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Molecular mechanism of Wnt signal pathway in multiple myeloma cell line H929 cell autophagy

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Abstract. – OBJECTIVE: Pathogenic mechanism of multiple myeloma is still unclear yet. Abnormality in cell autophagy is closely correlated with various orthopedic diseases especially multiple myeloma. Therefore, this study investigated the mechanism of cell autophagy abnormality in multiple myeloma occurrence and clinical implications.

MATERIALS AND METHODS: Using multiple myeloma cell line H929 as the model, cells were treated with UV irradiation. Western blot was used to measure the autophagy of H929 cell, expression level of autophagy molecules and activation of autophagy signal pathway such as Wnt. Using autophagy activator, H929 cell autophagy was potentiated, followed by quantification of autophagy molecular expression and signal pathway such as Wnt activation. Agonist or antagonist of Wnt signal pathway was used to treat H929 cells followed by measuring autophagy molecules and Wnt pathway activation. The correlation between Wnt signal pathway or cell autophagy and occurrence of multiple myeloma was analyzed.

RESULTS: UV irradiation treatment on multiple myeloma cell line H929 induced autophagy and Wnt signal pathway activation. The inhibitor of Wnt signal pathway suppressed UV-induced H929 cell autophagy. However, over-expression of Wnt signal pathway enhanced UV-mediated autophagy of H929 cells. The condition of Wnt activation and autophagy level were positively correlated.

CONCLUSIONS: UV irradiation can induce autophagy of multiple myeloma cells, suggesting that management of cell autophagy might be one possible treatment for multiple myeloma.

Key Words:
UV irradiation, Wnt signal pathway, Multiple myeloma, Cell autophagy.

Introduction

Multiple myeloma (MM) has a higher incidence, and affects physiological and psychological functions of patients[1]. Therefore, investigation of MM pathogenesis is of critical importance. However, the pathogenesis of MM is still unclear yet involving multiple governing factors such as chemical reagents benzopyrene, viral carcinogen, chronic ulcer, ionization irradiation, inflammation and high dosage of UV[2-4].

Treatment strategy of MM is early diagnosis and treatment. Although traditional methods such as radiotherapy, chemotherapy and surgery have gained satisfactory effects, chemo- or radio-therapy frequently causes internal organ bleeding, immune suppression and dizziness[5]. The improvement of treatment accuracy and successful rate is a major challenge in medical science. In clinic, targeted treatment can effectively cure MM[6,7], although the selection of treatment target is a major challenge. Therefore, it is necessary to identify more effective molecular targets against MM. More importantly, treatment for MM using Wnt signal pathway is still lacking[8,9].

Wnt signal pathway has multiple functions. For example, it can inhibit MM cell growth, and is correlated with the metastasis of malignant tumors such as liver cancer or lung cancer[10]. These studies showed that Wnt signal pathway might be correlated with MM pathogenesis, although still requiring further investigation[11].

Cell autophagy is a regulatory process of cells via a series of autophagy related proteins and signal pathways[12,13]. Moreover, autophagy participates in aging related disease pathogenesis. Wnt signal pathway is a widely studied pathway for facilitating autophagy[14,15]. Currently there are few drugs targeting Wnt signal pathway, and the decrease of Wnt signal pathway protein cannot reach satisfactory treatment efficacy targeting tumors[16,17]. This study used MM cell line H929 as an *in vitro* model to investigate the possible regulatory role of UV on MM cells and related mechanisms, in order to provide evidences for selecting MM treatment targets.

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Materials and Methods

Cell Model and Reagents

MM cell line H929 was purchased from American Microbe Bank (Manassas, VA, USA). Test kits for cell autophagy were purchased from Beyotime (Shanghay, China). Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) culture medium were purchased from Hualan Bio (Shanghai, China). Other reagents were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Cell Culture

MM cell line H929 was resuscitated and re-suspended in high-glucose DMEM medium for culture[18].

Wnt Signal Pathway Activity Assay

Wnt signal pathway activity assay kit was used to test the activity of MM cell line H929[19]. In brief, cells were cultured and treated with UV irradiation. 2 mg/ml Wnt signal activity assay reagent (Beyotime, Nantong, China) was added into each well for 4 h culture. Dimethyl sulfoxide (DMSO) was added to treat cells for 5 min. After quenching the reaction, 24-well containing MM H929 cells was placed on the microplate reader for testing optical absorbance (A) values at a wavelength of 560 nm[20].

Western Blot for Cell Autophagy

MM cell line H929 after UV or Wnt signal pathway inhibitor/activator treatment was extracted for total protein suspension following the manual instruction. Protein concentration was quantified by BCA kit. Cell lysate was extracted and quantified by microplate reader to separate proteins by centrifugation. Equal volume of protein suspension containing 20 μg proteins was firstly boiled for 10 min, followed by Western blot analysis in 12% separation gel. Electrophoresis was performed under 60 V for 30 min, followed by 120 V for 90 min. After electrophoresis, the protein was transferred to the membrane under 90 mA for 180 min. The membrane was then blocked using 5% defatted milk powder for 60 min room temperature incubation. Primary antibody (mouse anti-human

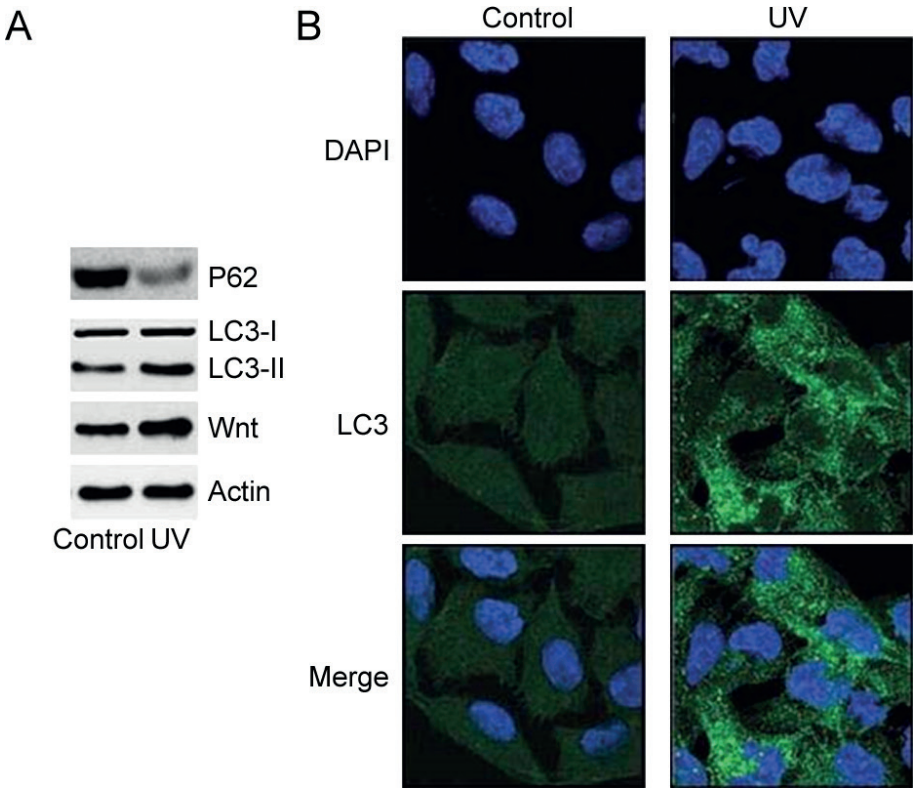

Figure 1. UV irradiation on MM cell line H929 induced cell autophagy and Wnt signal pathway activation. ***(A)*** Western blot results for cell autophagy; ***(B)*** Confocal images reflecting cell autophagy. Blue indicates DAPI staining for nucleus, green indicates LC3 staining for cell autophagy.

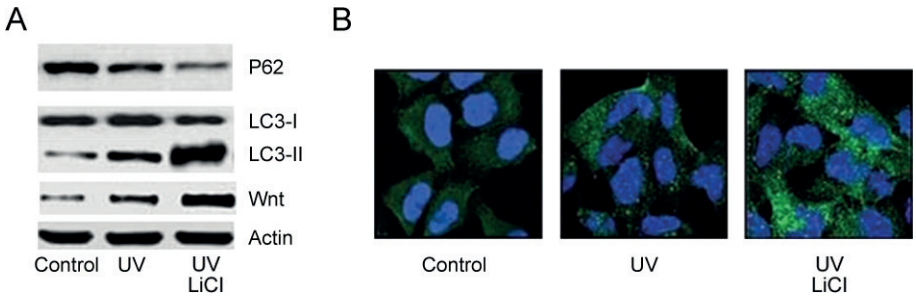

Figure 2. Activator of Wnt signal pathway enhanced UV induced autophagy MM cell line H929. ***(A)*** Western blot results for cell autophagy; ***(B)*** Confocal images reflecting cell autophagy. Blue indicates DAPI staining for nucleus, green indicates LC3 staining for cell autophagy.

Wnt monoclonal antibody or mouse anti-human actin monoclonal antibody as the internal control) was added for 4°C overnight incubation (1:1000 dilution). After Tris-buffered saline and tween 20 (TBST) rinsing, horseradish peroxidase conjugated secondary antibody (1:2500) was added for 37°C reaction for 3 hr, followed by TBST rinsing and development/exposure using ECL reagent. Gel imaging apparatus was used to analyze protein expression level to compare Wnt signal pathway expression level in MM cell lines among all groups[21].

Immune Fluorescent Analysis of Cell Autophagy

MM cell line H929 treated with UV or Wnt signal pathway inhibitor/activator followed by analysis of autophagy level by immune fluorescent assay.

Statistical Analysis

SPSS 15.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Student *t*-test was used for comparison among all groups of H929 cells. A significant difference was defined when $p < 0.05$ in replicated experiments.

Results

UV Irradiation on MM cell Line H929 Induced Cell Autophagy and Wnt Signal Pathway Activation

UV irradiation on MM cells H929 led to activation of Wnt signal pathway, as Wnt activity levels were 1.0 and 8.3 ± 0.4 in control and UV irradiation group, respectively (Figure 1). Cell autophagy was also enhanced after UV irradiation, as demonstrated by immune fluorescent analysis showing the relative autophagy levels was 0 and 79.3 ± 10.2% in control and UV group, respectively.

Wnt Signal Pathway Activator LiCl Enhanced UV Induced Autophagy MM Cell line H929

Wnt signal pathway activator LiCl enhanced UV induced autophagy of MM cell line H929 by about 61% (Figure 2).

Wnt Signal Pathway inhibitor DKK Suppressed UV Induced Autophagy MM Cell Line H929

Wnt signal pathway inhibitor DKK effectively suppressed UV induced cell autophagy of MM cell line H929 (Figure 3).

Correlation Between Wnt Signal Pathway Activation and Autophagy Level

Activation of Wnt signal pathway in MM cells was significantly positively correlated with autophagy level (p=0.011, correlation coefficient R=83.7%) (Figure 4).

Discussion

MM severely threatens patients' life quality and health. This study used MM cell line H929 as the model, in which regulatory role of UV on MM cells H929 and possible mechanisms are studied from molecular and protein levels. UV is a common autophagy inducer, and is correlated with hematological diseases such as leukemia. We thus selected UV as the approach to induce autophagy. Data showed that UV enhanced autophagy of human MM cells H929 line, as consistent with previous study showing the involvement of UV in cell autophagy[22]. Cell autophagy abnormality is also related with various diseases such as aging, cardiovascular disorder, neural degenerative diseases and MM[4]. However, the signal transduction

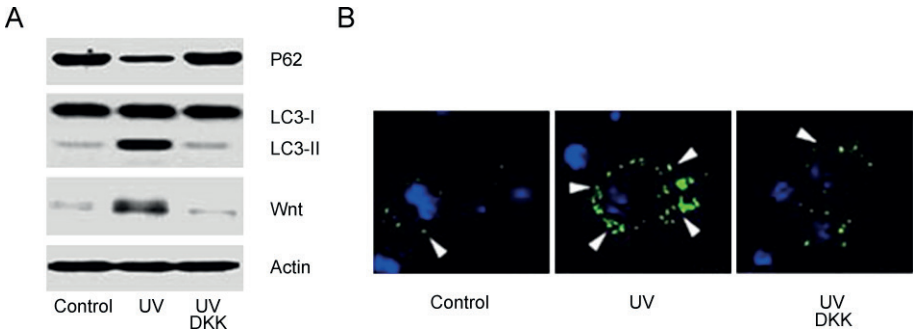

Figure 3. Wnt signal pathway inhibitor DKK suppressed UV induced autophagy MM cell line H929. ***(A)*** Western blot results for cell autophagy; ***(B)*** Confocal images reflecting cell autophagy. Blue indicates DAPI staining for nucleus, green indicates LC3 staining for cell autophagy.

pathway of autophagy in MM occurrence is still unclear yet. This study investigated the role of Wnt signal pathway induced cell autophagy in MM and potential clinical values.

Treatment strategy of MM is early diagnosis and treatment. Although traditional methods such as radiotherapy, chemotherapy and surgery have gained satisfactory effects, chemo- or radio-therapy frequently causes internal organ bleeding, immune suppression and dizziness[5]. The improvement of treatment accuracy and successful rate is a major challenge in medical science. In clinic, targeted treatment can effectively cure MM[6,7], although the selection of treatment target is a major challenge. Therefore, it is necessary to identify more effective molecular targets against MM. More importantly, treatment for MM using Wnt signal pathway is still lacking[8,9].

How does UV regulate growth and autophagy of MM cells is still unclear[23]. Wnt signal pathway can suppress MM growth, whilst Wnt signal pathway is correlated with tumor metastasis[24], suggesting its potential involvement in MM occurrence and progression[25].

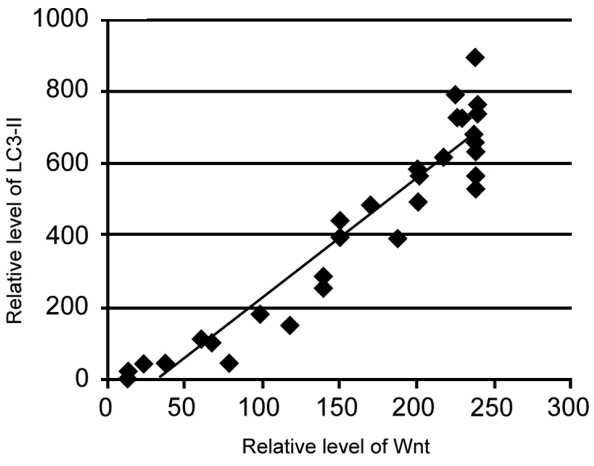

Figure 4. Correlation analysis between Wnt signal pathway activation and autophagy level.

Wnt signal pathway participates in cell autophagy. In current study, whether this pathway is under regulation of UV for further mediating autophagy of MM cell line H929 is still unclear[26,27]. This study showed that UV enhanced Wnt signal pathway level. After pre-treatment with Wnt signal pathway activator, MM cells H929 had elevated autophagy rate, whilst Wnt signal pathway inhibitor suppressed UV induced autophagy of MM cell line H929. These results showed consistent role of Wnt signal pathway in autophagy as demonstrated by a previous study[10], which showed that Wnt signal pathway could facilitate autophagy occurrence.

In this study, the role of Wnt signal pathway in UV-induced autophagy of MM cells H929 can be proved from three perspectives. Firstly, data showed significantly enhanced Wnt signal pathway protein in MM cell line H929 after UV irradiation. Secondly, after pre-treatment with Wnt signal pathway activator, UV irradiation on H929 cells resulted in enhanced cell autophagy. Thirdly, after pre-treatment using Wnt signal pathway, UV irradiation on MM cell line H929 showed suppressed cell autophagy. These results showed the role of Wnt signal pathway protein in UV induced autophagy of MM cell line H929. Targeting Wnt signal pathway proteins might be a new strategy for molecular targeted treatment of MM[26]. Currently, Wnt signal pathway has been shown to suppress tumor cell autophagy in other cancers[27]. Our results showed that UV could induce the autophagy of MM cell line H929 possibly via enhancing Wnt signal pathway.

This work has some limitations. Firstly, we did not collect MM tumor tissues and adjacent tissue samples from patients, making it impossible to explore the direct relationship between Wnt signal pathway and MM. Secondly, this study lacked MM tumor tissues after treatment, and cannot investigate the correlation between Wnt signal pathway and MM prognosis. Thirdly, no MM animal model was employed in this study for UV irradiation *in vivo*, lacking the UV targeted treatment efficacy on MM from the whole body level.

Conclusions

We showed that UV can induce MM tumor cell autophagy possibly via Wnt signal pathway, suggesting that modulation of Wnt signal pathway activation status might help to manage autophagy level of MM.

Conflict of Interest

The Authors declare that they have no conflict of interest.

Acknowledgments

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