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Research Paper

The migration of human lens epithelial cells induced by UV-irradiation *in vitro*☆

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Abstract

Objective: Ultraviolet (UV) radiation is one of the important cataract risk factors. However, the pathogenesis is still poorly understood. The migration of human lens epithelial cells(HLECs) plays a crucial role in the remodeling of lens capsule and cataract formation. The purpose of this study is to investigate the mechanism of UV inducing cataractogenesis. Methods:The toxicity of UV-irradiation on HLECs was assessed by Methyl thiazolyl tetrazolium(MTT) assay. The activity of matrix metalloproteinase-2(MMP-2) was observed by Gelatin zymography. The migration of HLECs was examined by Cell Track Motility. Results:UV-irradiation does great harm to HLECs, and may induce apoptosis in the cells when UV higher than 15 mj/cm^2. UV significantly increased MMP-2 activity in a time-dependent manner. In addition, the irradiation could induce the migration of HLECs. Conclusion:UV-irradiation could induce the migration of HLECs by increasing the activity of MMP-2.

Key words: UV, HLECs, migration, MMP-2

INTRODUCTION

Ultraviolet(UV) radiation from the sun, welding arcs, germicidal lamps and other sources, causes several health problems such as photokeratitis, photoconjunctivitis, pterygium, cataract, erythema, skin aging and skin cancer[1-2]. Unlike other diseases, one can predict that the lens can suffer damage even at very low levels of irradiation, as the lens is regarded as an accumulating site of oxidative insults from radiant energy, including photo-oxidation by UV. The fact that UV radiation can result in the lens opacification is supported by many researchers and some medical research laboratories[3-4]. However, the mechanisms that how UV causes damage to the ocular lens are not fully understood.

Recently studies show that the abnormal proliferation and migration of lens epithelial cells(LECs) at the equator and underneath the anterior lens capsule is induced by several risk factors, like alcohol, fat, diabetes and UV light, leading to the development of cataracts[5-7]. Repopulation of the posterior capsule by LECs migration can lead to capsule opacification(accompanied with capsule wrinkling, and contraction) and matrix production which can result in significant visual impairment. Matrix metalloproteinases(MMPs) are a family of zinc-dependent endopeptidases that act as key regulators of tissue remodeling[8] and play an important part in the development of many ocular diseases, including retinal disease, glaucoma, and corneal disorders[9]. MMPs and tissue inhibitors of matrix metalloproteinases have also been examined in both the normal and cataractous lens. And the dysregulation of MMP activity plays a very important role in cataract formation[10-12]. However, the role of MMPs and the mechanism by which they contribute to the cataractogenesis are still as yet not well understood. Our recerchers recently showed that UV

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could up-regulate the expression of MMP-1 which may be correlative with cortex cataract[13]. While, in current studies we have been focusing our study on MMP-2 because the main compositions for basal lamina of LECs are collagen type IV and laminin, which are known as the substrates for MMP-2 that may have be related to anterior subcapsular cataract[14]. The aim of this study was to investigate the regulation of MMP-2 by UV in the development of subcapsular cataracts caused by the migration of LECs.

MATERIALS AND METHODS

UV light apparatus

Consistent with previous studies[15], the UV irradiation apparatus used in this study consisted of four F36T12 UV tubes. A Kodacel TA401/407 filter was mounted 4 cm in front of the tubes to remove wavelengths≤ 290 nm(UVC). Irradiation intensity was monitored using an IL443 phototherapy radiometer and a SED240/UV/W photodetector. Before UV irradiation, cells were washed with PBS and then changed to 0.5 ml PBS in each well. Cells were irradiated at a desired intensity without a plastic dish lid screened. After UV irradiation, cells were put back to incubation in basal DMEM with treatments at various time points prior to harvesting.

Cell culture

Human lens epithelial cells(cell line) were obtained from Prof. YanShen Wan of Providence University, USA. Cells were maintained in Dulbecco's modified Eagles's medium(DMEM, Gibco Life Technologies) supplemented with 15% fetal bovine serum(Hyclone), penicillin/streptomycin(1∶100, Sigma) and 4mM L-glutamine and 0.19%HEPES, in a humid atmosphere incubator with 5% CO_2 at 37℃. Cells were reseeded in 6-well plates at a density of 0.2×10^6 cells/ml with fresh complete culture medium. Prior to treatment, cultures were grown to 70%~80% confluence and given another serum-starved condition overnight in DMEM medium, prior to treatment.

MTT assay of cell viability

MTT assays are based on the ability of viable cells to convert MTT, a soluble tetrazolium salt(thioazyl blue) into an insoluble formazan precipitate, which is quantitated by spectrophotometry following solubilization in dimethyl sulfoxide[16]. Briefly, subconfluent proliferating cells in 96-well dishes were treated with different a radiation intensity, and then postincubated for 48 h in fresh, culture medium. At this time, the cells(1×10^4 cells/ml) were solubilized and absorbance readings were taken using a Dynatech 96-well spectrophotometer(Billingshurst, UK). The amount of MTT dye reduction was calculated based on the difference between absorbance at 492 nm. Cell viability was expressed as the amount of dye reduction relative to that of the untreated control cells. 4 replicated wells were tested per assay condition, and each experiment was repeated at least 3 times.

Gelatin zymography

Zymography was performed by using a technique previously reported.[17] Briefly, cell culture media were centrifuged at 10,000g for 10 min at 4℃. Supernatants were diluted with zymogram sample buffer(Bio-Rad) without boiling in nonreducing conditions. Gels were running at 90V at 4℃ until the bromo-phenol blue reached the bottom of the plates. After electrophoresis, the gels were washed twice with 2.5%Triton X-100 (Sigma) in Tris solution containing 50 mmol/L Tris-HCl, 10 mmol/L $CaCl_2\cdot H_2O$,50 mmol/L NaCl, 1.36 mg/L $ZnCl_2$, pH 7.6) for 30 min to remove SDS from the gel. Gels were incubated overnight at 37℃ in zymogram incubation buffer containing 1.0% Triton X-100 in the Tris-HCl solution above, stained(Coomassie blue R-250; Bio-Rad) for at least 3 h and destained with destain solution(containing 80% methanol and 20% acetic acid). Enzymatic activity was identified as clear zones in a blue-stained background.

Cell track motility

As previously described[18], One milliliter of a 1×10^4 cells/ml suspension in 10%FBS-DMEM was added to each well of a 12-well tissue culture plate and cultured for 48 hours, and then coated with 10 mg/L mitomycin in order to inhibit the cell proliferation, at 37℃ for 2 h. A straight-edge razor blade was used to denude two 10 mm × 5 mm areas in each well of cells. After exposure to different radiation intensities, the cultures were washed 2 times with PBS to remove floating cells. The vehicle group had DMEM added, the control group had DMEM added with 10% FBS as with the radiation group. After the treatment, cells were cultured at 37℃ and 24 hours later migration of cells into a denuded area of the cultures was quantitated.

Statistical analysis

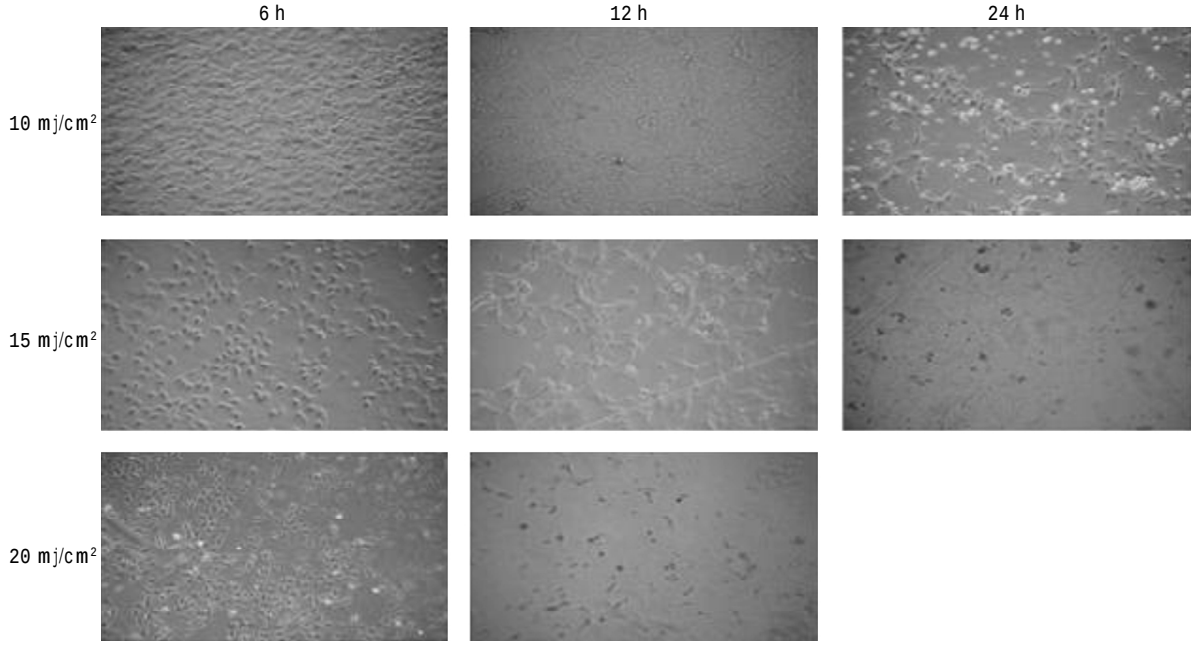
The values in the figures are expressed as the mean ± standard deviation(SD). In the experiments involving UV-induced cell migration and MMP-2 activity, the figure shown is the representative of the three different experiments. Statistical analysis of the data between the control and treated groups was performed by a Student t test. Values of $P < 0.05$ were considered as significant.

RESULTS

The UV attenuates the cell viability

In order to make sure of the proper UV irradiation, we chose 10 mj/cm², 15 mj/cm², 20 mj/cm². Firstly, we observed the morphous of cells when they were

exposured in UV. When cultures grew to 70%~80% confluence, the cells were serum-starved overnight in DMEM medium prior to treatment. And when the irridation was more than 15 mj/cm^2, the cells became more emaciated at 6h. After 12h some cells floated, whereas for most of cells we found apoptosis at 24 h. The morphous changed greatly, and the cells became slim and weak, while still surviving at 15mj/cm^2(Fig 1).



The morphous changes of HLECs after being exposed with different irradiation levels of UV. The cells viability had been decreased overwhelmingly, when they were exposed in UV higher than 15 mj/cm^2.

Fig 1 The morphous changes of HLECs after exposed in different irradiation of UV

The MTT also confirmed that when the irradiation was higher than 15 mj/cm^2, apoptosis would be found in the cells(Tab 1). So we chose the max irradiation 15 mj/cm^2 as our following experiment concentration.

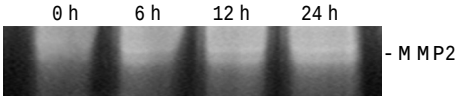
Tab 1 UV inhibited cell vitality detected by MTT assay

Time / Irradiation	6 h	12 h	24 h
control	0.426 ± 0.069	0.418 ± 0.023	0.436 ± 0.065
UVB 10(mj/cm^2)	0.431 ± 0.084	0.414 ± 0.068	0.363 ± 0.059
UVB 15(mj/cm^2)	0.400 ± 0.017	0.398 ± 0.042	0.378 ± 0.048
UVB 20(mj/cm^2)	0.344 ± 0.044	0.293 ± 0.023*	0.253 ± 0.033*

Compared with the control, *$P < 0.05$.

The activity of MMP-2 induced by UV in a time-dependent manner

As we know, there is a balance between the activity of MMP-2 and free TIMPs(the tissue inhibitors of matrix metalloproteinases) in normal conditions. Some factors, (especially UV) could break up the balance to make cataractogenesis progress. So to investigate the role played by UV on MMP-2 activity, we used gelatin zymography. The results revealed a significant increase of MMP-2 activity in UV-treated cells, in a time-dependent manner. The activity of MMP-2 in control is very low, as there is a balance. While, after being exposed in 15 mj/cm^2 for 6 h, the activation of MMP-2 had a rapid increase. And when cultured for 12 or 24 h, the activation of MMP-2 increased more(the result was shown in Fig 2).

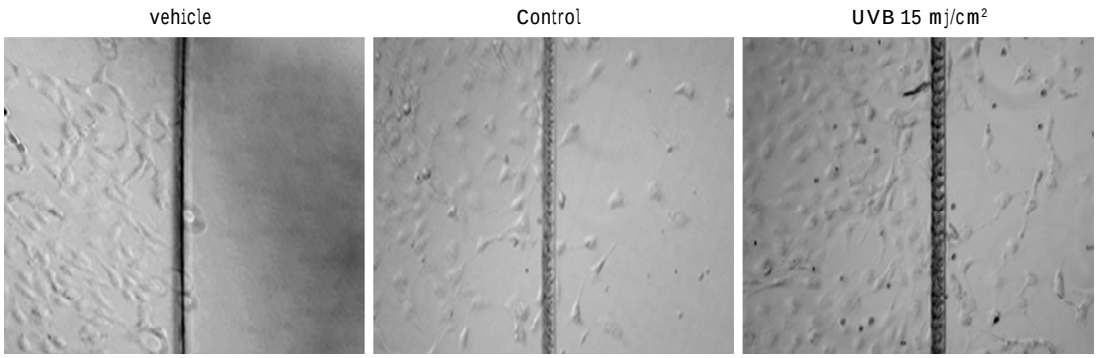


UV activated MMP-2 in a time-dependent manner. There is almost no expression of MMP-2 in control lane. With cultured time, MMP-2 activity increased significantly when exposed in UV.

Fig 2 MMP-2 activities was inspired by UV

UV induced the migration of HLECs

It's well known that the abnormal proliferation and migration of HLECs can cause subcapsular cataract (especially posterior capsule opacification, PCO). Our studies found that UV could induce the migration of HLECs. As shown following(Fig 3), we cultured the cells in 60mm plates. When the cells were 70~80% confluences, the proliferation was inhibited by mytomycin. And then three groups were performed as per the methods section. The results suggested that there was no migration of HLECs in vehicle group(cultured with DMEM only). As we know, there are many growth factors in FBS, so in the control group(cultured with



Vehile group :cultured with DMEM only. Control group: cultured with 10% FBS in DMEM. UVB 15 mj/cm² group: after exposed in UV and then cultured with 10%FBS in DMEM.

Fig 3 UV induced the migration of HLECs.

10% FBS in DMEM) some cells migrated. While, after being exposed to UV and then cultured with 10%FBS in DMEM, there were more cells found.

DISCUSSION

Cataracts, a major cause of blindness worldwide, are the clinical result of light-scattering by the lens. Epidemic investigations have shown that UV-irradiation plays a role in the development of this disorder. However, the mechanism that causes migration of HLECs and plays a crucial role in the development of the pathogenesis of cataract[19] is still not yet fully understood. The subcapsular cataract(especially the secondary cataract) following cataract surgery, is mainly caused by the activity of residual lens epithelial that has migrated and proliferated[20,21]. While, in our previous studies, we found that the abnormal migration and proliferation is at least associated with growth factors like epithelial growth factor(EGF)[22]. In previous studies we firstly discovered that UV could also induce the migration of HLECs leading to the presenting cataract. In our study, we inhibited the proliferation of HLECs firstly, and then to discover the migration ability of cells under different conditions. Only HLECs which cultured with DMEM kept static. Under the effect of growing factors in the serum, the cells were activated to move. A great deal of cells migrated under UV-irradiation. The present discovery suggested that the action spectrum derived by UV may help develop the hazard function for cataract formation.

So how did UV make the HLECs migrate? Currently, increasing evidence regarding the importance of MMPs in development and pathology has accumulated over the last 10 years. More recently, inducation of MMPs expression has been correlated with the formation of cataracts. When MMPs activity is increased with insufficient quenching activity by TIMPs, progressive degradation of ECM components occurs, thus leading to HLECs migration. These can be divided into collagenases, stromelysins, gelatinases and membrane type MMPs(MT-MMPs) which are expressed by various tissues and cell types. Every type could degrade one component of ECM. MMP activity is controlled at multiple points. Previous studies have localized MMPs and growth factors in cataractous and normal lenses[23,24], however, little attempt was made to observe the effect of UVB exposure on MMPs in a lens epithelial cell line. Nitin H. **et al**[25] revealed specific localization of MMP-1 within lens epithelium and cortical lens fibers of cortical cataract. Our previous studies also confirmed that UV induced the overexpression of MMP-1 through activating the MAPK signaling way[13]. While, as the LECs is composed primarily of collagen type IV and laminin, which are known as the substrates for MMP-2 and MMP-9, that may be correlated to anterior subcapsular cataract. Our results showed that UV could induce MMP-2 overexpression in a concentration-dependent manner, but interestingly, MMP-9 was not changed(data not shown). MMP-2 may play the leading role in the cells migration.

Nowdays, it's known that UV radiation is the production of reactive oxygen species(ROS), including hydrogen peroxide, which are proven to be deleterious to DNA, lipids and proteins in the lens, probably leading to cataract[26]. And our previous studies showed that UV-induced Dsg-2(which was highly organized intercellular junctions that provide mechanical integrity to tissues by anchoring intermediate filaments to sites of strong adhesion) down-regulation is mediated by ROS[27]. And several studies suggest that ROS plays a major role in the activation of MMPs[28,29]. So we proposed that UV-induced MMP-2 overexpression could be mediated by ROS. And this may constitute potential therapeutic targets in the treatment of subcapsular cataract.

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