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Salubrinal protects human skin fibroblasts against UVB-induced cell death by blocking endoplasmic reticulum (ER) stress and regulating calcium homeostasis

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The role of UVB in skin photo damages has been widely reported. Overexposure to UVB will induce severe DNA damages in epidermal cells and cause most cytotoxic symptoms. In the present study, we tested the potential activity of salubrinal, a selective inhibitor of Eukaryotic Initiation Factor 2 (eIF2) -alpha phosphatase, against UV-induced skin cell damages. We first exposed human fibroblasts to UVB radiation and evaluated the cytosolic Ca^{2+} level as well as the induction of ER stress. We found that UVB radiation induced the depletion of ER Ca^{2+} and increased the expression of ER stress marker including phosphorylated PERK, CHOP, and phosphorylated IRE1α. We then determined the effects of salubrinal in skin cell death induced by UVB radiation. We observed that cells pre-treated with salubrinal had a higher survival rate compared to cells treated with UVB alone. Pre-treatment with salubrinal successfully re-established the ER function and Ca^{2+} homeostasis. Our results suggest that salubrinal can be a potential therapeutic agents used in preventing photoaging and photo damages.

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1. Introduction

The prevalence of skin cancer is growing rapidly and has become an indisputable problem worldwide. In the United States, for instance, more than 3 million skin cancer cases are diagnosed every year. Among all the contributing factors in non-melanoma skin cancers, UV radiation is associated with more than 90% of these cases [1–3]. Individuals who are overexposed to ultraviolet (UV), particularly UVB, are at greater risk for solar erythema, edema, photoaging, and even skin cancer [4–6]. UVB (275-320 nm) is the most damaging component of UV irradiation and can generate excessive reactive oxygen species (ROS) which can mediate mitochondrial apoptosis in skin cells [7]. However, beside its involvement in ROS generation, UVB has also been reported to induce ER stress in HaCaT cells [8].

Endoplasmic reticulum (ER), a critical organelle, plays a central role in the proper folding and synthesis of proteins [9]. ER stress, which occurs when there is an accumulation of unfolded and misfolded proteins in the ER, can trigger the unfolded protein response (UPR) to restore Ca^{2+} homeostasis and redox balance [10]. In addition, Ca^{2+} signaling is also tightly linked to the cell fate in cell redox regulation [11,12].

Salubrinal, a small chemical agent, is known as a selective inhibitor of Eukaryotic Initiation Factor 2 (eIF2) -alpha phosphatase and plays a protective role from ER stress-induced cytotoxicity in a variety of cell types [13–15]. Nevertheless, little is known about its effects on UVB-induced cell damage in dermal fibroblasts, particularly in the context of UVB-induced ER stress and Ca^{2+} imbalance.

In our current study, we examined the protective effects of salubrinal on UVB irritated skin fibroblasts. Salubrinal is able to attenuate UVB-induced ER stress and protect human skin fibroblasts against UVB radiation-induced cell death in a dose dependent manner. Moreover. Salubrinal regulates the intracellular Ca^{2+} level in UVB irradiated human skin fibroblasts. Our results demonstrate a potential pharmacological role of salubrinal in the inhibition of UVB-induced cell damage in human dermal fibroblasts.

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2. Materials and methods

2.1. Chemicals and reagents

Salubrinal was purchased from Calbiochem (La Jolla, CA, USA). Anti-phospho-IRE1α (Ser 724) was purchased from Novus Biologicals (Littleton, Colorado, USA). All other phospho-antibodies and their non-phosphorylated control antibodies in this study were obtained from Cell Signaling Technology (Beverly, MA, USA).

2.2. Cell culture and UVB radiation

We used human foreskin fibroblasts BJ from American Type Culture collection (Manassas, VA, USA) in our experiments. Cells were maintained in Dulbecco's Modified Eagle medium (DMEM) with 10% FBS (Biomeda, Fostercity, CA, USA) and 1% penicillin in a humidified incubator with 5% CO_2 at 37 °C. All other cell culture regents were obtained from Gibco (Carlsbad, CA, USA). UVB radiation equipment and procedures were described in our previous publications [16–18].

2.3. Cell survival and cell death assays

MTT cell viability assay and cell death trypan blue staining assay were described in our previous publications [18–20].

2.4. TUNEL assay

TUNEL assay was performed with an in situ cell death detection kit (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instruction. Cells were TUNEL-stained, followed by counterstaining with DAPI. TUNEL positive cells were detected and counted with a confocal microscope.

2.5. Western blotting

Western blots were performed as previously described [17, 18] with slight modifications. Briefly, after different treatments, the whole cell lysates were prepared by sonication in CelLytic MT buffer (Sigma, St. Louis, MO, USA) and cleared by centrifugation. Samples consisting of 50ug proteins were resolved on a denaturing 8-12% SDS-PAGE gel, then transferred to polyvinylidene fluoride membranes by electrophoresis. The membrane was then blocked in PBST containing 5% dried milk at room temperature for 1 h, incubated with specific primary antibodies at 4 °C overnight. Blots were incubated with appropriate secondary antibodies at room temperature for 1 h the next day. The signals were detected by ECL reagent. Blots were analyzed by ImageJ software and normalized to corresponding loading controls.

2.6. Confocal imaging of intracellular Ca^{2+}

Changes in Ca^{2+} level were determined by the Ca^{2+} indicator Fluo-4/AM (Invitrogen) using confocal imaging. After the cell were seeded to sterile cover slips, BJ cells were loaded with fluorescent Ca^{2+} indicator dye Fluo-4/AM (Invitrogen) at 5 μM for 45 min prior to confocal imaging. Cells were washed three times with live cell imaging solution (Invitrogen) for confocal imaging using an Olympus Fluoview FV1000 microscope. Imaging was done by using excitation at 488 nm, emission at 515–565 nm and 40 × oil lenses. Twelve random microscopic fields were selected in each group. Fluo4 signal was quantified and analyzed by ImageJ software.

2.7. Statistical analysis

All data was expressed as the means ± SEM using unpaired Student's t-tests or two-way ANOVA with Bonferroni post-tests (GraphPad Software). The data represented at least three independent experiments and p values were calculated using GraphPad Prism software 7.0. A significant difference was considered at $p < 0.05$.

3. Results

3.1. Salubrinal protects human skin fibroblasts against UVB radiation-induced cell death in a dose dependent manner

We first examined BJ cell viability to determine whether there are any potential protective effects of salubrinal on UVB-induced skin cell damage. Human skin fibroblasts were pretreated with or without salubrinal for 1 h and then irradiated with UVB at different intensities (5, 10, 15, and 20 mJ/cm^2). The MTT assay revealed that UVB irradiation largely decreased skin cell survival, which was rescued by salubrinal pretreatment (Fig. 1A). To further determine what dose is effective against UVB radiation, skin cell viability in response to different doses of salubrinal was measured after UVB (15 mJ/cm^2) irradiation. Salubrinal demonstrated a protective effect in a dose dependent manner against UVB in BJ cells (Fig. 1B). A trypan blue staining assay demonstrated that salubrinal significantly decreases UVB-induced BJ cell death which is consistent with the previous data (Fig. 1D). TUNEL assay was also performed to verify the cell apoptosis. After the treatment of salubrinal (20μM), the number of TUNEL positive cells was significantly reduced compared to the control group in response to UVB (15 mJ/cm^2) irradiation (Fig. 1E). Taken together, these results suggest that salubrinal protects skin cells from UVB radiation.

3.2. UVB induces ER stress response in human skin fibroblasts

The aim of our study is to investigate the potential role of ER stress response in UVB induced skin cell death. First, we attempted to determine possible ER stress response in UVB-irradiated BJ cells by testing the expression of CHOP, cleaved caspase3, phosphorylated PERK (Thr 980), and phosphorylated IRE1α (Ser 724) by Western blotting. Our data suggests that UVB irradiation at 5 mJ/cm^2 leads to a significant ER stress in BJ cells. The expression level of ER stress markers including CHOP, cleaved caspase 3, and phosphorylated PERK/IRE1α, were significantly elevated compared to the untreated group (Fig. 2B). However, while UVB irradiation at low intensity (5 mJ/cm^2) does not cause induce cell death at the same level as high intensity irradiation (20 mJ/cm^2) by MTT assay, this low dose can still induce a significant ER stress response.

3.3. Salubrinal inhibits UVB radiation-induced elevation of ER stress

To determine the effect of salubrinal on UVB-induced ER stress in human skin fibroblasts, BJ cells were pre-treated with salubrinal (20μM) and exposed to UVB radiation (15 mJ/cm^2). The following western blot assay showed that the elevated expression levels of CHOP and phosphorylated PERK/IRE1α induced by UVB were significantly attenuated by pre-treatment of salubrinal (Fig. 3A and B). Our study clearly indicates that salubrinal protects skin fibroblasts from UVB-induced ER stress.

3.4. Salubrinal regulates the intracellular Ca^{2+} level in UVB irradiated human skin fibroblasts

To study the effect of salubrinal on intracellular Ca^{2+} level

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