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Molecular mechanism for P38 signaling pathway in autophagy of skin cancer cell line HS-1

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Abstract. – OBJECTIVE: Abnormal cell autophagy is correlated with aging, neurodegenerative disease, and skin cancer. The signal transduction pathway of autophagy in skin cancer is still unclear. This study aimed to investigate the role of P38 signal pathway-induced cell autophagy in skin cancer onset and potential clinical application value.

MATERIALS AND METHODS: Skin cancer cell line HS-1 was used as the model for ultraviolet (UV) irritation. Western blot tested autophagy signal molecules P38 activation in skin cancer cell line HS-1. Cells were then treated with P38 pathway agonist and antagonist to test autophagy condition and P38 pathway activation. Correlation analysis was performed to investigate the correlation between P38 pathway and cell autophagy level. RESULTS: UV irradiation treated skin cancer cell line HS-1 led to cell autophagy and P38 activation. AICAR and SB203580 potentiated and inhibited UV-induced HS-1 cell autophagy, respectively. P38 signal pathway activation condition was positively correlated with autophagy level.

CONCLUSIONS: UV irradiation can induce skin cancer cell autophagy via the P38 signal pathway, indicating that the regulation of the P38 signal pathway activation might be one potential strategy treating skin cancer.

Key Words:
UV irradiation, P38 signal pathway, Skin cancer cells, Cell autophagy

Introduction

Skin cancer has a relatively higher incidence and can affect the patient's physiological and mental functions[1]. Therefore, the study of skin cancer pathogenesis mechanism is of critical importance. However, such mechanism is still unclear yet with multiple factors involved including chemical reagent benzopyrene, viral carcinogenesis compound, chronic ulcer, electrical radiation, inflammation, and high dosage ultraviolet (UV) irradiation[2-4]. Early treatment of skin cancer is an optimal strategy and has obtained major advances and efficacy. However, radiation often causes internal hemorrhage of patients, in addition to suppressed immune function or dizziness[5]. The improvement of treatment specificity and efficacy is thus one major challenge in medical science, as precise treatment is an effective tool against skin cancer[6,7]. However, the selection of target is one major problem, which thus requires more effective molecular targets against skin cancer. More importantly, no targeted treatment against P38 signal pathway has been applied for skin cancer[8,9]. P38 signal pathway has pluripotent functions, such as the inhibition of skin cancer cell growth and the correlation with tumor metastasis[10], indicating the possible involvement of P38 signal pathway in skin cancer pathogenesis and progression[11]. This investigation thus used skin cancer cell line HS-1 cell as the model to investigate the possible regulatory role of UV on skin cancer cell line HS-1 cells. Autophagy is one self-regulatory process of cells and is achieved via a series of autophagy-related proteins and autophagy signaling pathways[12,13]. It is also involved in disease onset. P38 signal pathway protein is one group of autophagy facilitating factors that have been widely studied[14-16]. Currently, few medicines have been developed to target P38 signal pathway proteins, whose decrease has not received satisfactory results[17,18]. We also investigated the potential molecular target of P38 signal proteins. Therefore, we utilized skin cancer cell line HS-1 as the model, on which possible regulatory mechanism and roles of UV irradiation on skin cancer cells were investigated, in order to provide evidence for optimization of treatment targets of skin cancer.

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Materials and Methods

Reagent and Cell Model

Cell autophagy test kit and reagent were all purchased from Beyotime Biotech. (Shanghai, China). Fetal bovine serum (FBS) and cell culture medium were purchased from Hualan Bio. Engineering Inc. (Beijing, China). Other common reagents were purchased from Santa Cruz Biotechnology (Santa Cruz Biotechnology, CA, USA). This study utilized skin cancer cell line HS-1, which was purchased from American Microbial Bank (Manassas, VA, USA). This research was approved by the Ethics Committee of Zhongnan Hospital of Wuhan University, Wuhan, China.

Cell Culture

Skin cancer cell line HS-1 cells were resuscitated and re-suspended in high-glucose Dulbecco's Modified Eagle Medium (DMEM) medium as previously described[19].

Cellular P38 Signal Pathway Activity Assay

P38 signal pathway activity assay kit was used to test HS-1 cells following routine methods [20]. In brief, HS-1 cells were treated with UV irradiation and were mixed with cell activity assay buffer (2 mg/ml for P38 signal pathway activity assay). HS-1 cells were cultured for 4 h, followed by the addition of dimethyl sulfoxide (DMSO). The reaction was quenched for 5 min, followed by quantification under a microplate reader to test absorbance values at 560 nm. A growth curve of HS-1 cells was plotted[21].

Western Blotting For Cell Autophagy

Skin cancer cells HS-1 after UV irradiation and/or P38 signal pathway inhibitor/agonist treatment were prepared for cellular protein suspension for measuring concentration. The cell lysate was extracted and quantified by a microplate reader. Proteins were separated by centrifugation. An equal volume of cell protein suspension (containing 20 μg protein) was boiled in water-bath for 10 min for Western blotting using primary antibody (1: 1000 dilution) at 4°C overnight incubation. After Tris-buffered saline Tween-20 (TBST) washing, secondary antibody (1: 2000) was added for 37°C incubation for 3 h. With TBST washing, enhanced chemiluminescence (ECL) substrate was used to develop the membrane, which was blocked in 5% milk powder, primary antibody (anti-P38) and secondary antibody (anti-mouse IgG) were sequentially added for incubation, followed by three times of washing. Horseradish peroxidase was used for staining. Imaging analysis system (Qinxiang, China) was used to capture the image for analyzing protein expression level. P38 signal pathway expression levels in HS-1 cells were compared across all groups[22].

Immunofluorescence Analysis of Cell Autophagy

Skin cancer cell line HS-1 under UV irradiation or P38 signal pathway agonist/activator pre-treatment were tested for autophagy level by the immunofluorescent method.

Statistical Analysis

SPSS 15.0 software (SPSS Inc., Chicago, IL, USA) was used for analysis. The Student *t*-test was used for comparing between groups of HS-1 cells. The Student's *t*-test was used to compare the differences between the two groups. The Tukey's post-hoc test was used to validate the ANOVA for comparing measurement data among groups. A statistical significance was defined when $p<0.05$ for replicated studies.

Results

UV Irradiation of Skin Cancer HS-1 Cells Led to Autophagy and P38 Signal Pathway Activation

As shown in Figure 1, UV irradiation on skin cancer HS-1 cells lead to P38 signal pathway activation (p-P38 band gray values were 1.0 and 4.1 ± 0.6 for control and UV group, respectively), and potentiated cell autophagy (fluorescent levels were 0 and 82.3% ± 4.7% in control and UV group, respectively).

P38 Signal Pathway Agonist AICAR Potentiated UV-Induced Skin Cancer HS-1 Cell Autophagy

As shown in Figure 2, the P38 signal pathway agonist AICAR potentiated UV-induced autophagy of skin cancer cell line HS-1 by about 45%.

P38 Signal Pathway Inhibitor SB203580 Depressed UV-Induced Skin Cancer Cell Line HS-1 Autophagy Occurrence

As shown in Figure 3, the P38 signal pathway antagonist SB203580 could depress UV-induced HS-1 cell autophagy.

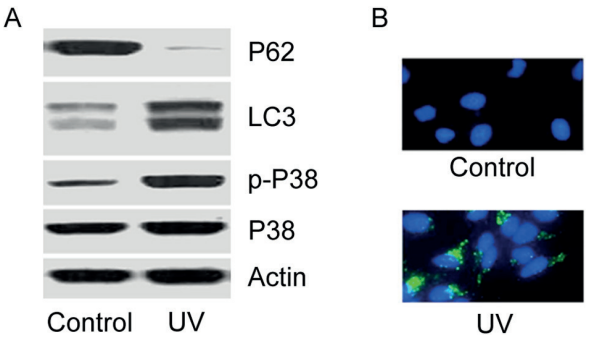

Figure 1. UV irradiation on skin cancer cell line HS-1 caused cell autophagy and activation of P38 signal molecules. (A) Western blot results of cell autophagy. (B) Confocal microscopy for cell autophagy. Blue, DAPI staining for nucleus. Green, LC3 antibody for cell autophagy.

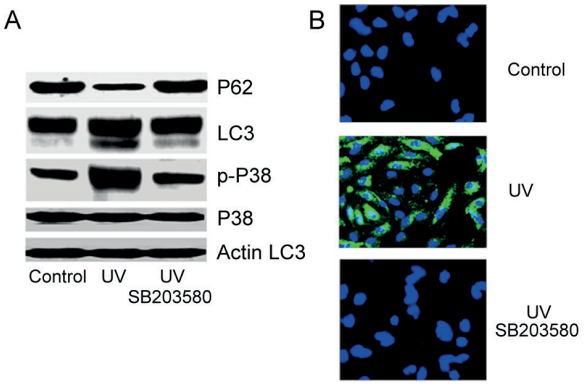

Figure 3. P38 signal pathway antagonist SB203580 depressed UV-induced HS-1 cell autophagy. ***(A)*** Western blot for cell autophagy. ***(B)*** Confocal microscopy of cell autophagy. Blue, DAPI staining for nucleus. Green, LC3 antibody for cell autophagy.

Correlation Between P38 Signal Pathway Activation and Autophagy Level

As shown in Figure 4, the P38 signal pathway activation status was positively correlated with P38 signal pathway activation (r=86.7%, p=0.0041).

Discussion

Skin cancer severely threatens the patient's health. We utilized skin cancer cell line HS-1 as the model, from which regulatory mechanism of UV irradiation on skin cancer cells and possible mechanisms were studied on molecular and protein levels. Data showed that UV irradiation potentiated autophagy of human skin cancer cell line HS-1, as consistent with previous reports showing the participation of UV in cell autophagy[23]. Cell autophagy has been shown to be correlated with some diseases such as aging, neurodegenerative diseases or skin cancer. However, the signal transduction mechanism of autophagy in skin cancer pathogenesis has not been illustrated. This work thus investigated the P38 signal pathway induced cell autophagy in skin cancer occurrence and potential clinical application values. The best strategy for clinical treatment of skin cancer is early diagnosis and treatment. Although traditional methods have gained satisfactory results, chemo-/radio-therapy often causes internal bleeding, immune suppression and dazzles[5]. The improvement of treatment specificity and efficacy is thus one major challenge in medical science, as precise treatment is an effective tool against skin cancer[6,7]. However, the selection of target is one major problem, which thus requires more effective molecular targets against skin cancer. Notably, no targeted treatment against P38 signal pathway has been applied for skin cancer[8,9]. How dose UV irradiation regulates skin cancer cell growth and autophagy is still unclear[24]. P38 signal pathway could inhibit skin cancer growth, whilst partial of this pathway is correlated with tumor metastasis[25], suggesting the possible involvement of P38 signal pathway in skin cancer occurrence and progression[26]. The P38 signal pathway participates in cell autophagy. In current knowledge, whether the P38 signal pathway is under UV regulation for regulating HS-1 cell autophagy is still unclear[27,28]. Our findings showed that UV irradiation potentiated P38 signal pathway activity. After treatment using P38 signal pathway agonist, HS-1 cell

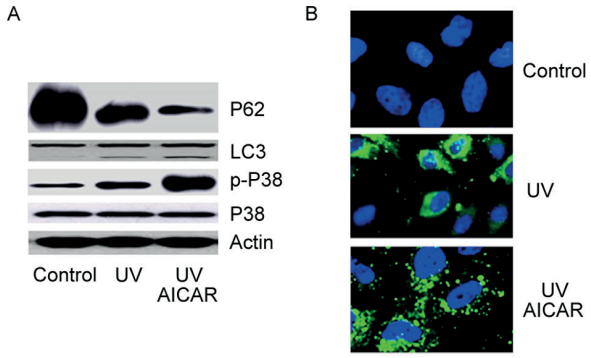

Figure 2. P38 signal pathway agonist potentiated UV-induced skin cancer HS-1 cell autophagy. ***(A)*** Western blot results of cell autophagy. ***(B)*** Confocal microscopy of cell autophagy. Blue, DAPI staining for nucleus. Green, LC3 antibody for cell autophagy.

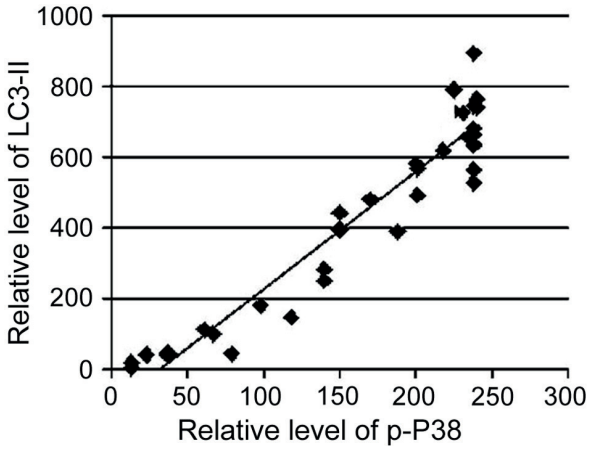

Figure 4. Correlation analysis between P38 signal pathway activation and autophagy level.

autophagy rate was elevated, whilst P38 signal pathway inhibitor suppressed UV-induced HS-1 cell autophagy. These results showed a consistent role of the P38 signal pathway in autophagy, as it can promote autophagy occurrence. In our study, the role of P38 signal pathway protein in UV-induced skin cancer cell line HS-1 autophagy can be proved by three pieces of information. Firstly, data showed significantly potentiated P38 signal pathway protein in UV-treated HS-1 cells. Secondarily, pre-treatment of P38 signal pathway activator enhanced UV-induced cell autophagy. Thirdly, after pre-treatment using P38 signal pathway inhibitor, UV-induced autophagy of HS-1 cells was suppressed. These results collectively suggested that P38 signal pathway protein played a role in UV-induced skin cancer HS-1 cell autophagy. Targeting P38 signal pathway protein might be a novel strategy for molecular treatment of skin cancer[26]. Yoon et al[28] of the P38 signal pathway in other cancers also showed suppression of cell autophagy. These results thus indicated that UV could induce skin cancer cell line HS-1 cell autophagy via enhancing P38 signal pathway. Certain weakness also existed in the current study. Firstly, no tumor or adjacent tissues were collected, making it impossible to study the direct correlation between the P38 signal pathway and skin cancer. Secondarily, skin tissues of cancer patients after treatment was also lacked, impeding the discussion of the correlation between P38 signal pathway activity and skin cancer prognosis. Thirdly, an animal model of skin cancer is expected for UV *in vivo* study to investigate the treatment efficiency of UV for skin cancer.

Conclusions

UV can induce skin cancer cell autophagy via the P38 signal pathway, probably consisting one molecular mechanism of skin cancer occurrence. We indicated that regulation of P38 signal pathway activation benefited the management of skin cancer cell autophagy, and the potency as one potential strategy for treating skin cancer.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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