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Interference of silibinin with IGF-1R signalling pathways protects human epidermoid carcinoma A431 cells from UVB-induced apoptosis

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ABSTRACT

Ultraviolet B (UVB) from sunlight is a major cause of cutaneous lesion. Silibinin, a traditional hepatic protectant, elicits protective effects against UVB-induced cellular damage. In A431 cells, the insulin-like growth factor-1 receptor (IGF-1R) was markedly up-regulated by UVB irradiation. The activation of the IGF-1R signalling pathways contributed to apoptosis of the cells rather than rescuing the cells from death. Up-regulated IGF-1R stimulated downstream mitogen-activated protein kinases (MAPKs), such as c-Jun N-terminal kinases (JNK) and extracellular signal-regulated protein kinases 1/2 (ERK1/2). The subsequent activation of caspase-8 and caspase-3 led to apoptosis. The activation of IGF-1R signalling pathways is the cause of A431 cell death. The pharmacological inhibitors and the small interfering RNA (siRNA) targeting IGF-1R suppressed the downstream activation of JNK/ERK-caspases to help the survival of the UVB-irradiated A431 cells. Indeed, silibinin treatment suppressed the IGF-1R-JNK/ERK pathways and thus protected the cells from UVB-induced apoptosis.

1. Introduction

Excess exposure to solar ultraviolet light (UV, 10–400 nm) is a key risk for skin injury [1]. UV irradiation, particularly UVB (280–320 nm), induces cellular DNA lesions and protein damages, resulting in cell death or premature ageing [1,2]. Potent substances have been searched for the protection from UVB damages. A natural flavonoid, silibinin (Fig. 1A), which has been clinically used for decades for treatment of the patients with liver diseases [3], has recently been shown to elicit striking anti-irradiative effects [4]. However, the precise mechanisms underlying the protective effects of silibinin against UVB irradiation remain poorly understood.

It was reported [5] that UVB irradiation elevated IGF-1R activity. IGF-1R, which is generally involved in the determination of cellular fate [6], is a member of the receptor tyrosine kinases (RTKs) that mediate the transduction of extracellular signals into the cytoplasm to elicit diverse cellular responses [7]. ERK1/2, JNK and p38 are the classic MAPKs that respond to the activation of RTKs [8]. The activation of ERK1/2, JNK and p38 in general contributes to cell proliferation [8]. However, under UVB stress, their activation frequently augments apoptosis [9–11]. In this study, the stimulation of IGF-1R-ERK/JNK signalling pathways was found to trigger the apoptosis of A431 cells under UVB stress. We found that in UVB-irradiated A431 cells silibinin saves the cells from apoptosis through inhibition of the IGF-1R activation followed by repression of the ERK1/2 and JNK phosphorylation.

2. Materials and methods

2.1. Cells and culture

The human epidermoid carcinoma A431 cell line, obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA), was cultured in Ham's F12 medium (Hyclone, Logan, UT, USA) supplemented with 10% foetal bovine serum (Beijing Yuanheng Shengma Research Institution of Biotechnology, Beijing, China), 100 μg/mL streptomycin and 100 U/ml penicillin, and was maintained at 37 °C with 5% CO_2 in a humidified atmosphere.

Abbreviations: UVB, ultraviolet B; IGF-1R, insulin-like growth factor-1 receptor; RTK, receptor tyrosine kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated protein kinase; JNK, c-Jun N-terminal kinase; ICAD, inhibitor of caspase-activated DNase; PARP, poly-ADP-ribose polymerase; FADD, Fas-associated death domain; siRNA, small interfering RNA.

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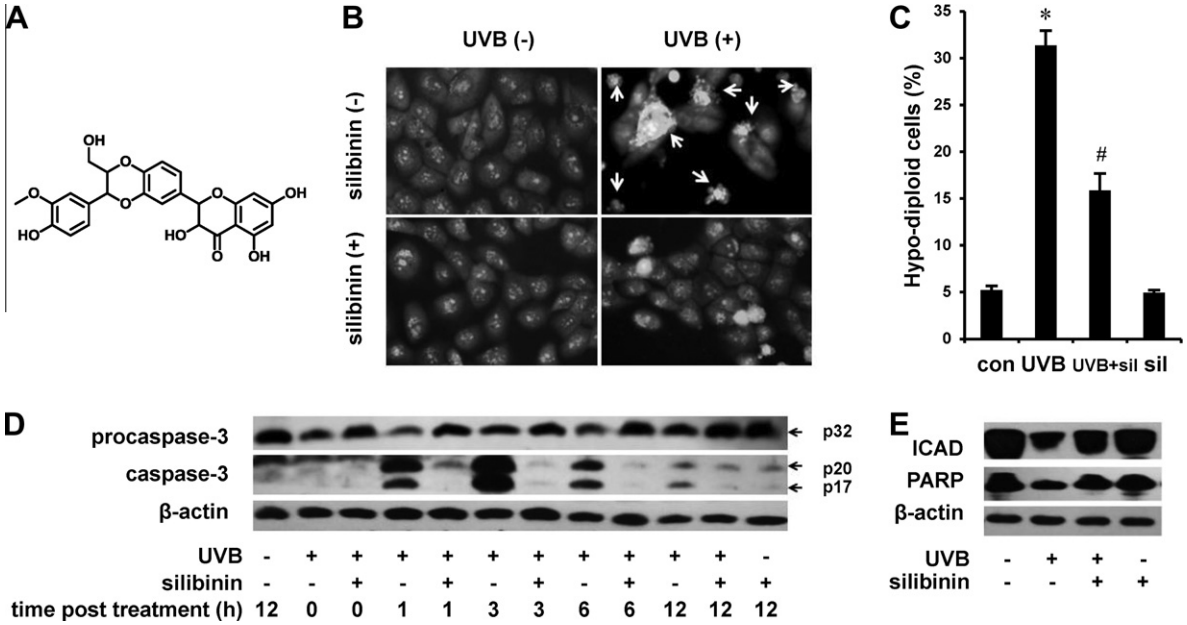

Fig. 1. Silibinin protects A431 cells from UVB-induced apoptosis. (A) The chemical structure of the natural flavonoid silibinin. (B) AO staining of representative A431 cells at 6 h (the time of UVB irradiation was defined as base-line) after each treatment. UVB, 90 J/m^2; silibinin, 125 μM. (C) Six hours after the indicated treatments, the cells were collected, fixed, stained with PI and analysed by flow cytometry. Percentages of cells with hypo-diploid DNA content were presented as mean values ± SEM. con, control; UVB, 90 J/m^2; sil, 125 μM silibinin; UVB + sil, cells treated with silibinin before and after UVB irradiation. (D) The cells after treatments were collected at the indicated time points and then analysed by western blotting for caspase-3 expression. E. The cells were harvested 6 h after each treatment, and the expression levels of the caspase-3 substrates, ICAD and PARP, was analysed.

2.2. UVB exposure

UVB lamps (220 V, 40 W) (Beijing Lighting Research Institute, Beijing, China), equipped with a UVB spectra radiometer (Photoelectric Instrument Factory of Beijing Normal University, Beijing, China), emitted UVB radiation ranging from 280 to 340 nm, with a peak at 314 nm. Silibinin treatment was performed 1 h prior to UVB exposure. When protein inhibitors were administered, they were added into the medium 1 h prior to the next step. To avoid possible UVB absorption by proteins, silibinin or other reagents in the culture medium, the medium was removed from the culture plates prior to irradiation and replaced simultaneously with fresh PBS. After UVB exposure, the medium that had been removed before irradiation was added back to the cell culture plates. The cells were harvested or analysed at 6 h after irradiation or at the indicated time points.

2.3. Reagents

Silibinin was obtained from the Beijing Institute of Biological Products (Beijing, China), and its purity was determined to be approximately 99% by HPLC measurement. The silibinin was dissolved in dimethylsulfoxide (DMSO) to make a stock solution and diluted with Ham's F12 in the experiments. The DMSO concentration in all cell cultures was kept below 0.1%, which elicited no detectable effects on cell growth.

Methylthiazolyldiphenyl-tetrazoliumbromide (MTT), propidium iodide (PI), RNase A, acridine orange (AO), tyrphostin AG1024, tyrphostin AG1478, PD98059, SP600125 and SB203580 were purchased from Sigma Chemical (St. Louis, MO, USA). Caspase-9 inhibitor III, caspase-8 inhibitor II and genistein were purchased from Merck (Darmstadt, Germany).

Primary antibodies against caspase-3, caspase-8, caspase-9, inhibitor of caspase-activated DNase (ICAD), poly-ADP-ribose polymerase (PARP), Fas-associated death domain (FADD), cytochrome *c*, EGFR, IGF-1R, growth factor receptor-bound protein 2 (GRB2), Son of Sevenless (SOS), phosphorylated c-Jun N-terminal kinases (p-JNK), phosphorylated extracellular signal-regulated protein kinases 1/2 (p-ERK), phosphorylated p38 (p-p38) and β-actin, as well as horseradish peroxidase-conjugated secondary antibodies, were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The Super Signal West Pico Chemiluminescent Substrate® used in conjunction with the horseradish peroxidase (HRP) enzyme was purchased from Thermo Scientific (Rockford, IL, USA).

2.4. Cell viability – MTT assay

Cells were seeded into 96-well cell culture clusters (Corning, NY, USA) at a density of 8×10^3 cells per well and cultured for 24 h. Next, the cells were subjected to the indicated treatments for 6 h. The cells were rinsed twice with ice-cold PBS and incubated with 100 μL of 0.5 mg/mL MTT solution at 37 °C for 3 h. The resulting supernatant was discarded. The residual cell layer was dissolved in 150 μL of DMSO, and the optical density was measured at a 490 nm wavelength using a microplate reader (Thermo Scientific Multiskan MK3, Shanghai, China). Cell viability was calculated using the following equation:

$$\text{Cell viability } (\%) = 100 \times (A_{490,\text{sample}} - A_{490,\text{blank}})/(A_{490,\text{control}} - A_{490,\text{blank}})$$

2.5. Fluorescent microscopy of nuclear morphologic changes

The nuclear morphology of the cells was assessed by staining cells with the fluorescent DNA-binding dye, AO. The cells were seeded into 24-well cell culture clusters (Corning, NY, USA) at a density of 2×10^4 cells per well. After culture for 24 h, the cells were subjected to the indicated treatments for 6 h, and then the cells were rinsed twice with ice-cold PBS, stained with AO (20 μg/mL) in the dark at 37 °C for 2 min, and imaged with a

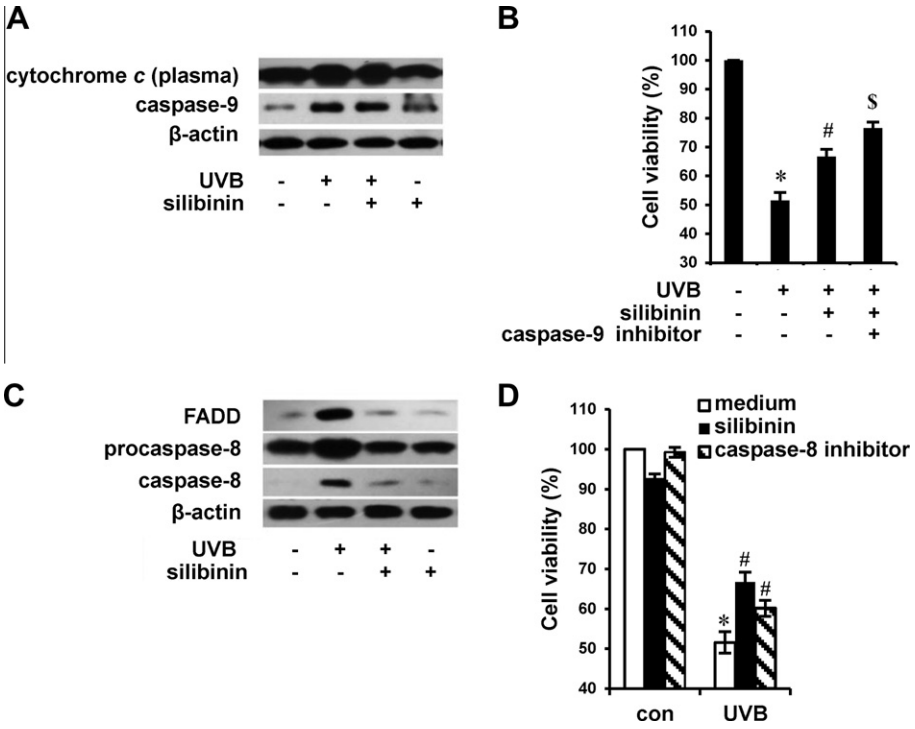

Fig. 2. Silibinin inhibits the activation of caspase-8 without affecting caspase-9 in UVB-irradiated A431 cells. (A) The effects of UVB irradiation and silibinin treatments on mitochondria-related apoptosis-associated proteins. (B) The additional inhibition of caspase-9 (3 μM caspase-9 inhibitor) enhanced the protective effects of silibinin against UVB-induced cell death. *Compared with control group, $P < 0.05$; #Compared with UVB treatment alone, $P < 0.05$; $Compared with UVB + silibinin treatment group, $P < 0.05$. (C) The effects of UVB irradiation and silibinin treatment on extrinsic-apoptotic pathway-associated proteins FADD and caspase-8. (D) The effect of caspase-8 inhibition (3 μM caspase-8 inhibitor) on cell viability. *Compared with control group, $P < 0.05$; #Compared with UVB treatment alone, $P < 0.05$.

fluorescence microscope (Olympus, Tokyo, Japan). The cells with condensed and fragmented nuclei were undergoing apoptosis.

2.6. Measurement of apoptosis by DNA fragmentation

PI is a fluorescent dye commonly used to quantify cellular DNA content. For the analysis of DNA fragmentation, cells subjected to the indicated treatments were collected (5×10^5 cells per sample), fixed with 70% (v/v) ethanol at 4 °C overnight, rinsed with ice-cold PBS twice and incubated with 1 mL of PI solution (PI 50 mg/L and RNase A 1 g/L) at 4 °C in the dark for 30 min. The samples were next analysed using a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Percentages of cells with hypo-diploid DNA content were used for the quantification of apoptotic cells [12].

2.7. Western blot

After the indicated treatments, both adherent and floating cells were collected at the indicated time points and lysed with RIPA lysis buffer (Beyotime, Haimen, Jiangsu, China) supplemented with PMSF (1 mM) for 30 min. Next, the cells were centrifuged at 12,000*g* for 10 min, and the protein content of the supernatants was determined using the Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA, USA). Equal amounts of the total protein lysates were separated using 10–13% SDS–polyacrylamide gels (SDS–PAGE) and transferred onto Millipore Immobilon-P Transfer Membrane® (Millipore Corporation, Billerica, MA, USA). The membranes were blocked with 5% skimmed milk at room temperature for 2 h, incubated first with appropriately diluted primary antibodies (blocking buffer dilution) at 4 °C overnight and then with corresponding HRP-conjugated secondary antibodies at room temperature for 2 h. The blots were visualised using the SuperSignal West Pico Chemiluminescent Substrate®.

2.8. Transfection of siRNA

Human IGF-1R-specific (si-IGF-1R) and negative control (si-con) siRNAs were purchased from Gene Pharma (Suzhou, China). Cells were transfected with 5 nM si-IGF-1R or si-con using 3 μg/mL siRNA-Mate™ (GenePharma, Suzhou, China) according to the manufacturer's protocols. The transfected cells were used for subsequent experiments 24 h later.

2.9. Statistical analysis

Comparisons between groups were determined using Student's *t*-test. All *P*-values were one-tailed and were considered significant when $P < 0.05$.

3. Results

3.1. In A431 cells, UVB induces apoptosis, which is markedly attenuated by treatment with silibinin

UVB irradiation (90 J/m^2) resulted in severe stress in A431 cells. AO staining of UVB-irradiated cells revealed cellular blebbing, shrinkage, nuclear fragmentation and condensation, which were characteristic features of apoptosis (Fig. 1B). Flow cytometric PI staining profiles showed that UVB irradiation significantly elevated percentages of cells with hypo-diploid DNA content (from 5.21 ± 0.44% to 31.36 ± 1.60%, $P < 0.05$) (Fig. 1C). At the protein level, apoptosis is characterised by the sequential activation of caspase cascades. Caspases are synthesised as inactive pro-enzymes comprising an N-terminal peptide (pro-domain) together with one large and one small subunit [13]. Among the caspases, caspase-3 plays frequently a role of death executor, catalysing the specific cleavage of many key cellular proteins such as ICAD and PARP to evoke apoptotic cell death. Caspase-3 is synthesised as

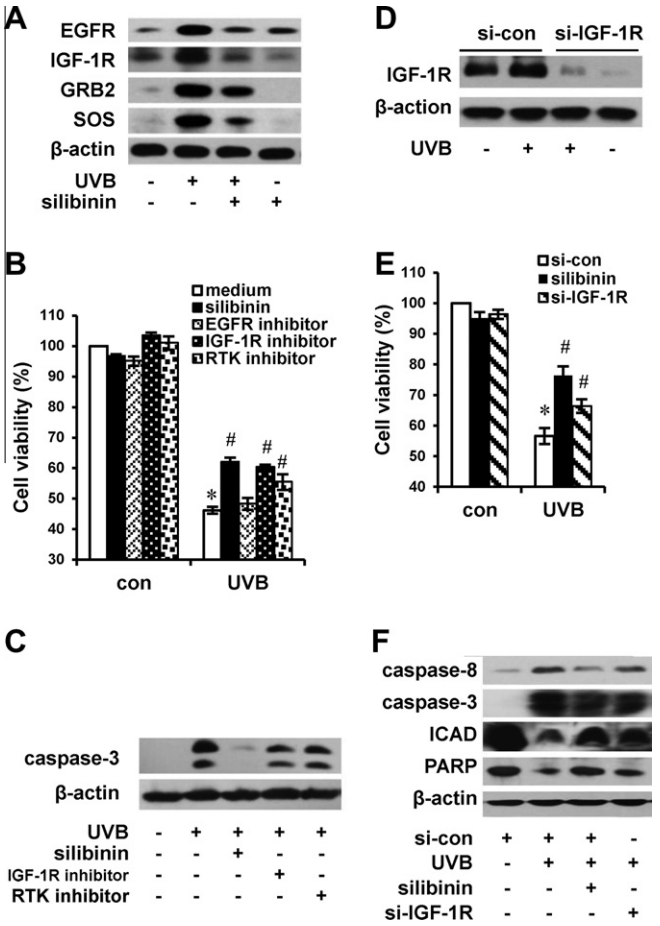

Fig. 3. Silibinin down-regulates IGF-1R signalling pathway to reduce cell apoptosis in UVB-irradiated A431 cells. (A) UVB irradiation up-regulated RTKs, EGFR and IGF-1R, and the adaptor proteins, GRB2 and SOS, in A431 cells. Silibinin treatment reduced their expression. (B) The effects of EGFR inhibitor, AG1478 (10 μM), IGF-1R inhibitor AG1024 (5 μM), and RTK inhibitor, genistein (10 μM) on the survival of UVB-irradiated A431 cells. *Compared with control group, $P < 0.05$; #Compared with UVB treatment alone, $P < 0.05$. (C) Inhibition of the IGF-1R signalling with AG1024 and genistein reduced caspase-3 activation induced by UVB irradiation. (D) The expression of IGF-1R after the transfection of A431 cells with si-IGF-1R. (E) Silencing of IGF-1R elevated cell viability in UVB-irradiated A431 cells *compared with control group, $P < 0.05$; #compared with UVB treatment alone, $P < 0.05$. (F) Silencing of IGF-1R inhibited expression of active caspase-8 and caspase-3 and reversed the cleavage of ICAD and PARP in UVB-irradiated cells.

an inactive 32 kDa pro-enzyme, and when stimulated by apoptotic signals, it is cleaved into the active form, which comprises the p20/p17 and p12 subunits [14,15]. Examination of protein expression clearly showed that after UVB irradiation, p32 pro-caspase-3 was cleaved into p20 and p17 caspase-3 (Fig. 1D). The substrates ICAD and PARP were cleaved accordingly (Fig. 1E). However, treatment with 125 μM silibinin rescued the apoptotic morphology (Fig. 1B), significantly reduced the hypo-diploid cell ratio (Fig. 1C), and inhibited the activation of caspase-3 (Fig. 1D and E), indicating that silibinin markedly repressed the apoptotic process initiated by UVB irradiation.

3.2. Silibinin inhibits apoptosis in A431 cells by down-regulating caspase-8 but not caspase-9

Caspase-9 and caspase-8 are believed to be initiator caspases at the top of caspase signalling cascades, which can activate downstream caspase-3 [16]. As shown in Fig. 2A and C, UVB irradiation activated both caspase-9 and caspase-8. Silibinin significantly inhibited the activation of the caspase-8 pathway, as indicated by the down-regulation of FADD and reduced cleavage of the caspase-8 (Fig. 2C). Inhibition of caspase-8 markedly improved the viability of UVB-irradiated cells (Fig. 2D), indicating that silibinin protected cells by repressing the activation of caspase-8.

However, silibinin elicited no obvious effect on the caspase-9 pathway. The release of cytochrome *c* from the mitochondria and the activation of caspase-9 were not altered by silibinin treatment (Fig. 2A). The inhibition of caspase-9 synergistically enhanced protective effects of silibinin against UVB irradiation (Fig. 2B).

3.3. Silibinin suppresses IGF-1R signalling pathway to rescue A431 cells from apoptosis

Silibinin was reported to modulate several membrane-bound receptors, including EGFR [17] and IGF-1R [18]. Meanwhile, IGF-1R-mediated signalling was reported to be involved in the cellular responses against UVB irradiation, influencing cell apoptosis, senescence and proliferation [6]. UVB irradiation markedly up-regulated the expression of EGFR and IGF-1R as well as those of GRB2 and SOS, the adaptor proteins recruited by RTKs. Silibinin reduced their expression (Fig. 3A). EGFR and IGF-1R are often linked to cell proliferation [17,18], whereas silibinin treatment protected UVB-irradiated A431 cells from apoptosis. Therefore, we examined which of these RTKs, EGFR and/or IGF-1R, would cause apoptosis in UVB-irradiated A431 cells.

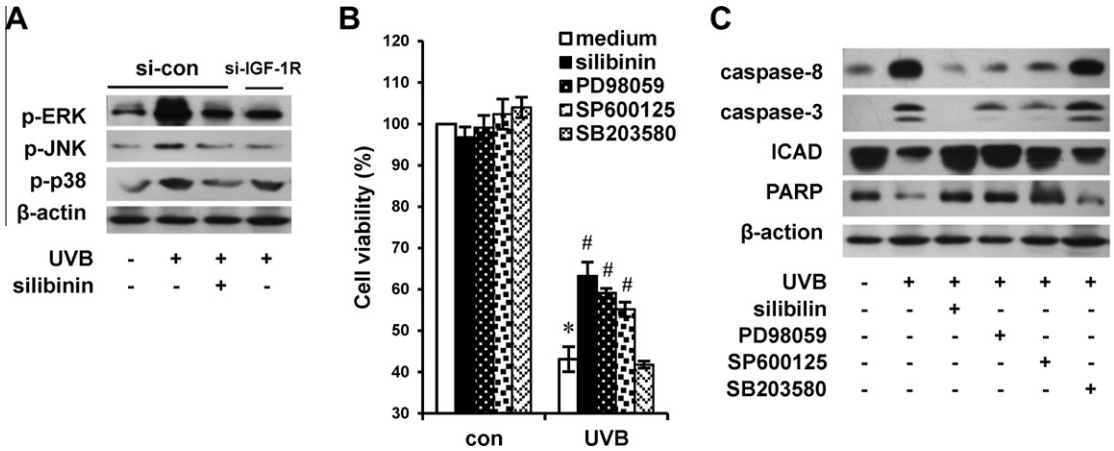

Fig. 4. Silibinin inhibits the activation of ERK1/2 and JNK in IGF-1R pathway in UVB-irradiated cells. (A) The expression of phosphorylated-ERK/JNK/p38 after the silencing of IGF-1R. (B) The effects of ERK1/2 inhibitor, PD98059 (10 μM), JNK inhibitor, SP600125 (5 μM), and p38 inhibitor, SB203580 (20 μM), on the viability of UVB-irradiated A431 cells. *Compared with control group, $P < 0.05$; #Compared with UVB treatment alone, $P < 0.05$. (C) The effects of MAPKs inhibitors on the activation of caspase-8 and caspase-3 and the cleavage of ICAD and PARP.

Inhibition of the EGFR activity with AG1478 [19] in UVB-irradiated A431 cells did not influence cell viability. However, inhibition of IGF-1R with AG1024 [18] unexpectedly improved cell survival, and treatment with RTKs inhibitor genistein [20] showed the similar effect as AG1024 (Fig. 3B). These findings suggested that the up-regulation of IGF-1R signalling pathway in UVB-irradiated A431 cells resulted in cell death, and silibinin might protect the cells by inhibiting the activation of IGF-1R signalling pathway. Consistently, assessment of caspase-3 expression further showed that, similar to silibinin, both the IGF-1R inhibitor and the RTKs inhibitor attenuated the activation of caspase-3 induced by UVB irradiation (Fig. 3C).

To confirm the effects of AG1024 in UVB-treated A431 cells, we transfected IGF-1R targeted siRNA (si-IGF-1R) into cells that were subsequently irradiated with UVB (Fig. 3D). Consistently, the transfection of si-IGF-1R significantly improved the viability of UVB-irradiated cells (Fig. 3E). The expression of active caspase-8 and caspase-3 was down-regulated by silencing of IGF-1R, and the cleavage of caspase substrates ICAD and PARP was inhibited as well (Fig. 3F). Taken together, these findings indicated that the suppression of IGF-1R-meidated signalling during UVB stress played a central role in the protective effect of silibinin in UVB-irradiated A431 cells.

3.4. Silibinin inhibits the activation of ERK1/2 and JNK, thereby protecting cells from caspase-8-mediated apoptosis

The activation of RTKs including IGF-1R stimulates MAPKs such as ERK1/2, JNK and p38, resulting in different responses [21]. It was reported that activation of ERK1/2, JNK or p38 in UVB-irradiated cells augmented apoptosis [9–11]. This study demonstrated that the expression of p-ERK, p-JNK and p-p38 elevated by UVB stimulation was suppressed by treatment with silibinin or si-IGF-1R (Fig. 4A), indicating that the activation of ERK1/2, JNK and p38 was stimulated by IGF-1R. Consistently, inhibition of ERK1/2 and JNK using PD98059 and SP600125 [22], respectively, promoted cell survival (Fig. 4B) and attenuated the activation of caspase-8 and caspase-3 (Fig. 4C). However, inhibition of p38 with SB203580 [22,23] was ineffective in protecting cells from UVB-induced apoptosis (Fig. 4B and C). Taken together, these results suggested that the inhibition of the activated ERK1/2 and JNK in IGF-1R pathway was involved in the protective effect of silibinin against UVB-induced cellular damage.

4. Discussion

Based on the previous works of our laboratory in the context of UVB-associated skin damage *in vivo* [24], this study demonstrated that silibinin could protect UVB-irradiated A431 cells from apoptosis *in vitro*. The most interesting finding in this study was that the activation of IGF-1R signalling pathway was deleterious in UVB-irradiated A431 cells, followed by ERK1/2 and JNK stimulation to induce cell apoptosis. The present study revealed that the down-regulation of IGF-1R-ERK/JNK pathway contributed to the protective effect of silibinin against UVB-induced apoptosis in A431 cells.

Silibinin also protected murine fibrosarcoma L929 cells as well as the primary murine dermal fibroblasts from UVB-induced apoptosis (data not shown). Treatment with AG1024, an inhibitor of IGF-1R, improved the survival rate of both cells (supplementary data). Therefore, the protective effect of silibinin through the down-regulation of IGF-1R signalling pathway found in this report could likely be extended to other cell lines including normal cells.

It is likely to expect that the IGF-1R signalling pathway contributes to the cell proliferation that might rescue cells by repression of apoptosis. In fact, IGF-1R signalling is essential for skin wound vascularization [7] and inhibitory against oxidative stress-induced mitochondrial dysfunction, cytochrome *c* release and apoptosis in induced pluripotent stem cells [25]. However, this study discloses an unexpected facet of this signalling pathway. Inhibition of the IGF-1R pathway with the inhibitor AG1024 or with IGF-1R-targeted siRNA improved A431 cell survival after acute UVB exposure. The pro-apoptotic effect of IGF-1R has recently been reported in a study on *Caenorhabditis elegans* [28]. Researchers identified that the activation of insulin/IGF-1-Ras/MAPK signalling pathways contributed to the non-canonical control of *C. elegans* germline apoptosis [26]. In *C. elegans*, the Ras/MAPK pathway promoted DNA damage-induced apoptosis of germ cell by cep-1-dependent and cep-1-independent mechanisms. In the study, insulin/IGF-1 signalling selectively promoted DNA damage-induced apoptosis of germ cell through the Ras pathway. Therefore, the authors hypothesised that the non-canonical insulin/IGF-1 signalling was required to sustain the hyper-activated Ras signalling [28]. The hypothesis is identical with the mechanism we propose from the present study as described below. In this study, ERK1/2 and JNK, both of which had been reported to induce cell apoptosis during UVB stress [9,10], were highly activated in UVB-irradiated A431 cells. Taking into consideration the finding that in normal status IGF-1R is responsible for promotion of cell proliferation, IGF-1R signalling has dual functions, depending on cell status: when cells bear UVB- or other stimulators-induced DNA and/or RNA damage, IGF-1R activated ERK1/2 and JNK to augment cell apoptosis. It was previously reported that silibinin suppressed the IGF-1R axis by increasing circulating levels of IGF-binding protein 3 (IGFBP-3) and decreasing levels of IGF-1 *in vivo* [27]. In this *in vitro* study, silibinin suppressed abnormal activation of the IGF-1R-mediated pathway in UVB-irradiated A431 cells, thereby inhibiting the activation of ERK1/2 and JNK, and finally rescued cells from UVB-induced apoptosis. These findings suggested that silibinin and RTK inhibitors could probably be developed to prevent UVB-induced skin damage.

In recent years, RTKs have been studied extensively in terms of their roles in cancer progression [28]. The repression of RTK signalling represents a therapeutic modality for the treatment of carcinoma. Whether the uncommon role of IGF-1R found in this study can be extended to other cell types during the irradiation remains to be determined. However, our results warn that in combination therapies for cancer, the inhibition of IGF-1R or of other RTKs might attenuate the pro-apoptotic effect of radiotherapy instead of potentiating it.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.01.109.

References

[1] J. Li, Z. Liu, C. Tan, X. Guo, L. Wang, A. Sancar, D. Zhong, Dynamics and mechanism of repair of ultraviolet-induced (6–4) photoproduct by photolyase, Nature 466 (2010) 887–890.

[2] D.A. Lewis, Q. Yi, J.B. Travers, D.F. Spandau, UVB-induced senescence in human keratinocytes requires a functional insulin-like growth factor-1 receptor and p53, Mol. Biol. Cell 19 (2008) 1346–1353.

[3] C. Dehmlow, J. Erhard, H. de Groot, Inhibition of Kupffer cell functions as an explanation for the hepatoprotective properties of silibinin, Hepatology 23 (1996) 749–754.

[4] S. Roy, G. Deep, C. Agarwal, R. Agarwal, Silibinin prevents ultraviolet B radiation-induced epidermal damages in JB6 cells and mouse skin in a p53-GADD45alpha-dependent manner, Carcinogenesis 33 (2012) 629–636.
[5] H.A. Hansson, R. Jonsson, K. Petruson, Transiently increased insulin-like growth factor. I. Immunoreactivity in UVB-irradiated mouse skin, J. Invest. Dermatol. 91 (1988) 328–332.
[6] D.A. Lewis, J.B. Travers, A.K. Somani, D.F. Spandau, The IGF-1/IGF-1R signaling axis in the skin: a new role for the dermis in aging-associated skin cancer, Oncogene 29 (2010) 1475–1485.
[7] S.Y. Aghdam, S.A. Eming, S. Willenborg, B. Neuhaus, C.M. Niessen, L. Partridge, T. Krieg, J.C. Bruning, Vascular endothelial insulin/IGF-1 signaling controls skin wound vascularization, Biochem. Biophys. Res. Commun. 421 (2012) 197–202.
[8] G.L. Johnson, R. Lapadat, Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases, Science 298 (2002) 1911–1912.
[9] J. Saegusa, D.K. Hsu, W. Liu, I. Kuwabara, Y. Kuwabara, L. Yu, F.T. Liu, Galectin-3 protects keratinocytes from UVB-induced apoptosis by enhancing AKT activation and suppressing ERK activation, J. Invest. Dermatol. 128 (2008) 2403–2411.
[10] C. Bivik, K. Ollinger, JNK mediates UVB-induced apoptosis upstream lysosomal membrane permeabilization and Bcl-2 family proteins, Apoptosis 13 (2008) 1111–1120.
[11] K. Nys, A. Van Laethem, C. Michiels, N. Rubio, J.G. Piette, M. Garmyn, P. Agostinis, A p38(MAPK)/HIF-1 pathway initiated by UVB irradiation is required to induce Noxa and apoptosis of human keratinocytes, J. Invest. Dermatol. 130 (2010) 2269–2276.
[12] B.K. Pliyev, M. Menshikov, Differential effects of the autophagy inhibitors 3-methyladenine and chloroquine on spontaneous and TNF-alpha-induced neutrophil apoptosis, Apoptosis 17 (2012) 1050–1065.
[13] G.M. Cohen, Caspases: the executioners of apoptosis, Biochem. J. 326 (Pt 1) (1997) 1–16.
[14] D. Green, G. Kroemer, The central executioners of apoptosis: caspases or mitochondria?, Trends Cell Biol 8 (1998) 267–271.
[15] S. Gamen, A. Anel, A. Pineiro, J. Naval, Caspases are the main executioners of Fas-mediated apoptosis, irrespective of the ceramide signalling pathway, Cell Death Differ. 5 (1998) 241–249.
[16] M. Chen, J. Wang, Initiator caspases in apoptosis signaling pathways, Apoptosis 7 (2002) 313–319.
[17] L. Qi, R.P. Singh, Y. Lu, R. Agarwal, G.S. Harrison, A. Franzusoff, L.M. Glode, Epidermal growth factor receptor mediates silibinin-induced cytotoxicity in a rat glioma cell line, Cancer Biol. Ther. 2 (2003) 526–531.
[18] H.J. Wang, S. Tashiro, S. Onodera, T. Ikejima, Inhibition of insulin-like growth factor 1 receptor signaling enhanced silibinin-induced activation of death receptor and mitochondrial apoptotic pathways in human breast cancer MCF-7 cells, J. Pharmacol. Sci. 107 (2008) 260–269.
[19] N.R. Pandey, G. Vardatsikos, M.Z. Mehdi, A.K. Srivastava, Cell-type-specific roles of IGF-1R and EGFR in mediating Zn2+-induced ERK1/2 and PKB phosphorylation, J. Biol. Inorg. Chem. 15 (2010) 399–407.
[20] J. Lee, J. Ju, S. Park, S.J. Hong, S. Yoon, Inhibition of IGF-1 signaling by genistein: modulation of E-cadherin expression and downregulation of beta-catenin signaling in hormone refractory PC-3 prostate cancer cells, Nutr. Cancer 64 (2012) 153–162.
[21] H.J. Schaeffer, M.J. Weber, Mitogen-activated protein kinases: specific messages from ubiquitous messengers, Mol. Cell Biol. 19 (1999) 2435–2444.
[22] S. Krifka, K.A. Hiller, C. Bolay, C. Petzel, G. Spagnuolo, F.X. Reichl, G. Schmalz, H. Schweikl, Function of MAPK and downstream transcription factors in monomer-induced apoptosis, Biomaterials 33 (2012) 740–750.
[23] B.M. Kim, K.H. Lee, I.S. Hong, S.H. Hong, P38 mitogen-activated protein kinase is a key regulator of 5-phenylselenyl- and 5-methylselenyl-methyl-2′-deoxyuridine-induced apoptosis in human HL-60 cells, Biochem. Biophys. Res. Commun. 417 (2012) 237–244.
[24] Q. Wang, Y. Ye, W. Liu, S. Jiang, S. Tashiro, S. Onodera, F. Gu, Y. Wang, T. Ikejima, Dual effects of silibinin treatment on autophagy-regulated dermal apoptosis retardation and epidermal apoptosis up-regulation in UVB-induced skin inflammation, J. Asian Nat. Prod. Res. 14 (2012) 688–699.
[25] Y. Li, H. Shelat, Y.J. Geng, IGF-1 prevents oxidative stress induced-apoptosis in induced pluripotent stem cells which is mediated by microRNA-1, Biochem. Biophys. Res. Commun. 426 (2012) 615–619.
[26] A.J. Perrin, M. Gunda, B. Yu, K. Yen, S. Ito, S. Forster, H.A. Tissenbaum, W.B. Derry, Noncanonical control of *C. elegans* germline apoptosis by the insulin/IGF-1 and Ras/MAPK signaling pathways, Cell Death Differ. 20 (2013) 97–107.
[27] C. Hoh, D. Boocock, T. Marczylo, R. Singh, D.P. Berry, A.R. Dennison, D. Hemingway, A. Miller, K. West, S. Euden, G. Garcea, P.B. Farmer, W.P. Steward, A.J. Gescher, Pilot study of oral silibinin, a putative chemopreventive agent, in colorectal cancer patients: silibinin levels in plasma, colorectum, and liver and their pharmacodynamic consequences, Clin. Cancer Res. 12 (2006) 2944–2950.
[28] J.S. Yuen, E. Akkaya, Y. Wang, M. Takiguchi, S. Peak, M. Sullivan, A.S. Protheroe, V.M. Macaulay, Validation of the type 1 insulin-like growth factor receptor as a therapeutic target in renal cancer, Mol. Cancer Ther. 8 (2009) 1448–1459.