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DUSP22 promotes senescence of HS-1 skin cancer cells through triggering MAPK signaling pathway

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Abstract. – OBJECTIVE: Skin cancer severely threatens the public health. Dual-specificity protein phosphatase 22 (DUSP22) characterizes with multiple roles regulating cell growth, proliferation and gaining. This study aims to investigate the regulatory role of DUSP22 on aging of skin cancer cells and clarify molecular mechanisms, along with potential application value.

PATIENTS AND METHODS: HS-1 skin c... cells were transfected with DUSP22 by lip... transfection approach. Western blot assay... s used to evaluate the effects of DUSP22 on a... protein of HS-1 cells, and activation of mi... gen-activated protein kinase cas... (MAPK... signal pathway. Real-time PC... R) and Western blot assay were us... o ex... he the DUSP22 expression in HS-1... ncer ce... Mean-while, the correlation betw... P53... aging was also investig... d.

RESULTS: Transfe... of DU... plasmid significantly elevate... SP22 expre... in HS-1 skin cancer cell... ed to un-tra... fected cells (p<0.05), ar... ctivat... APK to induce cell aging. Transfe... ion of smal... fere RNA (siRNA) DUSP22... nificantly supp... sed DUSP22 expressio... HS-1 c...ncer cells, inhibited MAPK signal pa... ay, a... decreased aging proteins P53 and... co... red t... e untreated group (p<0.05). D... was ... nregulated in HS-1 skin... cer c... wit... APK signal pathway in... nd lo... ng protein P53 expression. DU... 22 expr... sion was positively correlated w... protein P53 (p<0.05).

... DUSP22 facilitated HS-1 s... cer cell aging via activating MAPK signal p... ay, possibly providing novel strategy agains... cancer.

Key Words
DUSP22, MAPK, Skin cancer cells, Cell aging.

In...duction

...kin cancer is one major death reason in ...matology, ...th 0.07% incidence and can ...erely affect... blic health. Skin cancer has a ...plicated m...genesis mechanism[1,2]. Recent stu... d that abnormal cell proliferation and de... ed number of aging cells are one major determinant factor for skin cancer. Early ... is critical for skin cancer. Classical ...eatments have satisfactory, but with various weakness and flaw. For example, there are various complications such as lower appetite, mental refractory, body fatigue, nausea and vomiting, skin vessel bleeding may occur after chemo- or radio-therapy[5,6]. The strategy to improve precision of skin cancer treatment and successful rate is major challenge. In clinical, precise medication is important for skin cancer treatment, although the selection of target is the major challenge. Therefore, more effective molecular drug targets are required in clinics for skin cancer[7,8]. Recently dual-specificity protein phosphatase 22 (DUSP22) has become the potential treatment target for multiple tumors. DUSP22 gene is one member of non-receptor protein tyrosine phosphatase family sharing similar amino acid sequence. DUSP22 protein has phosphatase activity, especially for facilitating activation of mitogen activating protein (MAP)[9,10]. Therefore, DUSP22 may exert important response of cells targeting environmental threaten via negative regulation, to maintain its normal function[11]. DUSP22 has pluripotent functions, such as the inhibition of esophageal cancer cell growth or relation with tumor metastasis[12-14]. These studies suggested the possible involvement of DUSP22 in tumor

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occurrence and progression. The participation of DUSP22 in skin cancer occurrence or progression, however, is still unclear. Current researches[15,16] showed that cell aging may exert important roles in occurrence and progression of skin cancer. Cell aging is one progressive aging process during cell cycle, which is frequently accompanied with elevated expression of aging related proteins such as P21 and P53, and blue staining of cells in beta-galactosidase, which has become one major index detecting cell aging[17,18]. However, molecules of cell aging that directly affect skin cancer occurrence are still unclear. Therefore, this work used HS-1 skin cancer cell model, on which the possible regulatory function of DUSP22 on HS-1 skin cancer cells was investigated for clinical implication.

Patients and Methods

Reagent, Cell Model and Clinical Samples

P53 and P21 test antibody for cell aging was purchased from Beyotime Biotech. (Shanghai, China). Fetal bovine serum (FBS) and cell culture medium were purchased from Biological Engineering Inc., (Xinxiang, China). Liposome cell transfection reagent was produced by Invitrogen/Life Technologies (Carlsbad, CA, USA). 3-(4,5-dimethyl-2-thiazolyl)-5-diphenyl-2-H-tetrazolium bromide (MTT) for testing cell viability was purchased from Ding Tech. (Beijing, China). Other reagents were from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Small interference RNA (siRNA) DUSP22 (5'-ATAAT CGCAG TC CAC 3'; and 5'-AATAA GAAC GAC GGTA A-3') and control siRNA (5'-CC TATGA GGACG CTTGC-3' and TTATG TC GCACA GAT-3'), and DUSP22 plasmids were purchased from Shanghai Pharm Co. Ltd. (Shanghai, China) to amplify target genes. Expression vectors were used to a SP22 protein. Skin cancer cell model HS-1 was purchased from American Microbe Stock (MA, USA). Based on inclusive exclusive criteria of skin cancer[19,20], we selected cancer patients from October 2016 to Sep 2 the Fifth Affiliated Hospital of Medical University (Daqing, Heilongjiang, China). We selected 50 patients and 50 controlled volunteers aging between 46 and 80 years (average age = 66.5±6.5 years). This study fitted Ethical requirements and all subjects (including skin cancer patients and healthy volunteers) signed informed consents. Among 50 skin cancer patients, 11 of them received surgery. Tumor was resected 0.5 cm at basal carcinoma site from tumor edge based on tumor size, infiltration degree. Skin graft was then performed at surgical site. This study was approved by the Ethics Committee of The Fifth Affiliated Hospital of Harbin Medical University (Daqing, Heilongjiang, China).

Cell Culture

HS-1 skin carcinoma cells were resuscitated and re-suspended in Dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum culture medium[21].

Transfection

Liposome transfection reagent (Huamei Bio, Beijing, China) was used to transfect DUSP22 and controlled plasmid into HS-1 skin cancer cells following routine methods. Cells were cultured at 70% density. In brief, 2 μl (1 μg/μl) DUSP22 and controlled plasmid were re-suspended in Lipo2000 liposome for transfection and cell culture.

Immunoblotting of Cell Aging

HS-1 skin cancer cells after transfecting DUSP22 or siRNA were extracted for protein following manual instruction of protein extraction kit. Protein sample was prepared for quantification by micro-plate reader to separate proteins by centrifugation. Protein samples were analyzed by electrophoresis. After Tris-buffered saline and Tween-20 (TBST) rinsing, primary antibody was added for 4°C overnight incubation at 1:1000 dilution. TBST was used to rinse mouse originated primary antibody (1:2000 for actin, 1:1000 for DUSP22, 1:1000 for MAPK, 1:1000 for P53 and 1:1000 for P21, Santa Cruz Biotech., Santa Cruz, CA, USA). Secondary antibody (1:2500, Santa Cruz Biotech., Santa Cruz, CA, USA) was added for 37°C for 3 h. TBST was then used for rinsing, followed by development in enhanced chemiluminescence (ECL) substrate. 5% defatted milk powder was used to block, anti-MAPK primary antibody and anti-mouse IgG secondary antibody were sequentially used for incubation. After three times of rinsing, horseradish peroxidase (HRP) was added for staining. Gel imaging apparatus was used for protein quantification by grey value analysis. Gel imaging system (Qinxinag, China) was used for capturing pictures and to analyze protein expression level. MAPK expression level of MAPK in HS-1 cells was compared among groups[23].

MAPK Activity Assay

Aging of HS-1 cell cancer cells was described by MAPK activity kit (Beyotime Biotech., Shanghai, China) following routine method. In brief, HS-1 skin cancer cells were cultured and transfected with DUSP22 or controlled plasmid used methods abovementioned. HS-1 skin cancer cells after transfecting DUSP22 or controlled plasmid were lysed for chromogenic reaction using p protein as the substrate. Model 545 microplate reader was used for 20 min incubation in triplicates. Under room temperature, HS-1 skin cancer cells were place in 24-well plate, for measuring absorbent values of each group on micro-plate reader[24]. MAPK relative activity was calculated as follows: using absorbent values of DUSP22 controlled plasmid transfected cells as the basal level, control values subtracted activity of DUSP22-transfected cells.

Effects of Interference or Over-Expression of DUSP22 on Aging of HS-1 Skin Cancer Cells

To test the effect of interference or over-expression of DUSP22 on aging of HS-1 cancer cells, Lipo2000 liposome transfection reagent was used to transfect HS-1 cells with DUSP22 siRNA or DUSP22 over-expression plasmid following the manual instruction of transfection kit. Cultured cells were transfected using 2 μl (0.5 μg/μl) siRNA DUSP22 or DUSP22 plasmid suspension in Lipo2000 followed by further culture.

Real-Time PCR Assay

Real-time PCR was performed to test DUSP22 level in skin tissues following routine method. Reaction system and conditions followed the manual instruction of test kit. Primer sequences were: 5'-CGAAT CTCCT GCGAC AGTGG-3' and 5'-AGGA CGAAT CGAAG AGTGC-3'; 5'-CTCA AGA GGTTC GGGTC GCACA GT G-3' and 5'-CTCAA CTGTC G TGAAT GGATT C-3'.

Statistical Analysis

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

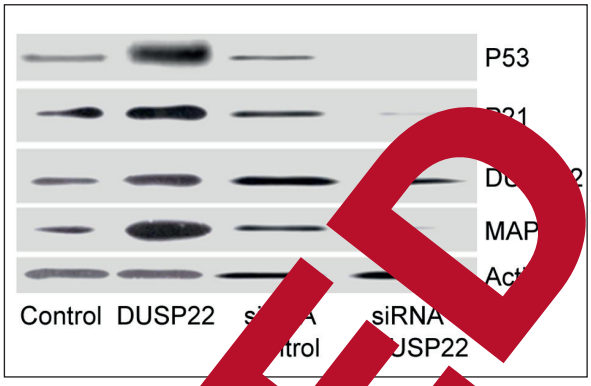

Figure 1. Effects of DUSP22 plasmid or siRNA-DUSP22 plasmid transfection on expression levels of MAPK signal pathway and aging proteins P53 or P21.

Results

Effects of DUSP22 Plasmid or siRNA-DUSP22 Plasmid Transfection on Expression Levels of MAPK Signal Path and Aging Proteins P53 or P21

As Figure 1, transfection of DUSP22 plasmid significantly elevated DUSP22 expression level in HS-1 skin cancer cells, activating MAPK inducing cell aging. Transfection of siRNA DUSP22 significantly depressed DUSP22 expression level in HS-1 skin cancer cells, inhibiting MAPK signal pathway and decreasing expression levels of aging proteins P53 and P21.

Real-Time PCR Results of DUSP22 in Skin Cancer Patient's Skin Tissues

Figure 2 showed decreased DUSP22 level in skin tissues of skin cancer patients compared to those in healthy volunteers ($p<0.05$). After treatment, DUSP22 level in skin tissues was elevated, indicating possible relationship between DUSP22 level and occurrence or prognosis of skin cancer.

Western Blot Results for DUSP22 Protein in Skin Tissues of Skin Cancer Patients

The functional mechanism of DUSP22 in skin cancer remains to be further illustrated. As shown in Figure 3, Western blot results showed lower DUSP22 level in skin cancer tissue ($p<0.05$ compared to skin of healthy volunteers). After treatment, DUSP22 level in skin tissues was elevated, indicating possible correlation between DUSP22 level and occurrence or prognosis of skin cancer.

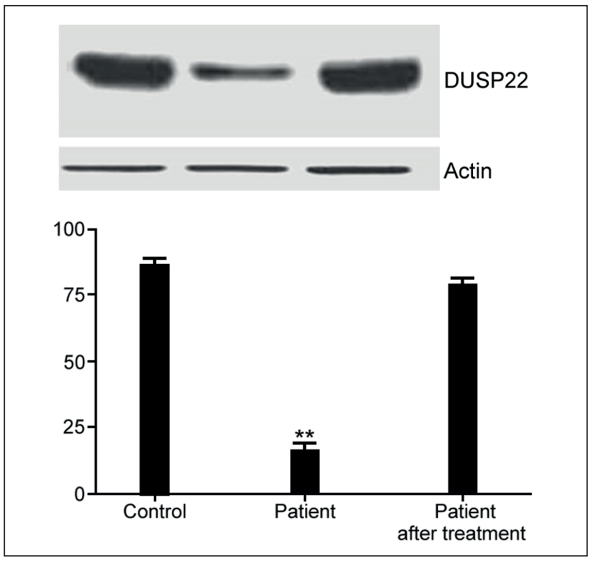

Figure 2. Real-time PCR results of DUSP22 in skin cancer patient's skin tissues. **p<0.01 compare to control group.

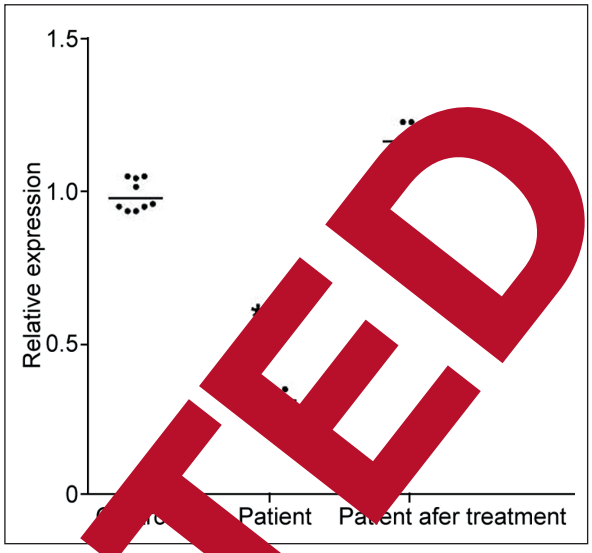

Figure 3. Western blot results of DUSP22 in skin tissues from skin cancer patients. **p<0.01 compared to control group.

Western Blot Results for Skin Tissue Aging of Skin Cancer

Cell aging is accompanied with alternation of aging proteins, especially P21 and P53 proteins with elevated expression. To further investigate the role of cell aging in skin cancer, we explored expression level of aged proteins in skin cancer tissues. As shown in Figure 4, Western blot results showed inhibited cell aging in skin cancer tissues compared to those from healthy volunteers, in addition to higher levels of proteins P21 and P53.

Results of DUSP22 Level and Aging Protein Level in Skin Cancer Tissues

To further explore the correlation between DUSP22 level and aging protein P53 in skin cancer tissues, Pearson correlation analysis was used to analyze the correlation between skin cancer tissue DUSP22 level and aging protein P53/P21 levels. As shown in Figure 5 and Figure 6, Pearson correlation analysis revealed close correlation between DUSP22 level in skin cancer tissues and aging proteins P53 and P21 levels.

Discussion

Skin cancer severely threatens public health, making the early diagnosis and assay as one major challenge for both clinicians and researchers[25]. We investigated the possible correlation between DUSP22 and skin cancer, hoping to provide valuable information for disease test. DUSP22 has dual roles in mediating cell growth, proliferation and aging. This study indicated that DUSP22 may facilitate cell aging.

Currently important molecules for skin cancer diagnosis include CD44, ECM protein

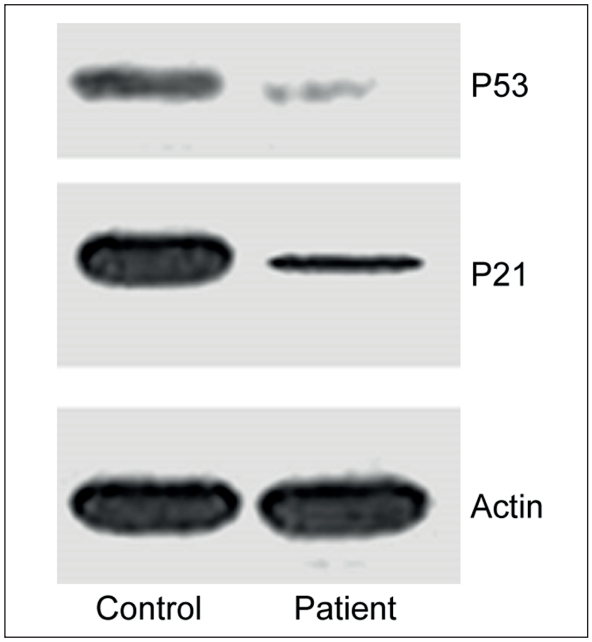

Figure 4. Western blot results of tissues aging in skin cancer patients.

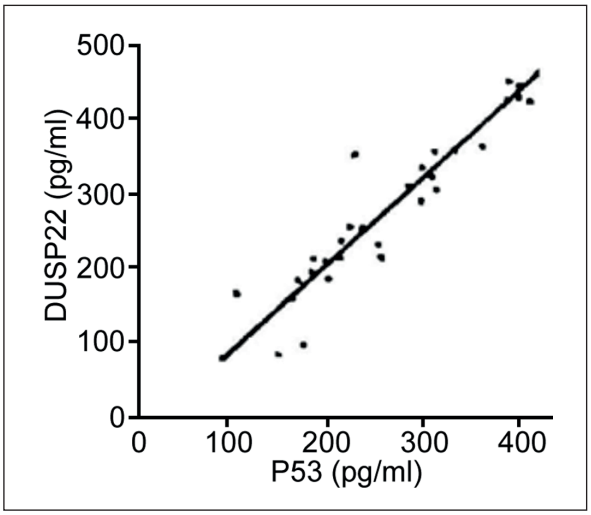

Figure 5. Test results of DUSP22 level in skin cancer tissues and aging protein P53 level.

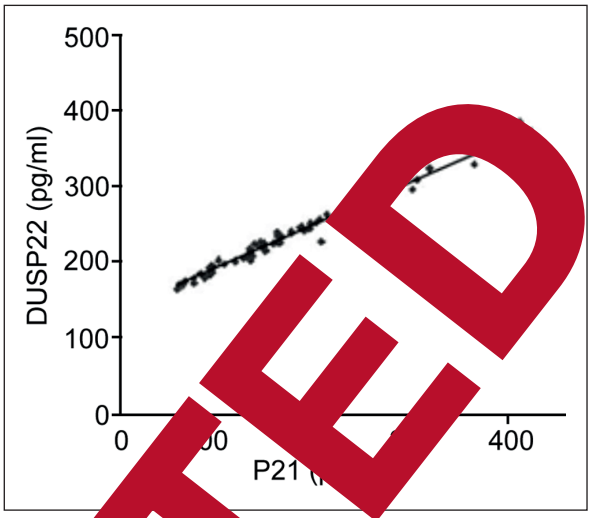

Figure Test resu kin cancer tissue DUSP22 level and aging protein P21 e on.

laminin α5 and proteoglycan. DUSP22 is closely correlated with various physiopathological processes in clinics[26,27]. For example, DUSP22 is closely correlated ith occurrence and progression of digestiv cancer, whilst DUSP14 and DUSP3 are i d with inflammatory response and prognosi multiple tumors including esophageal carcino and lung cancer. These researche [14-16] indicat certain relationship betwee 2 and disease onset or progressio makin it one potential molecular mark flecti onset and progression. The echanism of DUSP22 in skin ca rema be further elucidated. This wor lized HS-1 cancer cell as the model h regulato ole of DUSP22 on HS- in ca cells and possible mechanism w investigat om molecular and protein l s. Data showed transfection of DUSP2 used ing of HS-1 skin cancer cells, as sister th previous studies, which also sugg partic ion of DUSP22 in cell aging[4]. K p n can facilitate cell as urre whether MAPK is under SP22 ulation further mediation of HS-1 in can ll growth remains unclear[24,28]. We ve ection of DUSP22 elevated M level. After transfecting DUSP22 and enha MAPK expression, HS-1 skin cancer cell agi e was elevated. On the other hands, transfection of siRNA DUSP22 inhibited MAPK activity, suppressing HS-1 skin cancer cell aging. This study for the first time demonstrated

se facts: (1) nsfection of DUSP22 plasmid ficantly ated DUSP22 expression in HS r cells, and also activated MAPK for cel g. (2) Transfection of DUSP22 siRNA ignificantly decreased DUSP22 expression in in cancer cells, thus suppressing MAPK gnal pathway for decreasing expression level of aging proteins P53 and P21. (3) DUSP22 is down-regulated in HS-skin cancer cells, accompanied by suppression of MAPK signal pathway and lower P53 expression level. (4) DUSP22 is positively correlated with expression level of aging protein P53. This study has certain weakness. Firstly, relatively smaller sample size (including tissue number of skin cancer) may bias the conclusion. Further analyses should thus expand samples size including both cancer tissues and control tissues for further substantiation[26]. Secondarily, although skin DUSP22 level is correlated with cell aging protein P53 expression, no direct evidence has been shown at animal or individual levels. Thirdly, this work did not classify skin cancer patients based on tumor severity for further investigating correlation between DUSP22 level and cell aging protein P53 expression level.

Conclusions

We showed that DUSP22 could facilitate HS-1 skin cancer cell aging via activating MAPK signal pathway, possibly providing novel strategy for anti-skin cancer.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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