Dosage Suppressor of NNF1(DSN1) Promotes Tumour Progression of Oral Squamous Cell Carcinoma(OSCC) Through Remaining High Level of Chromosome Instability

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Abstract

Introduction: Dosage suppressor of NNF1 (DSN1) is a component of the kinetochore protein complex which is critical for normal chromosome segregation. Researches showed that DSN1 was upregulated in colorectal carcinoma and hepatocellular carcinoma. However, the biofunction and potential mechanism was still unknown.

Materials and methods: We searched The Cancer Genome Atlas (TCGA) database and analyse DSN1 expression pattern in head and neck carcinoma. qRT-PCR and western blot were applied to measure the expression level of DSN1 in OSCC patients and OSCC cell lines. The biological influence of DSN1 was studied using CCK-8, colony formation assay, Transwell migration and invasion assays, the chromosome instability and DNA repair ability were also detected.

Results: DSN1 was upregulated in OSCC tissues in both TCGA database and our in-house database. The proliferation, migration and invasion ability decreased in Cal-27 cell line after knocking down of DSN1. The epithelial-mesenchymal transition was activated in DSN1 overexpressed cells. DSN1 confers radio-resistance to OSCC and DSN1 remains high level of chromosome instability and low level of DNA repair.

Conclusion: Taken above, our results indicate that the upregulated DSN1 is positively associated with prognosis in OSCC. Our findings indicate that DSN1 promotes OSCC cell proliferation, migration and invasion and radio resistance and remains OSCC with high level of chromosome instability.

Introduction

Oral cancer is the sixth most malignancy worldwide and oral squamous cell cancer represents 90% during all the subtypes[1]. Current therapy was only effective for early stage OSCC and the 5-year overall survival was less than 50%[2]. Studies indicated that tobacco addiction and excessive alcohol consumption and HPV infection were the most responsible factors contributing to the tumorigenesis and development of OSCC[1, 3]. Current target therapy was far from satisfactory currently. New predicting biomarker and therapy target was still in urgent need.

Chromosome instability was reported to contribute to the program of many kinds of cancers including OSCC[4, 5]. It was partially responsible for the heterogeneity and chemo-resistance[6]. Mis12 was reported to play key roles in the microtubule formation during mitosis. It is a component of kinetochores which was composed of NNF1 NSL1 and DSN1. DSN1 was crucial for the assembly of the Mis12 complex[7, 8]. DSN1 was reported to be involved in the progression and prognosis of many cancers[8]. However, the potential function and the underlying mechanism in OSCC was still unknown.

In this study, we analysed the expression level of DSN1 in OSCC patients and uncover its' biological functions and underlying mechanism in OSCC cell lines. Our results demonstrated for the first time that
DSN1 is upregulated in OSCC tumours. Knocking down of DSN1 could repress BC cell proliferation, migration and invasion.

Results

**DSN1 is upregulated in human OSCC and correlate with poor prognosis**

We scanned the RNA sequencing datasets from TCGA and identified DSN1 was one of the dysregulated genes in Head and Neck cancers which was never studied before (Fig. 1A, N = 44, T = 519, P < 0.05). We next detect the expression level of DSN1 in randomly selected 20 paired normal tissues and cancer tissues from our in-house database. The results showed that DSN1 was upregulated in cancers compared with the normal tissues (Fig. 1B, n = 20, P < 0.001). We then divided the patients into 4 subtypes according to the TNM stage. Results indicated that patients with developed stage of oral cancers harbour higher level of DSN1 (Fig. 1C, P < 0.001). We next apply immunoblot to detect DSN1 in 4 randomly picked paired normal tissues and cancer tissues, DSN1 was upregulated in all cancers (Fig. 1D). We next detect the RNA level and protein level of DSN1 in OSCC cell lines and normal epithelial cells. DSN1 was upregulated in both RNA level and protein level in OSCC cell lines (Fig. 1E, P < 0.001, Fig. 1F). We next applied overall survival analysis of the 20 patients. Patients were divided into two groups according to the mean RNA level of the whole cohort. Patients with higher level of DSN1 suffer from early death (Fig. 2).

**DSN1 promotes the proliferation of OSCC.**

We previously indicated that DSN1 was upregulated in OSCC cancer tissue. To uncover the bio-function of DSN1 in the progression of OSCC cells, according to the different expression pattern of OSCC cell lines. We established DSN1 stable knocking down and overexpression cell lines in Cal-27(Sh-1/Sh-2) and SCC-9(OV) cell lines. The relative RNA level was detected in Fig. 3A (P < 0.001) and protein level was detected using immune blot in Fig. 3B. We next detect the proliferation ability in the cells described above and found that cells with lower level of DSN1 developed restrict proliferation ability in both CCK-8 assay (Fig. 3C, P < 0.001) and colony formation assay (Fig. 3D,3E,P < 0.001), indicating that DSN1 promotes the proliferation in both short and long time.

**DSN1 promotes the migration and invasion and EMT of OSCC.**

Migration and invasion were one of the most important features of cancer cells. It helped cancer cells migrates to distant metastases, invaded into the normal tissue and escape from immune surveillance. We next uncover the biofunction of DSN1 on migration and invasion. We previously indicated that DSN1 was upregulated in OSCC cancer tissue. To uncover the bio-function of DSN1 in the migration and invasion of OSCC cells, we applied wound healing assay to measure the 2D migrates ability and trans-well assay and invasion chamber assay to measure the 3D migrates and invasion ability. The results indicated that cells
with higher level of DSN1 healed more quickly and migrates and invades more easily (Fig. 4A-E, p < 0.001). Epithelial mesenchymal translation was one of the key features when cancer cells migrate and invade. We next detect the EMT markers and found that mesenchymal markers such as N-cadherin, Snail, Slug and Vimentin decreased while the epithelial marker E-cadherin increased with the knocking down of DSN1 (Fig. 5A).

**DSN1 confers radio-resistance to OSCC cells**

We next treat the OSCC cells with Infrared Radiation and detect the apoptosis rate using flow cytometry and detect apoptosis rate accordingly. Results showed that DSN1 inhibited the apoptosis rate of cells (Fig. 6A,B). We next detect the apoptosis markers in the cells described above. The activate form of the caspase cascade increased, indicating that the cells were more sensitive to IR with the knocking down of DSN1 (Fig. 6C).

**Knocking down of DSN1 reduced the chromosome instability and promotes DNA repair reprogram**

DSN1 was critical for the assembling of kinetochores which plays key role in chromosome instability. We next detect the chromosome instability (CIN) related genes by qPCR and analysed the weighted score as CIN score[9]. CIN score was lower in DSN knocking down cell line and increased in DSN1 overexpression cells (Fig. 7A, P < 0.001). We next detect the microtubule polymerisation rate, which was crucial for the separate of the chromosome[10, 11]. Results showed that the microtubule polymerisation rate increased to meet the higher chromosome instable demands (Fig. 7B, P < 0.001), additionally, PALB2 and CHK1 was lower in DSN1 knocking down cell lines. We next detect the active form of CHK1 and ATR, which were important check point for Intra-S-phase after chromosome instability happened and was also important for the repair of DNA damage[12, 13]. p-CHK1 and p-ATR increased in DSN1 overexpress cell lines (Fig. 7C). Taken together, our results indicated that DSN1 increased the chromosome instability and DNA repaired ability.

**Conclusion**

DSN1 is upregulated in OSCCs and positively associated with poor prognosis. Our findings indicate that DSN1 promotes OSCC cell proliferation, migration and invasion and radio resistance and remains OSCC with high level of chromosome instability which responsible for the tumor cancer survival.

**Discussion**

OSCC was one of the most common malignant worldwide, although many risk factors such as alcohol addiction and HPV infections were reported to be engaged in the tumorigenesis and progression of OSCC, the exact mechanism of the tumorigenesis was still unknown.
Chromosomal instability was one of the most crucial factors contributing to the progression of tumours and may be the most responsible reason for the heterogeneity of tumours. The Long Noncoding RNA CCAT2 induced Chromosomal instability and promote the progression of colorectal cancers[14]. BUB1 Overexpression was reported to Promotes Mitotic Segregation Errors and Chromosomal Instability in myeloma[15].

Apoptosis was a highly programmed cell death which was regulated by multi signallings such as cell stress, DNA damage and immunity surveillance. Chemo-therapy and radio-therapy were apoptosis based therapy. However, cancers cells developed chemo-therapy resistance and radio-therapy resistance by reducing the apoptosis program. Oncogene activation and tumor suppressor mutation contributes to the anti apoptosis program of OSCC. GanT61 inhibits the chemo-therapy resistance by reducing the GLI1 expression[16], Ilimaquinone induced cancer cell apoptosis to exert its’ anti-tumour therapy function[17].

In this research, we analysed the expression pattern of DSN1 in OSCC and its’ correlation with the prognosis of patients and found that DSN1 overexpression could promote the EMT of OSCC and confers radio-therapy resistance to OSCC cells.

Methods

Patient information and clinical sample collection

Totally, 20 paired OSCC samples and paired normal tissues were randomly collected from patients who underwent surgery at the The Affiliated Stomatological Hospital of Nanchang University, between January 2012 and December 2013. All OSCC tissue samples were confirmed by pathology. This study was approved by the Ethics Committee of The Affiliated Stomatological Hospital of Nanchang University. Clinical samples were collected from patients after written and informed consent was obtained.

Cell Culture And Cell Transfection Assay

HaCaT and CAL 27 were cultured with complete DMEM (Sigma) medium containing 10% fetal bovine serum (Invitrogen) and 1% penicillin–streptomycin while other cell lines were cultured with complete DMEM-F12 medium.

Western Blotting

Western blotting

Equal protein was separated using 12% SDS-PAGE gel. After transferring and blocking, the membranes were then incubated with primary antibodies, washed with TBST and then incubated with secondary antibodies (anti-rabbit IgG) for 1h at room temperature. Target proteins were detected using the ECL (EMD Millipore, MA, USA) method.
Cell Proliferation Assay

500 Cells per well were seeded into 96-well plates. The absorption values of different cell lines were detected at 0,1,2,3 days. The experiments were repeated three times, and the data are shown as the mean ± standard deviation (SD).

Colony Formation Assay

To measure long-term effects, certain cells were seeded in a six-well plate. After incubating for 14 days, colonies were stained with crystal violet (Sigma-Aldrich, St. Louis, MO, USA), and the number of colonies was counted. The CFA assay was applied for three times and the data were shown as the mean ± standard deviation (SD).

Statistical analysis

All data analyses were performed with SPSS 20.0 statistical software. The differences between groups were compared by Student’s $t$-test. Kaplan-Meier methods were used to analyse OS, and $p < 0.05$ was considered to be a significant difference from the control.

Declarations

Ethics approval and consent to participate

Studies were performed conforming to the Declaration of Helsinki and got approval the Ethics Committee of The Affiliated Stomatological Hospital of Nanchang University. Clinical samples were collected from patients after written informed consent was obtained.

Consent for publication

Not applicable

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Competing interests

The authors declare no competing interests.

Availability of data and materials

All data was available in this article.

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Author contribution

WW designed the article. All the authors were engaged in the experiments application, data collection and analysis, literal writing and editing.

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**Figures**
Figure 1

DSN1 was upregulated in OSCC: A: The relative level of DSN1 in the TCGA database (Student’s two-tailed paired test * p<0.05). B: The relative level of DSN1 in our in-house database. (Student’s two tailed paired test*** p<0.001). C: The relative level of DSN1 of patients with different TNM stages in our in-house database. (Student’s two tailed paired test*** p<0.001). D: The protein level of DSN1 in 4 paired normal tissue and cancer tissues. E: The relative RMA level of DSN1 in OSCC cell lines. (Student’s two tailed test *** p<0.001). F: Western blot of DSN1 in the OSCC cell line.
DNS1 was negatively correlated with the prognosis of OSCC patients. Patients were divided into two groups according to the mean RNA level of the whole cohort. The overall survival analysis was applied to measure the predicting value of DSN1.
DSN1 promotes the proliferation of BC

A: The relative level of DSN1 in Cal-27 knocking down cell line and SCC-9 overexpression cell line. (Student's two tailed paired test*** p<0.001)

B: Western blot of DSN1 in different cell lines.

C: The relative viability of different cell lines (Student's two tailed paired test*** p<0.001)

D: The representative image of colony formation assay of Cal-27 and SCC-9 cell lines.

E: The analysis of colony formation assay of Cal-27 and SCC-9 cell lines.

Figure 3
Figure 4

DSN1 promotes the migration and invasion of OSCC A: The representative image of wound healing assay of Cal-27 and SCC-9 cell line and the corresponding statistical analysis in B(Student's two tailed paired test*** p<0.001). C: The representative image of migration and invasion chamber assay and the statistical analysis in D, E (Student's two tailed paired test*** p<0.001).
Figure 5

DSN1 promotes the EMT and Wnt pathway in OSCC. A: The Western blot of EMT markers in different cell lines.
Figure 6

DSN1 confers radio-resistance to OSCC cell lines. A: The flow cytometry detecting apoptosis in different cell lines. B: The apoptosis rate in different cell lines. (Student's two tailed paired test*** p<0.001) C: Western blot of apoptosis markers in different cell lines.
Figure 7

DSN1 induced translation stress and increased DNA repair ability A: The CIN scores in different cell lines (Student's two tailed paired test*** p<0.001). B: The microtubule polymerisation rate in different cell lines. (Student's two tailed paired test*** p<0.001) C: Western blot of DNA repair markers in different cell lines.

C

|         | Cal-27 | SCC-9 |
|---------|--------|-------|
|         | NC     | NC    |
| PALB2   | 150    |       |
| CHK1    | 55     |       |
| ATR     | 300    |       |
| pCHK1   | 55     |       |
| pATR    | 300    |       |
| β-actin | 42     |       |