Cellular and Molecular Biology

Fu *et al. Cell. Mol. Biol.*2016, 62 (7): 36-41
ISSN: 1165-158X
doi: 10.14715/cmb/2016.62.7.7

Original Research

The role of apoptosis inducing factor in the apoptosis of retinal pigment epithelium cells induced by oxidative stress

Y. Fu[#], M. Tang[#], L. Bai, Y. Fan[*]

Department of Ophthalmology, Shanghai general Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai, China

Abstract: To explore the role of apoptosis inducing factor (AIF) in the apoptosis of retinal pigment epithelium (RPE) cells induced by oxidative stress**.** RPE cell apoptotic models were constructed by H_2O_2, Annexin V/PI flow cytometry and MTT assay were used to determine the changes of RPE cell apoptosis and proliferation. The production of reactive oxygen species (ROS) and mitochondrial membrane potential were observed. QRT-PCR and western blot were used to determine the expression of AIF to evaluate whether AIF-mediated non caspase pathway participated in the RPE cell apoptosis induced by H_2O_2. After adding melatonin, the production of ROS, RPE apoptotic rate and the changes of AIF expression were also detected. The production of ROS and apoptotic rate of RPE cells increased with the increase of H_2O_2 concentration and treating time. The expression of AIF increased evidently in cytoplasm, while not in mitochondria. After intervention with melatonin, the production of ROS was inhibited and the apoptotic rate decreased. However, the expression of AIF was not inhibited correspondingly. AIF may participate in but not be dominant in the process of RPE cell apoptosis induced by oxidative stress.

Key words: Apoptosis inducing factor, **r**etinal pigment epithelium, mitochondria, melatonin, apoptosis.

Introduction

Age-related macular degeneration (AMD) is a progressive degenerative eye disease that causes painless loss of central vision in those aged 55 years-old or over (1). It is characterized by the appearance of drusen in the macula, accompanied by choroidal neovascularization (CNV) or geographic atrophy (2). It is a disease normally associated with the elderly, and is therefore a growing problem worldwide. The retinal pigment epithelium (RPE) maintains the choriocapillaris in the normal eye and is involved in the pathogenesis of CNV in age-related macular degeneration (3, 4). RPE arises from neuroectoderm and plays a key role in support of photoreceptor functions (5). RPE cells possess the potential to trans-differentiate into myofibroblasts after stimulation with transforming growth factor beta (TGF-β) and are implicated in the pathogenesis of proliferative vitreoretinopathy (6). The production of extracellular matrix material (ECM) by RPE may influence or mediate some of the many important functions of this tissue (7). ECM *in vitro* which was located between the basal surface of the RPE and the culture plate (8). Abnormal ECM caused the changes of capillary behavior in choroid membranes of RPE and ultimately resulted in the atrophy of retina and RPE, and the growth of CNV (9).

High levels of apoptosis in RPE cells was observed in oxidative stress and blue light damage models. Apoptosis is a type of cell death whose morphological appearance relies on the activation of caspase-family cysteine proteases. Mitochondria play a major role in apoptosis triggered by many stimuli (10). High level overexpression of the anti-apoptotic protein Bcl-2 prevented Bax redistribution to the mitochondria, caspase activation and apoptosis following exposure to staurosporine or etoposide (11). Recent studies found a pathway, apoptosis inducing factor (AIF), which is independent of caspase pathway. AIF is a phylogenetically ancient mitochondrial intermembrane flavoprotein endowed with the unique capacity to induce caspase-independent peripheral chromatin condensation and large-scale DNA fragmentation (12). It is ubiquitously expressed, both in normal tissues and in a variety of cancer cell lines. Studies showed AIF played a key role in the apoptosis of a variety of retinal cells (13). However, the role of AIF-mediated caspase-independent pathway in the apoptosis of RPE cells induced by reactive oxygen species (ROS) is still unknown. Therefore, further study on AIF is necessary.

Melatonin, produced by the pineal gland at night, has a role in regulation of the sleep-wake cycle (14). Recent studies showed that melatonin counteracted mitochondrial oxidative stress and increased the activity of the mitochondrial oxidative phosphorylation (OXPHOS) enzymes both *in vivo* and *in vitro* (15). Here, melatonin was used as a key antioxidant to inhibit oxidative damage and apoptosis. In our study, we aimed to explore the role of AIF in the apoptosis of RPE cells.

Materials and Methods

Cell culture

Human retinal pigment epithelial (RPE) cell line CRL2302 (Rock- ville, MD, USA), which was storaged

Received January 13, 2016; **Accepted** May 30, 2016; **Published** June 30, 2016

*** Corresponding author:** Ying Fan, Department of Ophthalmology, Shanghai general Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai, Haining Road 100, Hongkou District, Shanghai, China, 200080. Email: xunxu111@sina.com

[#] The two authors contributed equally.

Table 1. Primes in the study.

	Nucleotide sequences	Length
AIF	F: 5'-ATAGACTCAGATTTTGGTGGCTTCC-3' R: 5'-CCAGTCATATTTTCTCCAGCCAATC-3'	450 bp
GAPDH	F: 5'-ACCACAGTCCATGCCATCAC-3' R: 5'-TCCACCACCCTGTTGCTGTA-3'	173 bp

in -80°C liquid nitrogen, was recovered, inoculated, cultured and digested to obtain RPE single cell suspension. The cells were routinely cultured in DMEM/F_{12} supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified cell incubator with an atmosphere of 5% CO_2 at 37°C. Cells growing at an exponential rate were used for the follow-up experiments.

Cell proliferation detection

RPE cells were seeded in 96-well culture plates with 2.5×10^4 cells every hole (100 μL) and cultured for 72 hours. Cells were treated with DMEM/F_{12} medium with 10% fetal bovine serum, serum-free DMEM/F_{12} medium, serum-free DMEM/F_{12} medium with various concentrations of H_2O_2 (0, 500, 1000 or 1500 μM) for 12, 24 or 48 hours, respectively. Then cells were added 20 μL MTT (5 mg/ml PBS stock solution) every hole and incubated for another 4 hours, the liquid culture was aspirated off and 150 μL dimethylsulfoxide (DMSO) every hole was added. The absorbances were recorded at 590 nm. Each experiment was repeated three separate times.

Mitochondrial membrane potential detection

RPE cells were treated with serum-free DMEM/F_{12} medium with various concentrations of H_2O_2 (0, 500, 1000 or 1500 μM) for 12, 24 or 48 hours, respectively. Cells were digested to obtain RPE single cell suspension and added rhodamine 123 ($10\mu g \cdot mL^{-1}$), incubated for another 30 minutes, then washed twice with PBS, and then examined by flow cytometry (Dako Cytomation, Glostrup, Denmark) assay.

Reactive oxygen species (ROS) detection

When RPE cells, initially seeded onto slides within culture dishes, reached 80% confluence, culture medium was removed, cells were washed twice with PBS and then cultured in serum-free DMEM/F_{12} medium with various concentrations of H_2O_2 (0, 500, 1000 or 1500 μM) and melatonin (0 or 100 μM, Sigma USA). After incubation for 2 hours, cells were washed twice with PBS, treated for 1 hour with 2 nM 2,7-dichlorodihydrofluorescein diacetate (H_2DCFDA, Fluka, St. Paul, MN, USA) and 500 nM MitoTracker Red CM-H_2XRos (MTR; Molecular Probes, Eugene, OR, USA), then washed twice with PBS, and examined by fluorescent microscope (AxioVert S100, Zeiss, Exposure time: 500ms).

Apoptosis detection

RPE cells were washed twice with PBS, and serum-free DMEM/F_{12} medium, containing H_2O_2 (0 or 1000 μM) and melatonin (0 or 100 μM) was added. The number of apoptotic cells was determined 24 or 48 hours posttreatment, using Annexin V / Propidium iodide (Molecular Probes, Eugene, OR, USA) flow cytometry assay.

AIF detection

When RPE cells reached 80% confluence, culture medium was removed, cells were washed twice with PBS and then cultured in serum-free DMEM/F_{12} medium with various concentrations of H_2O_2 (0, 500, 1000 or 1500 μM) . After incubation for another 9 hours, culture medium was removed, cells were washed twice with PBS again and PCR was performed to detect AIF mRNA. Primers used in the study were listed in Table 1.

RPE cells were cultured in serum-free DMEM/F_{12} medium with various concentrations of H_2O_2 (0, 500 or 1000 μM). After incubation for 3, 9 or 24 hours, cellular cytoplasm and mitochondria were separated according Bio Vision Mitochondria/Cytosol Fractionation Kit (Biovision, USA). Western-blot was performed to evaluate AIF protein, using monoclonal AIF antibody (Cell Signaling TECHNOLOGY, USA).

RPE cells were cultured in serum-free DMEM/F_{12} medium with various concentrations of H_2O_2 (0, 1000 μM) and melatonin (0 or 100 μM) for 3 or 9 hours, PCR was performed to detect AIF mRNA. Cellular cytoplasm and mitochondria were separated and Western-blot was performed to evaluate AIF protein.

Statistics analysis

Differences within groups in all assays were tested by ANOVA and Dunnett's *t*-test. *P* values less than 0.05 were considered statistically significant. The statistical analysis was implemented by SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). All experiments were repeated three times.

Results

Cell proliferation was inhibited by H_2O_2

The proliferation of RPE cells was determined by MTT assay. As Figure 1A showed, the A value decreased gradually with the increase of H_2O_2 concentration, and the inhibition of H_2O_2 on cell proliferation was enhanced over time under the same H_2O_2 concentration. Moreover, the results of ROS staining showed green fluorescence intensity, which represented ROS, was enhanced with the increase of H_2O_2 concentration (Figure 2). Most of green fluorescence and red fluorescence were coincide, which indicated mitochondria was the main part that producing ROS in cells.

Mitochondrial membrane potential changed by H_2O_2

The results of mitochondrial membrane potential detection showed the fluorescence intensity of rhodamine 123 had no obvious changes after treated with various concentrations of H_2O_2 (500, 1000 or 1500 μM) H_2O_2 for 12 or 24 hours, but it increased significantly after treated with H_2O_2 for 48 hours. (Figure 1B).

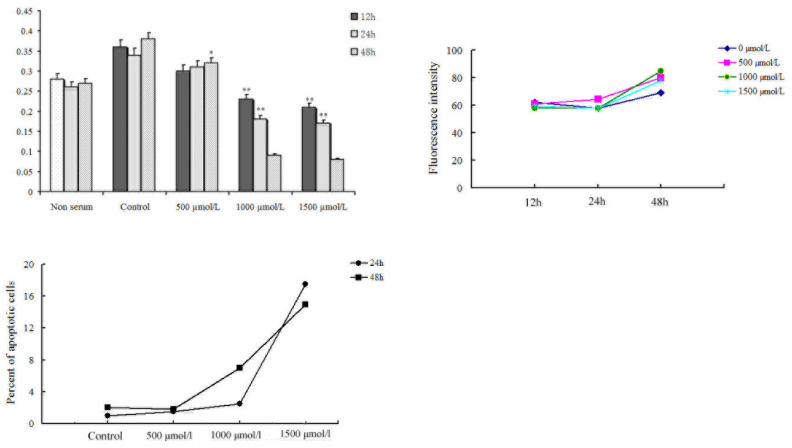

Figure 1. A. The proliferation of RPE cells after treating with DMEM/F12 culture solution containing 10% fetal bovine serum, DMEM/F12 without serum, DMEM/F12 culture solution containing 10% fetal bovine serum and 500 μmol/L, 1000 μmol/L and 1500μmol/L H_2O_2 for 12, 24 and 48h, respectively. * *P<0.05* and ***P<0.01*, compared with the control, the value in 500 μmol/L, 1000 μmol/L and 1500μmol/L H_2O_2 group had statistical differences. $^{\#}$ *P<0.05* and $^{\#\#}$*P<0.01,* compared with RPE cells treated for 12h and 24h in each group, the vaule in 48 had statistical differences. B. The fluorescence intensity of rhodamine 123 in RPE cells after treating with 500 μmol/L, 1000 μmol/L and 1500μmol/L H_2O_2 for 12, 24 and 48h. C: The apoptosis situation of RPE cells after treating with 500 μmol/L, 1000 μmol/L and 1500μmol/L H_2O_2 for 24 and 48h.

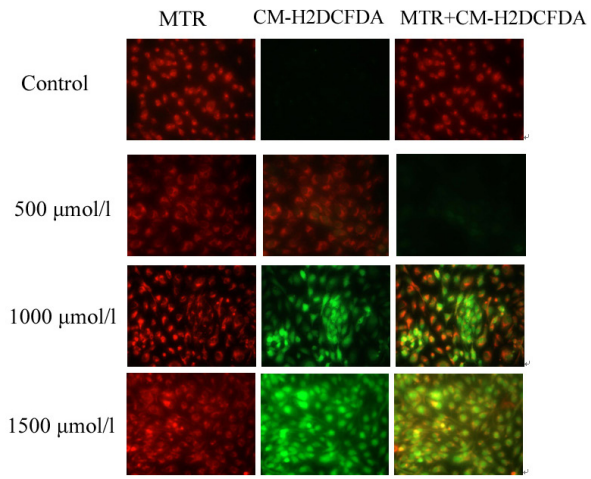

Figure 2. The changes of fluorescence intensity in the control, 500 μmol/L, 1000 μmol/L and 1500μmol/L H_2O_2 group by ROS staining.

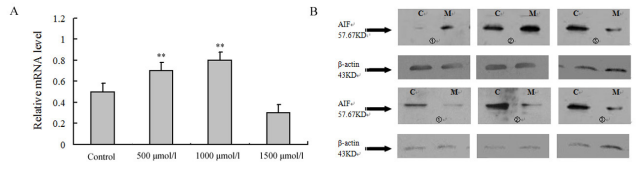

Figure 3. A. The mRNA level of AIF in RPE cells after treating with 0, 500, 1000 and 1500 μmol/L H_2O_2 for 24h. ***P<0.01*, compared with the control, the relative mRNA level of AIF in 500 and 1000 μmol/L group had statistical differences. B. The protein expression of AIF in RPE cells after treating with 0, 500, 1000 and 1500 μmol/L H_2O_2 for 3h, 9h and 24h.

Melatonin reduced ROS

100 μM melatonin could evidently inhibit the production of ROS in RPE cells induced by 500, 1000 and 1500 μM H_2O_2. It indicated melatonin could reduce oxidative stress induced by H_2O_2 in the models (Figure 4).

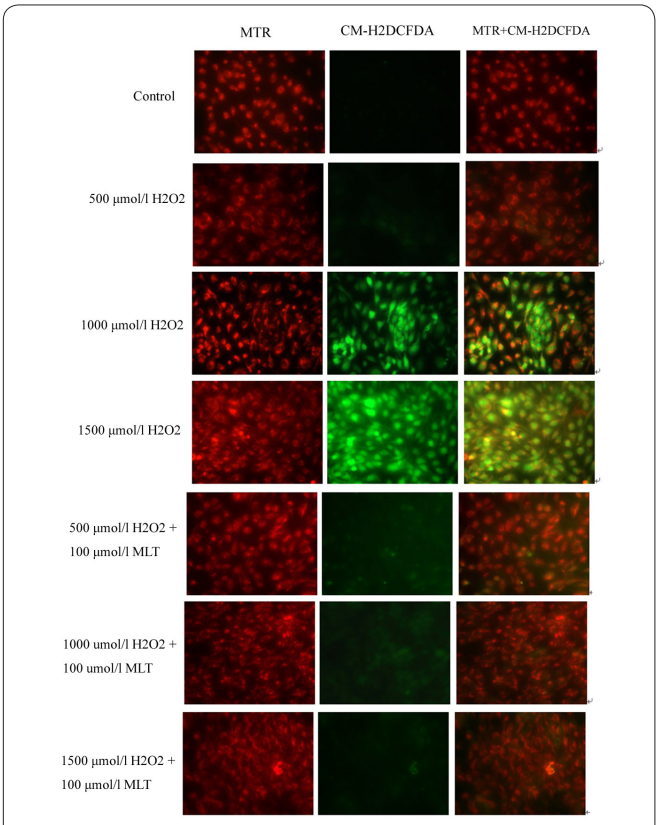

Figure 4. The production of ROS in RPE cells after treating with 500μmol/L H_2O_2, 1000μmol/L H_2O_2 a, 1500 μmol/L H_2O_2, 500 μmol/L H_2O_2 + 100μmol/L melatonin, 1000 μmol/L H_2O_2 + 100μmol/L melatonin and 1500 μmol/L H_2O_2 + 100μmol/L melatonin for 24h, respectively.

Melatonin reduced RPE cell apoptosis

The RPE cell apoptotic rate increased with treating time increased. The apoptotic rate in 1000 μM H_2O_2 and 100 μM melatonin group was significantly lower than that in 1000 μM H_2O_2 group (p<0.05, Figure 5A).

H_2O_2 influenced the level of AIF

The level of AIF-mRNA in 500 μM H_2O_2 and 1000

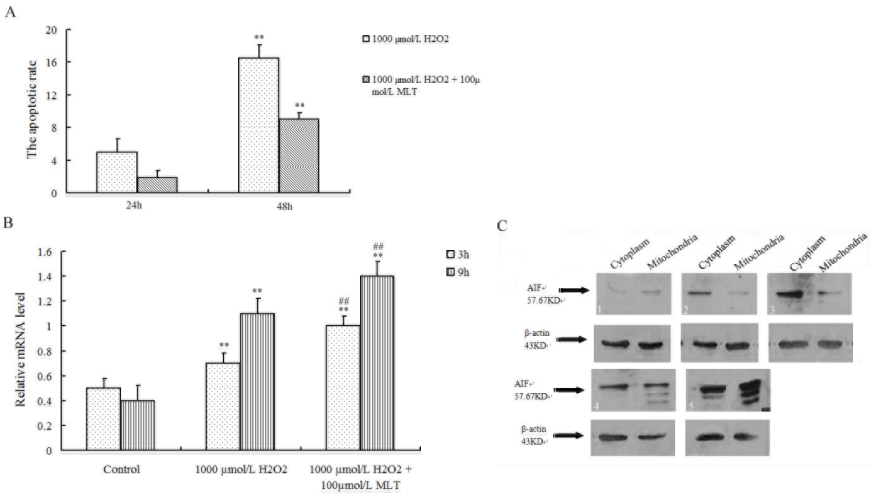

Figure 5. A. The apoptotic rate of RPE cells after treating 1000 μmol/L H_2O_2 and 1000 μmol/L H_2O_2 + 100μmol/L melatonin for 24h and 48h by flow cytometry. $^{**}P<0.01$, compared with 24h in 000 μmol/L H_2O_2 and 1000 μmol/L H_2O_2 + 100μmol/L melatonin group, the value in 48 had statistical differences. B. The relative mRNA level of AIF in the control for 3h and 9h, 1000 μmol/L H_2O_2 for 3h and 9h, and 1000 μmol/L H_2O_2 + 100μmol/L melatonin for 3h and 9h. $^{**}P<0.01$, compared with the control for 3h and 9h, the value in 1000 μmol/L H_2O_2 for 3h and 9h, and 1000 μmol/L H_2O_2 + 100μmol/L melatonin for 3h and 9h had statistical differences. $^{\#\#}P < 0.01$, compared with 1000 μmol/L H_2O_2 for 3h and 9h, the value in 1000 μmol/L H_2O_2 + 100μmol/L melatonin for 3h and 9h had statistical differences. C. The protein expression of AIF in the control, 1000 μmol/L H_2O_2 for 3h and 9h, and 1000 μmol/L H_2O_2 + 100μmol/L melatonin for 3h and 9h. 1: the control; 2: 1000 μmol/L H_2O_2 for 3h; 3: 1000 μmol/L H_2O_2 for 9h; 4: 1000 μmol/L H_2O_2 + 100μmol/L melatonin for 3h; 5: 1000 μmol/L H_2O_2 + 100μmol/L melatonin for 9h.

μM H_2O_2 group was significantly higher than that in the control. The level in 1500 μM H_2O_2 group was the lowest (Figure 3A). The protein expression of AIF in the control was low and focused on the mitochondria. The expression of AIF in cytoplasm increased when H_2O_2 concentration increased from 500 μM to 1000 μM, while the expression value in mitochondria increased in 500 μM H_2O_2 group and decreased in 1000 μM H_2O_2 group. Moreover, the expression value of AIF in cytoplasm increased with the increase of treating time, while the value in mitochondria had no obvious changes (Figure 3B).

Melatonin influenced the level of AIF

The mRNA level of AIF in 1000 μM H_2O_2 + 100 μM melatonin group for 3 hours and 1000 μM H_2O_2 + 100 μM melatonin group for 9 hours group were significantly higher than that in 1000 μM H_2O_2 group for 3 hours and 1000 μM H_2O_2 group for 9 hours, respectively. Moreover, the level increased when the time increased from 3 to 9 hours (Figure 5B). The protein expression of AIF after treating with 100 μM melatonin was much higher than that in the related control group. At early stage, the level of AIF increased mainly in cytoplasm. The expression of AIF increased both in cytoplasm and mitochondria with treating time of 9 hours (Figure 5C).

Discussion

Oxidative damage and inflammation are postulated to be involved in age-related macular degeneration (AMD) (16,17). The free radical theory proposes that ageing is the cumulative result of oxidative damage to the cells and tissues of the body that arises primarily as a result of aerobic metabolism (18). RPE cells were located between photoreceptor cell layer and choroid blood capillary layer. It could produce high concentration of oxygen free radicals and therefore the oxidative damage of RPE cells was appeared firstly in a lot of eye diseases. Oxidative stress is believed to contribute to the pathogenesis of many diseases, including AMD (19). Therefore, we selected H_2O_2 oxidative stress to induce RPE apoptosis. Mitochondria were rich in RPE cells and the changes of mitochondria function played a key role in metabolism of RPE cells. Chronic administration of MPTP is associated with evidence of apoptotic cell death in the substantia nigra (20). Rh123 is a cell-permeable dye that localizes to the mitochondrial lumen when the inner membrane is hyperpolarized (21). Rh123, which can bind specifically to mitochondria, has been used in numerous investigations to estimate MMP with some modifications (22). Our study showed Rh123 fluorescence intensity increased with the increase of H_2O_2 concentration. The number of necrotic cells were too much in 1500 μM H_2O_2 group which influenced the results and the value was lower than that in 1000 μM H_2O_2 group. Annexin V/PI double dye combining with flow cytometry showed the percent of apoptotic cells increased with the increase of treating time by H_2O_2. Those indicated H_2O_2 could effectively cause oxidative damage in RPE cells and resulted in the apoptosis of RPE cells. The production of ROS in mitochondria increased greatly under the induction of oxidation revulsant H_2O_2 and resulted in the apoptosis of RPE cells.

AIF is a mitochondrial flavoprotein, which translocates to the nucleus during apoptosis and causes chromatin condensation and large scale DNA fragmentation (23). Our results showed the levels of AIF in 500 μM H_2O_2 and 1000 μM H_2O_2 groups were markedly higher than the control, but the level in 1500 μM H_2O_2 group was low. It might be high RPE cell death rate due to high concentration of H_2O_2 decreased the synthesis of AIF. We found when RPE cell apoptosis was induced by H_2O_2, the synthesis of AIF precursor protein in cytoplasm increased and then located in mitochondria becoming mature molecular, after that, the mature AIF mole-

cular entered into cytoplasm again from mitochondria. From the results of AIF, we concluded the changes of AIF expression in RPE apoptotic process induced by H_2O_2 participated in the apoptosis of RPE apoptosis.

Melatonin, or N-acetyl-5-methoxytryptamine, is a compound derived from tryptophan that is found in all organisms from unicells to vertebrates (24). melatonin can inhibit the release of mitochondria cytochrome C and show protective effect on caspase pathway apoptosis in mitochondria (25,26). The protection of melatonin on apoptotic RPE models induced by H_2O_2 had been proved in our study and it could evidently reduce the apoptosis of RPE cells. However, the expression of AIF in mitochondria and cytoplasm increased. It indicated melatonin had no obvious inhibition on AIF. This reflected AIF might be not dominating in the RPE cells apoptosis induced by oxidative stress. The mechanism about melatonin influencing the expression of AIF and the effect on RPE cell apoptosis need further research.

In conclusion, the apoptosis in RPE cells induced by H_2O_2 contained both Caspase pathway(27) and AIF-mediated non-Caspase pathway. Melatonin can obviously decrease Caspase pathway apoptosis(26,27) and RPE cell apoptosis as a whole, but showed no obvious effect on the production and release of AIF. Therefore, AIF-mediated non-Caspase pathway may participate in but not be dominant in the process of RPE cell apoptosis in oxidative stress.

Acknowledgments

This study was surpported by the Subject of Shanghai Municipal Health Bureau of China (20114118, 20124094).

References

1. Hill D. Age-related macular degeneration. Innovait, 2015, 8.
2. La CM, Kiilgaard JF, Nissen MH. Age-related macular degeneration: epidemiology and optimal treatment. Drugs & Aging, 2002, 19(2):101-33.
3. Boulton M, Dayhaw-Barker P. The role of the retinal pigment epithelium: topographical variation and ageing changes. Molecular Pharmacology, 2001, 15(Pt 3):96-105.
4. Algvere PV, Berglin L, Gouras P, & Sheng Y. Transplantation of fetal retinal pigment epithelium in age-related macular degeneration with subfoveal neovascularization. Albrecht Von Graæes Archiv Für Ophthalmologie, 1994, 232(12):707-16.
5. Klimanskaya I. Retinal pigment epithelium. Methods in enzymology, 2006. 418: p. 169-194.
6. Gamulescu MA, Chen Y, He S, Spee C, Jin M, & Ryan SJ, et al. Transforming growth factor β 2 -induced myofibroblastic differentiation of human retinal pigment epithelial cells: Regulation by extracellular matrix proteins and hepatocyte growth factor. Experimental Eye Research, 2006, 83(1):212-22.
7. Campochiaro PA, Jerdon JA, Glaser BM. The extracellular matrix of human retinal pigment epithelial cells in vivo and its synthesis in vitro. Investigative Ophthalmology & Visual Science, 1986, 27(11):1615-21.
8. Li W, Stramm LE, Aguirre GD & Rockey JH. Extracellular matrix production by cat retinal pigment epithelium in vitro: Characterization of type IV collagen synthesis. Experimental Eye Research, 1984, 38(3):291-304.
9. Zarbin MA. Current concepts in the pathogenesis of age-related macular degeneration. Archives of ophthalmology, 2004. 122(4): p. 598-614.
10. Desagher S, Martinou JC. Mitochondria as the central control point of apoptosis.. Trends in Cell Biology, 2000, 10(10):369-77.
11. AMurphy KM, Ranganathan V, Farnsworth ML, Kavallaris M, & Lock RB. Bcl-2 inhibits Bax translocation from cytosol to mitochondria during drug-induced apoptosis of human tumor cells. Cell Death & Differentiation, 2000, 7(1):102-11.
12. Candé C, Cohen I, Daugas E, Ravagnan L, Larochette N, & Zamzami N, et al. Apoptosis-inducing factor (AIF): a novel caspase-independent death effector released from mitochondria. Biochimie, 2002, 84(2–3):215-222.
13. Hisatomi T, Sakamoto T, Murata T, Yamanaka I, Oshima Y, Hata Y, Ishibashi T, Inomata H, Susin SA, Kroemer G. Relocalization of apoptosis-inducing factor in photoreceptor apoptosis induced by retinal detachment in vivo. American Journal of Pathology, 2001, 158(4):1271-8.
14. Garfinkel D, Laudon M, Nof D, & Zisapel N. Improvement of sleep quality in elderly people by controlled-release melatonin. Lancet, 1995, 346(8974):541-4.
15. MartíN M, MacíAs M, León J, Escames G, Khaldy H & Acuña-Castroviejo D. Melatonin increases the activity of the oxidative phosphorylation enzymes and the production of ATP in rat brain and liver mitochondria. International Journal of Biochemistry & Cell Biology, 2002, 34(4):348-57.
16. Hollyfield JG, Bonilha VL, Rayborn ME, Yang X, Shadrach KG & Lu L, et al. Oxidative damage-induced inflammation initiates age-related macular degeneration. Nature Medicine, 2008, 14(2):194-198.
17. Hanus J, Anderson C, Wang S. RPE necroptosis in response to oxidative stress and in AMD. Ageing Research Reviews, 2015, 24:286-298.
18. Wickens AP. *Ageing and the free radical theory.* Respiration physiology, 2001. 128(3): p. 379-391.
19. Liang FQ, Godley BF. Oxidative stress-induced mitochondrial DNA damage in human retinal pigment epithelial cells: a possible mechanism for RPE aging and age-related macular degeneration. Experimental Eye Research, 2003, 76(4):397-403.
20. Mochizuki H, Hayakawa H, Migita M, Shibata M, Tanaka R & Suzuki A, et al. An AAV-derived Apaf-1 dominant negative inhibitor prevents MPTP toxicity as antiapoptotic gene therapy for Parkinson's disease. Proceedings of the National Academy of Sciences, 2001, 98(19):10918-23.
21. Verstreken P, Ly CV, Venken K & Koh TW. Synaptic mitochondria are critical for mobilization of reserve pool vesicles at Drosophila neuromuscular junctions. Neuron, 2005, 47(3):365-78.
22. Yan C, Huang D, Zhang Y. The involvement of ROS overproduction and mitochondrial dysfunction in PBDE-47-induced apoptosis on Jurkat cells. Experimental & Toxicologic Pathology Official Journal of the Gesellschaft Für Toxikologische Pathologie, 2011, 63(5):413-7.
23. Miramar M, Costantini PL, Saraiva L, Haouzi D, Brothers G & Penninger J, et al. NADH oxidase activity of mitochondrial apoptosis-inducing factor. Journal of Biological Chemistry, 2001, 276(19):16391-8.
24. Yang D, Elner SZ, Till G, Petty H & Elner V. Pro-inflammatory cytokines increase reactive oxygen species through mitochondria and NADPH oxidase in cultured RPE cells. Experimental Eye Research, 2007, 85(4):462-472.
25. Fontán L, Melnick A. Molecular Pathways: Targeting MALT1 paracaspase activity in lymphoma. Clinical Cancer Research An Official Journal of the American Association for Cancer Research, 2013, 19(24):6662-8.
26. Rosen RB, Hu DN, Chen M, Mccormick SA, Walsh J & Roberts AJE. Effects of melatonin and its receptor antagonist on retinal pig-

ment epithelial cells against hydrogen peroxide damage. Molecular Vision, 2012, 18(18):1640-8.
27. Fu Y, Tang M, Fan Y, Zou H, Sun X & Xu X. Anti-apoptotic effects of melatonin in retinal pigment epithelial cells. Frontiers in Bioscience, 2012, 17(17):1461-8.