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Original Article

NEDD9 is a novel prognostic marker in gastric cancer patients

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Abstract: Objective: Our previous studies have shown that NEDD9 was an important oncogene, which upregulated in gastric cancer development and progression. However, the prognostic value of NEDD9 in gastric cancer is rare. The aim of this study was to evaluate the association between NEDD9 expression and survival in gastric cancer patients. Methods: The expression of NEDD9 in gastric cancer and matched adjacent non-tumor tissues was measured by immunohistochemistry. Results: We found that NEDD9 was highly expressed in gastric cancer tissues, but weakly expressed in adjacent non-tumor tissues. Further analysis of the clinicopathological characteristics showed that NEDD9 staining was significantly correlated with TNM stage and degree of differentiation. Besides, patients with a higher NEDD9 expression had a significantly shorter median survival time (MST). Conclusion: Our findings suggest that NEDD9 might prove to be a novel molecular biomarker for the predicting progression with gastric cancer patients.

Keywords: NEDD9, gastric cancer, prognosis, immunohistochemistry

Introduction

Neural precursor cell expressed, developmentally downregulated 9 (NEDD9), also known as human enhancer of filamentation-1 (HEF1) or Crk-associated substrate lymphocyte type (Cas-L), was first cloned from the brain of embryonic mice in 1992 [1, 2]. In recent years, experiments have shown that NEDD9 expressed in several tumor cells, and associated with tumor proliferation, apoptosis, differentiation and invasive ability [3-7]. In our previous study, we have shown that NEDD9 was an important oncogene, which often upregulated in gastric cancer development and progression. Furthermore, the expression of NEDD9 could induce abnormal expression of vimentin and E-cadherin. Based on these results, we speculated that knockdown of NEDD9 expression in gastric cancer might provide a novel therapeutic strategy [8]. However, the prognostic value of NEDD9 has not been evaluated before. Therefore, in this study, we show the expression of NEDD9 in large sample gastric cancer and the relationship between the clinicopathological characteristics and prognosis.

Materials and methods

Clinical specimens and patient data

Gastric cancer tissue samples were obtained from 435 patients undergoing a gastroectomy for gastric cancer at the Third Affiliated Hospital of Soochow University between January 2011 and December 2011 and were confirmed by a pathologist. Cancer tissues were cut in wedge shapes and normal tissues resection margin is at least 5 cm away from tumor. All of the gastric cancer patients were not treated with preoperative chemotherapy or radiotherapy. The patients provided informed consent for their participation in the study, which was approved by the Ethical Committee of Soochow University, China. TNM staging and clinicopathological classification were determined according to the National Comprehensive Cancer Network (NCCN-2015v3). Patients were followed-up annually by telephone or at outpatient clinic till May, 2016 or death.

Immunohistochemistry

Immunohistochemistry (IHC) was performed according to the previously described methods

Table 1. Demographic and pathologicalparameters of gastric cancer patients

Parameters		Number (%)
Sex	Male	310 (71.3%)
	Female	125 (28.7%)
Age (y)	≥60	240 (55.2%)
	<60	195 (44.8%)
Borrmann	I	30 (7.9%)
	II	269 (71.0%)
	III	68 (17.9%)
	IV	12 (3.2%)
pT	T1	56 (12.9%)
	T2	82 (18.8%)
	T3	194 (44.6%)
	T4	103 (23.7%)
pN	N0	84 (19.3%)
	N1	167 (38.4%)
	N2	169 (38.8%)
	N3	15 (3.5%)
TNM stage	I	63 (14.5%)
	II	138 (31.7%)
	III	221 (50.8%)
	IV	13 (3.0%)
Differentiation	Poor	283 (65.1%)
	Moderate	108 (24.8%)
	Well	44 (10.1%)

[9]. Briefly, 4 μm-thick paraffin-embedded tissue sections were routinely dewaxed and hydrated, then treated with 3% peroxide for 10 minutes. The slides were covered with a blocking solution for 1 h at room temperature and incubated with mouse-anti-human NEDD9 monoclonal antibody (1:500 dilution; Abcam, Cambridge, MA, USA). After rinsing with phosphate-buffered saline (PBS; pH 7.4) solution, the sections were further incubated with a goat-anti-mouse secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at 37°C. After washing with PBS, the sections were treated with DAB at room temperature for 4 minutes and counterstained with hematoxylin. Negative control was designed with PBS instead of antibody. NEDD9 expression was quantified using Image-Pro Plus version 6.0 (Media Cybernetics, Inc., Bethesda, MD, USA).

Statistic analysis

The differences between groups were analyzed using the nonparametric test. Cumulative survival curves were constructed using the method of Kaplan-Meier and the difference was evaluated by the log-rank test. All data analyses were done using SPSS software (version 17.0, Chicago, IL, USA). A *P* value of <0.05 was considered statistically significant.

Results

Characteristics of study population

The demographic and pathological characteristics of gastric cancer patients are listed in **Table 1**. The mean age of all patients was 61.75± 10.19 (range, 25-86 years), and 125 patients were female. Of all tumors, 63 (14.5%), 138 (31.7%), 221 (50.8%) and 13 (3.0%) had stages I, II, III, and IV tumors, respectively, and 283 (65.1%), 108 (24.8%) and 44 (10.1%) had poor-, moderate-, and well-differentiated tumors, respectively. In the advanced gastric cancer patients, 30 (7.9%), 269 (71.0%), 68 (17.9%), 12 (3.2%) had I, II, III, and IV according to the Bormann grade.

NEDD9 is highly expressed in gastric cancer tissues

To test the NEDD9 expression in gastric cancer and matched adjacent non-tumor tissues, immunohistochemistry was performed in 435 pairs of gastric tissues. We found that NEDD9 protein was highly expressed in gastric cancer tissues, but weakly expressed in adjacent non-tumor tissues (**Figure 1**).

Further analysis of the clinicopathological characteristics in 435 gastric cancer showed that NEDD9 staining was significantly correlated with TNM stage (P=0.003), pT grade (P=0.000), pN grade (P=0.000). With respect to the degree of differentiation, NEDD9 expression was much higher in poorly-differentiated tumors than well-differentiated tumors (P=0.000). Other characteristics, like sex (P=0.383), age (P=0.822) were not associated with NEDD9 expression. In addition, the expression of NEDD9 was also no significant difference between Bormann grade (P=0.136) in the advanced gastric cancer (**Table 2**).

Association between NEDD9 expression and gastric cancer survival

Overall, patients with gastric cancer were followed up for a median (range) of 57 (51-63) months. The 5-year cumulative survival indicat-

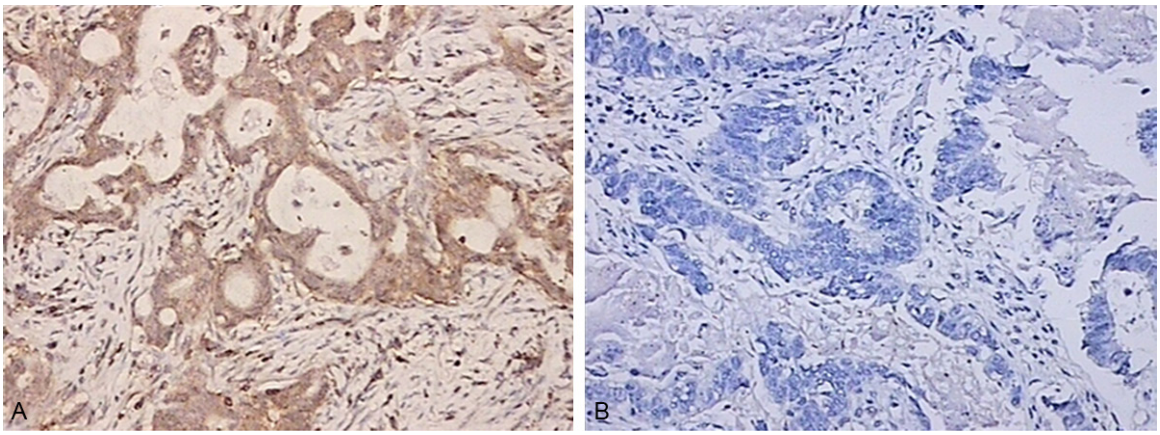

Figure 1. NEDD9 was highly expressed both in gastric cancer tissues. (A) Gastric cancer tissues with positive NEDD9 immunohistochemical reaction (B) adjacent normal tissues negative for NEDD9, magnification, ×200.

Table 2. Association between NEDD9 expression and clinic-pathological features of gastric cancer patients

Clinic-pathological features		NEDD9 expression		χ^2	*P*
		Low	High		
Sex	Male	65	245	0.761	0.383
	Female	31	94		
Age (y)	≥60	52	188	0.050	0.822
	<60	44	151		
Borrmann	I	8	22	5.539	0.136
	II	39	230		
	III	11	57		
	IV	4	8		
pT	T1	34	22	72.746	0.000
	T2	26	56		
	T3	29	165		
	T4	7	96		
pN	N0	32	52	32.515	0.000
	N1	46	121		
	N2	18	151		
	N3	0	15		
TNM stage	I	23	40	13.716	0.003
	II	35	103		
	III	37	184		
	IV	1	12		
Differentiation	Poor	36	247	62.174	0.000
	Moderate	32	76		
	Well	28	16		

ed by Kaplan-Meier survival curves and log-rank test of low and high NEDD9 expression was shown in **Figure 2**. We found that NEDD9 expression was significantly associated with the poor survival of gastric cancer (P=0.000). The median estimated cumulative survival was significantly lower in NEDD9 high expression group (39 months; 95% CI: 35.722-42.278 months), compared with NEDD9 low expression group (45 months; 95% CI, 41.560-48.819 months).

Discussion

NEDD9, which is located on chromosome 6p25-24, encoding an 843 amino acid protein, and was related to p130CAS. Although it lacks any known enzymatic function, it contains many functional modules for protein interaction [10]. In recent years, it has been demonstrated that NEDD9 is high expressed in several types of cancer. Kim M found that NEDD9 has been a biomarker of invasiveness in melanoma due to its role in the regulation and activation of transcriptional pathways relevant for metastasis and cancer progression, including FAK and Src [4]. Li Y et al found that NEDD9 was overexpressed in colonic cancers and promoted invasion via Wnt signaling [5]. In GB, NEDD9 is a downstream effector of FAK that causes an increase in migration capacity [3]. Our present study here demonstrates that NEDD9 is overexpressed in the gastric cancer tissues, and none or very weak staining of NEDD9 is detected in the adjacent non-tumor tissues.

The tumor of invasion and metastasis is a complex continuous process. Previous studies have found that the adhesion of cell and matrix could promote NEDD9-FAK-Src-Crk complex, and then activate the GTP kinase of Ras and Rho family via enrichment of DOCK180 and C3G

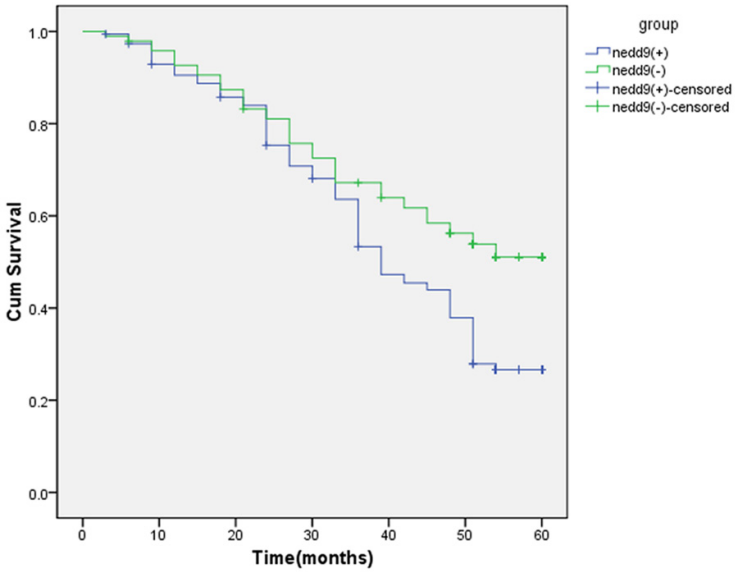

Figure 2. Kaplan-Meier survival curves for 435 gastric cancer patients by expression of nedd9.

[11-13]. Another paper point out GTP kinase could regulate cell migration, invasion, and tumor formation [14]. And our previous study found that NEDD9 protein expression was significantly correlated with the TNM stage, tumor differentiation and lymph node metastasis in 60 gastric cancer patients. Further study found the expression of vimentin was enhanced after upregulating NEDD9 expression, and the other hall marker of EMT, E-cadherin was inhibited after upregulating NEDD9 expression [8]. Now we enlarged the sample, we demonstrated that the upregulation of NEDD9 is correlated with TNM stage, lymph node metastases, and differentiation grade again. The results observed in this study were also consistent with the previous study [15-17].

TNM stage, lymph node metastases, and differentiation grade have been considered to be the most important factors in the survival, management, and prognosis of cancer patients. Papers had been validated that NEDD9 was a promising biomarker for the prognosis of NSCLCs and colorectal cancer patients [18, 19]. In the present study, we show that the gastric cancer patients with a high level of NEDD9 have a significantly shorter survival time than those patients with a low level of NEDD9. The result is in agreement with earlier reports that elevated expression of NEDD9 has been implicated with the poor prognosis in a variety of tumor types. Thus, it is deserved to further detect its value as a prognosis marker of gastric cancer patients.

In conclusion, our study shown that NEDD9 was high expressed in gastric cancer, but weakly expressed in adjacent non-tumor tissues, besides, the NEDD-9 expression was associated with the poor survival in gastric cancer patients. Based on these results and our previous study, we speculated that NEDD9 might prove to be a novel molecular biomarker for the predicting progression and poor prognosis with gastric cancer patients.

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Disclosure of conflict of interest

None.

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