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Original Article

NOVA1 acts as an oncogene in osteosarcoma

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Abstract: Osteosarcoma is one of the most common bone tumors in young patients. NOVA1 (neuro-oncological ventral antigen 1) is a neuron-specific RNA binding-protein and belongs to the Nova family. Previous studies showed that NOVA1 played crucial roles in the development of several tumors. The objective of our study was to study the role of NOVA1 in the osteosarcoma. In our study, we showed that NOVA1 expression was upregulated in osteosarcoma cell lines and tissues. The expression of NOVA1 was upregulated in 22 (22/30; 73%) osteosarcoma cases compared to that in the adjacent tissues. Overexpression of NOVA1 promoted osteosarcoma cell viability, colony formation and invasion. Furthermore, knockdown of NOVA1 suppressed osteosarcoma cell viability, colony formation and invasion. These data suggested that NOVA1 acted as an oncogene in the development of osteosarcoma.

Keywords: Osteosarcoma, RNA binding-proteins, NOVA1, oncogene

Introduction

Osteosarcoma is one of the most common bone malignant tumors in young patients [1-4]. Despite the development of various treatment strategies which combine multiagent chemotherapy, surgery and radiotherapy, the five year survival rate of osteosarcoma patients is still unsatisfied [4-8]. About 80% of osteosarcoma patients will develop metastatic disease or local relapse after surgical treatment [9-12]. The detail molecular mechanisms underlying osteosarcoma progression and carcinogenesis remain uncovered [13-16]. Hence, it's urgent to find novel markers and develop new treatment strategies for osteosarcoma.

NOVA1 (neuro-oncological ventral antigen 1) is a neuron-specific RNA binding-protein and one member of Nova family [17-22]. It was first identified in a neurologic disease characterized by failure of the motor suppression [23]. NOVA1 is proved to play significant roles in the development of motor system and motoneurons survival [23]. It can regulate the alternative process of various mRNAs that is crucial for synaptic activity [24]. Recent evidences have showed that NOVA1 plays crucial roles in the development of several tumors [18, 21, 25-27]. For example, Kim et al [20]. demonstrated that NOVA1 expression was inhibited in the microenvironment of gastric cancer, and attenuated NOVA1 expression in the gastric cancer cells was correlated with cancer progression and poor prognosis. Shen et al [21]. showed that miR-339 was upregulated in gastric cancer tissues and ectopic expression of miR-339 inhibited gastric cancer cell invasion, migration and viability through targeting NOVA1. Ectopic expression of NOVA1 in the miR-339-overexpressing gastric cancer cells impaired the inhibitory function of miR-339. However, the role of NOVA1 in osteosarcoma is still unknown.

In this study, we demonstrated that NOVA1 expression was upregulated in osteosarcoma cell lines and tissues. Overexpression of NOVA1 promoted osteosarcoma cell viability, colony formation and invasion. Furthermore, Knockdown of NOVA1 suppressed osteosarcoma cell viability, colony formation and invasion. These data suggested that NOVA1 acted as an oncogene in the development of osteosarcoma.

Materials and methods

Specimens and cell lines cultured and transfection

Surgically excised tissues from 35 cases with osteosarcoma tissues and adjacent normal tissues were collected in the Heilongjiang provincial hospital. All the samples were collected after obtaining the written informed consent

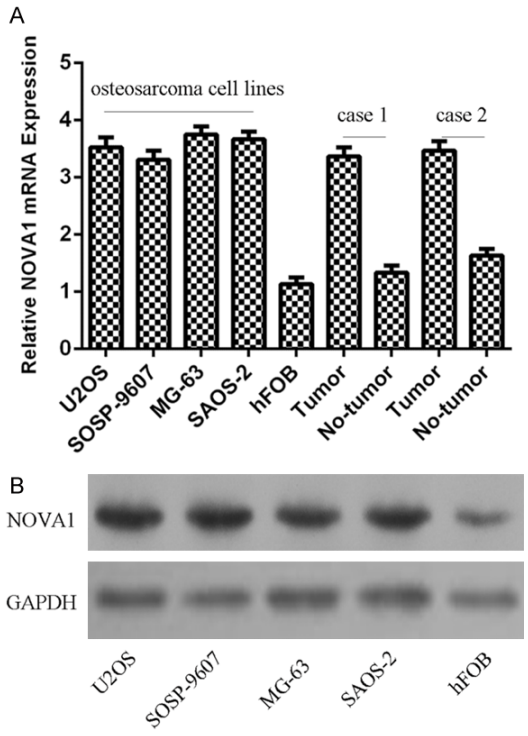

Figure 1. NOVA1 expression was upregulated in osteosarcoma cell lines. A. The mRNA expression of NOVA1 in the osteosarcoma tissues and cell lines was determined by using qRT-PCR. B. The protein expression of NOVA1 was determined in the osteosarcoma cell lines (MG-63, U2OS, SOSP-9607 and SAOS-2) and hFOB by using Western blot.

following to the protocol was approved by the Institutional Review Board of the Heilongjiang provincial hospital. The tissues were snap-frozen in the liquid nitrogen and stored. The following osteosarcoma cell lines were used in our study: MG-63, U2OS, SOSP-9607 and SAOS-2 and osteoblast cell line (hFOB). These cells were collected from the Cell bank Center of the Institute of the Chinese Academy of Medical Sciences (Beijing, China) and were cultured in the DMEM (Dulbecco's modified Eagle's medium; Gibco; Invitrogen) supplemented with FBS (fetal bovine serum; GIBCO, USA), streptomycin, and penicillin. NOVA1 vector and the control vector were purchased from the GenePharma (Shanghai, China). The cells were transfected with vector using the Lipofectamine 2000 (Invitrogen, USA) according to the instruction.

Real-time quantitative PCR (qPCR)

Total RNA from cells or tissues was extracted by using the Trizol reagent (Invitrogen, CA, USA) according to the instruction. qRT-PCR was performed to detect the mRNA expression of NOVA1 and GAPDH on the ABI 7500 System (ABI) using the SYBR Green Mix (Takara). Ct ($2^{-\Delta\Delta Ct}$) method was used to measure the relative expression of each group. The following sequences: NOVA1, (forward: GGGTTCCCATAGACCTGGAC; reverse: CGCTCAGTAGTACCTGGGTAA); GAPDH, (forward: TGCACCACCAACTGCTTAGC; reverse: GGCATGGACTGTGGTCATGAG).

Western blotting

Cells or tissues were lysed with lysis buffer and the total proteins concentration was measured by using the BCA Protein Assay. Total protein was separated on the 12% SDS-PAGE and transferred to PVDF membranes (Bio-Rad). The membrane was blocked with FBS for 1 hour and then incubated the primary antibodies (NOVA1, GAPDH, Sigma, USA) overnight and band was visualized by the chemiluminescence.

Viability, invasion, colony formation assay

Cell viability was detected by the MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Twenty μl MTT solution was added to the cell culture medium for about 4 hours incubation. Then, the absorbance of each well was measured at 450 nm. For cell invasion assay, Matrigel coated transwell chambers (Millipore, USA) was used. Cells were cultured in the serum-free medium on the upper chamber and medium containing FBS as the chemo-attractant was put to the bottom well. The invasive cell was stained with the crystal violet after 48 hours. For colony formation assay, cells were cultured on the 6-well plate and kept in the DMEM medium containing 10% FBS for 2 weeks. Colony was fixed and stained and the number of colony was counted.

Statistical analysis

Statistical analysis was measured by the SPSS version 17.0 (Chicago, IL, USA). Data are presented as the mean ± SD (standard deviation). $P<0.05$ is considered to be statistically significant. The difference between groups was determined using Student's t-test or one-way ANOVA.

Results

NOVA1 expression was upregulated in osteosarcoma cell lines

We firstly detected the NOVA1 expression level in osteosarcoma cell lines. Our result showed

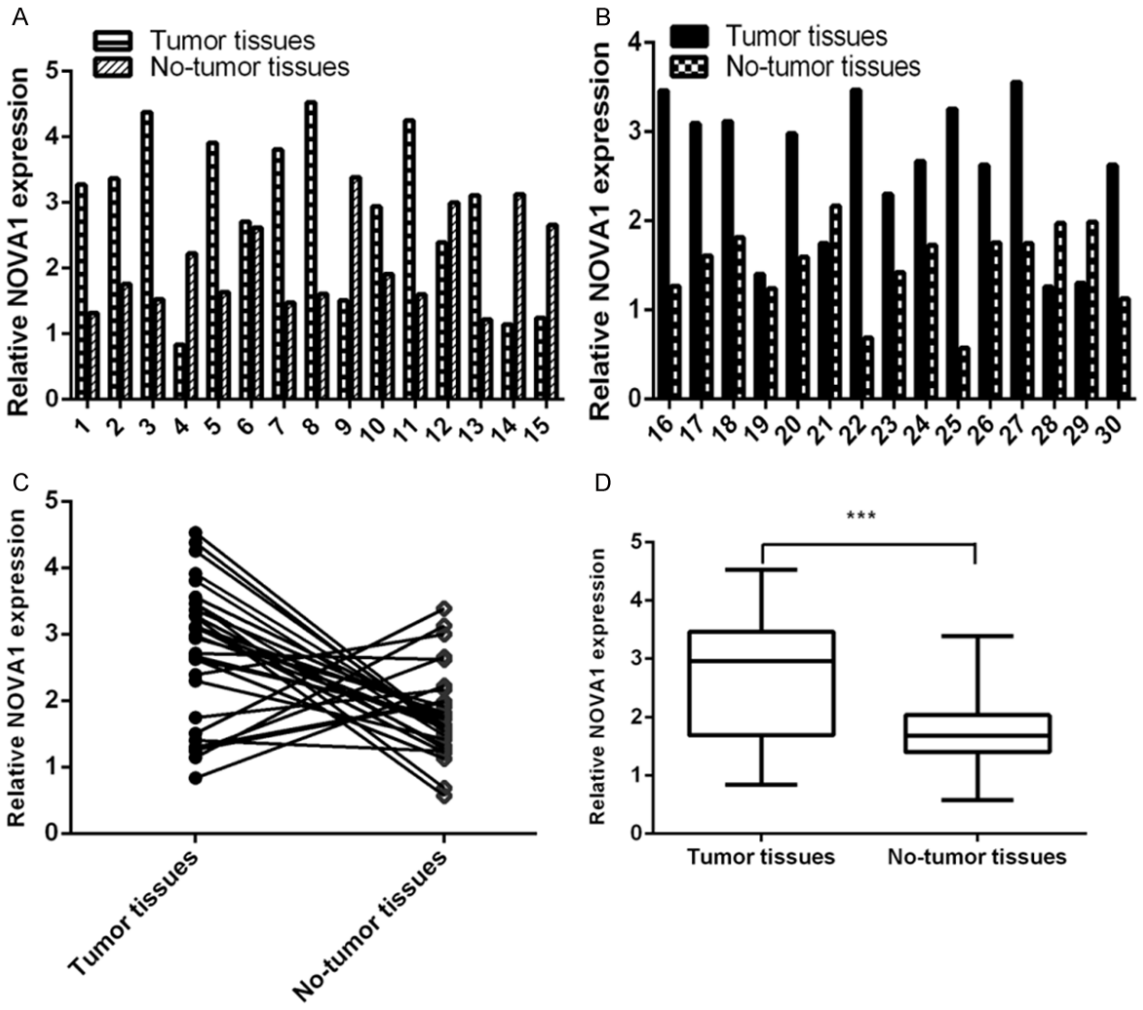

Figure 2. NOVA1 expression was upregulated in osteosarcoma tissues. A. The expression of NOVA1 in the osteosarcoma tissues was detected by using qRT-PCR. B. The expression of NOVA1 in the osteosarcoma tissues was detected by using qRT-PCR. C. The expression of NOVA1 was upregulated in 22 cases (22/30; 73%) of osteosarcoma cases compared to that in adjacent tissues. D. The expression level of NOVA1 in osteosarcoma tissues was higher than that in the adjacent tissues. ***P<0.001.

that the expression level of NOVA1 was upregulated in osteosarcoma cell lines (MG-63, U2OS, SOSP-9607 and SAOS-2) and tissue compared to that in the osteoblast cell line (hFOB) (**Figure 1A**). Moreover, the NOVA1 protein expression was also upregulated in the osteosarcoma cell lines (MG-63, U2OS, SOSP-9607 and SAOS-2) compared to that in the hFOB (**Figure 1B**).

NOVA1 expression was upregulated in osteosarcoma tissues

We next measured NOVA1 expression in 30 osteosarcoma cases. The expression of NOVA1 was shown in the **Figure 2A** and **2B**. The expression of NOVA1 was upregulated in 22 cases (22/30; 73%) of osteosarcoma cases compared to that in adjacent tissues (**Figure 2C**). The expression level of NOVA1 in osteosarcoma tissues was higher than that in the adjacent tissues (**Figure 2D**).

Overexpression of NOVA1 promoted osteosarcoma cell viability

To study the biological function of NOVA1 in osteosarcoma cell, MG-63 cell was tranfected with NOVA1 vector and control vector. The mRNA and protein expression of NOVA1 was upregulated in the MG-63 cell after treated with NOVA1 vector (**Figure 3A** and **3B**). Ectopic expression of NOVA1 promoted the MG-63 cell

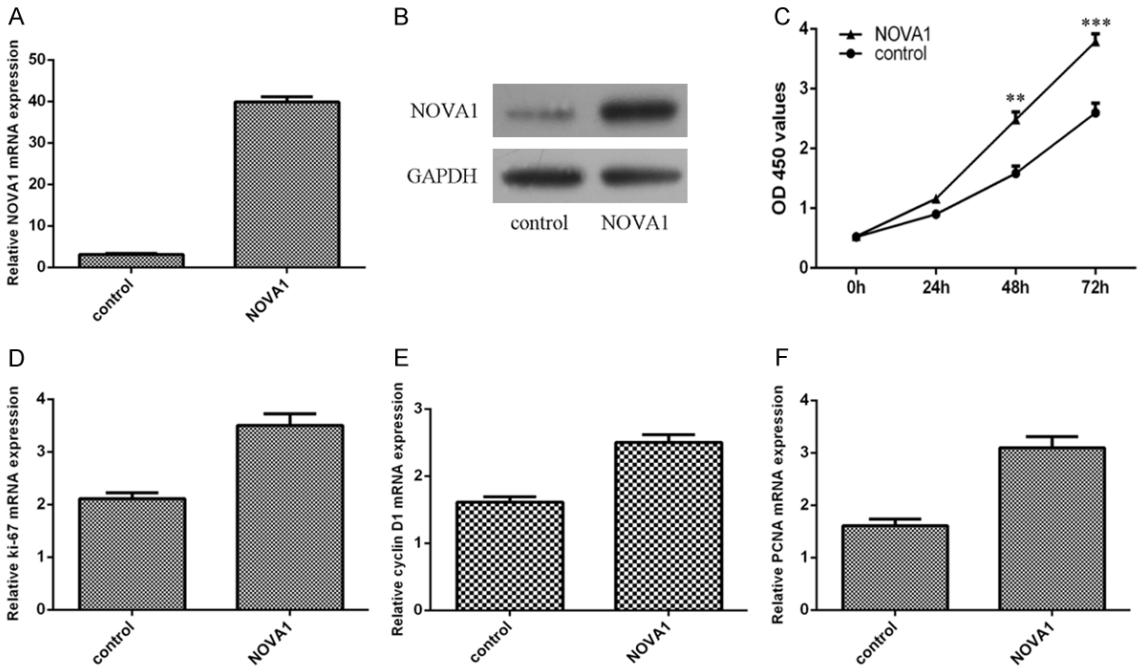

Figure 3. Overexpression of NOVA1 promoted osteosarcoma cell viability. A. The mRNA expression of NOVA1 in the MG-63 cell after treated with NOVA1 vector was measured by qRT-PCR. B. The protein expression of NOVA1 in the MG-63 cell after treated with NOVA1 vector was measured by Western blot. C. Ectopic expression of NOVA1 promoted the MG-63 cell viability. D. The expression of ki-67 was determined by qRT-PCR. E. The expression of cyclin D1 was determined by qRT-PCR. F. The expression of PCNA was determined by qRT-PCR. **P<0.01 and ***P<0.001.

viability (**Figure 3C**). Moreover, overexpression of NOVA1 increased the expression of ki-67, cyclin D1 and PCNA (**Figure 3D-F**).

Overexpression of NOVA1 promoted osteosarcoma cell colony formation and invasion

Ectopic expression of NOVA1 increased osteosarcoma cell colony formation (**Figure 4A**). In addition, we showed that overexpression of NOVA1 promoted the MG-63 cell invasion by using invasion assay (**Figure 4B**).

Knockdown of NOVA1 suppressed osteosarcoma cell viability

To furtherly detect the biological function of NOVA1 in osteosarcoma cell, MG-63 cell was tranfected with si-NOVA1 vector and control vector. The mRNA and protein expression of NOVA1 was downregulated in the MG-63 cell after treated with si-NOVA1 vector (**Figure 5A** and **5B**). Inhibition expression of NOVA1 suppressed MG-63 cell viability (**Figure 5C**). Moreover, knockdown of NOVA1 decreased the expression of ki-67, cyclin D1 and PCNA (**Figure 5D-F**).

Knockdown of NOVA1 suppressed osteosarcoma cell colony formation and invasion

Knockdown of NOVA1 decreased osteosarcoma cell colony formation (**Figure 6A**). In addition, we showed that inhibition expression of NOVA1 suppressed the MG-63 cell invasion through using invasion assay (**Figure 6B**).

Discussion

In this study, we demonstrated that NOVA1 expression was upregulated in osteosarcoma cell lines and tissues. The expression of NOVA1 was upregulated in 22 cases (22/30; 73%) of osteosarcoma cases compared to that in adjacent tissues. The expression level of NOVA1 in osteosarcoma tissues was higher than that in the adjacent tissues. Overexpression of NOVA1 promoted osteosarcoma cell viability, colony formation and invasion. Furthermore, Knockdown of NOVA1 suppressed the osteosarcoma cell viability, colony formation and invasion. These data suggested that NOVA1 acted as an oncogene in the development of osteosarcoma.

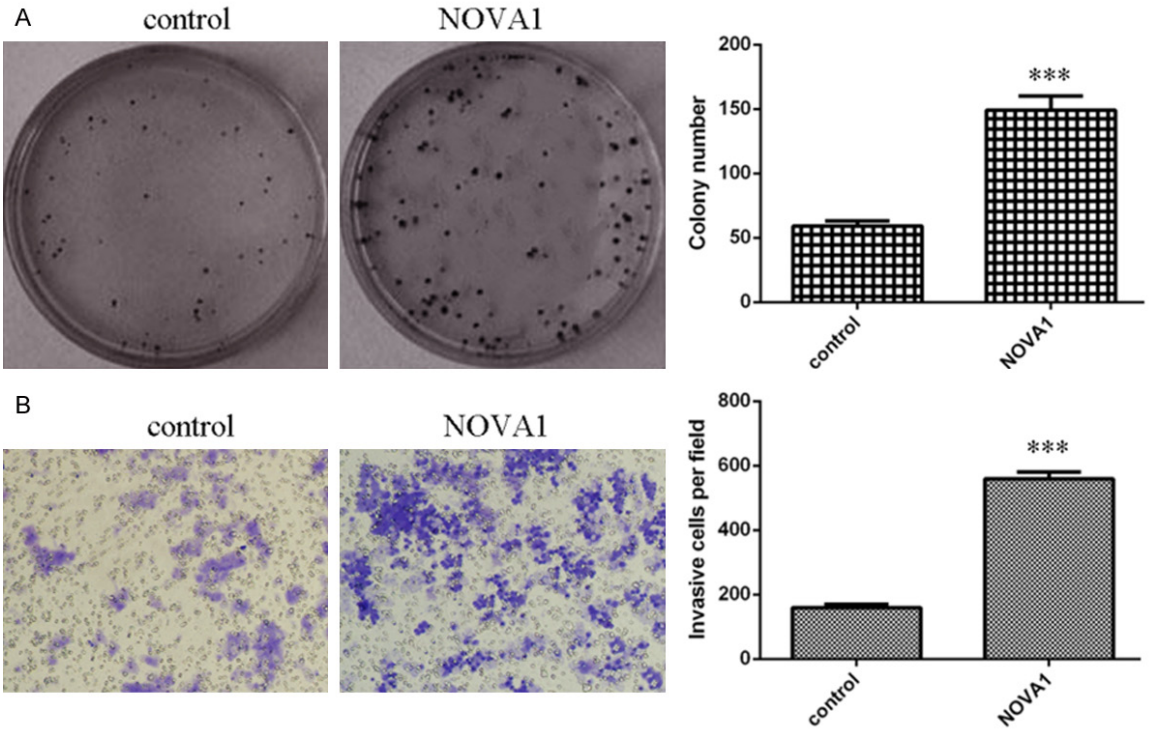

Figure 4. Overexpression of NOVA1 promoted osteosarcoma cell colony formation and invasion. A. Ectopic expression of NOVA1 increased osteosarcoma cell colony formation. The relative colony number was shown in the right. B. Overexpression of NOVA1 promoted the MG-63 cell invasion by using invasion assay. The relative invasive cell was shown in the right. ***P<0.001.

NOVA1 is one member of the Nova family, which was firstly identified as the target in autoimmune neurologic diseases characterized through the failure of motor suppression [23]. NOVA1 was essential for development of the mammalian motor system and survival of motoneurons through regulating the alternative process of various mRNAs that is crucial for synaptic activity [28-30]. Recent studies have demonstrated that NOVA1 plays important roles in the development of various tumors [17, 19, 26, 31, 32]. For example, Kim et al [20]. showed that NOVA1 inhibition was found in the microenvironment of gastric cancer and attenuated NOVA1 expression was correlated with gastric cancer progression and poor prognosis. Yoon et al [18]. showed that NOVA1 was enriched in stromal spindle cells and T lymphocytes, while NOVA1 expression was frequently downregulated in those types of cells and gastric cancer tissues. Shen et al [21]. demonstrated that miR-339 overexpression suppressed gastric cancer cells migration, viability, tumorigenicity and invasion through inhibiting NOVA1. Zhang et al [26]. demonstrated that higher NOVA1 expression was correlated with increased recurrence rate and poor survival of hepatocellular carcinoma. Overexpression of NOVA1 promoted hepatocellular carcinoma cell invasion, migration and viability. However, the role and biology function of NOVA1 are still unknown. In this study, we firstly detected the expression of NOVA1 in osteosarcoma cell lines. Our result demonstrated that the expression level of NOVA1 was upregulated in osteosarcoma cell lines (MG-63, U2OS, SOSP-9607 and SAOS-2) and tissue compared to that in the osteoblast cell line (hFOB). Furthermore, we measured the NOVA1 expression in the 30 osteosarcoma cases. The expression of NOVA1 was upregulated in 22 cases (22/30; 73%) of osteosarcoma cases compared to that in adjacent tissues. The expression level of NOVA1 in osteosarcoma tissues was higher than that in the adjacent tissues.

We next investigated the function role of NOVA1 in osteosarcoma cell. Ectopic expression of NOVA1 promoted osteosarcoma cell line MG-63 cell viability. In line with this, we show-

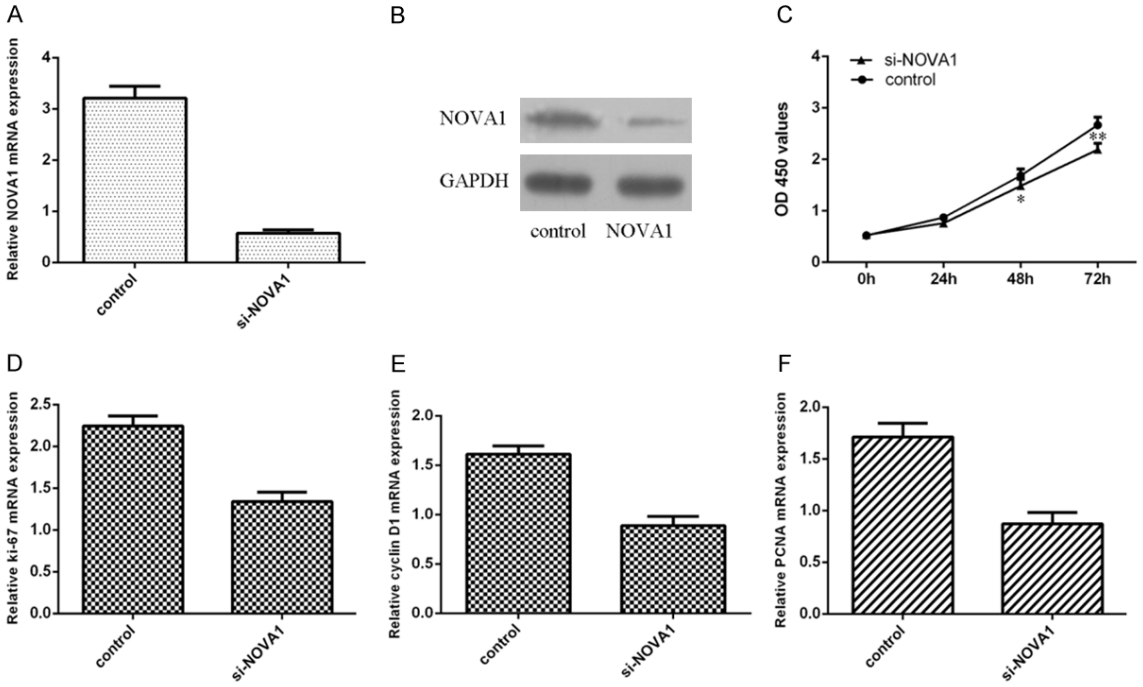

Figure 5. Knockdown of NOVA1 suppressed osteosarcoma cell viability. A. The mRNA expression of NOVA1 in the MG-63 cell after treated with si-NOVA1 was measured by qRT-PCR. B. The protein expression of NOVA1 in the MG-63 cell after treated with si-NOVA1 vector was measured by Western blot. C. Inhibition expression of NOVA1 promoted the MG-63 cell viability. D. The expression of ki-67 was determined by qRT-PCR. E. The expression of cyclin D1 was determined by qRT-PCR. F. The expression of PCNA was determined by qRT-PCR. *P<0.05 and **P<0.01.

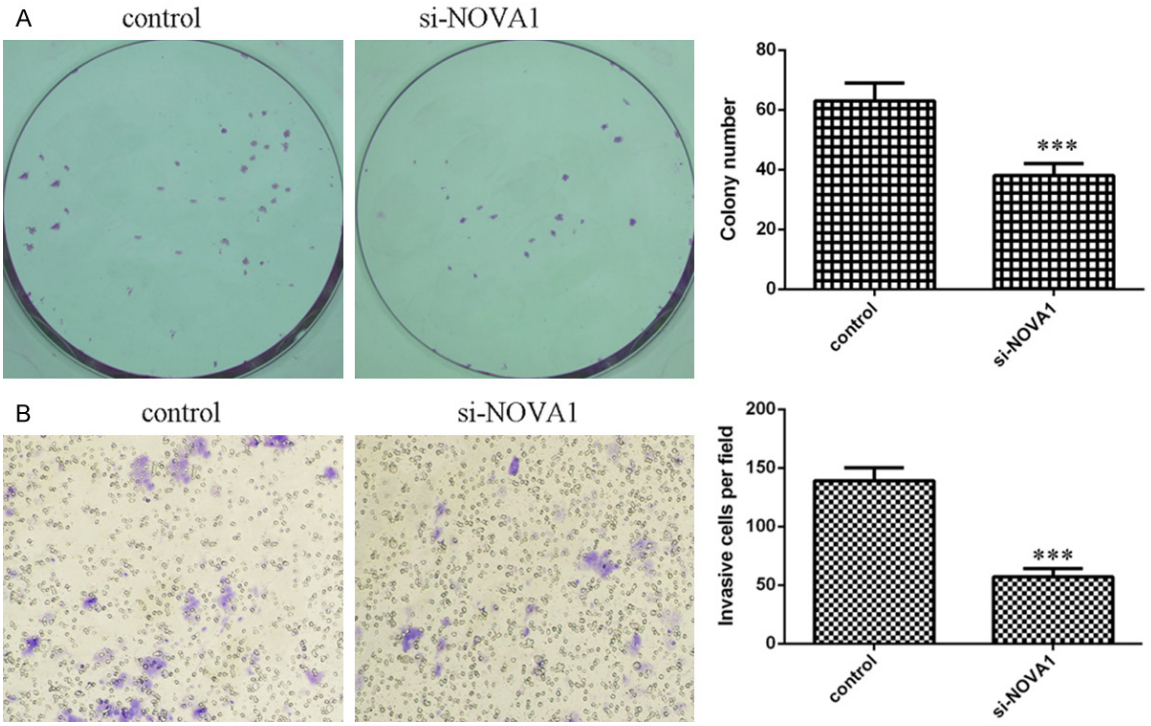

Figure 6. Knockdown of NOVA1 suppressed osteosarcoma cell colony formation and invasion. A. Knockdown of NOVA1 decreased osteosarcoma cell colony formation. The relative colony numer was shown in the right. B. Inhibition expression of NOVA1 suppressed the MG-63 cell invasion through using invasion assay. The relative invasive cell was shown in the right. ***P<0.001.

ed that overexpression of NOVA1 increased the expression of ki-67, cyclin D1 and PCNA. Furthermore, we demonstrated that overexpression of NOVA1 promoted the MG-63 cell colony formation and invasion. In addition, we showed that knockdown of NOVA1 suppressed osteosarcoma cell line MG-63 cell viability. We also showed that inhibition of NOVA1 decreased the expression of ki-67, cyclin D1 and PCNA. In addition, we demonstrated that suppression of NOVA1 inhibited MG-63 cell colony formation and invasion. These results suggested that NOVA1 played as an oncogene in the development of osteosarcoma.

In conclusion, NOVA1 acted as an oncogene in the initiation and development of osteosarcoma. Given that overexpression of NOVA1 promoted osteosarcoma cell viability and invasion in the osteosarcoma, NOVA1 may be a potential therapeutic target for osteosarcoma.

Disclosure of conflict of interest

None.

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