Article

Effect of geranylgeranylacetone on ultraviolet radiation type B-induced cataract in heat shock transcription factor 1 heterozygous mouse

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Abstract

Purpose:We investigated whether heat shock transcription factor 1 (HSF1) was involved in ultraviolet radiation type B (UVR-B)-induced lens opacity (cataract) using HSF1 heterozygous mice. We also examined the effects of geranylgeranylacetone (GGA), an inducer of heat shock proteins via activation of HSF, on the UVR-B-induced cataract.

Material and Methods:Male $HSF1^{+/-}$ and WT mice were unilaterally exposed to UVR-B (total: 1200mJ) at 16 weeks of age. At 48 h after the last UVR-B irradiation, the lens was isolated and the induction of the cataract was quantified as the cataract area ratio (opacity area/anterior capsule). GGA was orally administered at a dosage of 500 mg/kg once a day for two days before the first UVR-B exposure until the end of the experiment (21days in total).

Results:The HSF1 expression was more greatly decreased in the lens from $HSF1^{+/-}$ mice than in that from WT mice ($p<0.01$). UVR-B exposure could mainly induce cataracts in the anterior capsule in both $HSF1^{+/-}$ and WT mice, while the opacity of the lens was markedly enhanced in $HSF^{1+/-}$ mice compared to that in WT mice（$p<0.01$). GGA treatment could prevent the induction of lens opacity by UVR-B exposure in both WT and $HSF1^{+/-}$ mice as

compared with the non-administration group ($p<0.01$). No obvious alteration by the UVR-B radiation was seen in lens protein levels of αA-crystallin, αB-crystallin, or γ-crystallin with or without GGA administration among all groups of mice. In contrast to the crystallins, the lens protein level of HSP25 was decreased by UVR-B exposure in both $HSF1^{+/-}$ and WT mice, and was significantly recovered in WT mice by the GGA treatment ($p<0.01$). The induction of HSP25 was suppressed in $HSF1^{+/-}$ mice compared with that in WT mice.

Conclusions:These data suggest that HSF1 plays an important role in the occurrence of UVR-B-induced cataracts, possibly via regulation of HSPs such as HSP25.

Keywords

Cataract, heat shock protein transcription factor 1, heat shock proteins, geranylgeranylacetone, ultraviolet radiation type B

Introduction

Cataracts are one of the most common eye diseases and are a major cause of legal blindness in the world.[1] Surgical removal of the opacified lens is the only effective treatment since the underlying molecular mechanisms of cataracts are still unclear.

It is known that genetic factors or, smoking, and diabetes are known to play critical roles in the occurrence of cataracts.[2-7]

In all type of cells, various systems such as the heat shock response in response to external stimulation or stress are present. Heat shock transcription factors (HSFs) are known as regulators mediating heat shock responses.[8] Under heat shock or other stress conditions, these regulators can bind to the heat shock elements (HSEs) in the promoter region of heat shock proteins (HSPs) to enhance their expression. The HSF family consists of four members (HSF1–4) in vertebrates, and HSF1, 2, and 4 are primarily expressed in the mammalian tissues and organs.[9] HSF4 is associated with the differentiation of lens fiber cells and is a causal gene of congenital cataracts, and severe cataracts have been naturally observed in HSF4$^{-/-}$ mouse.[10] HSF1 can regulate the expression of many HSPs via HSE-HSF interaction, and it is present in the lens.[10] Whereas HSF1 is thought to be

involved in the maintenance of lens transparency, no obvious enhancement of cataract occurrence as a phenotype was naturally seen in the HSF1$^{-/-}$ mouse.[10] It is well known that many type of cells, including lens epithelial cells, under certain stresses such as heat shock as well as UV irradiation can induce HSPs to prevent denaturation of their proteins brought on by the stress.[11-14] Since these stresses could lead to the cataract as a causal effect, we hypothesized that HSF1 may play a role in the onset of the cataract under stress conditions. Thus, we examined the influences of UVR-B irradiation on the HSF1$^{+/-}$ mouse eye and induced a cataract in order to analyze the role of HSF1 under stress. We also administered geranylgeranylacetone (GGA), an inducer of HSPs, to clarify whether a cataract induced by UVR-B was affected by the induction of HSPs. In this study, we observed a high severity of cataracts induced by UVR-B irradiation in the HSF1$^{+/-}$ mouse eye compared to those in wild type (WT) mice concomitant with a reduction in HSP25 protein levels in the lens. Furthermore, administration of GGA markedly prevented the onset of cataracts in the mice, in which a high induction of HSP25 was seen in the lens. Since HSP25, also known as HSPB1, is a member of the small HSP proteins and is known as a protein partner of αA- and αB-crystallin for maintaining lens transparency[15], the protein level of HSP25 may be critical

for UVR-B-induced cataracts.

2. Materials and Methods

2.1 Transgenic Mice

Mice that are heterozygous for the targeted mutation of HSF1 ($HSF1^{+/-}$) at a genetic background of 129Sv were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). The mice were back-crossed with a C57BL/6Cr Slc mouse more than ten times, and maintained on a C57BL/6Cr Slc genetic background (SLC, Shizuoka, Japan). Since it is known that mice that are homozygous for the targeted mutation of HSF1 ($HSF1^{-/-}$) exhibit widespread phenotypic effects including incomplete prenatal lethality, slowed growth, female infertility, and impaired antigen presentation on some genetic backgrounds such as a C57BL/6, [16,17] all experiments were performed using $HSF1^{+/-}$mice and WT mice as a control at 16 weeks of age at the beginning of the experiments. The $HSF1^{+/-}$ mice were identified by PCR analysis of genomic DNA isolated from tail tips. The animals were housed in microisolator cages in a pathogen-free barrier facility. All experimentation was performed under approved institutional guidelines.

2.2 Protocol for geranylgeranylacetone (GGA) treatment

GGA was purchased from Eisai, Inc. (Tokyo, Japan). For oral administration in mice, the GGA granules were suspended in 5% gum Arabic solution for the oral administration. GGA was orally administered at a dosage of 500 mg/kg once a day for two days before the first UVR-B exposure until the end of the experiment (21days in total).

2.3 UVR-B exposure

UVR-B in the 302 nm wavelength region was formed with a transilluminator (TFML-20; UVP, Upland, CA, USA). UVR-B intensity was 200 mW/cmcm measured with a radiometer (UV-340; Custom, Tokyo, Japan) as exposure in the corneal plane. The transilluminator was covered with aluminum foil, except for a 5-mm hole. Each mouse was manually held (without anesthesia), such that the right eye was irradiated through the hole. Five minutes before UVR-B exposure, 0.5% tropicamide and 0.5% phenylephrine sulfate hydrate were introduced to both eyes to induce mydriasis. Prior to radiation, all animals were checked with a slit lamp to exclude pre-existing cataracts. Only one eye of each mouse was exposed in vivo to UVR-B for 100 s six times (twice a week for three weeks) for a latency period of 48

h from the last UVR-B exposure, based on data showing cataract progression after in vivo exposure to UVR-B, as described previously.[18]

2.4 Cataract morphology

The lens opacity was observed with a slit-lamp microscope (Kowa SL-15; Kowa, Nagoya, Japan). Immediately after lens extraction from the eyes, cataract morphology was recorded with a microscope photography system. Digital images of the anterior capsules were captured, and areas of opacities were measured using Image J software (National Institutes of Health, Bethesda, MD, USA). The ratio of the cataract area to the anterior capsule area was calculated as the cataract area ratio.

2.5 RNA preparation and Quantitative Real time RT-PCR

Total RNA from lens cells was prepared using Isogen reagent (Nippon Gene, Toyama, Japan). The cDNA were prepared from 1 μg of total RNA using Prime Script reverse transcriptase (Takara Bio, Kyoto, Japan), according to the manufacturer's instructions. PCR amplification was carried out using ExTaq polymerase (Takara Bio) at 30 cycles, each cycle

consisting of denaturation at 94°C for 0.3 min, annealing at 62～63°C for 0.3 min, and extension at 72°C for 1 min. The mRNA levels of HSF1 glyceraldehydes-3-phpsphate (GAPDH) were determined by real time reverse transcription-polymerase chain reaction (RT-PCR). Quantitative real time-PCR (RT-PCR) was performed using the KAPA SYBR® FAST Universal qPCR Kit (Kapa Biosystems, Wilmington, MA, USA) according to the manufacture's protocol. RT-PCR was preformed using HSF1 Primers (F: 5'AAGTACTTCAAGCACAACAA-3' and R: 5'- GAGATCAGGAACTGAATGAGC-3'), GAPDH (F: 5'-AGGTCATCCATGCAACTTG-4' and R: 3 '-TTCAGCTCTGGATGACCTT-5'), and HSP25 (F: 5'- ATCCGACAGACGGCTGATCGC-3' and R: 5'-GCACCGAGAGATGTAGCCAT-3'). Data were normalized against GAPDH expression and were expressed as fold difference between WT mice and $HSF1^{+/-}$ mice.

2.6 Western Blot Analysis

Western blot analysis was performed to determine the expression of αA-crystallin, αB-crystallin, γ-crystallin, and HSP25. Mice lenses were rinsed in ice-cold PBS, and total cellular protein extracts were prepared using lysis buffer A (10 mM HEPES, 10 mM KCL, 0.1

mM EGTA, 1 mM DTT, 0.6% IGEPAL, and 0.5 mM PMSF) containing protease inhibitors (protease inhibitor cocktail; Sigma, St.Louis, MO, USA). The protein concentration was determined by the Bradford assay (protein assay CBB solution, Nacalai Tesque). Equal amounts of total protein (αA-crystallin: 2 μg, αB-crystallin: 10 μg, γ-crystallin: 5 μg) were loaded onto 12–15% Bis-Tris gels (12% lower gel: 2.5ml lower Tris buffer, 4.0 ml 30% acrylamide/bis mixed solution, 3.5 ml distilled water, 10μL TEMED, 60μL 10%APS; 15% lower gel: 2.5 ml lower Tris buffer, 5.5 ml 30% acrylamide/bis mixed solution, 2.5 ml DDW, 10 μl TEMED, 60 μl 10% APS; upper gel: 2.5 ml upper Tris buffer, 2.0 ml 30% acrylamide/bis mixed solution, 5.5 ml distilled water, 20 μl TEMED, 120 μl 10% APS) separated by polyacrylamide gel electrophoresis, and transferred to a PVDF membrane (GE Healthcare). Membranes were then probed with primary antibodies: anti-αA-crystallin (1:1000; Stressgen, Victoria, Canada; cat. no. SPA-221), anti-αB-crystallin (1:2000; Stressgen; cat. no. SPA-223), anti-γ-crystallin (Santa Cruz Biotechnology, Santa Cruz, CA, USA; cat. no. sc-22746), anti-HSP25 (Santa Cruz Biotechnology; cat. no. sc-1048), and anti-GAPDH (Chemicon International, Temecula, CA, USA). Secondary anti-goat IgG horseradish peroxidase conjugated antibody (Santa Cruz Biotechnology; cat. no. sc-2020)

was used. Western blot analysis using an antibody against Alpha-A crystallin shows two major bands in the lens extract since alternative splicing in Alpha-A crystallin protein may occur, as described in previous work.[19,20] The relative protein expression was normalized to the values for GAPDH. All bands detected by Western blots were analyzed using freely available Image J software.

2.7 Statistical analysis

Data are expressed as the mean ± standard error. Statistical analysis was performed using the unpaired Student's t-test and one-way ANOVA followed by a post hoc comparison using Scheffe's protected least significant difference. Statistical tests were performed using Kaleida Graph version 4.1 software (Synergy Software, Reading, PA, USA).

3. Results

We measured the mRNA levels of HSF1 in the lenses from WT mice and $HSF1^{+/-}$ mice using RT-PCR. The expression levels of HSF1 were more greatly decreased in $HSF1^{+/-}$ mice than in WT mice ($p<0.01$; Fig 1). In Fig 2 we can see a typical slit-lamp picture of a lens from an

HSF1+/- mouse at 16 weeks, 48 hrs after the last irradiation, in which lenticular opacities were mainly observed at the anterior sub-capsular area. The cornea has slight opacity, but it seems that no obvious differences were seen due to UVR-B irradiation among the four groups were seen. Thus, we believe that a significant effect on lenticular opacity is unlikely due to corneal opacity. There were no lenticular opacities in either group of mice when not irradiated. In the $HSF1^{+/-}$ mice, the opacity area ratio was significantly higher than that in the WT mice ($p<0.01$) (Fig 3a, 3c, and 3e). These opacity area ratios were decreased in both the WT and $HSF1^{+/-}$ mice with GGA administration ($p<0.01$) (Fig 3b, 3d, and 3e).

We then determined lens protein levels of αA-crystallin, αB-crystallin, γ-crystallin, and HSP25 before UVR-B irradiation. There were no significant differences in these protein levels between $HSF1^{+/-}$ mice and WT mice. There were also no differences in the protein levels of αA-crystallin, αB-crystallin, or γ-crystallin in the lens after the UVR-B irradiation for the $HSF1^{+/-}$ mice and WT mice (Fig 4A and B). We also examined the effect of GGA treatment on the protein levels, such as those of αA-crystallin, αB-crystallin and γ-crystallin, in the lens after the UVR-B irradiation between $HSF1^{+/-}$ mice and WT mice (Fig 4C and D). There were no differences in these protein levels in the lens after the UVR-B irradiation

between HSF1$^{+/-}$ mice and WT mice with or without GGA treatment (Fig 4C and D). In contrast to those of the crystallins, the lens protein level of HSP25 was significantly decreased in both the HSF1$^{+/-}$ and WT mice after UVR-B irradiation ($p<0.01$) (Fig 5A and B). The lens protein level of HSP25 in HSF1$^{+/-}$ after UVR-B irradiation was significantly lower than that in WT mice after UVR-B irradiation ($p<0.05$) (Fig 5A and B). The protein level of HSP25 decreased by UVR-B irradiation was enhanced by GGA treatment in both the HSF1$^{+/-}$ and WT groups ($p<0.01$) (Fig 5C and D).

4.Discussion

In the present study, we examined the involvement of HSF1 in cataracts induced by UVR-B irradiation using HSF1$^{+/-}$ mice. The expression level of HSF1 was attenuated in the lens from HSF1$^{+/-}$ mice compared with that from WT mice. Furthermore, the severity of the lenticular opacity in response to UVR-B stress was markedly enhanced in HSF1$^{+/-}$ mice compared to in WT mice concomitant with a marked reduction in HSP25 level in the lens. It is known that knockout mice deficient for HSF1 develop essentially normally and prosper under non-stress conditions,[21] and no obvious enhancement of cataract occurrence as a

phenotype was naturally seen in the HSF1$^{-/-}$ mouse,[10] although HSF1 is thought to be involved in the maintenance of lens transparency. Thus, these results, along with our findings, indicate that HSF1 may be associated with the development of cataracts induced by certain stresses such as UV irradiation. Furthermore, our results suggest that heterozygousness for targeted mutation of HSF1 can reduce the level of HSF1 in the lens, and haploinsufficiency of HSF1 can deteriorate cataracts induced by UVR-B irradiation. We also observed the marked reduction in HSP25 levels in the lens after UVR-B irradiation from WT mice and HSF1$^{+/-}$ mice while the levels of crystallins such as αA-, αB- and γ-crystallin were unchanged. It is known that stress induction of HSP25 is dependent on HSF1 and that GGA, an HSF1 activator, can enhance its expression when certain stresses are induced.[22,23] Since GGA treatment led to the enhancement of reduced HSP25 in the lens in both WT and HSF1$^{+/-}$ mice, these results may imply that a reduction in HSP25 is critical for the occurrence of cataracts, and enhancement of HSP25 may play an important role in the effectiveness of GGA in UVR-B-induced cataracts.

HSP25, also known as HSPB1, belongs to the small molecule HSP family that is part of the relatively small protein group (15~30kDa) among stress proteins induced by heat shock.[24]

HSPB1 is ubiquitously present in cells,[24] and it is localized in all regions of the lens.[25] HSPB1 is known to have an anti-apoptotic effect.[26] In the UVR-B-induced cataract, the occurrence of apoptosis in lens epithelial cells is shown as a possible cause of the cataract.[27] Furthermore, the occurrence of apoptosis of human lens epithelial cells was inhibited by treatment with HSPB1 protein fused with protein transduction domain at the N-terminus.[28] Although the existence of apoptotic cell death in the lens epithelium is unclear in this study, the cellular protective effect of HSP25 may be associated with the beneficial effect of GGA on the cataract induced by UVR irradiation.

Although we performed the experiments using high dose UVR-B, lenticular opacity was only seen as a mild central anterior subcapsular cataract in the mice. Similar to a mild phenotype of cataracts, we observed few cases of corneal opacity in the UVR-B irradiated mice in this study. We used a transilluminator (TFML-20; UVP, Upland, CA, USA) for UVR-B irradiation (spectral peaks of 302 nm) in this study. It is known that the most harmful wavelength for the lens is located around 300 nm, which is UVR-B.[29] Although the spectral peaks of UV generated by the transilluminator are 302 nm, the manufacturer instructions suggest that UVR is a mixture of UVR-A (approximately 40~50%) and UVR-B

(approximately 50~60%) (UVP website, http://www.uvp.com/spectralcharts.html). Thus, the mild damage on the cornea as well as the lens induced by UV irradiation in our experiments may be explained by the relatively high amount of UVR-A.

In this study, we determined the levels of αA-crystallin, αB-crystallin, γ-crystallin, and HSP25 in the mouse lens after UVR-B irradiation. Since many HSPs are present in the lens,[25] they may possibly contribute to protect from lenticular opacity, and GGA may also induce these HSPs. For example, HSP70 is present in lens epithelial cells[25] and is known to be induced by GGA treatment.[30] It is reported that genetic polymorphism of HSP70 is associated with protection against aging-related cataract onset.[31] We also attempted to detect protein levels of HSP70 in the lens after UVR-B irradiation using several different, commercially available anti-HSP70 antibodies. However, no convincing results were obtained in our Western blot analysis. Further study will be necessary for the determination of HSP70 contribution.

The molecular mechanism(s) involved in the reduction in HSP25 by UVR-B irradiation are unclear. Bagchi et al[32] reported that the ability of HSP induction in response to heat stress is dependent on its replicative activities as well as on HSF1 activity. Marked reduction in

HSP25 was seen in the lens from HSF1$^{+/-}$ mice, in which lower HSF1 activity by haploinsufficiency of HSF1 is observed, compared to that in WT mice. Thus, the low level of HSP25 observed in this study may be due to HSF1 activity in the epithelium after UVR-B irradiation. When we determined the level of HSP25 in the lens from WT as well as HSF1$^{+/-}$ mice, we observed that the level varied among the samples extracted from different days, although each experimental condition (such as age of mice, experimental procedures like protein extraction as well as UV irradiation) was identical. Although the exact underlying mechanism is uncertain, we believe that the level of HSP25 in mouse lens may be unstable and sensitive to environmental factors. Further study is needed to clarify the mechanism(s) involved. One possible explanation of lower HSP25 levels in the lens from HSF1$^{+/-}$ mice is alteration of the localization of HSP25 from soluble fraction to insoluble fraction such as insoluble inclusion body. Since point mutation, phosphorylation, or cleaved fragments of other small HSPs changed their localization, UVR-B irradiation may induce post-translational modification in HSP25.[33,34]

Declaration of Interests

The authors declare no conflict of interest associated with this manuscript. The authors alone are responsible for the content and writing of the paper.

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Fig 1. The expression of HSF1 in $HSF1^{+/-}$ mice lens.

Values are expressed as the means ± SD for six $HSF1^{+/-}$ and three WT mice.

Fig 2. Cataract development was observed 48 hr after the last exposure to UVR-B. Lens opacity was observed mainly at the anterior sub-capsular with a slit-lamp microscope ($HSF1^{+/-}$ mouse at age 16 weeks).

Fig 3. Stereomicroscopic images of lens opacities were obtained 48 h after the last exposure to UVR-B.

(a) WT mice without GGA treatment (WT GGA[-]), (b) WT mice with GGA treatment (WT GGA[+]), (c) $HSF1^{+/-}$ mice without GGA treatment ($HSF1^{+/-}$ GGA[-]), and (d) $HSF1^{+/-}$ mice with GGA treatment ($HSF1^{+/-}$ GGA[+]). The opacity areas are surrounded by dashed lines, and the anterior capsule area is surrounded by a solid line. The cataract area ratios were 13.3±0.5% in the WT GGA(-) group (n=3), 3.7±0.5% in the WT GGA(+) group (n=3), 29.1±1.9% in the HSF1+/- GGA(-) group (n=3), and 9.2±2.7% in the HSF1+/- GGA(+) group (n=3).

Fig 4. Lens protein levels of αA-crystallin, αB-crystallin, and γ-crystallin after UVR-B irradiation (A and B), and effect of GGA treatment on lens protein levels of αA-crystallin,

αB-crystallin, and γ-crystallin in HSF1$^{+/-}$ and WT mice after UVR-B irradiation (C and D).

No differences were observed in lens protein levels for αA-crystallin, αB-crystallin, or γ-crystallin between HSF1$^{+/-}$ and WT mice after UVR-B irradiation (A and B) with or without GGA treatment (C and D).

Fig 5. Lens protein levels of HSP25 after UVR-B irradiation (A and B), and effect of GGA treatment on lens protein levels of HSP25 in HSF1$^{+/-}$ and WT mice after UVR-B irradiation (C and D).

In contrast to the crystallins, the lens protein level of HSP25 was significantly decreased in both HSF1$^{+/-}$ and WT mice after UVR-B irradiation ($p<0.01$) (A and B). The lens protein level of HSP25 in HSF1$^{+/-}$ after UVR-B irradiation was significantly lower than that in WT mice after UVR-B irradiation ($p<0.05$)(A and B). We compared the effect of GGA treatment on protein levels of HSP25 between HSF1$^{+/-}$ and WT mice after UVR-B irradiation(C and D). The protein level of HSP25 decreased by UVR-B irradiation were enhanced by GGA treatment in both the HSF1+/- and WT groups ($p<0.01$)(C and D).

Figure titles

Fig 1. The expression of HSF1 in $HSF1^{+/-}$ mice lens.

Fig 2. Cataract development was observed 48 hr after the last exposure to UVR-B.

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