Eaf2 protects human lens epithelial cells against oxidative stress-induced apoptosis by Wnt signaling

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Abstract. The tumor suppressor protein ELL-associated factor 2 (Eaf2) serves an important role in lens development and maturation; however, its role in oxidative stress-induced cataract formation remains unclear. In the present study, an *in vitro* apoptosis model was constructed by treating HLE-B3 cells with 50 μM hydrogen peroxide (H_2O_2), and was confirmed by flow cytometry. Subsequently, overexpression of *Eaf2* was induced in H_2O_2-induced HLE-B3 cells by ligating Eaf2 cDNA to a pcDNA3.0 plasmid and the role of Wnt3a in the function of Eaf2 was also assessed by inhibiting the expression of the gene in *Eaf2*-overexpression cells. The expression levels of glycogen synthase kinase 3β, β-catenin, Eaf2, caspase 3, Wnt3a, B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X protein were examined using reverse transcription-quantitative polymerase chain reaction and western blot analysis. Immunocytochemistry was used to locate Eaf2 and Wnt3 protein expression in the H_2O_2-induced HLE-B3 cells. The results indicated that Eaf2 was able to effectively suppress H_2O_2-induced apoptosis of HLE cells via inhibition of caspase 3 production and activation of Wnt3a signaling. In addition, knockdown of Wnt3a in *Eaf2*-overexpression cells evidently counteracted the effect of *Eaf2* in antagonizing H_2O_2-induced apoptosis. Taken together, these findings suggested that Eaf2 may suppress oxidative stress-induced apoptosis of HLE-B3 cells exerted through the activation of Wnt3a signaling.

Introduction

The reduction in lens transparency caused by cataracts induces severe visual impairment (1), which can seriously effect

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patients' quality of life. Currently, cataract-induced blindness accounts for ~50% of blindness cases worldwide (2). It is well-recognized that damage resulting from oxidative stress in the ocular lens is a major cause of cataracts (3). The lens is a transparent organ that consists of a single layer of epithelial cells, which are the main target of oxidative stress (4). Oxidative stress caused by exposure to hydrogen peroxide (H_2O_2) results in DNA damage, and impairs the function of cells and tissues (5). H_2O_2, which is a non-free radical member of the active oxygen family, generates hydroxyl radicals that irreversibly damage the lens epithelium, resulting in cell death and cataract formation (6). Some patients with cataracts have markedly increased levels of H_2O_2 within their lenses (7), indicating the possible involvement of H_2O_2 in the genesis of nuclear cataracts in humans. Therefore, a better understanding of the mechanisms underlying H_2O_2-induced apoptosis of lens cells may provide information regarding the cause of cataract formation.

ELL-associated factor 2 (Eaf2) is a protein that was discovered based on its ability to bind with a second protein (eleven-nineteen lysine-rich leukemia protein; ELL), which is independently coded by a gene that is upregulated by androgen in the rat prostate gland and is also named human U19 (8). As a regulator of transcription, Eaf2 interacts with ELL to efficiently stimulate the extension activity of RNA polymerase II (9). It has previously been suggested that Eaf2 serves as a tumor suppressor in prostate cancer (10). Furthermore, knockdown of *Eaf2* has been reported to promote tumorigenesis in mouse models of adenocarcinoma and hepatocellular carcinoma (11). In addition to its role in cancer, Eaf2 serves important roles in embryonic development via its involvement in non-canonical Wnt signaling (12,13), which is thought to be essential for convergence and extension movements, as well as the midline convergence of organ precursors (14,15). Liu *et al* (16) demonstrated that Eaf2 acts as an upstream modulator of non-canonical Wnt signaling to mediate convergence and extension movements. In addition, the Wnt family of secreted signaling proteins has important roles in organogenesis, tissue homeostasis and tumor formation (17). Overexpression of Wnt3a has been reported to promote the proliferation of human lens epithelial (HLE) cells (18). Furthermore, Eaf2 is required for normal eye development and the regulation of crystalline lens development and maturation in *Xenopus laevis* (12,13).

Recently, a related study identified an important role for Eaf2 in ultraviolet-induced cataract formation (19). However, the mechanism underlying the effects of Eaf2 on lenses undergoing oxidative stress remains unknown.

The present study aimed to investigate the role of Eaf2 in HLE cells undergoing H_2O_2-induced apoptosis, and to determine the underlying molecular mechanism. The results indicated that in HLE cells, Eaf2 protects against H_2O_2-induced cell death by inhibiting caspase 3 enzymatic activity and activating the Wnt3 signaling pathway.

Materials and methods

Cell culture. HLE-B3 cells were purchased from the Cell Bank of the Chinese Academy of Science (Shanghai, China). HLE-B3 cells were cultured in minimum essential medium (MEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 20% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) in a humidified atmosphere containing 5% CO_2 at 37˚C.

Induction of apoptosis in HLE-B3 cells. H_2O_2-induced HLE-B3 cells used in subsequent experiments were gradually deprived of serum via an overnight culture in MEM containing 2% FBS, prior to being treated with 50 μM H_2O_2 for various time periods (4, 8 and 12 h) at 37˚C.

Vector construction and cell transfection. To induce Eaf2 overexpression, the full-length cDNA (NC_000003.12) for the human *Eaf2* gene was obtained from the total RNA of human HLE-B3 cells isolated using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and cloned into a pcDNA3.0 vector (Invitrogen; Thermo Fisher Scientific, Inc.) to generate pcDNA-Eaf2, which was subsequently designated as oeEaf2. The pcDNA3.0 empty plasmid was used as a negative control, which was designated as oeCon. Vector transfection was performed using Lipofectamine 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol for 48 h. Cells overexpressing *Eaf2* also underwent *Wnt3a* knockdown. The *Wnt3a* short hairpin (sh)RNA plasmid (5'-AGAAGUAUCCGA GUGGGGCCA-3') used to knockdown *Wnt3a* was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA) and was designated as shWnt3a. Subsequently, cells overexpressing *Eaf2* were identified and transfected with shWnt3a and control shRNA (shCon; 5'-ACGUGACACGUUCGGAGAATT-3') using Lipofectamine® 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol for 48 h. Cells were then cultured in 6-well plates at an inoculation density of $2x10^4$ cells/well. The resulting constructs were confirmed using RT-qPCR and western blot analysis, and were used for subsequent experiments.

Lactate dehydrogenase (LDH) release assay. To detect apoptosis, the release of LDH was assessed using an LDH assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocol. This method is used to measure the loss of membrane integrity by quantifying the release of LDH into the medium.

Flow cytometric analysis of apoptosis. Following exposure to the respective treatments, HLE-B3 cells were washed with PBS and subjected to a PI/Annexin V-FITC Apoptosis Detection kit (JingMei Biotech, Beijing, China): Briefly, 5 μl Annexin V-fluorescein isothiocyanate and 5 μl propidium iodide (PI) were added to the suspended cells, which were gently vortexed and incubated for 15 min at room temperature in the dark. Following incubation, the cells were analyzed by flow cytometry (Accuri C6; BD Biosciences, Franklin Lakes, NJ, USA). Flow cytometric analysis was performed in triplicate.

RNA isolation and RT-qPCR analysis. Total RNA was extracted from cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. cDNA was synthesized from the RNA using SuperScript II RT (200 U/ml; Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. β-actin served as an endogenous control in the qPCR analysis. The following primers were used: β-catenin, forward 5'-GCC ACAAGATTACAAGAACGG-3', reverse 5'-TGGGCACCA ATATCAAGTCC-3'; caspase 3, forward 5'-TGGTTCATC CAGTCGCTTTG-3', reverse 5'-AATTCTGTTGCCACC TTTCG-3'; Eaf2, forward 5'-AGGTGACCATAACTCTGC CAAAT-3', reverse, 5'-AGCCGACATTCTCCAGTATCA-3'; Wnt3a, forward 5'-GTCCACGCCATTGCCTCAG-3', reverse, 5'-GACACCATCCCACCAAACTCG-3'; B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax), forward 5'-GCAAAC TGGTGCTCAAGGC-3', reverse 5'-GCACTCCCGCCA CAAAGA-3'; and Bcl-2, forward 5'-TGTGGCCTTCTT TGAGTTCG-3' and reverse 5'-ATCCCAGCCTCCGTT ATCC-3'; β-actin, forward: 5'-CTTAGTTGCGTTACACCC TTTCTTG-3', reverse: 5'-CTGTCACCTTCACCGTTCCAG TTT-3'. The RT-qPCR analyses were performed in triplicate on a Bio-Rad Connect Real-Time PCR platform (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using a 20 μl reaction mixture containing 10 μl 2X SYBR Premix Ex Taq, 0.5 μl each primer (2.5 μM), 5 μl cDNA and 4.0 μl ddH$_2$O. The RT-qPCR procedure was conducted as follows: Initial denaturation at 95˚C for 1 min, followed by 40 cycles of denaturation at 95˚C for 5 sec and annealing extension at 60˚C for 20 sec, followed by 72˚C for 20 sec. Human β-actin was used as an internal loading control for RT-qPCR. The $2^{-\Delta\Delta Cq}$ (20) method was used to determine the relative mRNA expression levels of human β-catenin, caspase 3, Eaf2, Wnt3a, Bcl-2 and Bax. These levels were normalized to those of β-actin.

Western blot analysis. Treated HLE-B3 cells were harvested via centrifugation (14,000 x g 4 min) at 4˚C. Subsequently, the pelleted cells were lysed in a 2X SDS lysis buffer containing 100 mM Tris-HCl (pH 6.8), 10 mM EDTA, 4% SDS and 10% glycine. The lysed cells were then centrifuged at 15,000 x g for 15 min at 4˚C, the supernatant fractions were collected and protein concentrations were assessed using the bicinchoninic acid protein assay. Supernatant aliquots containing ~30 μg protein were separated by 12% SDS-PAGE. The proteins were then transferred onto polyvinylidene fluoride membranes by electrophoresis at 300 mA for 2 h. The membranes were then blocked with 5% non-fat milk, and were washed with TBST and probed overnight at 4˚C with 1:1,000 dilutions of primary antibodies, all from Cell Signaling Technology,
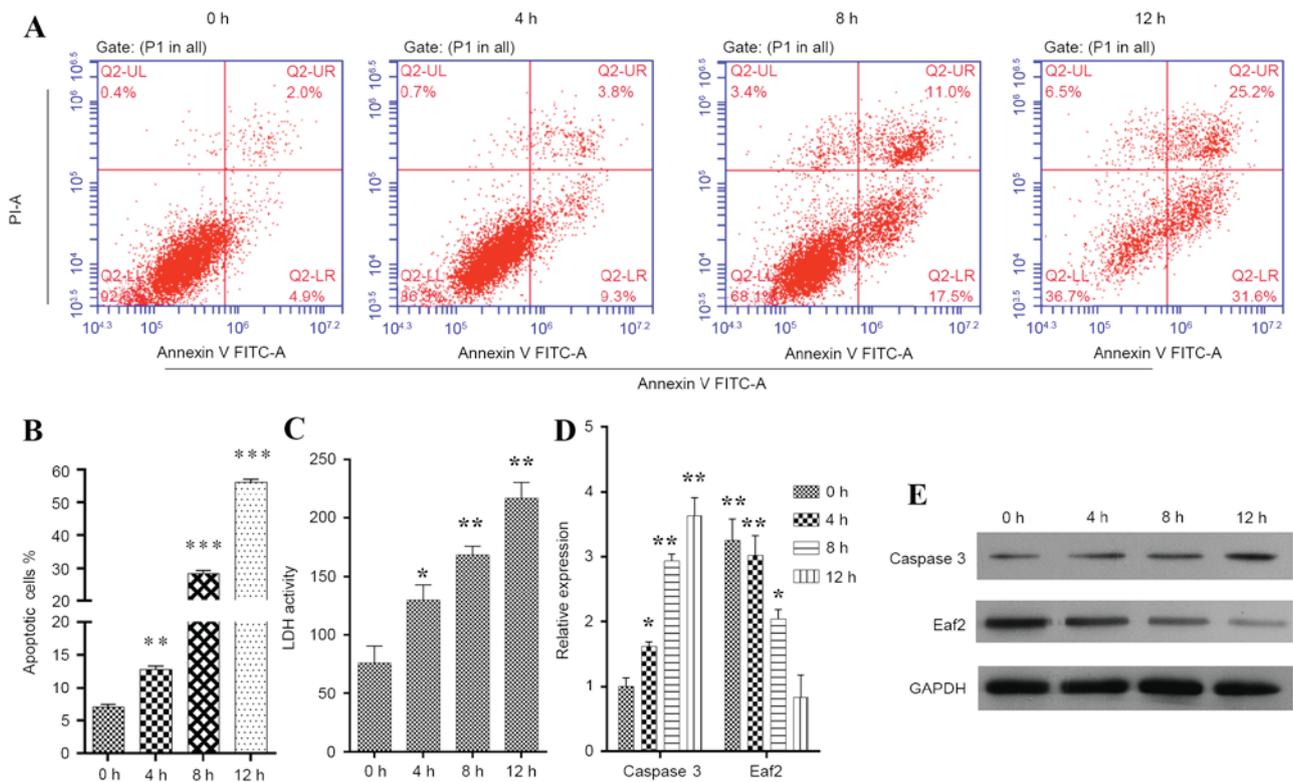
Figure 1. Construction of the H_2O_2-induced apoptotic HLE-B3 cell model. (A) Representative images showing the time-dependent uptake of Annexin V/PI by H_2O_2-induced HLE-B3 cells. (B) Quantification of overall apoptotic cells in a population of HLE-B3 cells, as determined by flow cytometry. Annexin V^+/PI^-, early apoptotic cells; Annexin V^+/PI^+, late apoptotic cells. (C) Quantification of LDH activity in a population of HLE-B3 cells. (D) mRNA expression levels of caspase 3 and Eaf2 in H_2O_2-induced HLE-B3 cells, as determined by reverse transcription-quantitative polymerase chain reaction analysis. (E) Protein expression levels of caspase 3 and Eaf2 in H_2O_2-induced HLE-B3 cells, as determined by western blot analysis. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. control. Eaf2, ELL-associated factor 2; FITC, fluorescein isothiocyanate; H_2O_2, hydrogen peroxide; HLE, human lens epithelial; LDH, lactate dehydrogenase; PI, propidium iodide.

Inc., Danvers, MA, USA: β-catenin (8480), GSK3e (12456), p-GSK3β (5558), Bcl-2 (15071), Bax (5023), caspase 3 (9662), Eaf2 (14159), Wnt3a (2721), GAPDH (5174). Subsequently, the blots were incubated with the corresponding horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology, Inc.) for 45 min at 37˚C. Immunodetection was performed using the Super Enhanced Chemiluminescence Detection reagent according to the manufacturer's protocol (Thermo Fisher Scientific, Inc.).

Immunocytochemistry. Then, 24 h post-transfection, immunostaining was performed as previously described (3). Briefly, the treated HLE-B3 cells were fixed in 4% paraformaldehyde in PBS for 15 min at room temperature, washed with PBS, and then permeabilized with 0.1% Triton X-100 in PBS for 5 min. Subsequently, the cells were blocked for 1 h with PBS containing 5% bovine serum albumin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) at room temperature and were incubated with primary antibodies (Eaf2; ab28357; Abcam and Wnt3a; ab28472; Abcam) for 1 h at room temperature. The cells were then incubated with the corresponding secondary antibodies (BM3894; Wuhan Boster Biological Technology, Ltd., Wuhan, China) for 1 h at room temperature. DAPI (Invitrogen; Thermo Fisher Scientific, Inc.) was used for counterstaining. Images of the immunostained cells were captured using a fluorescence microscope (VANOX-S; Olympus Corporation, Tokyo, Japan).

Statistical analysis. All statistical data were analyzed using GraphPad Prism 6.0 software (GraphPad Software, Inc., La Jolla, CA, USA), and results are expressed as the mean ± standard deviation (n=3). One-way analysis of variance was performed followed by post hoc least significant difference test for multiple comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

Construction of an H_2O_2-induced apoptotic HLE cell model. HLE-B3 cells were induced to enter apoptosis by exposure to H_2O_2 for various time periods (4, 8 and 12 h). The apoptotic cells were detected by flow cytometry (Fig. 1A). Determination of the proportion of Annexin V- and PI-stained gated cells revealed that the population contained cells in both early stage (Annexin V^+/PI^-) and late stage (Annexin V^+/PI^+) apoptosis. The statistical analysis indicated that, when compared with the control cells, cell populations exposed to 50 μM H_2O_2 for 4 (P<0.01), 8 (P<0.001) and 12 h (P<0.001) exhibited increased apoptosis (Fig. 1B). Similar patterns were also recorded in an LDH release assay (Fig. 1C). To further investigate the mechanism underlying H_2O_2-induced apoptosis, the expression levels of caspase 3 and Eaf2 were detected in HLE-B3 cells. The results indicated that the mRNA (Fig. 1D; P<0.01) and protein expression levels (Fig. 1E) of caspase 3 were markedly increased in HLE-B3 cells exposed to H_2O_2. Conversely, in HLE-B3 cells exposed to H_2O_2,
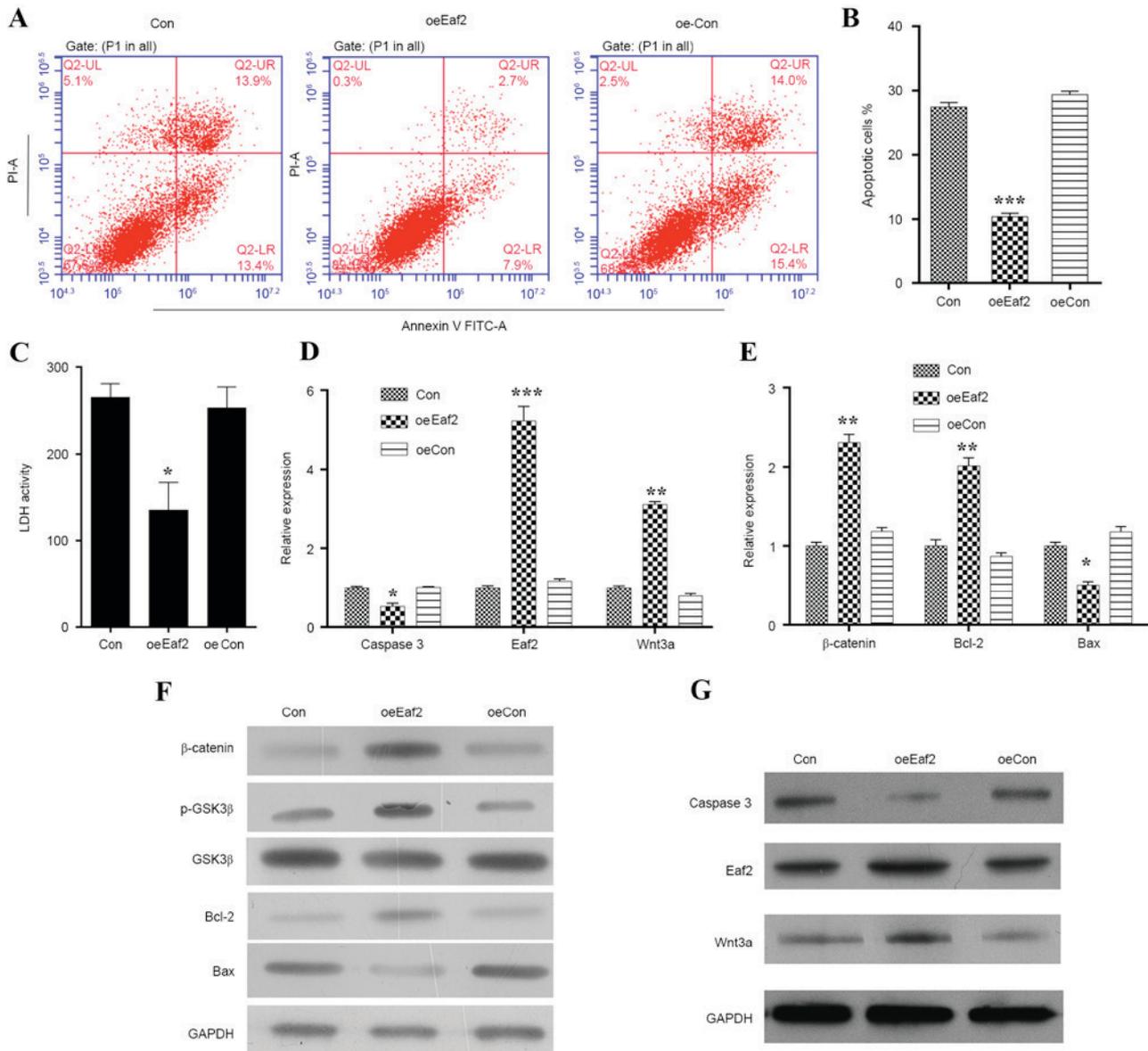
Figure 2. Effects of *Eaf2* overexpression on H_2O_2-induced apoptosis of HLE-B3 cells. (A) Representative images of Annexin V/PI uptake by H_2O_2-induced HLE-B3 cells following Eaf2 overexpression, as determined by flow cytometry. (B) Quantification of apoptotic cells in H_2O_2-induced HLE-B3 cells overexpressing *Eaf2*. (C) Quantification of LDH activity in H_2O_2-induced HLE-B3 cells overexpressing *Eaf2*. Reverse transcription-quantitative polymerase chain reaction indicating the effects of Eaf2 overexpression on the mRNA expression levels of (D) caspase 3, Wnt3a and Eaf2, and (E) β-catenin, Bcl-2 and Bax in H_2O_2-treated HLE-B3 cells. Western blot analysis indicating the effects of *Eaf2* overexpression on the protein expression levels of (F) β-catenin, p-GSK3β, GSK3β, Bcl-2 and Bax, and (G) Wnt3a, Eaf2 and caspase 3 in H_2O_2-treated HLE-B3 cells. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. Con or oeCon. Con represents the control group consisting of normal HLE-B3 cells; oeCon represents the negative control group consisting of HLE-B3 cells transfected with empty pcDNA3.0 plasmid; oeEaf2 represents the experimental group consisting of HLE-B3 cells transfected with pcDNA3.0-Eaf2. Bax, B-cell lymphoma 2-associated X protein; Bcl-2, B-cell lymphoma 2; GSK3β, glycogen synthase kinase 3β; Eaf2, ELL-associated factor 2; FITC, fluorescein isothiocyanate; H_2O_2, hydrogen peroxide; HLE, human lens epithelial; LDH, lactate dehydrogenase; p, phosphorylated; PI, propidium iodide.

the mRNA (Fig. 1D; P<0.05) and protein expression levels (Fig. 1E) of Eaf2 were markedly downregulated, as determined by RT-qPCR and western blot analysis. Therefore, it may be hypothesized that Eaf2 protects HLE cells from entering apoptosis. H_2O_2 treatment for 12 h increased the number of early and late stage apoptotic cells by 6- and 12-fold, respectively, making those cells lose the potential to recover viability during subsequent analysis. Consequently, HLE-B3 cells that underwent H_2O_2 treatment for 8 h were used in subsequent experiments.

Overexpression of Eaf2 alleviates H_2O_2-induced apoptosis of HLE cells. To further investigate the effects of Eaf2 on

H_2O_2-induced HLE-B3 cells, cells were induced to overexpress *Eaf2* by transfection with a pcDNA-Eaf2 plasmid, after which various experiments were conducted (Figs. 2 and 3). Apoptosis was analyzed by Annexin V/PI double staining (Fig. 2A); the results indicated that a significantly lower percentage of cells overexpressing *Eaf2* had entered apoptosis (Fig. 2B). Overexpression of *Eaf2* also decreased LDH activity (Fig. 2C), suggesting that the apoptotic process was suppressed. To investigate whether Eaf2 improves HLE-B3 cell survival rates by inhibiting apoptosis, the cellular levels of caspase 3 (a protease marker for apoptotic cell death) were analyzed by RT-qPCR and western blotting. The results demonstrated that,
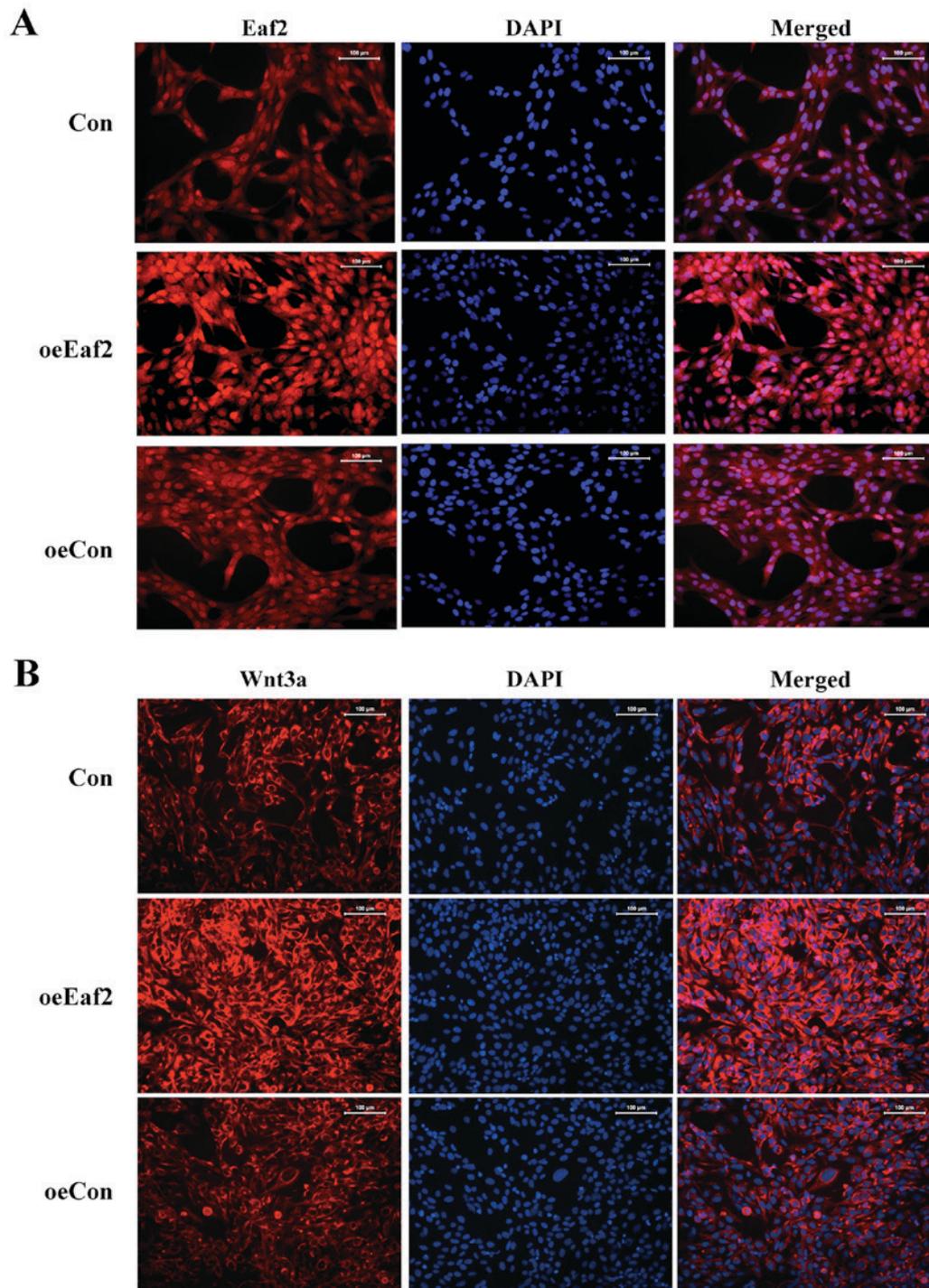
Figure 3. HLE-B3 cells as observed by fluorescence microscopy. Immunocytochemistry techniques were used to examine the expression levels of (A) Eaf2 and (B) Wnt3a in hydrogen peroxide-treated HLE-B3 cells overexpressing *Eaf2*. Con represents the control group consisting of normal HLE-B3 cells; oeCon represents the negative control group consisting of HLE-B3 cells transfected with empty pcDNA3.0 plasmid; oeEaf2 represents the experimental group consisting of HLE-B3 cells transfected with pcDNA3.0-Eaf2. Eaf2, ELL-associated factor 2; HLE, human lens epithelial.

when compared with the control cells, the mRNA (Fig. 2D; P<0.01) and protein expression levels (Fig. 2G) of caspase 3 were markedly decreased in cells overexpressing *Eaf2*. Since it has been reported that a hyperactive Wnt pathway serves a role in early eye development (21), the present study examined the expression levels of β-catenin, phosphorylated (p)-GSK3β and Wnt3a expression in HLE-B3 cells overexpressing *Eaf2*. As presented in Fig. 2D-G, cells overexpressing *Eaf2* exhibited elevated expression levels of β-catenin, p-GSK3β, Wnt3a and

Bcl-2 while decreased the expression of Bax, thus suggesting a close association between Eaf2 and Wnt3a signaling. An immunofluorescence assay further confirmed the upregulation of Wnt3a in Eaf2-overexpressing cells compared with the control cells (Fig. 3).

Eaf2 inhibits H_2O_2-induced apoptosis in HLE cells by regulating Wnt3a. To further investigate whether Eaf2 inhibits apoptosis of HLE-B3 cells by affecting Wnt3a signaling, *Wnt3a* expression
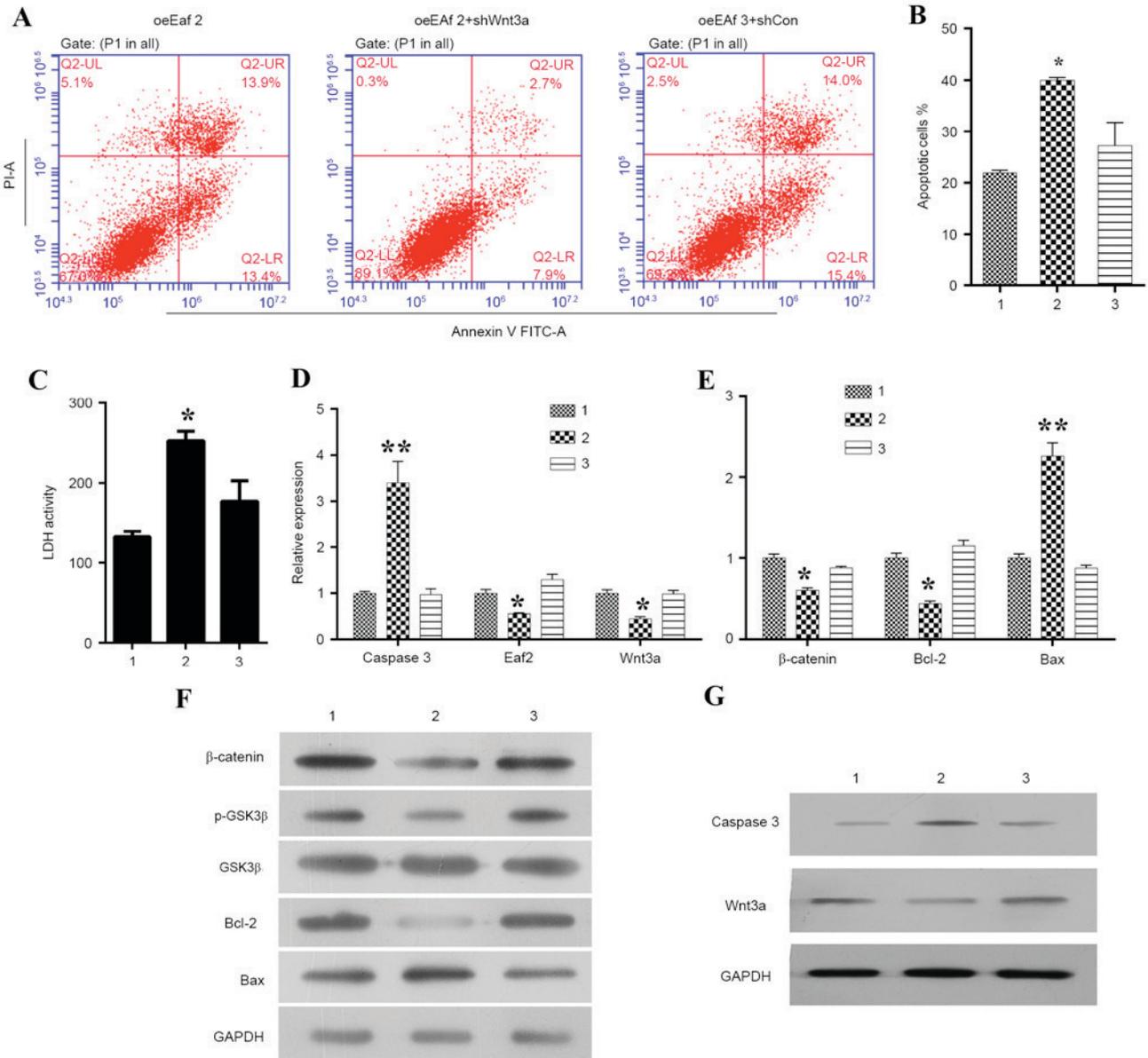
Figure 4. Effects of Wnt3a silencing on Eaf2 expression in HLE-B3 cells. (A) Representative images of Annexin V/PI uptake in Eaf2-overexpressing HLE-B3 cells following Wnt3a knockdown. (B) Quantification of apoptotic cells in *Eaf2*-overexpressing HLE-B3 cells following Wnt3a knockdown. (C) Quantification of LDH activity in *Eaf2*-overexpressing HLE-B3 cells following *Wnt3a* knockdown. Reverse transcription-quantitative polymerase chain reaction was conducted to determine the effects of Wnt3a silencing on the mRNA expression levels of (D) caspase 3, Eaf2 and Wnt3a, and (E) β-catenin, Bcl-2 and Bax in HLE-B3 cells overexpressing Eaf2. Western blot analysis was conducted to determine the effects of *Wnt3a* silencing on the protein expression levels of (F) β-catenin, p-GSK3β, GSK3β, Bcl-2 and Bax, and (G) caspase 3 and Wnt3a in HLE-B3 cells overexpressing Eaf2. $^*P<0.05$, $^{**}P<0.01$ vs. control. Group 1 represents the oeEaf2 group consisting of HLE-B3 cells overexpressing Eaf2; group 2 represents the oeEaf2 + shWnt3a group consisting of Eaf2-overexpressing cells transfected with Wnt3a shRNA; group 3 represents the oeEaf2 + shCon group consisting of Eaf2-overexpressing cells transfected with control shRNA. Bax, B-cell lymphoma 2-associated X protein; Bcl-2, B-cell lymphoma 2; GSK3β, glycogen synthase kinase 3β; Eaf2, ELL-associated factor 2; FITC, fluorescein isothiocyanate; HLE, human lens epithelial; LDH, lactate dehydrogenase; p, phosphorylated; PI, propidium iodide; shRNA, short hairpin RNA.

was specifically knocked down in cells overexpressing *Eaf2*, and the cells were then examined by Annexin V/PI double staining (Fig. 4A). A statistical analysis indicated that the knockdown of *Wnt3a* significantly increased the overall percentage of *Eaf2*-overexpressing HLE-B3 cells undergoing apoptosis (Fig. 4B; P<0.05). In addition, knockdown of *Wnt3a* reversed the *Eaf2* overexpression-induced suppression of LDH release, thus suggesting a key role for Wnt3a signaling in the function of Eaf2: That the role of Eaf2 in HLE-B3 cells depended on the activation of Wnt3a (Fig. 4C). Furthermore, the results suggested that *Wnt3a* knockdown promoted cell apoptosis by increasing

the expression levels of caspase 3 and Bax while decreasing the expressions of β-catenin, p-GSK3β, Bcl-2 and Wnt3a (Fig. 4D and G). As presented in Fig. 5, an immunofluorescence analysis of Wnt3a expression was conducted in the various cell groups and the results showed that suppressed expression and distribution of *Wnt3a* was achieved in HLE-B3 cells.

Discussion

Oxidative stress is a major cause of cancer and cell death, and is thought to serve a major role in cataract formation (22).
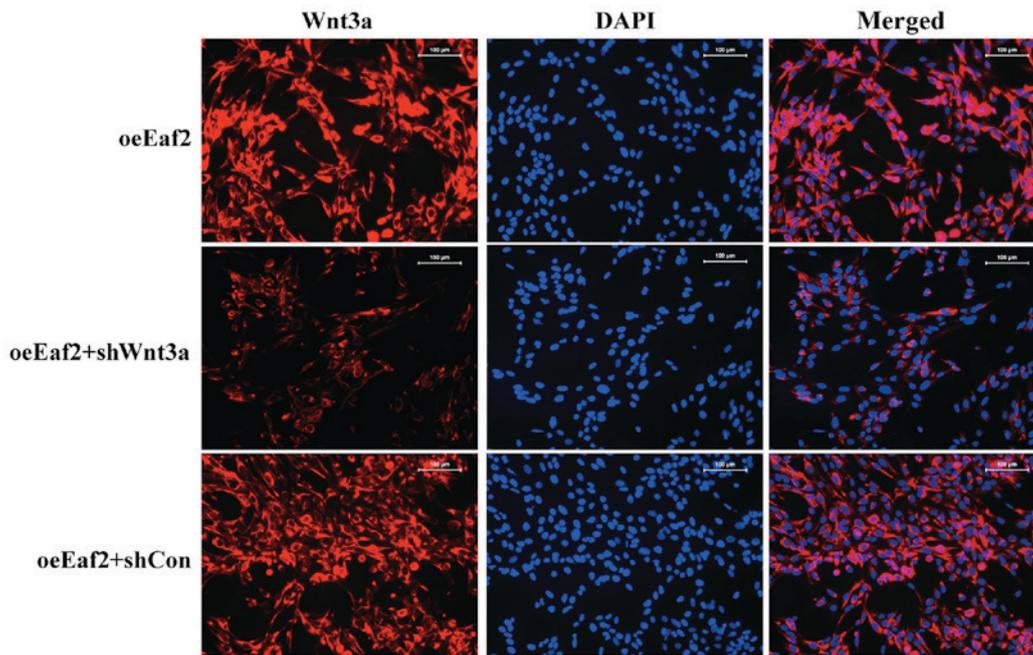
Figure 5. H$_2$O$_2$-treated HLE-B3 cells overexpressing Eaf2, as observed by fluorescence microscopy. Immunocytochemistry was used to examine Wnt3a expression in H$_2$O$_2$-treated *Eaf2*-overexpressing HLE-B3 cells following Wnt3a knockdown. The oeEaf2 group consists of HLE-B3 cells overexpressing *Eaf2*; the oeEaf2 + shWnt3a group consists of *Eaf2*-overexpressing cells transfected with *Wnt3a* shRNA; the oeEaf2 + shCon group consists of *Eaf2*-overexpressing cells transfected with control shRNA. Eaf2, ELL-associated factor 2; H$_2$O$_2$, hydrogen peroxide; HLE, human lens epithelial; shRNA, short hairpin RNA.

Previous studies have demonstrated that H$_2$O$_2$-induced oxidative stress can stimulate apoptosis of HLE cells, which form a single layer in the ocular lens (3,5,23). Furthermore, this type of cellular stress may be considered the initiating factor for non-congenital cataract formation (24). In the present study, the role of the tumor suppressor Eaf2 was determined in H$_2$O$_2$-induced apoptosis of lens cells. The results of the *in vitro* study suggested that Eaf2 protects HLE cells from H$_2$O$_2$-induced apoptosis by regulating the expression levels of apoptosis-associated proteins. This conclusion was based on an analysis of data obtained from loss and gain-of-function experiments. Cellular apoptosis is an important physiological and pathological process, which results in the destruction of cell membranes and condensation of chromosomes (25). Furthermore, the activation of caspase proteases is regarded as the central mechanism of apoptosis. The levels of caspase 3 can be used to accurately reflect the levels of apoptosis in a cell population, particularly the levels of early stage apoptosis (26). Meanwhile, Bax and Bcl-2 expression serve as key roles in cell apoptosis. The present study observed that HLE cells began undergoing apoptosis in a time-dependent manner following exposure to H$_2$O$_2$. Furthermore, their entry into apoptosis was accompanied by the activation of caspase 3 and Bax, and suppression of Bcl-2. In HLE cells, oxidative stress leads to apoptosis, which is a common cellular mechanism underlying cataract formation (27).

It has previously been reported that Eaf2 is spatially regulated in the lenses of embryonic mice (12). Another study demonstrated that Eaf2 is highly enriched in the developing eye, and is essential for normal eye development (13). In the present study, gradually decreasing levels of Eaf2 expression were detected over time in HLE cells undergoing H$_2$O$_2$-induced apoptosis. A further analysis indicated that in HLE cells

overexpressing Eaf2, the rate of H$_2$O$_2$-induced apoptosis was decreased, thus suggesting that it protects HLE cells against oxidative stress-induced apoptosis.

Overexpression of *Eaf2* resulted in a marked increase in the expression levels of Wnt3a in H$_2$O$_2$-induced cells. Conversely, knockdown of *Wnt3a* decreased *Eaf2* expression, and thereby promoted apoptosis of HLE cells. Wnt3a signaling is reportedly involved in the regulation of ocular cell proliferation and eye development (21,28). A previous study demonstrated that *Wnt3a* overexpression promotes the proliferation of HLE-B3 cells by increasing the percentage of cells in S phase (18). Furthermore, the loss of Eaf2 function has been reported to result in a loss of eye function, and the loss of Wnt-4 function can be reversed by Eaf2 (13). Based on these findings, it may be concluded that Eaf2 suppresses H$_2$O$_2$-induced apoptosis via its effects on Wnt3a signaling. In addition, immunocytochemistry studies also revealed that Eaf2 can influence the expression of Wnt3a, and affect Wnt3a expression levels. This finding may further explain how Eaf2 regulates the percentage of lens cells undergoing H$_2$O$_2$-induced apoptosis.

In conclusion, to the best of our knowledge, these data are the first to demonstrate that *Eaf2* gene transcription products can protect HLE cells from oxidative damage caused by exposure to H$_2$O$_2$. In addition, the results of the present study demonstrated that when overexpressed, Eaf2 can reduce H$_2$O$_2$-induced cell death by decreasing the expression levels of caspase 3 and Bax, and increasing Wnt3a and Bcl-2 expression. These results provide a theoretical foundation for the development of novel drugs for the treatment of cataracts. The present study focused on cell apoptosis and the suppressive effects of Eaf2 on apoptosis; however, further studies are required to explain how Eaf2 affects apoptosis.

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