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DUSP1 promotes senescence of retinoblastoma cell line SO-Rb5 cells by activating AKT signaling pathway

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Abstract. – OBJECTIVE: Retinoblastoma seriously threats to human health and life. Molecular targeted therapy of retinoblastoma supplies the direction of research in the future. This study aims to investigate the impact of DUSP1 on human retinoblastoma SO-Rb5 cell senescence.

MATERIALS AND METHODS: Angiotensin II (AGII) was used to induce human retinoblastoma SO-Rb5 cell senescence model. DUSP1 over-expression plasmid and small interfere RNA (siRNA) were transfected into SO-Rb5 cells by Lipofectamine. Dual specificity phosphatase 1 (DUSP1), p53, p16, and protein kinase B (Akt) signaling expressions were detected with Western blot assay. SH-6 was applied to inhibit Akt signaling in SO-Rb5 cells. Cell senescence was evaluated by using β-galactosidase test.

RESULTS: DUSP1 level increased in SO-Rb5 cells induced by AGII. Senescence protein p53 and p16 significantly upregulated in SO-Rb5 cell senescence model, together with β-galactosidase staining. DUSP1 plasmid transfection significantly enhanced DUSP expression, triggered SO-Rb5 cell senescence, and inhibited Akt signaling activation. DUSP1 siRNA exhibited the opposite effects. SH-6 significantly increased SO-Rb5 cell senescence induced by AGII through inhibiting Akt signaling.

CONCLUSIONS: DUSP1 facilitated human retinoblastoma SO-Rb5 cell senescence induced by AGII through inhibiting Akt signaling pathway.

Key Words:
DUSP1, Akt, Retinoblastoma, Cell senescence.

Introduction

Retinoblastoma is an important factor for the cancer death in ophthalmology[1]. However, the mechanism of retinoblastoma is still unclear. It was considered that cell growth and proliferation were enhanced in retinoblastoma. At present, the reduction of cell apoptosis and senescence are thought to be the important reasons[2]. Molecular targeted therapy is the research direction of retinoblastoma in the future. Current strategy of retinoblastoma mainly focused on cell apoptosis, while few studies investigated cell senescence in the treatment of retinoblastoma[3,4]. Cell senescence refers to the degeneration process of the cell growth, proliferation, and differentiation following cell cycle progression and time-lapse[5,6]. Cancer cell growth and viability do not decrease over time, therefore leading to excessive cell proliferation, break the balance, and cause cancer[7,8]. It was suggested that Sir-related enzymes (DUSP) protein family members play a key role in cell senescence[9,10]. DUSP family has the highly conserved amino acid sequence and similar structure. However, different DUSP members may have various functions[11,12]. The role of DUSP remains to be discussed. DUSP family proteins exhibit extensive biological functions. For example, dual specificity phosphatase 2 (DUSP2) reduced the speed of cell proliferation, while dual specificity phosphatase 1 (DUSP1) was closely associated with lung cancer metastasis[13,14]. It suggests that DUSP1 may also be involved in the occurrence and progress of retinoblastoma[15]. Therefore, this research selected human retinoblastoma SO-Rb5 cells to explore the potential mechanism of DUSP1 in retinoblastoma, which may provide theoretical basis for the choice of targeted therapy in retinoblastoma.

Materials and Methods

Retinoblastoma Cell Model and Reagents

Human retinoblastoma cell line SO-Rb5 was obtained from Microbial culture preservation Center (Beijing, China). Cell senescence reagen-

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ts were purchased from Tiangen Biotech Co. Ltd. (Beijing, China). Lipofectamine was purchased from Hualan Biology (Beijing, China). Antibiotics, medium, and fetal calf serum (FCS) were derived from Beyotime Biotech. (Shanghai, China). Antibodies were got from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Small interfere RNA (siRNA) DUSP1 (5'-GGCTACTCCACAATGTGTGC-3' and 5'-GCATGATACTTCTCAACGAGT TGCC-3') and DUSP1 over-expression plasmid were synthesized by Shanghai GenePharma Co. Ltd. (Shanghai, China). This study was approved by the Ethics Committee of Mudanjiang Medical University Hongqi Hospital (Mudanjiang, China).

Cell Senescence Modeling

Human retinoblastoma SO-Rb5 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) medium in suspension[16]. Angiotensin II (AGII) was added to cells at concentration of 10 μg/μl.

Lipofectamine Transfection

SiRNA DUSP1, DUSP1 over-expression plasmid, and control were transfected to SO-Rb5 cells using the Lipofectamine reagents according to the previously reported method[17]. SO-Rb5 cells were cultured with density at 90%. SiRNA DUSP1, DUSP1 plasmid, and control were suspended in lipo2000 and added to the cells. The medium was changed after 48 h for the following experiments.

Cell Senescence Assay

SiRNA DUSP1, DUSP1 over-expression plasmid, and control were transfected to SO-Rb5 cells using the lipofectamine reagents according to the previously reported method[18]. β-galactosidase was used to test cell senescence. The results were observed under the microscope for statistical analysis.

Western Blot Assay

SiRNA DUSP1, DUSP1 over-expression plasmid, and control were transfected to SO-Rb5 cells. The cell protein was collected for Western blot analysis. After electrophoresis, transferring membrane, blocking, and antibody incubation, the membrane was photographed on gel imaging system to compare DUSP1 and protein kinase B (Akt) expression[15].

Statistical Analysis

All data analyses were performed on SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The data were presented as mean ± standard deviation (SD). The student's *t*-test was used to compare the differences between two groups. Tukey's post-hoc test was used to validate the ANOVA for comparing measurement data among groups. $p<0.05$ was depicted as statistical significance.

Results

DUSP1 Enhanced, While Akt Signaling Blocked in SO-Rb5 Cell Senescence Model

As shown in Figure 1, p53 and p16 protein levels significantly increased in SO-Rb5 cells induced by AGII, revealing that SO-Rb5 cell senescence model was successfully established and could be used for the following experiments.

The Impact of DUSP1 Plasmid and siRNA on SO-Rb5 Cells

As shown in Figure 2, DUSP1 plasmid and siRNA transfection up-regulated and reduced DUSP1 expression in SO-Rb5 cells, respectively.

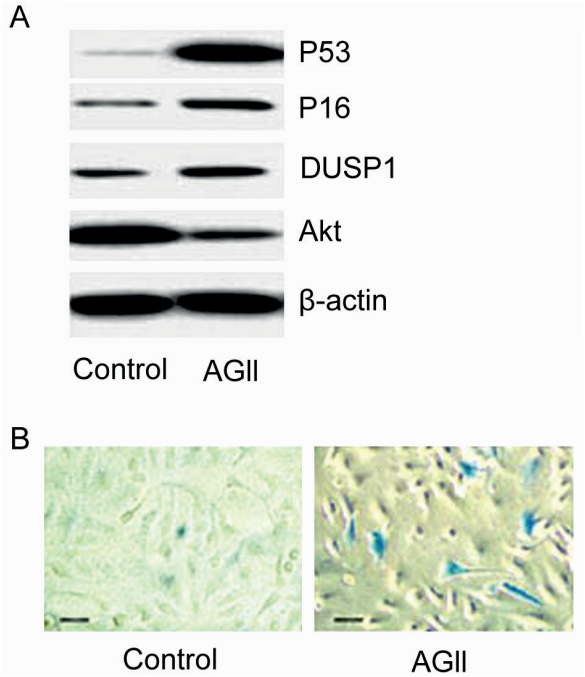

Figure 1. DUSP1 enhanced, while Akt signaling blocked in SO-Rb5 cell senescence model. **A,** Western blot images. **B,** β-galactosidase staining. DUSP1 expression was elevated in SO-Rb5 cell senescence model, suggesting that DUSP1 may be involved in retinoblastoma cell senescence induced by AGII (**A**). Akt signaling was inhibited in SO-Rb5 cell senescence model, indicating that Akt signaling may be associated with retinoblastoma cell senescence induced by AGII (**A**).

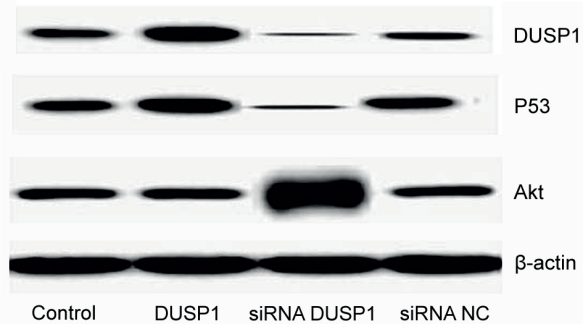

Figure 2. The impact of DUSP1 plasmid and siRNA on SO-Rb5 cells. DUSP1 plasmid and siRNA transfection enhanced and attenuated SO-Rb5 cell senescence induced by AGII, respectively, revealing that DUSP1 participated in SO-Rb5 cell senescence induced by AGII. DUSP1 plasmid and siRNA transfection inhibited and strengthened Akt signaling in SO-Rb5 cell senescence induced by AGII, respectively, revealing that DUSP1 participated in SO-Rb5 cell senescence induced by AGII.

This result suggests that DUSP1 could be used to explore the effects of DUSP1 in cell senescence.

SH-6 Restrained SO-Rb5 Cell Senescence

To investigate the role of Akt signaling in cell senescence, SH-6 was adopted to explore its effects on cell senescence. As shown in Figure 3, Western blot assay revealed that Akt signaling inhibition significantly enhanced SO-Rb5 cell senescence induced by AGII, suggesting the critical role of Akt signaling in cell senescence.

Discussion

This study adopted SO-Rb5 cell line to investigate the role of DUSP1 in retinoblastoma cell senescence from molecule and protein levels. It was showed that DUSP1 enhanced senescence biomarker expressions in SO-Rb5 cells, indicating that DUSP1 participated in cell growth and senescence[19,20]. However, the specific mechanism of DUSP1 in regulating retinoblastoma cell growth and senescence has not been fully elucidated[3]. DUSP 2 reduced tumor cell proliferation, while DUSP 1 was associated with tumor metastasis[21,22], suggesting that DUSP1 may also be involved in the occurrence and development of retinoblastoma[23-25]. Previous studies suggested that Akt protein may inhibit cell senescence[26]. The role of DUSP1 in regulating Akt and SO-the Rb5 cell growth and senescence was still unclear[27-29]. Our data showed that DUSP1 transfection reduced Akt level and elevated cell senescence rate. Inhibition of Akt enhanced SO-Rb5 cell senescence induced by AGII. Akt is recognized as cell aging inhibiting factor. We did not explore the impact of Akt on tumorigenesis. However, changing Akt level by regulating DUSP1 revealed the critical role of Akt inSO-Rb5 cell senescence induced by AGII. SH-6 markedly increased SO-Rb5 cell senescence induced by AGII through inhibiting Akt signaling. In this study, three different results verified the influence of DUSP1 and Akt protein in retinoblastoma cell senescence induced by AGII. Firstly, DUSP1, p53, and p16 expression elevated, while Akt signaling was suppressed in SO-Rb5 cell senescence model. Secondarily, DUSP1 plasmid transfection obviously enhanced DUSP expression, triggered SO-Rb5 cell senescence, and inhibited Akt signaling activation. DUSP1 siRNA exhibited the opposite effect. Thirdly, SH-6 markedly increased SO-Rb5 cell senescence induced by AGII through inhibiting Akt signaling. It suggested that DUSP1 and Akt play key roles in retinoblastoma cell senescence induced by AGII. Molecular targeting DUSP1 and Akt may be the new strategy in retinoblastoma treatmen[[26]. Akt also plays an anti-senescence role in other cancers[22-28]. It was revealed that DUSP1 induced SO-Rb5 cell senescence through down-regulating Akt[28]. There are three aspects of shortcomings. Firstly, this study did not collect retinoblastoma tumor tissue and para-carcinoma tissue in clinic to test DUSP1 and Akt protein levels by Western blot. Therefore, we failed to investigate the relationship among retinoblastoma cell senescence, DUSP1, and Akt from clinical aspect. Secondarily, this research did not enrolled retinoblastoma tissue from patients received surgery, thus failed to explore the relationship between cell senescen-

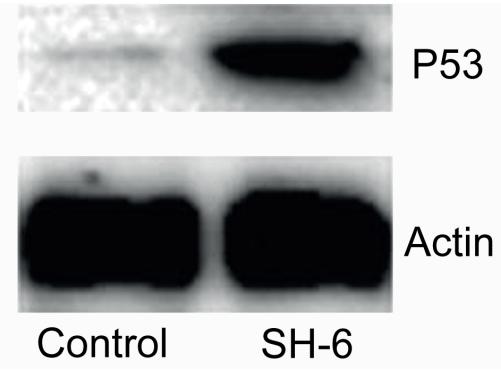

Figure 3. SH-6 enhanced SO-Rb5 cell senescence induced by AGII.

ce and retinoblastoma treatment. Thirdly, this study did not establish animal retinoblastoma model, so as to explore the curative efficacy of targeting DUSP1 on retinoblastoma at animal level.

Conclusions

We found that DUSP1 promoted human retinoblastoma SO-Rb5 cell senescence induced by AGII via inhibiting Akt signaling pathway. It may provide new strategy for the treatment of retinoblastoma. Akt may be a potential treatment target for retinoblastoma.

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Conflict of Interest

The Authors declare that they have no conflict of interest.

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