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Original Article

CALML3 mediates oxidative stress-induced apoptosis in human lens epithelial cells through PI3K/Akt pathway

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Abstract: The pathogenic factors of cataract are multivariate, among which oxidative stress is of great importance in the development of various types of cataract through causing damage to the lens epithelial cells, especially apoptosis. The cellular calcium (Ca^{2+}) homeostasis is a necessary condition to maintain lens transparency in the lens epithelial cells. The purpose of our investigation was to identify the function of Calmodulin-like protein 3 (CALML3) in H_2O_2-induced apoptosis of human lens epithelial (HLE) cells and the underlying molecular mechanisms involved. Human lens epithelial cell line, HLEB-3, was treated with a series of concentrations of H_2O_2 (100, 300 and 500 μM) and following focused on assessment of cell viability, apoptosis, accumulation of intracellular ROS and intracellular Ca^{2+} concentration by the MTT assay and flow cytometry analysis, respectively. The protein expression levels of CALML3, Caspase-3, Caspase-9, Cytochrome c (Cyt-c), p-Akt and Akt was measured by western blotting. In the present study, CALML3 overexpression inhibited cell apoptosis, generation of ROS, increased intracellular Ca^{2+}, and the release of Cyt-c from the mitochondria into the cytosol caused by H_2O_2 in HLEB-3 cells. CALML3 overexpression also attenuated the increased expression of Caspase-3 and Caspase-9 in HLEB-3 cells induced by H_2O_2. Moreover, CALML3 overexpression attenuated the reduced activation of Akt induced by H_2O_2. To investigate the underlying mechanism by which CALML3 overexpression may inhibit H_2O_2-induced HLEB-3 cell injury through activating Akt signaling, PI3K inhibitor LY294002 was introduced in H_2O_2-induced HLEB-3 cells after CALML3 overexpression. We found that LY294002 reversed the protective effect of CALML3 overexpression against H_2O_2-induced injury in HLEB-3 cells. In conclusion, our data showed that CALML3 overexpression leads to a significant reduction of apoptosis after H_2O_2 treated, suggested that CALML3 upregulation may protect against H_2O_2-induced cataractogenesis. The underlying mechanism by which CALML3 overexpression protects against HLEB-3 cell apoptosis could be related to activation of PI3K/Akt pathway.

Keywords: Human lens epithelial cells, oxidative stress, calmodulin-like protein 3, apoptosis, PI3K/Akt

Introduction

Cataract is one of the most common senile diseases. It is still the main cause of human visual impairment and blindness in aged population worldwide [1], accounting for 47.8% of all causes of blindness [2]. Along with the rapid advance of techniques, surgery has become a safe and effective method in the treatment of cataract, but so far, high cost and inevitable risks for the surgical complications still could not be avoided completely [3, 4]. Understanding of the pathogenesis of cataract has been an obstacle to cataract. There is evidence thatapoptosis in lens epithelial cells is a common cellular basis for the development of non-congenital cataract both in human and in animals [5, 6]. As a result, looking for new strategies for preventing the apoptosis of lens epithelial cells may provide new clues for understanding the mechanism of the occurrence and development of cataract.

It is commonly believed that oxidative stress plays a key role in the mechanism of cataractogenesis in both experimental animal models [7, 8] and cultured lens systems [9, 10]. The abnormal production of ROS leads to oxidative stress, which induces cell death in a cell type-independent manner by regulating a succession of intracellular signal transduction pathways [11, 12]. Hydrogen peroxide (H_2O_2), a

major intracellular ROS, causes peroxidation of lipid, oxidation and aggregation in protein and damage to DNA in the aqueous humor, and reduces the levels of antioxidant in the lens, ultimately promotes the damage to the lens epithelial cells, contributing to the following development of cataract [13]. Therefore, the inhibition of oxidative stress and apoptosis of lens epithelial cells remains to be the main issue in cataract therapy development.

Calcium (Ca^{2+}) is of great importance in the cell survival, and excessive loading of mitochondrial Ca^{2+} may lead to apoptosis via inducing the discharge of pro-apoptotic molecules from the mitochondrial intermembrane space to the cytoplasm and damaging to the function of mitochondria [14, 15]. The concentration of Ca^{2+} in lens epithelial cells is crucial to lens physiology, and it is increased in patients with cataract and in most of the animal models, which may associate with oxidative stress, resulting in gradually deteriorated Ca^{2+} homeostasis [16, 17]. The intracellular Ca^{2+} signal was transmitted by Ca^{2+} sensitive protein, such as calmodulin and Ca^{2+} binding-related protein family members. Although lack or abnormal expressions of these proteins that associated with abnormal cell growth were observed, the possible mechanisms of these Ca^{2+} binding-related proteins are still not clear. Previous study has reported that Calmodulin-like-protein CALML3 is an epithelial-specific calcium-sensing protein and is expressed specifically in normal epithelial cells. It is regulated by short-term blockade of the MaxiK channel associated with an increase in basal Ca^{2+} concentration [18] and has a relatively low expression in tumorigenesis [19]. However, the causal role of CALML3 in cataracts due to the apoptosis of lens epithelial cells and the possible mechanisms involving signaling pathway in this condition remain to be determined.

Because of the importance of oxidative stress-induced lens epithelial cells apoptosis in cataractogenesis, we present a study here to evaluate the function of CALML3 overexpression in HLEB-3 cell apoptosis, ROS generation and Ca^{2+} overload induced by H_2O_2, and we further evaluated the possible mechanisms involved. Overall, our results may provide a new target for treatment of cataract. Furthermore, our study provides the clear clue that CALML3 may act as a target for treatment of cataract.

Materials and methods

Cell culture

HLEB-3 cells purchased from ATCC (Rockville, MD) were cultured in Dulbecco's modified Eagle's medium (DMEM) obtained from Gibco (Grand Island, NY) containing heat-inactivated 10% FBS and cultured with 5% CO_2 at 37°C in a humidified atmosphere. When grown to 75%-80% confluence in a 60 mm culture dish, the cells were incubated with the different concentrations of H_2O_2 for 12 h or transfected with CALML3 expressing vector for 48 h prior to H_2O_2 treatment. After 12 h H_2O_2 treatment, the cells were collected for the subsequent experiments.

Cell viability assay

The cell viability of HLEB-3 was measured by MTT assay. Briefly, HLEB-3 cells were seeded in a 96-well plate at the density of 1×10^5 cells per plate and cultured with 5% CO_2 at 37°C overnight. After incubated with 100, 300, and 500 μM H_2O_2 for 12 h, the cells were added with 20 μl of MTT solution (5 mg/ml) with 5% CO_2 at 37°C for 4 h. The cell viability was calculated by using an ELISA plate reader at a wavelength of 490 nm.

Flow cytometric analysis using annexin V and PI

The apoptosis of HLEB-3 was measured by an Annexin V-FITC/PI staining kit (BD Biosciences, CA, USA) according to the manufacturer's instructions. Briefly, HLEB-3 cells were cultured in the 6-well plates at the density of 1×10^6 cells per plate and exposed to 100, 300, and 500 μM H_2O_2 for 12 h. After washes with cold PBS, the cells were centrifuged with 1000 g for 5 min and following stained with 195 μl of Annexin V-FITC and 15 μl of PI for 15 min in darkness at 25°C. Apoptosis was evaluated by the flow cytometry assay through a FACScan system flow cytometric analysis (Becton-Dickinson FACS Calibur, San Joes, CA, USA) equipped with Cell Quest 3.3 software.

Detection of ROS and calcium concentration

Cells were grown on the 6-well plates at the density of 1×10^6 cells per plate and exposed to 100, 300, and 500 μM H_2O_2 for 1 h, followed by trypsinized, centrifuged and resuspended twice with PBS. For ROS detection, HLEB-3 cells were

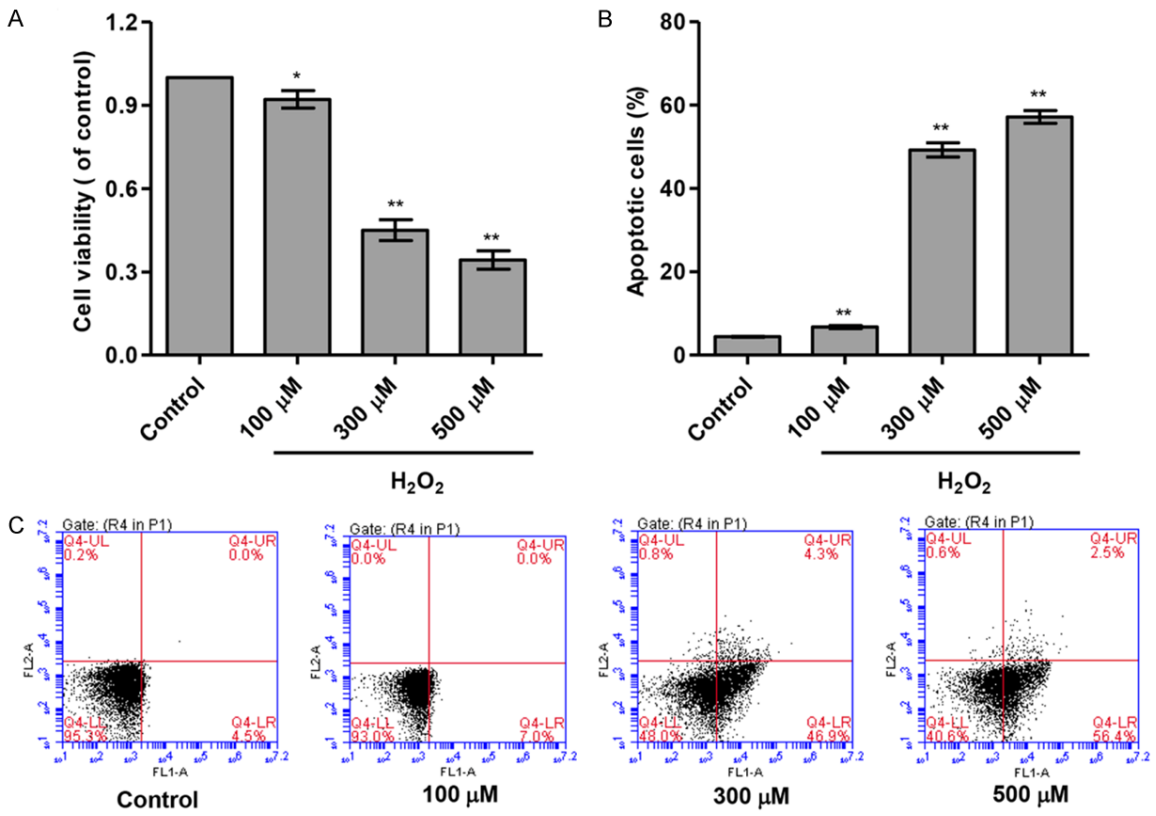

Figure 1. H_2O_2 reduced cell viability and induced apoptosis of HLEB-3 cells. After exposed to different doses of H_2O_2 (100, 300, and 500 μM) for 12 h, the viability of HLEB-3 cells was measured by MTT assay (A) and the cell apoptosis was measured by flow cytometry (B, C). *P<0.05, **P<0.01 compared with control.

added with DHE (50 μM) at 37°C for 15 min. For intracellular Ca^{2+} content detection, HLEB-3 cells were added with Fluo-3 (10 μM, Sigma) at 37°C for 30 min. The production of ROS and calcium was monitored using flow cytometry as above described.

Overexpression of CALML3

The CALML3 coding sequence was cloned into the pLVX-AcGFP-C1 lentivirus vector. Lipofectamine 2000 (Invitrogen, Shanghai, China) was used to the constructs of human CALML3 mRNA transfection according to the manufacture's instruction. Empty lentivirus vector was used as the negative control (NC). Cells were analyzed at 48 h after transfection.

RNA isolation and quantitative RT-PCR

Whole RNA was extracted from HLEB-3 cells by RNAiso Plus (TaKaRa, Dalian, China) according to the manufacturer's instructions. RNA (1 μg) was used to synthesize first strand complementary DNAs with the Reverse Transcription System (Promega, Madison, WI). Real-time PCR was performed using a DyNAmo Flash SYBR Green qPCR kit (Finnzymes Oy, Espoo, Finland) and performed using the Applied Biosystems 7300 Sequence Detection System (Applied Biosystems). The relative mRNA expression of target gene compared with GAPDH was calculated using the $2^{-\Delta\Delta Ct}$ method. To comparerelative amount of mRNA levels, the expression of CALML3 was given as ratio to GAPDH. The sequences of the primers were designed by the Primer Express software and listed as followed: CALML3 (sense): 5'-GGCCAGGTCAATTATGAAG-3'; CALML3 (antisense): 5'-TCAGGGAAGAAGG-AGAAAG-3'; GAPDH (sense): 5'-CACCCACTCCT-CCACCTTTG-3'; and GAPDH (antisense): 5'-CCACCACCCTGTTGCTGTAG-3'.

Protein extraction and western blotting

Total protein from the HLEB-3 cells was extracted in RIPA Lysis buffer (Beyotime, China) supplemented with a protease inhibitoron ice for 30 min. The protein extract was quantified using the bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Inc.). 15 μl of proteins were separated through a 10% SDS-PAGE gel, and the gel was electrophoretic

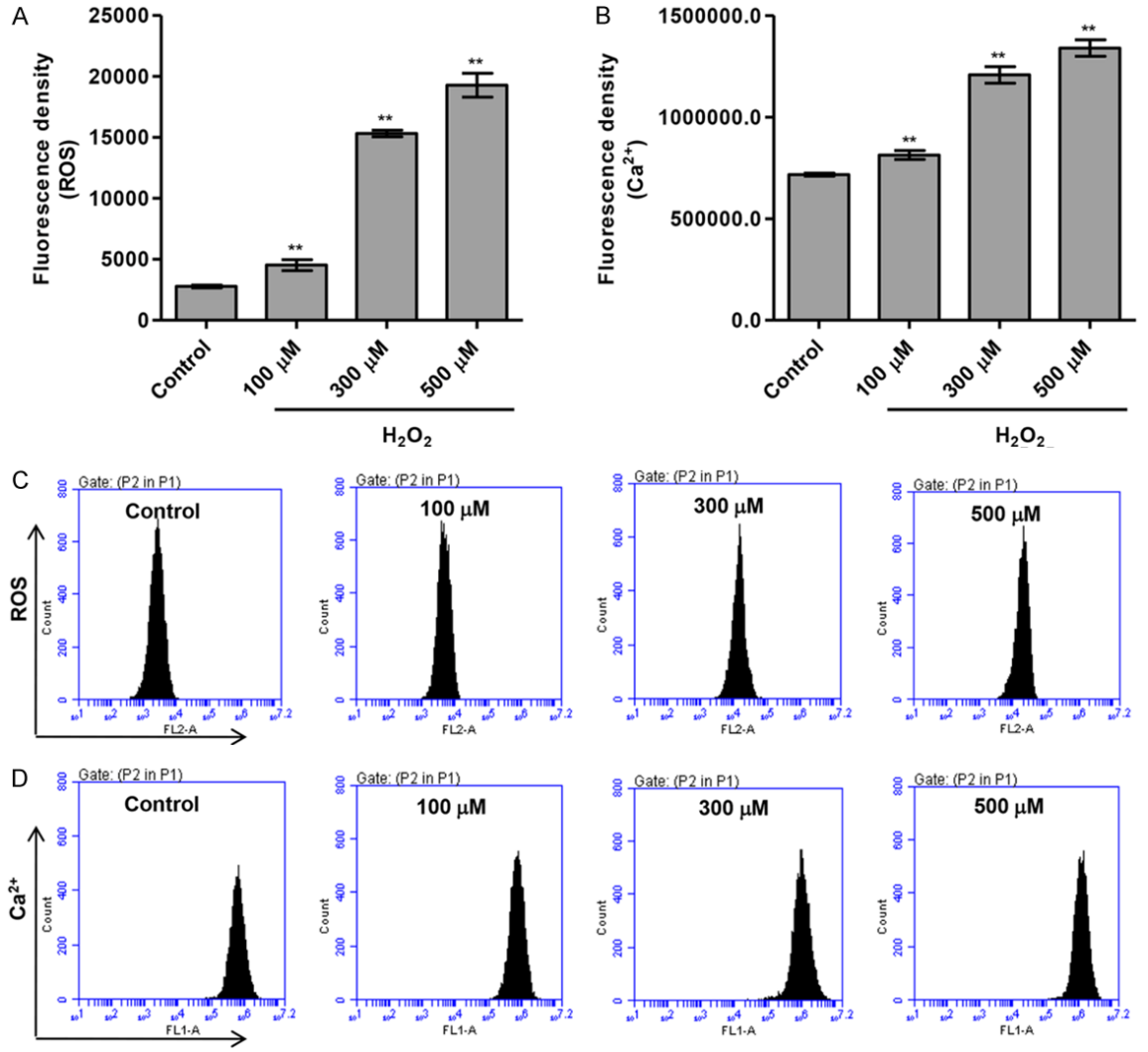

Figure 2. H_2O_2 increased ROS generation and Ca^{2+} level of HLEB-3 cells. After exposed to different doses of H_2O_2 (100, 300, and 500 μM) for 1 h, the ROS generation (A, C) and Ca^{2+} level (B, D) of HLEB-3 cells was measured by flow cytometry, respectively. **P<0.01 compared with control.

moved onto membranes of nitrocellulose (EMD Millipore, Billerica, MA, USA). After blocking in 5% non-fat milk for 2 h at room temperature, these membranes got immunoblotted overnight in 4°C with anti-CALML3, anti-Caspase-3, anti-Caspase-9, anti-Cyt-c, anti-p-Akt, anti-Akt, and anti-GAPDH, followed by 3 washes of 5 to 7 minuteseach in TBST. Horseradish peroxidase-conjugated second antibodies were used to incubate membranes after they were washed (1:1000; Beyotime Institute of Biotechnology, Inc.) for one hour at 37°C. The blots were detected using enhanced chemiluminescence according to the manufacturer'sinstructions (Pierce, Rockford, IL, USA) and exposed to X-ray film and quantified in Chemi Doc XRS Imaging System, Bio-Rad (USA).

Statistical analysis

All the data are presented as means ± SD. All the assays were performed in triplicate, and each experiment was repeated three times. Data were analyzed by One Way ANOVA followed by Tukey's post hoc test using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). A two-tailed difference with P<0.05 was considered statistical significance.

Results

H_2O_2 inhibits the viability and induces apoptosis of HLEB-3 cells

The oxidizing agent H_2O_2 has been applied to the classic design implicating the oxidative

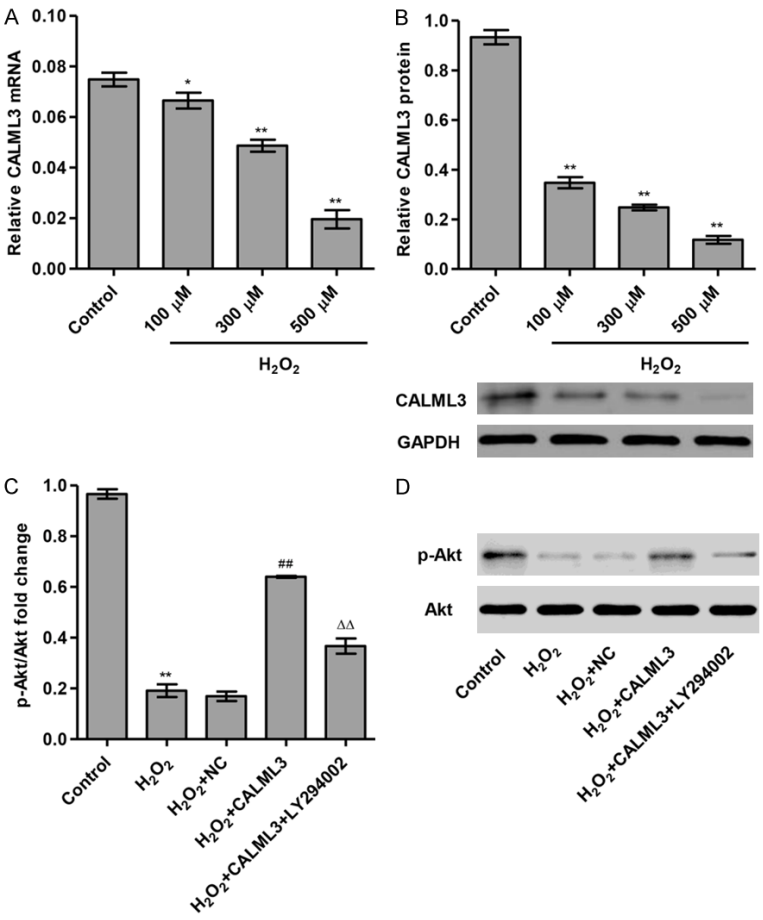

Figure 3. H_2O_2 reduced CALML3 expression and Akt activation in HLEB-3 cells. After exposed to different doses of H_2O_2 (100, 300, and 500 μM), the expression of CALML3 in HLEB-3 cells was measured by Real-time PCR (A) and western blot (B), and the expression of p-Akt and total-Akt was measured by western blot (C, D). To investigate the underlying mechanism by which CALML3 overexpression may inhibit H_2O_2-induced HLEB-3 cell injury through activating Akt signaling, PI3K inhibitor LY294002 was introduced in H_2O_2-induced HLEB-3 cells after CALML3 overexpression. $^{*}P<0.05$, $^{**}P<0.01$ compared with control. $^{\#\#}P<0.01$ compared with H_2O_2. $^{\Delta\Delta}P<0.01$ compared with H_2O_2+CALML3.

stress models in lens epithelial cells, which causes rapid membrane permeation and depolarization of the mitochondrial membrane potential, and was therefore selected for our investigation. Firstly, a succession of dose-dependent experiments was performed to detect the cytotoxicity of H_2O_2 to HLEB-3 cells. Exposed to H_2O_2 at 100, 300, and 500 μM for 12 h, HLEB-3 cells showed decreased cell viability in a H_2O_2 dose-dependent manner compared with control cells (92.1%±3.1%, 45.0%±3.8%, and 34.2%±3.3% of control value; **Figure 1A**). Meanwhile, Exposed to H_2O_2 at 100, 300, and 500 μM for 12 h, HLEB-3 cells showed increased cell apoptosis in a H_2O_2 dose-dependent manner compared with control cells (6.7%±0.4%, 49.3%±1.7%, and 57.2%±1.5% versus 4.4%±0.1%; **Figure 1B** and **1C**).

H_2O_2 promotes ROS generation and intracellular calcium level in HLEB-3 cells

HLEB-3 cells were exposed to 100, 300, and 500 μM H_2O_2 for 1 h and significantly increased the ROS production by 1.6-, 5.5-, and 6.9-fold in comparison with control cells, respectively (**Figure 2A** and **2C**). In the presence of normal extracellular calcium concentration, Fluo-3-loaded HLEB-3 cells were treated with the oxygen radical H_2O_2. As shown in **Figure 2B** and **2D**, stimulation with 100, 300, and 500 μM H_2O_2 caused 1.1-, 1.7- and 1.9-fold increase of Ca^{2+} level compared to nontreated cells, respectively.

H_2O_2 inhibits CALML3 expression and Akt activation in HLEB-3 cells

As shown in **Figure 3A** and **3B**, treatment of cells with 100, 300, and 500 μM H_2O_2 induced a dose-dependent decrease of CALML3 mRNA and protein. Cell stimulation with 100, 300, and 500 μM H_2O_2 caused a detectable decrease of CALML3 mRNA with 88.9%, 65.1%, and 26.2% above control (**Figure 3A**). Consistent with the results presented above, cell stimulation with 100, 300, and 500 μM H_2O_2 caused a detectable decrease of CALML3 protein with 37.2%, 26.6%, and 12.6% above control (**Figure 3B**).

LY294002 treatment inhibits CALML3 overexpression-induced Akt activation in HLEB-3 cells

Additionally, cell stimulation with H_2O_2 caused obvious reduction of Akt phosphorylation level, whereas it had no effect on total Akt expression. However, CALML3 overexpression signifi-

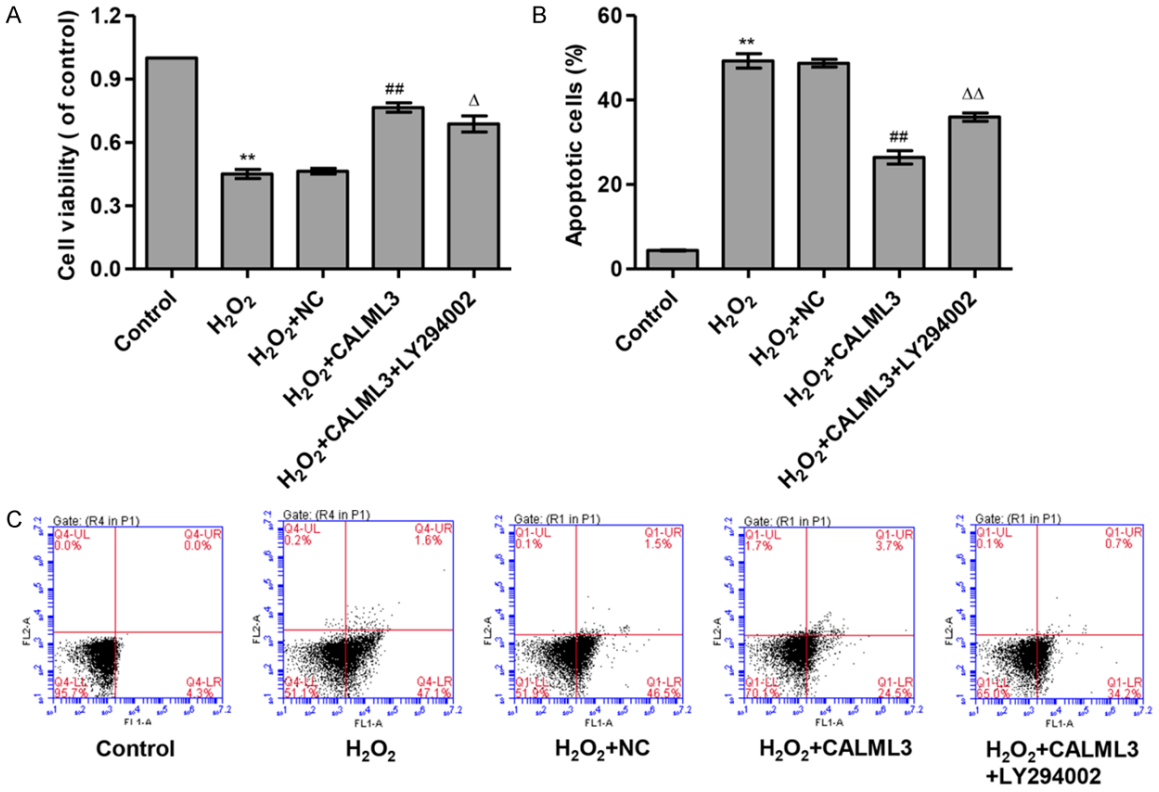

Figure 4. CALML3 overexpression inhibited H_2O_2-induced HLEB-3 cell apoptosis, which was reversed by LY294002 treatment. After transfected with CALML3 expressing vector prior to incubated with H_2O_2 (300 μM), the viability of HLEB-3 cells was measured by MTT assay (A) and the cell apoptosis was measured by flow cytometry (B, C). $^{**}P<0.01$ compared with control. $^{\#\#}P<0.01$ compared with H_2O_2. $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$ compared with H_2O_2+CALML3.

cantly reduced H_2O_2-induced decrease of p-Akt/Akt. To investigate the underlying mechanism by which CALML3 overexpression may inhibit H_2O_2-induced HLEB-3 cell injury through activating Akt signaling, PI3K inhibitor LY294002 was introduced in H_2O_2-induced HLEB-3 cells after CALML3 overexpression. LY294002 treatment significantly inhibited CALML3 overexpression-induced Akt activation in HLEB-3 cells (**Figure 3C** and **3D**).

CALML3 overexpression inhibits H_2O_2-induced HLEB-3 cell apoptosis, which is reversed by LY294002 treatment

The cell viability of HLEB-3 transfected with CALML3 expressing vector before exposure to 300 μM H_2O_2 was increased by 69.9% compared to H_2O_2 treatment alone whereas LY294002 treatment inhibited the increased cell viability induced by CALML3 overexpression by 20.3% (**Figure 4A**). An obvious decrease in apoptosis was found in HLEB-3 cells transfected with CALML3 expressing vector compared with H_2O_2 treatment alone (26.4% versus 48.7%; **Figure 4B** and **4C**). However, LY294002 treated cells showed significant increase of apoptosis compared with CALML3 overexpression in H_2O_2-induced HLEB-3 cells (35.9% versus 26.4%; **Figure 4B** and **4C**).

CALML3 overexpression inhibits H_2O_2-induced ROS generation and intracellular calcium in HLEB-3 cells, which is reversed by LY294002 treatment

HLEB-3 cells treated with CALML3 expressing vector resulted in the decreased production of ROS by 64.4% compared to HLEB-3 cells with H_2O_2 treatment alone (**Figure 5A** and **5C**). However, LY294002 treatment markedly increased the ROS levels compared to CALML3 overexpression in H_2O_2-induced HLEB-3 cells by 97.6% (**Figure 5A** and **5C**). Moreover, HLEB-3 cells treated with CALML3 expressing vector resulted in the decreased Ca^{2+} level by 28.4% compared to HLEB-3 cells with H_2O_2 treatment alone (**Figure 5B** and **5D**). However, LY294002 treatment markedly increased the Ca^{2+} level compared to CALML3 overexpression in H_2O_2-

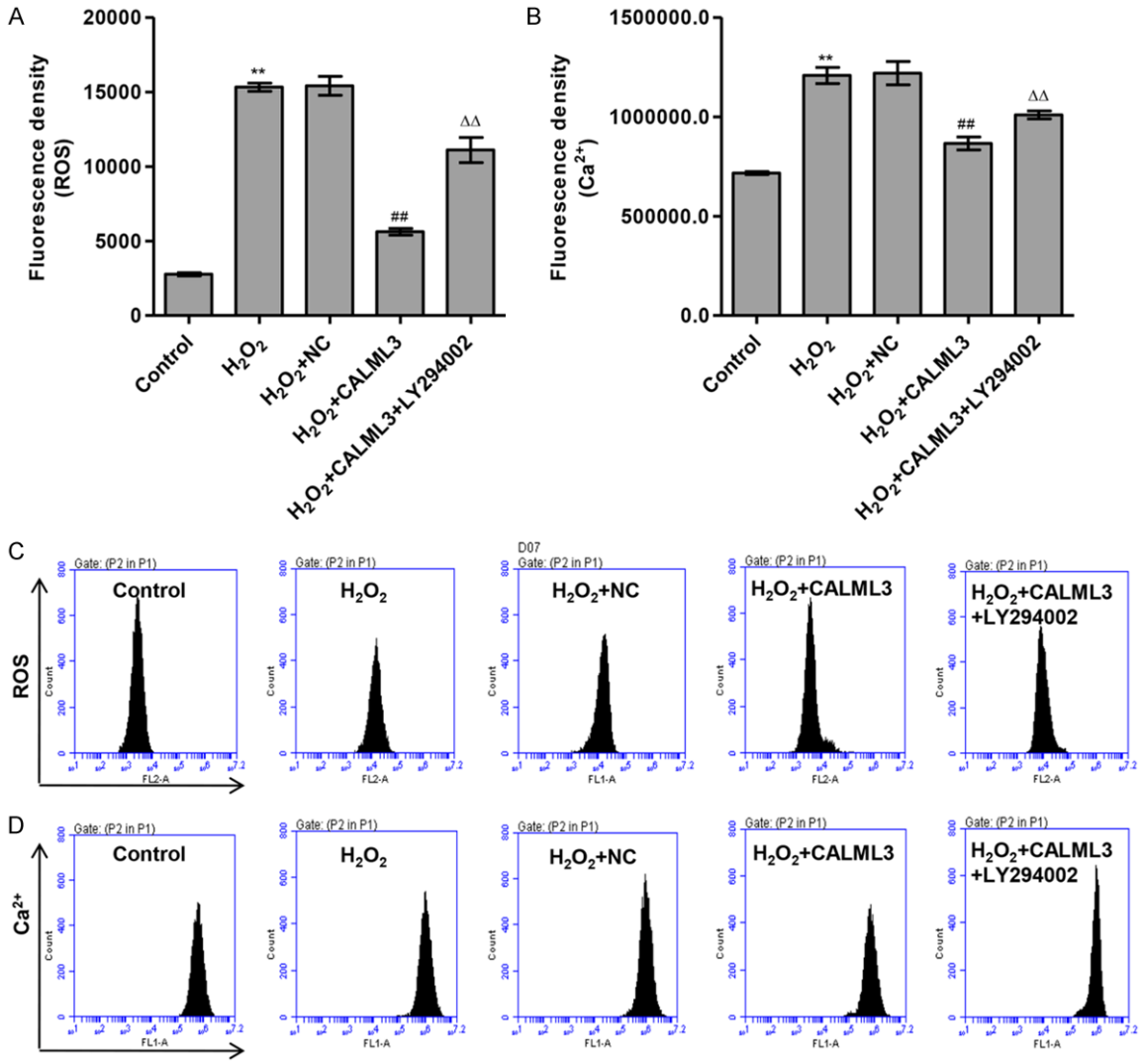

Figure 5. CALML3 overexpression inhibited H_2O_2-induced ROS generation and intracellular calcium in HLEB-3 cells, which was reversed by LY294002 treatment. After transfected with CALML3 expressing vector prior to incubated with H_2O_2 (300 μM), the ROS generation (A, C) and Ca^{2+} level (B, D) of HLEB-3 cells was measured by flow cytometry, respectively. **P<0.01 compared with control. $^{\#\#}$P<0.01 compared with H_2O_2. $^{\Delta\Delta}$P<0.01 compared with H_2O_2+CALML3.

induced HLEB-3 cells by 16.6% (**Figure 5B** and **5D**).

CALML3 overexpression inhibits H_2O_2-induced Cyt-c, Caspase-9 and Caspase-3 expression in HLEB-3 cells, which is reversed by LY294002 treatment

To further investigate the role of H_2O_2 in mitochondrial apoptosis, the protein expression levels of Caspase-3, Caspase-9 and Cyt-c were measured by western blot assay. As shown in **Figure 6A** and **6B**, H_2O_2 treatment significant increase in the protein expression of Cyt-c, Caspase-9 and Caspase-3, except CALML3. Furthermore, CALML3 overexpression inhibited the increased protein expression of Caspase-3, Caspase-9 and Cyt-c induced by H_2O_2 in HLEB-3 cells, while LY294002 treatment reversed the effects of CALML3 overexpression on CALML3, Caspase-3, Caspase-9 and Cyt-c expression in H_2O_2-induced HLEB-3 cells (**Figure 6A** and **6B**).

Discussion

The pro-apoptotic effect of Ca^{2+} is modulated by a variety of factors that are sensitive to calcium, and Ca^{2+} chronically elevated and alterations in its homeostasis initiate the mitochon-

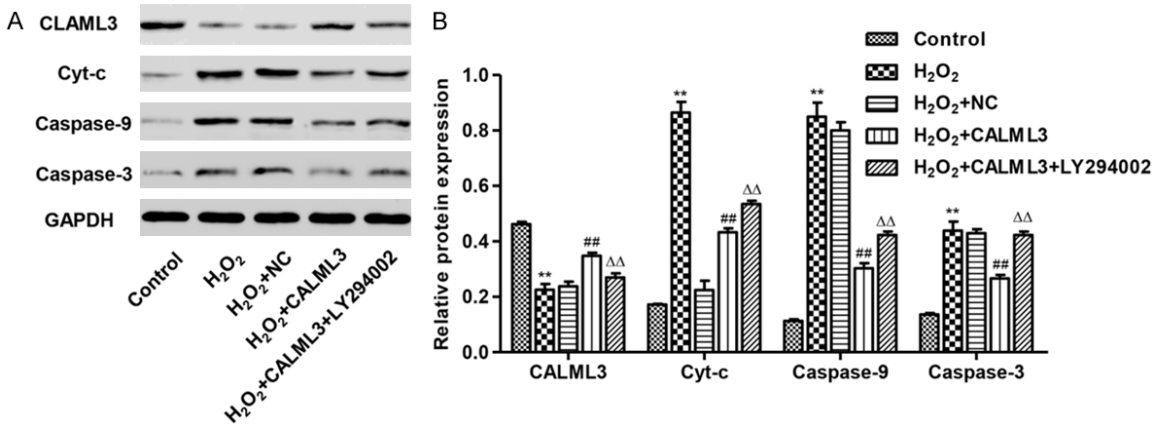

Figure 6. CALML3 overexpression inhibited H_2O_2-induced Cyt-c, Caspase-9 and Caspase-3 expression in HLEB-3 cells, which was reversed by LY294002 treatment. A, B After transfected with CALML3 expressing vector prior to incubated with H_2O_2 (300 μM), the expression of Caspase-3, Caspase-9 and Cyt-c expression in HLEB-3 cells was measured by western blot assay. $^{**}P<0.01$ compared with control. $^{\#\#}P<0.01$ compared with H_2O_2. $^{\Delta\Delta}P<0.01$ compared with H_2O_2+CALML3.

drial apoptotic pathway [20]. This study was designed to determine the effects of the H_2O_2 and the epithelial-specific calcium-sensing protein CALML3 on mitochondrial apoptosis in the human lens epithelial (HLE) cell line HLEB-3, which is a satisfactory cellular model for studying cataract *in vitro*. Our findings showed that H_2O_2 evoked apoptosis through the increase of ROS and Ca^{2+} in HLEB-3 cells, inducing activation of Caspase-3 and Caspase-9 and Cyt-c release, which is associated with inactivation PI3K/Akt signaling pathway.

Using H_2O_2 (a main ingredient of ROS) *in vitro* to induce cell oxidative damage model is commonly used [21, 22]. H_2O_2 was therefore applied in this study to induce oxidative damage model which mimicked the characters of cataract pathogenesis *in vitro*, so as to explore the protection effects of CALML3 involved and its probable mechanisms. There are increasing evidences that apoptosis of lens epithelial cells which induced by oxidative stress is of great importance in cataract formation, and inhibition of apoptosis has important implications in the treatment of cataract. In this study, H_2O_2 treatment in HLEB-3 cells significantly inhibited cell viability, induced cell apoptosis and ROS generation in a dose-dependent manner as well as increased Ca^{2+} level. Maintaining the dynamic balance of calcium is essential for the transparency of the lens and a lot of mechanisms are involved, including Ca^{2+} ATPase, Ca^{2+} channels, Ca^{2+}-binding proteins and Na^+/Ca^{2+} exchange [23]. The adverse effects of oxidative stress induced by ROS production are largely caused a large accumulation of intracellular Ca^{2+}, resulting in impairment of mitochondrial function [20]. In addition, apoptosis caused by the upregulation of intracellular Ca^{2+} is associated with protein degradation, which activates Ca^{2+}-dependent enzymes that mediate apoptosis including lipases and proteases. In the present study, overexpression of Calmodulin-like-protein CALML3, an epithelial-specific calcium-sensing protein, significantly inhibited cell apoptosis, ROS generation and increased Ca^{2+} level induced by H_2O_2 in HLEB-3 cells. H_2O_2 evokes increased Ca^{2+} in the absence of extracellular calcium, indicating that H_2O_2 mobilizes calcium from intracellular stores leading cells into an apoptotic state [24].

In the present study, H_2O_2 dose-dependently induced HLEB-3 cell apoptosis through releasing Cyt-c from the mitochondrial intermembrane space into the cytoplasm and activation of Caspases-3 and Caspases-9. Among Caspases, Caspases-3 is the most common executor of apoptosis in response to multifarious stimulation including H_2O_2 [25, 26]. Caspase-9 plays an important role in mitochondria-initiated intrinsic apoptosis pathway. In response to apoptotic stimuli, the permeability of mitochondrion is abnormal and following results in the release of Cyt-c from mitochondrial intermembrane space [27], which induces Caspase-9 activation and subsequently in turn promotes

cellular self-destruction through activating a cascade of Caspase such as Caspase-3 [28]. It is not completely clear for the mechanism of H_2O_2-induced endothelial Caspase activation, which may be due to a direct effect of oxidative stress or is regulated by mitochondrial apoptosis mechanisms, and CALML3 overexpression may inhibit the mechanism.

To gain further insight into the mechanisms by which CALML3 regulates apoptosis induced by H_2O_2, the role of PI3K/Akt signaling pathway was evaluated in the present study. In the H_2O_2 excitation process, PI3K/Akt activation seems to regulate different cellular responses [25]. Activation of PI3K/Akt contributes to a series of events that promotes cell survival and prevents apoptosis, specifically in HLE cells, while increasing evidences suggest that inactivation of Akt induced by H_2O_2 was found in several cell types [29, 30]. Therefore, we focused on the activation of kinases such as PI3K/Akt. Our results showed that CALML3 overexpression markedly attenuated the decreased phosphorylated Akt in HLEB-3 cells induced by H_2O_2. In contrast, CALML3 overexpression-induced activation of Akt and decreased expression of Caspase-3, Caspase-9 and Cyt-c was completely corrected by treatmentof LY294002, a PI3K-inhibitor, suggesting an important role of PI3K/Akt activation in the protective effect of CALML3 overexpression against H_2O_2-induced apoptosis, which may through inhibiting activation of Caspase-9 and Caspase-3 [31, 32]. Taken together, our findings conclusively indicate that CALML3 overexpression inhibits HLEB-3 cell apoptosis induced by H_2O_2 by activating the PI3K/Akt signaling pathway.

In conclusion, our data showed that CALML3 overexpression contributes to an obvious inhibition of cytotoxicity and apoptosis after H_2O_2 treated, suggesting a protective effect of CALML3 upregulation against cataractogenesis. The underlying mechanism by which CALML3 overexpression protect against apoptosis could be related to the reduction of ROS production, Ca^{2+} overload and expression of Caspase-3, Caspase-9 and Cyt-c through activating PI3K/Akt pathway. Furthermore, our study provides the clear clue that CALML3 may act as a target for treatment of cataract.

Disclosure of conflict of interest

None.

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