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CSN5/Jab1 facilitates non-small cell lung cancer cell growth through stabilizing survivin

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ABSTRACT

CSN5/JAB1 is a critical subunit of the COP9 signalosome (CSN) and is essentially involved in diverse types of cancer, but little is known about the role of CSN5 in non-small cell lung cancer (NSCLC). In the current study, we found that CSN5 expression was higher in NSCLC tissues compared to the corresponding non-tumor tissues. High CSN5 expression level is closely correlated with tumor progression and poor survival in NSCLC patients. We also found that knockdown of CSN5 remarkably suppressed cell growth by inducing cell cycle arrest and apoptosis promotion in NSCLC cells. Mechanistic investigations revealed that CSN5 directly bound survivin and decreased its ubiquitination to enhance the protein stability of survivin. Additionally, our results confirmed that the tumor-promoting effects of CSN5 in NSCLC cells is at least partly through stabilization of survivin. Overall, our data suggested that CSN5 functions as an oncogenic gene in NSCLC, which could be a potential diagnostic and therapeutic target for NSCLC.

1. Introduction

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer and the leading causes of cancer induced death [1]. Despite advances in the early diagnosis and treatment of NSCLC, the prognosis of NSCLC patients remain poor, with the five-year overall survival rates currently at 15% [2]. It is of paramount importance to understand the underlying pathological mechanisms contributing to NSCLC for developing novel therapeutic strategies and improving the prognosis of NSCLC [3,4].

CSN5, the fifth CSN subunit, was initially identified as c-Jun-activation-domain binding protein-1 (Jab1) that mediates AP-1-dependent gene transcription via stabilization of c-Jun [5,6]. Due to this function, CSN5 is involved in the control of various cellular processes, such as cell proliferation and apoptosis [7,8]. Recently, CSN5 overexpression was linked to various cancer including breast cancer [9], hepatocellular carcinoma [10] and renal cell carcinoma [11]. Additionally, target inhibition of CSN5 has potential for anti-tumor therapy [12]. However, the expression pattern and potential role of CSN5 in NSCLC remain unclear.

Here, we found that high CSN5 level is significantly associated with progression and poor prognosis in NSCLC patients. We also found that CSN5 silence inhibited cell growth by inducing cell cycle arrest and apoptosis promotion in NSCLC cells. Subsequent mechanistic studies demonstrated that CSN5 exerted its function by stabilizing survivin, an anti-apoptosis protein in various cancers [13–15]. Thus, CSN5 may serve as a predictor and a potential therapeutic target for NSCLC patients.

2. Materials and methods

2.1. Patients and tumor specimens

Matched cancerous and noncancerous tissues were obtained from 102 patients with NSCLC in the Department of General surgery, The General Hospital of The People's Liberation Army, immediately snap-frozen in liquid nitrogen, and stored at −80 °C for RNA and protein extraction. A pathologist confirmed that all the specimens were derived from normal tissues. Informed consent was obtained from the patients, and the research procedure was approved by the Ethics Committee of The General Hospital of The People's Liberation Army.

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2.2. Cell lines and cell culture

Four NSCLC cell lines (H226, H292, H460 and A549) and the immortalized normal human bronchial epithelial cell line (BEAS-2B) were obtained from ATCC (Rockville, Md., USA). The NSCLC cells were maintained in RPMI-1640 (HyClone) supplemented with 10% FBS (Gibco) and antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin) (Invitrogen). BEAS-2B cells were maintained in BEGM (Lonza). All cell lines were maintained in an incubator with a humidified atmosphere of 95% air and 5% CO_2 at 37 °C.

2.3. RNA extraction and quantitative real-time PCR (qRT-PCR)

The relative RNA levels of genes were assessed by quantitative real-time PCR. In brief, total RNA was isolated with the standard TRIzol-based protocol (Invitrogen, USA). RNA was reverse transcribed using the PrimeScript RT Reagent Kit (Invitrogen, USA) and qPCR was performed using SYBR Premix Ex Taq (TaKaRa, China), following the manufacturer's instructions. The gene-specific primers were as follows: CSN5 (sense: 5′-AGCGAGGTAAAGTTGCGTCT-3′, antisense: 5′- AGGTTTTCTGGGCCATACCG-3′), GAPDH (sense: 5′-CTGGGCTACACTGAGCACC-3′, antisense: 5′-AAGTGGTCGTTGAGGGCAATG-3′).

2.4. Western blot assay

Western blot analysis for specific protein expression was performed as previously described [16]. The antibodies used were as follows: anti-CSN5 (1:1000, Santa Cruz), anti-survivin (1:1000, Abcam), anti-Ub (1:100, Abcam) and anti-GAPDH (1:1000, Santa Cruz). The signals were detected by enhanced chemiluminescence (Pierce, USA).

2.5. Constructs and plasmids

The RNA duplexes for shRNA-mediated CSN5 silencing (sh-CSN5) were synthesized by Genepharma Company (Shanghai, China). Full-length human CSN5 or survivin cDNA was compounded by Genepharma and ligated into pcDNA3.1 vector (*p*-CSN5 or *p*-survivin). An empty vector was used as the negative control. Transfections of the sh-CSN5, negative control shRNA (sh-NC), *p*-CSN5, *p*-survivin and negative vector in NSCLC cells were performed using Lipofectamine 2000 Transfection Reagent (Invitrogen, USA) following the manufacturer's recommended protocol.

2.6. Cell proliferation and cell cycle assays

For cell proliferation assays, multiple cultures of NSCLC cells were plated in 96-well plates at a density of 5.0×10^3 cells/well. At the indicated time points, viable cells were tested by cell counting kit-8 (CCK-8) according the manufacturer's instructions. For the colony formation assay, NSCLC cells (at the density of 1000 cells per well) were seeded into 6-well plates. After 2 weeks, cells were washed with phosphate buffered saline (PBS), stained with 0.1% crystal violet for 15 min.

For cell cycle assays, the PC cells stable silenced NEAT1 or negative control (2×10^5 cells/well) were seeded in a 6-well plate. After 48 h incubation, the cells were fixed in 70% ethanol at 4 °C overnight. Then, the cells were analyzed by using a Cycletest™ Plus DNA Reagent Kit (BD, USA) following the manufacturer's protocol.

2.7. Cell apoptosis analysis

The apoptosis rate of cells was tested by Apoptosis Detection Kits (BD, USA) following the manufacturer's instructions. In brief, 5×10^5 cells were harvested by centrifugation at 1000*g* for 5 min and resuspended in 200 μL binding buffer, followed by a 15 min incubation with 5 μL Annexin V-FITC and 5 μL propidium iodide (PI) in the dark at 37 °C. Then, the flow cytometry analysis was employed for detecting apoptotic events.

2.8. Co-immunoprecipitation (Co-IP) and in vivo ubiquitination assay

Co-immunoprecipitation assay was performed as previously described [17]. For *in vivo* ubiquitination assay, NSCLC cells were transfected with or without constructs. The transfected cells were incubated for 30 h and then exposed to MG132 (15 mmol/L) treatment for 4 h before harvesting. The cell lysate was immunoprecipitated with anti-survivin antibody, and the ubiquitination of survivin was detected with an anti-Ub antibody.

2.9. Xenograft nude mouse model

To examine the effect of CSN5 expression in tumor formation, male BALB/c nude mice (4–6 weeks old) were used for all *in vivo* xenograft studies. All animal experiments were approved by the Animal Care and Use Committee of The General Hospital of The People's Liberation Army. Exponentially growing cells were injected subcutaneously into the flanks of nude mice (2×10^6 cells per animal). Tumors size were measured every 5 days by caliper to determine tumor volume using the formula: $V = [\text{length}/2] \times [\text{width}^2]$. All mice were killed 35 days after seeding of tumor cells, and the tumor weights measured.

2.10. Statistical analysis

All data are expressed in terms of means ± the SEM. Significant differences were analyzed using Student's t-test and two-tailed distribution. The Kaplan-Meier method was used to calculate the survival curve, and log-rank test to determine statistical significance. P values < 0.05 were considered statistically significant.

3. Results

3.1. Expression of CSN5 was up-regulated in NSCLC tissues

To investigate the role of CSN5 in NSCLC, we first explored the expression pattern of UBR5 in human NSCLC tissues by qRT-PCR and Western blotting. As shown in Fig. 1A, CSN5 expression was significantly up-regulated in 68.6% (70/102) NSCLC tissues compared with non-tumor tissues. The Western blotting results also revealed that elevated expression of CSN5 was observed in NSCLC tissues compared with corresponding adjacent non-tumor tissues (Fig. 1B and C).

Then, we analyzed the relationship between CSN5 expression and clinicopathological parameters in NSCLC patients detected. As shown in Table 1, high CSN5 expression was significantly associated with larger tumor size ($p = 0.017$), advanced TNM stage ($p = 0.025$) and lymph node metastasis ($p = 0.017$). Furthermore, the Kaplan-Meier survival curves (Fig. 1D and E) revealed that high level of CSN5 expression had poor overall and disease-free survival than patients who had low level of CSN5 expression. Moreover, Kaplan–Meier survival analysis was performed using GEPIA (http://gepia.cancer-pku.cn/) for a TCGA Lung cancer (LUAD) dataset (478 patient samples). As shown in Fig. 1F, high expression of CSN5 were significantly negatively correlated with patient overall survival (Logrank $p = 0.012$), suggesting potentials of CSN5 as prognostic indicators. Collectively, these data suggest that the expression of CSN5 is significantly upregulated in NSCLC tissues

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