.8% low-melting-temperature agarose (Sigma) at 37°C. They were then placed onto a frosted microscope slide that was already covered with a thin layer of 0.8% normal melting agarose (Sigma) to promote adhesion of the second layer. The slides were covered with a coverslip and kept at 4°C for 5 minutes. After removing the coverslip, the slides were covered with a second layer of 0.8% low-melting agarose containing the sample cells on the surface. To protect the cells, this layer was covered with 0.8% normal-melting agarose and then covered with a coverslip and kept at 4°C for 5 minutes. The coverslip was removed, and the cells were incubated for 1 hour in the dark with freshly prepared lysing solution (1% N-lauroylsarcosine sodium, 2.5 M NaCl, 100 mM EDTA, 10 mM Tris [pH 10.0], and 1% Triton X-100; Sigma). The sample slides were placed into electrophoresis buffer (1 mM EDTA with 300 mM NaOH; Sigma) for 20 minutes at 4°C in the dark, and then electrophoresed with 17 V for 20 minutes at 4°C in the dark. These slides were put into 4 M Tris at pH 7.5 for 5 minutes to neutralize the NaOH. After staining with 20 μg/ml ethidium bromide (Sigma), the sample gel was covered with a coverslip and photographed on 35-mm film at 200× magnification with a fluorescence microscope (VANOX-S; Olympus, Lake Success, NY) and enlarged to 5 × 7 in. prints to measure the comet tails.

Preparation of LEDGF

In our previous study, cos7 cells and mouse LECs, which secrete a fusion protein between GST and LEDGF as GST-LEDGF, were created.21−29 The GST-LEDGF subsequently expressed in the prokaryotic expression system (pGST-LEDGF, Escherichia coli BL21) was purified with a GST column (Amersham Pharmacia) chromatography and dissolved in PBS with 100 U/ml heparin (Sigma) for stabilization.30 Heparin (equivalent concentration to heparinized GST-LEDGF) was added to the control culture medium. In using GST-LEDGF, the antitoxic effect of GST itself31 had to be considered. To resolve this issue, the protective effect of GST was evaluated in the presence of an equivalent concentration of GST and heparin environment as a control for the H₂O₂ and UVB experiments. Cell survival and proliferation transferred membrane was stained with 0.1% Ponceau S (Sigma, St. Louis, MO). The relative density of Hsp27 bands was determined by NIH Image (version 1.61; provided in the public domain by the National Institutes of Health, Bethesda, MD, and available at http://www.nb.nih.nih.gov).

H₂O₂ Exposure or UVB Irradiation

To investigate the H₂O₂-induced damage on cell survival, a higher concentration of H₂O₂ was used, because cell growth was unaffected at lower H₂O₂ concentrations (<150 μM). In contrast, a lower concentration of H₂O₂ (50 μM) was used, because DNA strand breaks are more sensitive to low oxidative stress. The cells were seeded in DMEM containing 15% FBS for 24 hours, to allow attachment to the culture plates. After confirming cell attachment, the DMEM containing serum was replaced by serum-free DMEM containing 10 ng/ml GST-LEDGF-heparin, GST-heparin, or heparin alone and cultured for 2 days. To investigate H₂O₂-induced cell death, the cells were cultured in medium containing 200 or 400 μM H₂O₂.

The attached cells were also exposed to two levels of UVB radiation (0.05 J/cm² or 0.09 J/cm²). A broad-spectrum lamp (Spectroline EB-160C; Spectronics Corp., Westbury, NY) was the source of the UVB irradiation. The UVB dose was measured and monitored by a radiometer and sensor (UVX and J10, respectively; UV-2, San Gabriel, CA) and altered by varying the duration of exposure for each set of experiments. Generally, less than 2 minutes of UVB exposure was required for the radiation dosage used.
were determined in the presence of GST-heparin or heparin alone as a control.

Statistical Analysis

The results are expressed as means ± SD. Statistical significance was determined by a one-factor analysis of variance (ANOVA) followed by the Fisher post hoc test for multiple comparisons. \( P < 0.01 \) was considered significant.

RESULTS

Immunocytochemical Localization of LEDGF in Human RPE Cells

To determine whether LEDGF is present in RPE cells, 25-μg protein samples from cultured RPE cells were separated by SDS-PAGE. Protein blot analysis with an Ab to LEDGF revealed a strongly stained band with a molecular weight of 60 kDa (Fig. 1).

Immunohistochemical staining of RPE cells with an Ab to LEDGF showed intense staining in and around the nucleus. We also observed a weak staining in the cytoplasm (Fig. 2). These results showed that LEDGF was predominantly located in and around the nucleus of the RPE cells.

Stimulation of Proliferation and Survival of RPE Cells

RPE cells (15,000) were cultured in 24-well plates in DMEM containing 15% serum for 24 hours. After serum deprivation, the cells were cultured further in serum-free DMEM with various concentrations of GST-LEDGF-heparin (0.1, 10, and 1000 ng/ml), GST-heparin (concentration equivalent to that present in 1000 ng/ml GST-LEDGF-heparin) or heparin alone as a control. The cells were counted after 0, 7, 14, and 21 days in culture to obtain the number of live cells. The cells in the heparin environment proliferated for 1 week then gradually died over the next 2 weeks. GST-LEDGF-heparin enhanced both proliferation and survival of the RPE cells for the first week in a dose-dependent manner (Fig. 3), and the cells survived for the next 2 weeks. However, all the cells died by the fourth week, despite continuous addition of fresh GST-LEDGF (data not shown).

Protection of RPE Cells against Oxidative Stress

To study the protective effect of LEDGF against oxidative stress, the RPE cells that were attached to culture well for 24 hours before culturing, were exposed to either 10 ng GST-LEDGF-heparin, or equal amounts of GST-heparin or heparin alone in serum-free DMEM for 2 days. The cells were then cultured further in the presence of 200 or 400 μM H₂O₂. The cells were counted after 0, 3, 6, 9, and 12 hours of culturing. Significant differences in the number of live cells were observed in RPE cells cultured in the presence of GST-LEDGF-heparin, GST-heparin, or heparin control for each dose of H₂O₂.

![Figure 1](image1.png)

**Figure 1.** Protein blot analysis showing that LEDGF was present in the human RPE cells. Protein samples (25 μg) from cultured RPE cells were separated by SDS-PAGE gel and transferred to nitrocellulose membranes. (A) The transferred membranes were stained with Ponceau S. Lanes 1 and 2 are duplicate samples. (B) Immunoreaction with rabbit Ab to LEDGF (lane 1) or with the Ab to LEDGF neutralized with GST-LEDGF (lane 2). Arrow: LEDGF band (60 kDa).

![Figure 2](image2.png)

**Figure 2.** Localization of LEDGF in RPE cells. The cells were cultured for 1 day in DMEM containing 15% FBS. Attached cells were immunostained with Ab to LEDGF (A) or with Ab to LEDGF neutralized with GST-LEDGF (B). LEDGF was observed in and around the nucleus. The specificity of antibody to LEDGF was apparent by the absence of staining when this antibody was neutralized with GST-LEDGF.

![Figure 3](image3.png)

**Figure 3.** Effect of LEDGF on survival and growth of RPE cells in culture. The cells were cultured in serum-free DMEM in the presence of 1000 ng/ml (●), 10 ng/ml (▲), and 0.1 ng/ml GST-LEDGF-heparin (▲); GST-heparin (○; concentration equivalent to that present in 1000 ng/ml GST-LEDGF-heparin); and heparin alone (○). Each data point represents the mean of four experiments. Error bars indicate SD. A dose-dependent response was seen with GST-LEDGF-heparin. A significant difference was observed between GST-LEDGF-heparin and the control treatments and between GST-heparin and heparin alone.
The effect on cell survival in the presence of GST-LEDGF-heparin was greater than that of GST-heparin or heparin alone. GST-LEDGF prolonged cell survival under exposure to oxidative stress. Because the cytotoxicity of \( H_2O_2 \) has been shown to vary significantly with the concentration of cells, we cultured RPE cells for 1 day and performed the same experiment, and we obtained similar results (data not shown). These results suggest that GST-LEDGF protected RPE cells against oxidative stress.

To evaluate the protective effect of LEDGF on DNA damage induced by \( H_2O_2 \), DNA strand breaks were evaluated by single-cell electrophoresis. The RPE cells attached to wells were washed with serum-free DMEM and cultured further in the same medium containing 10 ng/ml GST-LEDGF-heparin, GST-heparin, or heparin alone for 2 days. The cells were then exposed to 50 \( \mu M \) \( H_2O_2 \) in Hanks’ balanced salt solution (HBSS; Gibco) for 20 minutes. After this treatment, catalase was added, and the cells were incubated for 5 minutes to neutralize the residual hydrogen peroxide. The treated cells were harvested immediately and a DNA single-strand breakage assay was performed. The DNA breakage of the human RPE cells cultured in the presence of GST-LEDGF-heparin was significantly lower than in GST-heparin or heparin control (Fig. 5).

**Protection of RPE Cells from UVB Irradiation**

The effect of UVB irradiation on cell survival was also assessed by exposing the precultured RPE cells to 10 ng/ml GST-LEDGF-heparin, GST-heparin, or heparin alone for 2 days. The cells were irradiated with either 0.05 or 0.09 J/cm\(^2\) UVB. To evaluate cell viability, cell numbers were counted at 0, 1, and 3 days. The cell survival was greater in the presence of GST-LEDGF-heparin than in control cultures (i.e., those treated with GST-heparin or heparin) at both doses of UVB radiation. There was near complete survival (90%–95%) of RPE cells exposed to 0.05 J/cm\(^2\) at both culture periods of days 1 and 3 (Fig. 6A). At a higher dose of radiation (0.09 J/cm\(^2\)), cell survival was lower under all experimental conditions. However, there was significantly greater survival in the GST-LEDGF-heparin environment than in the control groups during both culture periods (Fig. 6B).

To study the effect of LEDGF on UVB-induced DNA damage, the attached cells were cultured in serum-free DMEM containing 10 ng/ml GST-LEDGF-heparin, GST-heparin, or heparin for 2 days. The RPE cells were then exposed to 0.05 or 0.09 J/cm\(^2\) UVB (irradiation time was <2 minutes, depending on the dose) and the cells were immediately harvested in PBS as fresh suspensions. The DNA strand breaks were monitored by single-cell gel electrophoresis (Fig. 7A). The DNA breakages were fewer in cells cultured in the presence of GST-LEDGF-heparin than in GST-heparin or heparin control at both doses of UVB irradiation. These results showed that LEDGF protected against DNA damage induced by UVB irradiation (Fig. 7B).

**Induction of Hsp27 Expression**

Our earlier studies demonstrated that Hsp27, Hsp90, and \( \beta \)-crystallin are elevated in the cells treated with LEDGF.\(^{22,26}\) We hypothesized that RPE cells also express Hsps under oxidative and UVB-induced stress. To test this, RPE cells attached to the culture dish were cultured in serum-free DMEM containing 10 ng GST-LEDGF-heparin or an equal amount of GST-heparin for 2 days. To estimate the levels of Hsps, 10 \( \mu g \) proteins from cultured RPE cells were separated by SDS-PAGE. Protein blot analysis was performed with Abs to Hsp27, Hsp90, and \( \beta \)-crystallin. The level of Hsp27 in culture with GST-LEDGF-heparin was 1.6 times higher than in GST-heparin (Fig. 8). Although, \( \beta \)-crystallin and Hsp90 were expressed in RPE cells, these Hsps were not elevated by GST-LEDGF-heparin (data not shown).
DISCUSSION

Our findings demonstrated that RPE cells could survive for 3 weeks in the presence of LEDGF and that the protective effect of LEDGF against DNA damage induced by \( \text{H}_2\text{O}_2 \) and UVB was dose-dependent. The results show that the expression of Hsp27 was upregulated in the LEDGF environment. These results suggest that LEDGF plays an important role as a survival

**FIGURE 6.** Effect of LEDGF on survival of RPE cells against UVB-induced stress. RPE cells were cultured for 2 days in serum-free DMEM containing GST-LEDGF-heparin, GST-heparin, or heparin alone and then irradiated with 0.05 (A) or 0.09 \( \text{J/cm}^2 \) (B) UVB. Cell viability was determined after 1 or 3 days of additional culture period. Each bar shows the mean of four experiments: white, GST-LEDGF-heparin; gray, GST-heparin; black, heparin alone. Error bars indicate SD. *Significant differences were observed between GST-LEDGF-heparin and the controls and between GST-heparin or heparin alone.

**FIGURE 7.** Effect of LEDGF on DNA strand breaks induced by UVB. RPE cells were cultured for 2 days in serum-free DMEM containing GST-LEDGF-heparin, GST-heparin, or heparin alone. These cells were then exposed to 0.05 or 0.09 \( \text{J/cm}^2 \) of UVB and DNA strand breaks were determined immediately. (A) Photomicrographs of DNA breakage. Left column: cells irradiated with 0.05 \( \text{J/cm}^2 \); right column: cells irradiated with 0.09 \( \text{J/cm}^2 \) (Aa, Ab) heparin alone; (Ac, Ad) GST-heparin; ( Ae, Af) GST-LEDGF-heparin. The control cultures without irradiation were similar to those shown in Figure 5 (Aa-Ad). (B) DNA migration calculated from 50 cells. Each bar shows the mean of four experiments: white, GST-LEDGF-heparin; gray, GST-heparin; black, heparin alone. Error bars indicate SD. *Significant differences were observed between GST-LEDGF-heparin and the control treatments and between GST-heparin and heparin alone.

**FIGURE 8.** Effect of LEDGF on the regulation of Hsp27 in RPE cells. Expression of Hsp27 was upregulated in RPE cells treated with GST-LEDGF-heparin. RPE cells were cultured for 2 days in serum-free DMEM containing GST-heparin or GST-LEDGF-heparin. Protein samples (10 \( \mu\text{g} \)) from cultured cells were separated on SDS-PAGE gel, transferred to nitrocellulose membranes, and immunoreacted with anti-Hsp27 Ab. (A) The loaded protein samples on the nitrocellulose membranes were stained with Ponceau S. Lane 1: GST-heparin; lane 2: GST-LEDGF-heparin. (B) Expression of Hsp27 in cultured RPE cells in the presence of GST-LEDGF-heparin (lane 2) was 1.6 times higher than in the presence of GST-heparin (lane 1). Arrow: Hsp27 band (27 kDa).
factor and probably acts by upregulating the expression of Hsp27 as shown in LECs \(^2\) and retinal photoreceptor cells. \(^2\)

In our previous study we demonstrated that LEDGF is effective when applied exogenously (endocrine). \(^2\) The proliferative and protective effects in a serum-free environment were enhanced by the exogenous application of LEDGF in a dose-dependent manner to RPE cells. It has been reported that certain molecules such as bFGF, epidermal growth factor (EGF), PDGF, and nerve growth factor (NGF) induce similar growth and survival responses when present as endocrine and intracellular factors. \(^3\) The presence of LEDGF intracellularly in the RPE cells was shown by protein blot and immunocytochemistry, and the expression of LEDGF messenger RNA was demonstrated in cos\(^7\) cells and LECs. \(^4\) In our earlier study, a fusion protein between green fluorescent protein (GFP) and LEDGF (GFP-LEDGF) was detected in the culture medium of LECs and cos\(^7\) cells. \(^5\) These findings suggest that LEDGF can be secreted from human RPE and taken up from outside the RPE cells. In addition, it has been shown that heparin protects LECs and retinal photoreceptors from phototoxicity by exogenous application of GST-LEDGF in a light-damaged rat model in vivo. \(^6\) The protective effect of GST-LEDGF on the survival of lens epithelial cells, cos\(^7\) cells, \(^7\) and retinal photoreceptor cells \(^8\) was examined. In these experiments, free LEDGF exhibited 10% to 20% higher potency than GST-LEDGF on the survival of these cells in culture. Assuming that GST-LEDGF is internalized into RPE cells, this fusion protein may be expected to have a greater survival effect than free LEDGF. In fact, free LEDGF rather than GST-LEDGF was found to have a greater potency. \(^9\) Furthermore, another fusion protein, GFP-LEDGF was shown to enhance the survival of lens epithelial cells or cos\(^7\) cells. \(^10\) Thus, extracellular LEDGF, and not GST, was responsible for the enhancement of survival effect on RPE cells in culture.

A number of proteins or enzymes have been shown to protect cells against \(H_2O_2\) or UVB-induced stress. We demonstrated the upregulation of Hsp27 and \(\alpha\)-crystallin in LECs \(^2\) and Hsp90 in retinal cells \(^7\) by LEDGF in vitro. We also showed that the expression of Hsp25 and \(\alpha\)-crystallin was increased by exogenous application of GST-LEDGF in a light-damaged rat model in vivo. \(^8\) The protective effect of Hsp27 against \(H_2O_2\) or UVB-induced stress in vitro has also been reported in other cell types. \(^9\) The results of the present study, which demonstrate that LEDGF protects RPE cells from \(H_2O_2\) or UVB-induced stress with simultaneous increased expression of Hsp27, are consistent with the protective role for this Hsp. A possible mechanism involved is suggested by the observations that Hsp27 binds to cytochrome \(c\) and negatively regulates apoptosis. \(^10\) In addition, \(\alpha\)-crystallin binds to precaspase-3 and also negatively regulates apoptosis. \(^10\) Cell death by oxidative stress and UV radiation has been reported to involve apoptosis. \(^11\) These results clearly suggest that Hsp27 is one of the candidate proteins that protects human RPE cells from damage induced by various types of environmental stress.

Metal-catalyzed reactions \(^12\) with \(H_2O_2\) are known to generate hydroxyl radicals. These radicals are highly reactive and interact with cellular constituents inflicting damage on proteins \(^7\) and DNA \(^7\) through the Fenton reaction. \(^9\) UVB causes free radical formation that can overwhelm cell antioxidant defense and cause cell damage. Hsps are believed to function as molecular chaperons to repair unfolding proteins impaired by various stresses. \(^5\) Hsp27 may play a role in the maintenance of damaged protein and increased cell survival. Hsp27 is also believed to inhibit apoptosis by inactivating caspases \(^7\) and thus enhances the resistance against apoptotic cell death. Damaged DNA is also repaired by base excision repair enzymes, such as uracil DNA glycosylase or DNA polymerase-\(\beta\). An association between Hsp27 and these DNA repair enzymes has been suggested. \(^5\) Thus, the upregulation of Hsp27 induced by LEDGF may play a role in DNA protection against \(H_2O_2\) or UVB stress.

In conclusion, LEDGF protected RPE cells against oxidative stress and UVB irradiation and protects DNA from \(H_2O_2\) or UVB-induced damage. LEDGF is an important survival factor, and high levels enhanced survival of RPE cells. On the contrary, low levels or absence of LEDGF leads to cell death. \(^9\) Thus, either addition of LEDGF exogenously or induced expression of LEDGF by other factors \(^8\) may enhance survival of cells under various kinds of stress.

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References


