Alternative Splicing of SMAD4 and Its Function in HaCaT Cells in Response to UVB Irradiation

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Received: 2018.01.05; Accepted: 2018.05.09; Published: 2018.08.06

Abstract

Alternative splicing is one of the most common mechanisms of human gene regulation and plays a crucial role in increasing the diversity of functional proteins. Many diseases are linked to alternative splicing, especially cancer. SMAD4 is a member of the SMAD family and plays a critical role in mediating of TGF-β signal transduction and gene regulatory events. Smad4 is a tumour suppressor and acts as a shuttling protein between nucleus and cytoplasm. The splicing variants of Smad4 have been found in many cancers. The present study performed nested PCR to detect alternative splicing of Smad4 in HaCaT cells lines in response to UVB irradiation. The UVB induced a novel Smad4B isoform that led to decrease the Smad4 expression. The hnRNPA1 splicing factor is responsible for Smad4 alternative splicing in response to UVB. The UVB increased the expression of SF2 and hnRNPA1 Splicing factors. The hnRNPA1 overexpression induced Smad4B by regulating Smad4 alternative splicing. The Smad4B isoform supported the function of Smad4 full length in UVB resistance with certain limitation. The western blot analyses showed that the overexpressed Smad4 full length significantly increased N-cadherin expression while Smad4B overexpression decreased the expression the N-cadherin (P<0.05). Furthermore, overexpression of the isoform in HaCaT cells decreased cell invasion as compared to Smad4 full-length overexpression. These results will be helpful to understand the importance of Smad4 alternative splicing in skin tumorigenesis.

Key words: Alternative splicing, Cancer, hnRNPA1, Proteins, Smad4, UVB

Introduction

The skin is the outermost organ of the body and can be damaged by environmental conditions such as sunlight and pollution [1]. It is continuously exposed to various environmental stress including ultraviolet irradiation. The ultraviolet radiation (UV) has both effects, beneficial and harmful. It increases pigmentation and vitamin D synthesis but also causes skin cancer and inflammation [2]. The ultraviolet radiation is the part of electromagnetic radiation having the wavelength range UVA 315-400nm, UVB 280-315nm and UVC 100- 400nm. The oxygen and ozone layer are essential to absorb UVC radiation entirely and approximately 90 percent of UVB [3]. However, the excessive exposure of UVB and UVA cause various type of DNA damage. The DNA damage may have numerous harmful consequences like mutation, cell death, photoaging, and cancer. UVA and UVB are not only absorbed by DNA but also by the chromophore which is present in the skin. This process may result in DNA damage indirectly due to the formation of reactive oxygen species. However, the human cells have an elaborate system to control the harmful effects of reactive species by antioxidant and DNA damage repair mechanism [4]. UVB primary source of Vitamin D which maintain calcium hemostasis and another critical process [5]. Excessive exposure to UVB cause skin cancer, including basal cell carcinoma, squamous cell
cancer, nonmelanoma and cutaneous malignant melanoma [6]. Literature study reveals that UVA and UVB wavelength triggers DNA damage and responsible for the majorities of this lesion [7]. The two primary DNA lesion appear due to UV irradiation, cyclobutane pyrimidine dimmer (CPDs) and 6-4 pyrimidine photoproducts (6-4 pps) [8,9]. The removal of CPDs is carried out by a complex and highly conserved mechanism called nucleotide excision repair (NER) in placental mammals. The loss or mutations in the NER regulatory pathway has been shown to result in a dramatic increase in cutaneous carcinogenesis in patients with xeroderma pigmentosa patients [10]. Keratinocytes have a defensive mechanism known as DNA damage response (DDR) against cutaneous carcinogenesis, which may have different physiological results such as cell cycle arrest, activation of DNA, apoptosis or senescence. Depending on the degree of DNA damage and the ability of keratinocytes to repair the damage [11,12].

Alternative splicing of the pre-mRNA is a crucial mechanism by which a gene can generate different mRNA transcripts and contribute to protein diversity, isoforms of proteins with different functions. Alternative splicing occurs in almost all human genes [13]. Approximately 95 percent of the human multi-exon genes are spliced alternatively [14]. Cancer is a complex disorder which is associated with a variety of genetic and epigenetic aberrations. Cells acquire eight common characteristics during cancer development. They include maintenance of proliferative signalling, elimination of growth suppressor, resistance to cell death, the possibility of replicative immortality, induced angiogenesis, and activation of invasion and metastasis. Many of these functions may be related directly resulting in abnormal gene regulation, dysregulation of alternative splicing during tumour progression [15]. The destruction of TGF-β signalling network at Smad level is commonly found in human cancers. Several sporadic epithelial tumours show missense, nonsense, frameshift, and point mutations at SMAD4 locus. The four alternatively spliced tumours associated Smad4 isoform have been found in a thyroid tumour. Smad4 is often mutated and deregulated by abnormal splicing in thyroid cancer [16]. The transforming growth factor beta (TGF-β) is a member of a large family of structurally related proteins that normally function to regulate embryonic development and cell homeostasis, including the cell proliferation regulation, differentiation, matrix production and apoptosis in a cell [17]. Alteration in the transforming growth factor beta (TGF-β) signalling pathway can cause human diseases, for example, developmental disorders, vascular disease, and cancer. TGF-β acts as a tumour-suppressing in the early stages of epithelial carcinogenesis, but TGF-β induces tumour development in advanced phases by inducing tumour growth, epithelial-mesenchymal transition (EMT), invasion, and metastasis [18]. The surface of the cell contains two kinds of the transmembrane kinase (serine/threonine) type I and II receptors. The TGF-β ligand binds to TBR1 receptor and transmitting the signal from the surface of the outer membrane to the interior of the cell, activating the signalling cascade.

The heterogeneous nuclear ribonucleoprotein (hnRNP) and pre-mRNA splicing factor (SF2) regulate pre-mRNA splicing excellently. Previously it has been reported that hnRNPA1 and SF2 regulate gene alternative splicing. The overexpression of hnRNPA1 and SF2 in many cancer cells denote its relation with the motility and proliferation of cancer cells [19,20].

The present study determined Smad4 alternative splicing in HaCaT cell in response to UVB irradiation. We also studied whether hnRNAP A1 or SF2 splicing factor is involved in UVB induce alternative splicing of Smad4.

Materials and Methods

Cell culture

HaCaT cells is a spontaneously immortalized human keratinocyte cell line that has been widely used in scientific research for skin biology and differentiation studies. The HaCaT cells were maintained in RPMI-1640 (Hyclone) supplemented with 10% fetal bovine serum (FBS, Hyclone), 1% antibiotics penicillin-streptomycin (P/S, Hyclone) and were incubated at standard condition (37°C, 5% CO₂ humidified incubator). Cell physiology and behaviour was progressively examined with the help of an inverted microscope.

UVB Irradiation

HaCaT cells were subcultured for 48 hours (80% confluence) in 60mm cell culture dishes in standard medium. UVB irradiation was carried out by using two 8-watts UVB lamps (SANKEY, Japan). The UVB intensity was measured by UV meter (photoelectric Instrument Factory of Beijing Normal University, China). The cells were washed with phosphate buffer saline (PBS) two-time and then irradiated at a different dose of UVB 30,50,100 and 400mj/cm² without the plastic lid of the dishes. After UVB irradiation, the cells were incubated in a humidified incubator for six hours and RNA were extracted after incubation. The low dose of UVB (3.5,7 mj/cm²) at 24 hours of incubation was also studied.

The HaCaT stable cell lines of hnRNPA1 and SF2 splicing factor were donated by Xichao Xu and were

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maintained in RPMI-1640 (Hyclon) supplemented with 10% fetal bovine serum (FBS) 1% penicillin-streptomycin and 1.0 ug/ml puromycin. The culture cell was incubated at 37°C in 5% CO₂.

**RNA extraction and RT- PCR**

Total RNA was isolated using TRlzol reagent (Tiangen) according to manufacturer instructions. The RNA was reverse transcribed by using Prime Script™ RT reagent with gDNA Eraser (Takara, Japan). The cDNA was used in nested PCR.

**Nested PCR**

The nested PCR was used to ensure the specificity. Two pairs of primers were used for a single locus. The second pairs (nested primers) were used to bind to the first step product. The total 20ul volume reaction has been amplified for 28 cycles by using the primer pair:

- 5'/ AAGGATCAAAATTGCTTCAGA 3'/ (Sense)
- 5'/ CAGTCTAAAGGTTGTGGGTC 3/ (Anti-sense)

With denaturation 94°C for 30 secs, annealing at 56°C for 30sec and elongation at 72°C for 90 secs in nested first step. The following primers were used in nested second step with the denaturation at 94°C for 30 secs, annealing at 56°C for 30 sec and elongation was reduce at 72°C for 1 minute.

- 5'/ GGTCGGAAAGGATTTCC 3/ (Sense)
- 5'/ CCAACTGACACACCTTTGCCTA 3/ (Anti-sense)

The GAPDH was used as an internal control. The PCR products were loaded on 1% agarose gel.

**Plasmid Construction and Sequencing Analysis**

The cDNA was re-amplified to obtain the full length of Smad4 and Smad4B. 0.5% agarose gel was used to resolve the amplified product: The Smad4F and Smad4B bands were censored and purified by using gel extraction and purification kit (Omega). Dr Liao Yi donated the plasmid (Plenti-CMV). SalI and BamH1 restriction enzymes were used to censor the Plenti-CMV and BamH1, and XhoI restriction enzymes were used for full length Smad4 and Smad4B isoform. The entire products were run on a 0.5% gel and purified by using gel extraction kit (Omega). The purification product was used in plasmid construction of Plenti-CMV-Smad4F, Plenti-CMV-Smad4B, and Plenti-CMV-GFP by a T4 ligase enzyme. The transformation was done by using E. Coli Top 10 competent cells through heat shock at 42°C and the bacteria were grown on LB medium plate for one night. Overnight, some colonies were picked to confirm the positive colonies by PCR. The amplified bands corresponding to the correct size of the colonies was added to 10ml Amp⁺LB media culture flask. The flask was placed in transient thermostaker or incubator at 37°C and 150 RPM shaking for one night. Overnight extract the bacteria by centrifugation and send to thermos fisher scientific company for BGI sequencing. The required plasmid was extracted by plasmid extraction kits (Omega) using company protocol. The plasmid concentration was checked by nano-dropper three times and takes the average. The plasmid concentrations were Plenti-CMV-Smad4F (647.9ng/ul) and Plenti-CMV-Smad4B (597.85ng/ul). The formula diluted the plasmid = total volume x desired concentration ÷ Start concentration.

**Formation of Stable Cell Lines**

Stable cells formation

HEK 293T packaging cells were suspended in three 60mm dishes. After 24 h the cells were 80% confluent. HEK 293T cells were co-transfected with lentiviral Plenti-CMV-Gene of interest (Smad4F, Smad4B Isoform, and GFP) as well as lentiviral helper vectors pCMV-VSV-G plasmid and pCMV-dr8.2 dvpr plasmid. After 48h incubation, the viral supernatant fraction was collected. The 10ml collected medium was centrifuged 2000RPM for 5 minutes at 4°C. HaCaT cells were suspended in three 60mm dishes. After 24 h, the HaCaT cells were treated with 1.5ml collected lentiviral particles and add 6ul polybrene as transfection reagent. The medium was changed after 24 h and selected with the puromycin-containing medium over one week.

**Cell Proliferation**

Cell proliferation was analyzed by using CCK-8 assay (Beyotime, China). Briefly, same numbers of cells were seeded in forty-eight well plate. After 24 hours of incubation, the cells were irradiated with UVB. The cell viability was measured at 24h, 48h Pre-and post UVB by CCK-8 Assay.

**Cell migration and invasion**

The wound healing assay was used to study cell direction and migration rate. The HaCaT stable cell line was seeded in six-well plates overnight to make a monolayer. After 24h a scratch was made by pipet tip and wash with PBS twice to remove the dead cells. After 6, 12 and 24h the cell direction and migration rate was studied through inverted inflorescence microscope.

**Western blotting**

HaCaT stable cells were seeded in 60mm dishes. After 24h, the cells were treated with UVB 50mj/cm². After 6 hours of UVB irradiation, cells were extracted a lysed by using RIPA lysis buffer (50 mMTris-HCl
pH7.5, 1% NP-40, 0.5 mM EDTA, 0.1% SDS, 150 mMNaCl, 0.5% Sodium deoxycholate) with PMSF (Beyotime, China). The cell lysate was centrifuged at 13,000 RPM for 15 min at 4°C. BCA assay (Beyotime, China) was used to measure the protein concentration. The protein samples were separated by 15% SDS-PAGE and then transferred to polyvinylidene fluoride (PVDF) membranes (PALL, USA). PVDF membrane was blocked in 1XTBST with 5% nonfat milk for one hour and then incubated with primary antibodies at 4°C overnight. After overnight incubation, the membrane was washed three times with 1xTBST and incubated with corresponding secondary antibodies for 1h at room temperature. Following a triple washing step with 1XTBST, the bands were visualized with the chemiluminescence method (Millipore, USA). Antibodies including GAPDH, Smad4, BcI-2, Bax, and Caspases anti-rabbit or anti-mouse secondary antibodies were purchased from santa cruz and cell signalling (USA).

Results

UVB Irradiation leads to Smad4 splicing into Smad4-B in Keratinocyte cell

The given study determined whether UVB could induce alternative splicing of Smad4 in HaCaT cell line. Nested PCR was used to detect the Smad4 splicing after UVB treatment according to the previous protocol (2). The results showed that UVB induced only one isoform of Smad4 in HaCaT cells. DNA sequencing confirmed the isoform sequence. The translation capability was detected by PPM5 software. The expression of Smad4 decreases led to increases of Smad4B (Isoform) ultimately Smad4F (Full length) expression was entirely loss with a high dose of UVB. We also studied low dose of UVB at 24 hours. The same isoform Smad4B was obtained with low dose of UVB (Figure 1). 

hnRNPA1 splicing factor is involved in Smad4 alternative splicing

Next, we investigated whether a change in expression of splicing factors (hnRNPA1 and SF2) will affect the alternative splicing of Smad4. Western blot confirmed the overexpression of hnRNPA1 and SF2 in the HaCaT stable cell line (Figure 2A). It was determined that hnRNPA1 is responsible for alternative splicing of Smad4 to Smad4B (Figure 2B). The hnRNPA1 and SF2 overexpressed cells were subjected to nested PCR to detect the UVB induced alternative splicing of Smad4. The given results showed that overexpression of hnRNPA1 increases the expression of Smad4B isoform as compared to control and SF2 splicing factor (Figure 2).

UVB regulates the expression of hnRNPA1 and SF2

We further investigated whether UVB has an effect on hnRNPA1 and SF2 splicing factors and also studied whether hnRNPA1 or SF2 involved in UVB induced alternative splicing of Smad4. It was determined that UVB increases hnRNPA1 and SF2 splicing factors. The expression of hnRNPA1 and SF2 splicing factors increase at 6h and decrease after 24h post UVB irradiation. These two protein expressions

Figure 1. (A) Alternative splicing of Smad4 in response to a high dose of UVB at six hours. The UVB induced Smad4 B isoform that led to decrease Smad4F expression (B) Smad4F expression decreased with the increase of Smad4B isoform (C) The alternative splicing of Smad4 at a low dose of UVB irradiation after 24h. The isoform significantly increased with the increasing of UVB dose. n=3 *P<0.05.
are not correlated with ROS level in the cell. The given study reported that hnRNPA1 involved in UVB induced alternative splicing of Smad4. The results showed that UVB increases the expression of hnRNPA1 and SF2 splicing factors (Figure 3A). Which indirectly resulted the alternative splicing of Smad4. The overexpression of hnRNPA1 resulted smad4 alternative splicing (Figure 3B).

**The Smad4B acts as a subsidiary of full length Smad4 during UVB irradiation.**

To investigate the function of Smad4B, the lentiviral Plenti-CMV-Gene of interest (Smad4F, Smad4B, and GFP), as well as lentiviral helper vectors pCMV-VSV-G plasmid and pCMV-dR8.2 dvrplasmid, were constructed and transfected into HaCaT cell line by using polybrene transfection reagent. The Smad4F and Smad4B were transiently transfected into HaCaT cell. RT-PCR and inflorescence microscope was used detect the successful transfection the required plasmid (Figure 4).

![Figure 2](image1.png)

**Figure 2.** Involvement of Splicing factors (hnRNPA1 and SF2) in alternative splicing of Smad4. (A) Overexpression of splicing factors hnRNPA1 and SF2. (B) Nested PCR results showed the involvement of hnRNPA1 in alternative splicing of Smad4 into Smad4B.

![Figure 3](image2.png)

**Figure 3.** Effect of UVB on splicing factors (hnRNPA1 and SF2). (A) UVB increased the expression of hnRNPA1 and SF2 splicing factors. The UVB significantly increase the expression of hnRNPA1 and SF2 after six hours. (B) The hnRNPA1 splicing factor overexpression due to UVB irradiation resulted in alternative splicing of Smad4.

![Figure 4](image3.png)

**Figure 4.** (A) RT-PCR was used to detect the expression of Smad4 in GFP control, Overexpression of Smad4 and Smad4B isoform in HaCaT stable cell. (B) Inflorescence microscope was used to detect the transfection efficiency of Lentivirus. A GFP control B. Plenti-CMV-Smad4F C. Plenti-CMV-Smad4B. The transfection efficiency was more than 90 percent.
The effect of Smad4F and Smad4B was observed on cell proliferation pre-and post UVB treatment by using CCK-8 cell proliferation assay. The results showed that the Smad4B and Smad4F overexpression had no significant effect on cell proliferation (Figure 5 A, B). To detect the UVB effect on isoform, the cell was treated with 3mj/cm² with UVB. After 24, 48 hours of UVB treatment there was no significant difference between isoform and Smad4 overexpression (Figure 5 C, D). The Smad4B acted as a subsidiary of Smad4F against UVB irradiation (Figure 5).

The pro-apoptotic and anti-apoptotic proteins (Bcl2 and BAX, caspase 9, Caspase-6, Caspase-12, and Cleaved Caspase-3 was detected by western blot. The western blot results indicated that overexpression of Smad4F and Smad4B had no significant difference on Bcl2, Bax, and other Caspases. The isoform showed little effect on Bax, Bcl2 and caspases but had no significant difference (Figure 6).

**Smad4F and Smad4B have no significant difference in cell migration**

Wound healing assay was used to study the effect of Smad4B and Smad4F on cell migration. The results of wound healing assay showed that Smad4F and Smad4B had no significant difference in cell
migration (Figure 7). The western blot results revealed that there was no significant differential effect on E-cad expression, but the expression of N-cad significantly decreased in Smad4B as compared to Smad4F overexpression and GFP control (Figure 8). The N-cad function in cell adhesion is more critical than E-cad for motility and invasion (21). The isoform decreases cell invasion while Smad4F overexpression increases (Figure 7) (Figure 8).

Discussion

Alternative splicing plays a significant role in creating the diversity of the protein by including or excluding specific exons. Majorities of the transcript are degraded in the nucleus or encode for unstable protein [22,23]. Alternative splicing is a central process for gene regulation. Most of the multi-exon genes in humans are alternatively spliced, and the transcript displays different fantastic function in physiological and pathological condition [24]. It has been found that aberrant splicing is associated with various diseases, including cancer. Mutations in splicing regulatory elements in the nucleotide sequence and changes in the cellular splicing regulatory device both lead to changes in the splicing pattern of many cancer-associated genes [25].

The present study analysed for the first time the alternative splicing of Smad4 in HaCaT cells in response to UVB irradiation and found that UVB can induce only one isoform (Smad4B) of Smad4 in HaCaT cell at 6 hours of the incubation period (Figure 1). The given isoform (Smad4B) was more potent against UVB irradiation as compared to full length of Smad4. The same isoform was obtained at low dose

Figure 7. The wound healing assay was used to determine the effect of isoform and Smad4 overexpression on HaCaT cell migration, but no significant difference was found in cell migration. The P-Value was P>0.05.

Figure 8. E-cadherin and N-cadherin expression. The Smad4 and Smad4 B isoform was no significant effect on expression of E-Cad, but the Smad4B decreased the expression of N-cad as compared to Smad4F and GFP control. The Smad4 overexpression significantly increases N-cad expression while the isoform Smad4B decreases the N-cad expression. *P<0.05. The UVB significantly decrease the expression of E-cad and N-cad. *P<0.01.
The two nuclear RNA binding proteins, the heterogeneous nuclear ribonucleoprotein family (hnRNP) and the serine/arginine-rich protein (SR) family play a key role in regulating alternative splicing and mRNA stability [32]. The heterogeneous nuclear ribonucleic acid (hnRNA) family of proteins forms a diverse RNA-binding protein group that deals with various functions of metazoan [33]. The two splicing factors (hnRNPA1 and SF2) were examined in the present study. Some previous study reported that hnRNPA1 and SF2 splicing factors involved in UVB regulated gene alternative splicing [1,34,35,36]. Some authors reported that hnRNPA1 and SF2 also upregulated in various human cancers. It can be related to cancer cell proliferation and metastasis [14,37]. The hdf2 alternative splicing under UVB in human keratinocyte has been reported [37]. The overexpression of hnRNPA1 and SF2 were detected by western blot (Figure 2A). It was found that hnRNPA1 is responsible for regulating the alternative splicing of Smad4. The production of Smad4B led to decrease the expression of Smad4F. The overexpression of hnRNPA1 stimulates the alternative splicing of Smad4 while SF2 overexpression does not affect (Figure 2B). The UVB significantly increase the expression of hnRNPA1 and SF2 splicing factors (Figure 3A). The overexpressed hnRNPA1 is directly proportional to alternative splicing of Smad4 in response to UVB (Figure 3B). There was no definite effect of SF2 splicing factor on alternative splicing of Smad4. It was determined that the overexpression of Smad4F and Smad4B isoform have no significant difference in cell proliferation, after UVB irradiation and without UVB (Figure 4). Alternative splicing increases the numbers and variety of proteins expressed in eukaryotic cells. Recently the relationship between human genetic diseases and cancer are associated with different abnormal alternative splicing enhanced [38]. There are four Smad4 isoform have been reported in a thyroid tumour. All the isoform loss different portion of the linker region such as ΔEx6, ΔEx5-6, ΔEx4-6, and ΔEx4-7. The Smad4 often mutates and deregulated abnormally in thyroid tumours, and these changes may contribute to the onset of thyroid neoplasms. ΔEx5-6 is previously reported in primary neuroblastoma [39] in the MDA-MB231 mammary cell [40] and HaCaT cell line [26]. The isoform ΔEx4-6 is also reported in neuroblastoma [39]. The present study reported lacking almost the entire linker region. The ΔEx4-7 variants are reported in HaCat cell [26] and papillary thyroid carcinoma [16].

Figure 9: UVB-induced Smad4 splicing (Smad4) led to the decrease the full length of Smad4 and increase of Smad4B. The Smad4B lacks Exor463-7 and part of Exon 3 compared with Smad4 full length.
The Wound healing assay was used to determine the effect of Smad4B and Smad4F overexpression on HaCaT cell migration, but no significant differences were found on cell migration (Figure 7). The Smad4B and Smad4F have no significant difference E-cadherin expression but decrease the expression of N-cad. E-cadherin is identified to play a role in tumour progression and metastasis because the loss of E-cadherin expression has been found to be associated with aggressive and undifferentiated phenotypes of many cancers, including pancreatic cancer. Another adhesion molecule, N-cadherin, is correlated with an increased invasion of cancer [41]. The previous study has shown that overexpression of N-cadherin in breast cancer is associated with invasiveness as a result of N-cadherin-mediated interaction between cancer and the stromal cell [42]. The transfection of N-cadherin promotes motility and invasion in breast cancer cells without a reduction in E-cadherin expression. It has been determined that N-cadherin, which acts as adhesion molecules, can be more critical than the E-cadherin for metastasis and invasion [21]. The Smad4F overexpression significantly increases the expression of N-cadherin while the Smad4B decrease the expression of N-cadherin (Figure 8).

Conclusion

Pathogenesis and treatment of skin cancer caused by UV radiation have been the focus of research recently, UV irradiation may cause activation of a series of intracellular signalling pathways, changes in protein levels as well as the variable genes Splicing, these stress changes have significant implications for the formation of skin cancer. Alternative splicing is a fundamental mechanism to yield many diverse proteins; multiple transcriptions can create numerous mRNA transcripts. The given study determined the alternative splicing of Smad4 and its function in HaCaT cell in response to UVB irradiation. It was investigated that UVB induced only one isoform of Smad4 under different doses of UVB irradiation. This isoform showed more resistance to UVB as compared the Smad4. A given isoform act as a supporter of Smad4 against UVB. At high dose of UVB irradiation, Smad4 suppress entirely while Smad4B still showing resistance. The overexpression of hnRNPA1 is responsible for UVB induced alternative splicing of Smad4 in HaCaT cells. The UVB increase the expression of hnRNPA1 and SF2 splicing factors. The overexpression of Smad4 full length increases N-cadherin expression while the isoform decreased N-cadherin expression. However, there was no effect on E-cadherin.

Acknowledgements

This work was supported by the Fundamental Research Funds for the Central Universities (No 106112017CDJXSYY0001, 2018CQDYSG0021) and the Graduated Scientific Research and Innovation Foundation of Chongqing, China (No CYB16039).

Competing Interests

The authors have declared that no competing interest exists.

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