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Effects of NGX6 expression on proliferation and invasion of nasopharyngeal carcinoma cells and survival of patients

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Abstract. – OBJECTIVE: To detect the expressions of nasopharyngeal carcinoma-associated gene 6 (NGX6) in nasopharyngeal carcinoma cells and tissues, and to investigate the effects of NGX6 on the proliferation and invasion of nasopharyngeal carcinoma cells and the survival of patients.

PATIENTS AND METHODS: The human nasopharyngeal carcinoma cells (HONE1) and immortalized human nasopharyngeal epithelial cells (NP69) were selected and cultured. The mRNA and protein expression levels of NGX6 were detected via quantitative reverse transcription polymerase chain reaction (qRT-PCR) and Western blot. The expression of NGX6 in HONE1 was up-regulated using the gene transfection technique. Moreover, the effects of NGX6 on the proliferation and invasion capacities of HONE1 were observed via methyl thiazolyl tetrazolium (MTT) assay and Transwell assay. 50 biopsy tissue specimens of nasopharyngeal carcinoma and 20 non-neoplastic nasopharyngeal biopsy tissue specimens were collected, and the immunohistochemical method was used to detect the protein expression of NGX6 in tumor tissues of patients with esophageal carcinoma. Finally, the follow-up data of patients were recorded, Kaplan-Meier method was used for survival analysis, and the difference in survival rates was detected using the Log-rank test.

RESULTS: The results of qRT-PCR and Western blot showed that the mRNA and protein expressions of NGX6 in HONE1 were significantly lower than those in nasopharyngeal carcinoma cells (NP69). After the overexpression of NGX6, the protein expression of NGX6 in HONE1 was significantly increased, but the proliferation and invasion capacities of HONE1 were significantly decreased. Besides, the immunohistochemical results revealed that the expression of NGX6 in tumor tissues of patients with nasopharyngeal carcinoma was significantly lower than that in normal tissues; the survival analysis showed that the level of NGX6 was positively correlated with the survival and prognosis of patients with nasopharyngeal carcinoma.

CONCLUSIONS: NGX6 is lowly expressed in nasopharyngeal carcinoma, and it can inhibit the proliferation and invasion of nasopharyngeal carcinoma cells, whose expression is positively correlated with the survival and prognosis of patients with nasopharyngeal carcinoma.

Key Words
NGX6, Nasopharyngeal Carcinoma, Proliferation, Invasion, Prognosis.

Introduction

Nasopharyngeal carcinoma is a kind of common malignant tumor in head and neck with a high incidence rate in South-East Asia and Southern China[1]. The pathogenesis of nasopharyngeal carcinoma is complex, including Epstein-Barr (EB) virus infection, carcinogen hazards, changes in expressions of a variety of oncogenes and tumor suppressor genes, etc.[2-4]. Nasopharyngeal carcinoma is clinically characterized with low differentiation and high metastasis. Besides, the invasion and metastasis of tumor cells are the most important factors influencing the treatment effect and causing the death of patients. Therefore, the study on the invasion and metastasis of nasopharyngeal carcinoma cells has a great clinical significance in the treatment of patients with nasopharyngeal carcinoma[5]. Nasopharyngeal carcinoma-associated gene 6 (NGX6) was cloned in the 9p21222 gene region by Cancer Institute of Xiangya Medical College. Studies have shown that NGX6 is a kind of candidate tumor suppressor gene of nasopharyngeal carcinoma[6], which contains 10 exons, has a full-length of 2134bp, encodes a total of 338 amino acids and has a protein molecular weight of 37kDa. It is mainly

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expressed in the membranous structures in cell membrane, nuclear membrane and cytoplasm[7,8]. Moreover, NGX6 can inhibit the proliferation, invasion and metastasis of nasopharyngeal carcinoma and other tumors, which may regulate the tumor cell cycle, affect the tumor cell migration and invasion, inhibit the tumor angiogenesis, etc., through the regulation of important signal transduction pathways in cells[9]. Researches revealed that NGX6 mRNA is highly expressed in both normal nasopharyngeal and colorectal tissues, but decreased or deleted in nasopharyngeal carcinoma and colorectal carcinoma tissues, and it was found that its down-regulation is closely related to the lymph node metastasis and clinical stage[10]. However, there have been no reports on the correlation of NGX6 expression in nasopharyngeal carcinoma cells with the cell proliferation and invasion, and the correlation of NGX6 protein expression in nasopharyngeal carcinoma tissues with the survival and prognosis of patients. In our work, the mRNA and protein expression levels of NGX6 in human nasopharyngeal carcinoma cells (HONE1) and immortalized human nasopharyngeal epithelial cells (NP69) were detected via quantitative reverse transcription polymerase chain reaction (qRT-PCR) and Western blot. The expression of NGX6 in HONE1 was up-regulated using the gene transfection technique, and the effects of NGX6 on the proliferation and invasion of HONE1 were studied. Besides, the immunohistochemical staining was used to detect the protein expression of NGX6 in nasopharyngeal carcinoma tissues. Finally, the follow-up data were recorded, and the correlation of NGX6 with the survival and prognosis of patients with nasopharyngeal carcinoma was analyzed.

Patients and Methods

Patients

A total of 50 biopsy tissue specimens were collected from patients with nasopharyngeal carcinoma treated in our hospital from January 2010 to December 2011. The patients were diagnosed as nasopharyngeal carcinoma via nasopharyngeal biopsy and treated for the first time. A total of 20 non-neoplastic nasopharyngeal biopsy tissue specimens were randomly selected during the same period as controls. All tissue specimens were fixed with 10% formalin solution, followed by dehydration, transparency and paraffin embedding to prepare the sections. This investigation was approved by the Ethics Committee of our hospital, and all patients or their families signed the informed consent.

Materials

HONE1 and NP69 (Shanghai Cell Bank, Chinese Academy of Sciences, Shanghai, China); Dulbecco's modified Eagle medium (DMEM) culture solution, serum-free medium (SFM) culture solution (Invitrogen, Carlsbad, CA, USA); fetal bovine serum (FBS) (Hyclone, South Logan, UT, USA); TRIzol kit (Invitrogen, Carlsbad, CA, USA); bicinchoninic acid (BCA) protein quantification kit and cell lysis buffer (Beyotime Institute of Biotechnology, Nantong, China); polyethyleneimine and dimethylsulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA); matrigel (BD Bioscience Pharmingen Inc., Franklin Lakes, NJ, USA); reverse transcription kit, qRT-PCR kit, primer synthesis and pcDNA3-NGX6 plasmid (TaKaRa, Dalian, China); Transwell chamber (Millipore, Billerica, MA, USA); NGX6 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primary antibodies and horseradish peroxidase (HRP)-labeled secondary antibody (Proteintech, Wuhan, China); immunohistochemical staining kit SP-9001 (Beijing Zhongshan Goldenbridge Biotechnology Co., Ltd., Beijing, China).

Cell Culture

HONE1 was cultured using DMEM culture solution (containing 10% FBS), while NP69 was cultured using SFM culture solution. They were incubated in an incubator containing 5% CO_2 at 37°C and the solution was replaced once every day, followed by digestion and passage after the cells were in the logarithmic growth phase.

Detection of mRNA Expressions of NGX6 in HONE1 and NP69 Via qRT-PCR

After digestion and centrifugation of cells in the logarithmic growth phase, the total RNA was extracted from cells using the TRIzol kit (Invitrogen, Carlsbad, CA, USA). The specimens with the absorbance ratio (A_{260}/A_{280}) between 1.8 and 2.0 were selected for reverse transcription. The complementary DNA (cDNA) obtained was taken as the template for PCR amplification. The primer sequences are shown in Table I and the specific reaction conditions were as follows: pre-denaturation at 94°C for 5 min, denaturation at 95°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s, a total of 40 cycles; the Ct value was output from the instrument and GADPH was used as the control. The results were analyzed using $2^{-\Delta Ct}$ method.

Table I. Primer sequences of qRT-PCR.

Gene	Primer name	Primer sequence
NGX6	Forward primer	5'-TGACCTGTTCCAAAGAGTCCCTG-3'
	Reverse primer	5'-GCAGCTTCCAGCACATATCGACT-3'
GADPH	Forward primer	5'-CCTGGTATGACAACGAATTTG-3'
	Reverse primer	5'-CAGTGAGGGTCTCTCTCTTCC-3'

Detection of Protein Expressions of NGX6 in HONE1 and NP69 via Western Blot

After digestion and centrifugation of cells in the logarithmic growth phase, the cell lysis buffer was added and the protein was extracted via centrifugation at 4°C. Then, the protein concentration was detected using the BCA protein concentration kit, followed by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). 50 μg protein samples were loaded into each well, followed by wet membrane transfer and sealing via 5% bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA). Next, the NGX6 and GAPDH antibodies (diluted at 1:1000) were dropped for incubation at 4°C overnight; HRP-labeled secondary antibody was added dropwise for incubation at room temperature for 2 h. Finally, the ECL developer was dropped for development in a darkroom for 1 min, and the gel imager (Bio-Rad Laboratories, Hercules, CA, USA) was used for scanning and photograph, with GAPDH as the internal reference.

Overexpression of NGX6 in HONE1 Using Gene Transfection Technique

The full-length cDNA of NGX6 and the pcDNA3.1-NGX6 overexpression plasmid were prepared by TaKaRa (Dalian, China). In the experiment, there were two groups: blank plasmid group and NGX6 transfection group. 500 μL serum-free DMEM culture solution was added into the sterile centrifuge tube and then 15 μL polyethyleneimine and 5 μg pcDNA3.1-NGX6 or blank pcDNA3 plasmid were added. The mixture was incubated for 15 min at room temperature and then added into each group of HONE1. After incubation for 12 h, the culture solution was replaced. After incubation for another 48 h, the protein expressions of NGX6 in the two groups were detected via Western blot.

Detection of the Effect of NGX6 Overexpression on Cell Proliferation Via MTT Assay

Cells were transfected according to the grouping and methods described as previously reported in Shen et al[4], and the cells were prepared into single cell suspension in a concentration of 1×10^4/mL. 100 μL cell suspension was absorbed into the 96-well plate, and 20 μL (5 μg/μL) MTT working solution was added after 0 h, 24 h, 36 h and 48 h for incubation in a dark place for 4 h. 100 μL dimethyl sulfoxide (DMSO) solution was added into each well. After the mixture was shaken for 10 min, the optical density (OD) value at the wavelength of 490 nm was measured using the microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). The cell proliferation rate was calculated using the following formula: proliferation rate = OD value (measurement time)/OD value (0 h).

Detection of the Effect of NGX6 Overexpression on Cell Invasion Via Transwell Assay

Cells were transfected according to the grouping and methods described, and the cells were prepared into single cell suspension in a concentration of 4×10^5/mL. 100 μL cell suspension was absorbed into the upper Transwell chamber coated with matrigel, and then 100 μL SFM was added, while 500 μL culture solution containing 30% FBS was added into the lower chamber. Then the chamber was removed from an incubator after culture for 48 h, and fixed with 4% formaldehyde solution, followed by crystal violet staining, and photograph under the microscope (TE2000-U, Nikon, Tokyo, Japan).

Detection of Protein Expression of NGX6 in Pathological Tissues Via Immunohistochemistry

According to the instructions of SP-9001 immunohistochemical kit, the paraffin sections were first dewaxed and the endogenous peroxidase was inactivated, followed by antigen retrieval using citric acid buffer and sealing via serum. Then, the NGX6 antibody (diluted at 1:200) was added in the refrigerator at 4°C overnight; the sections were washed with pre-cooled PBS for three times, the secondary antibody was added for incubation for 30 min, and the sections were washed again

with pre-cooled PBS for three times, followed by color development via diaminobenzidine (DAB) in a darkroom, gum sealing and photograph under the microscope (TE2000-U, Nikon, Tokyo, Japan). The dark brown sediment in cell membrane, cytoplasm and nuclear membrane indicated the positive NGX6 protein staining. Cells were scored according to the following scoring criteria: positive cells <5%: 0 point; 5-25%: 1 point; 26-50%: 2 points; >51%: 3 points; basically no staining: 0 point; light yellow staining: 1 point; brown yellow: 2 points; dark brown: 3 points; the above two points were added. ≤3 points: negative expression; >3 points: positive expression. The statistical results were analyzed.

Correlation of NGX6 With Survival and Prognosis of Patients With Nasopharyngeal Carcinoma

The patients with nasopharyngeal carcinoma were divided into NGX6 positive-expression group and NGX6 negative-expression group. The follow-up lasted for 5 years from the clinical diagnosis, and the patients were followed up once a month. According to the follow-up results, the effects of NGX6 on survival and prognosis of patients with nasopharyngeal carcinoma were analyzed.

Statistical Analysis

Statistical Product and Service Solutions 17.0 (SPSS Inc., Chicago, IL, USA) was used for the data processing in this study. Measurement data were presented as mean ± standard deviation and *t*-test was used for intergroup comparison. Kaplan-Meier method was used for survival analysis, and Log-rank test was used for the difference between the survival rates. $p \leq 0.05$ suggested that the difference was statistically significant.

Results

mRNA Expressions of NGX6 in HONE1 and NP69

The detection results of NGX6 mRNA are shown in Figure 1. The mRNA expression of NGX6 in HONE1 was significantly lower than that in NP69, and the difference was statistically significant ($p<0.01$)

Protein Expressions of NGX6 in HONE1 and NP69

The results of Western blot are showed in Figure 2. The protein expression of NGX6 in HONE1 was significantly lower than that in NP69, and the difference was statistically significant ($p<0.01$).

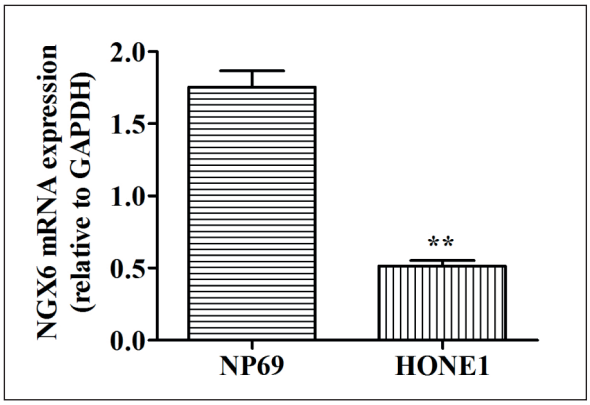

Figure 1. Detection of mRNA expressions of NGX6 in HONE1 and NP69 via qRT-PCR. The mRNA expression of NGX6 in HONE1 is significantly lower than that in NP69; compared with NP69, $^{**}p<0.01$.

Effect of NGX6 Transfection on NGX6 Protein Expression in HONE1

The effect of pcDNA3.1-NGX6 plasmid on the NGX6 expression in HONE1 was detected via Western blot. The results (Figure 3) showed that compared with blank plasmid group, NGX6 overexpression could significantly increase the protein expression of NGX6 in HONE1 ($p<0.01$).

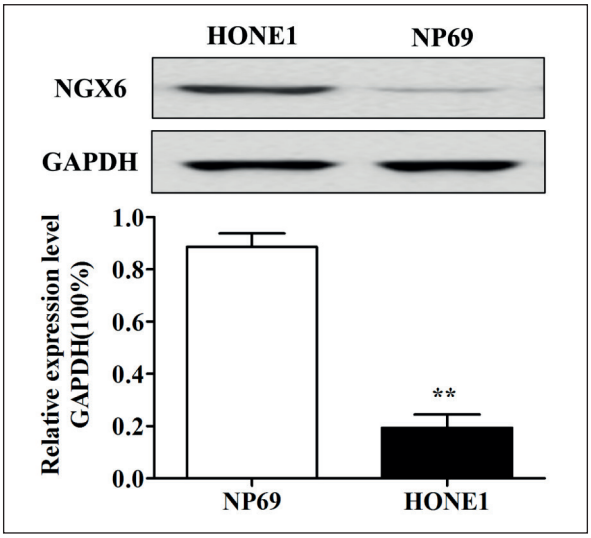

Figure 2. Detection of protein expressions of NGX6 in HONE1 and NP69 via Western blot. The protein expression of NGX6 in HONE1 is significantly lower than that in NP69; compared with NP69, $^{**}p<0.01$.

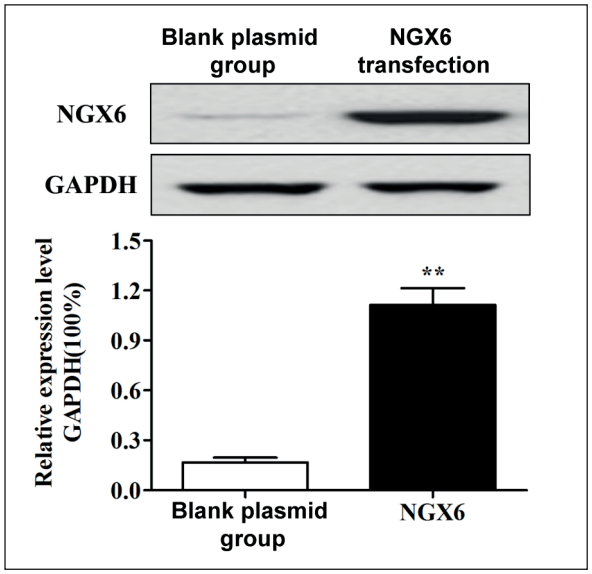

Figure 3. Detection of effect of NGX6 transfection on NGX6 protein expression in HONE1 via Western blot. The NGX6 expression in HONE1 transfected with pcDNA3-NGX6 plasmid is significantly increased. Compared with blank plasmid group, $^{**}p<0.01$.

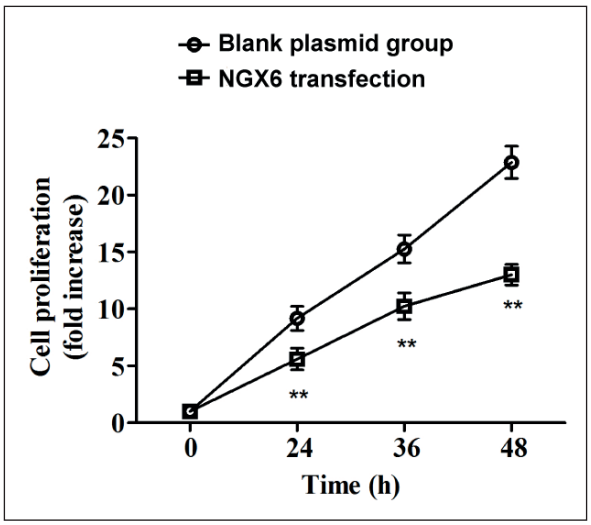

Figure 4. Detection of effect of NGX6 overexpression on HONE1 proliferation via MTT assay. The proliferation of HONE1 transfected with pcDNA3-NGX6 plasmid is significantly decreased. Compared with blank plasmid group, $^{**}p<0.01$.

Effect of NGX6 Overexpression on Proliferation Capacity of HONE1

The effect of NGX6 overexpression on HONE1 proliferation was detected via MTT assay. The results (Figure 4) revealed that the proliferation rate of HONE1 was significantly decreased after NGX6 overexpression, and the difference was statistically significant ($p<0.01$).

Effect of NGX6 Overexpression on Invasive Capacity of HONE1

The effect of NGX6 overexpression on the invasive capacity of HONE1 was investigated via Transwell assay. The results (Figure 5) showed that the number of invasive HONE1 was significantly reduced after NGX6 overexpression, and the difference was statistically significant ($p<0.01$).

Protein Expression of NGX6 in Pathological Tissues of Patients With Nasopharyngeal Carcinoma

As shown in Figure 6, the protein expression of NGX6 was positive and negative in both nasopharyngeal carcinoma and non-neoplastic nasopharyngeal carcinoma tissues; statistical results (Table II) showed that the positive expression rate of NGX6 protein in nasopharyngeal carcinoma tissues was 22.00% (11/50), while that in non-neoplastic nasopharyngeal carcinoma tissues was 90.00% (18/20).

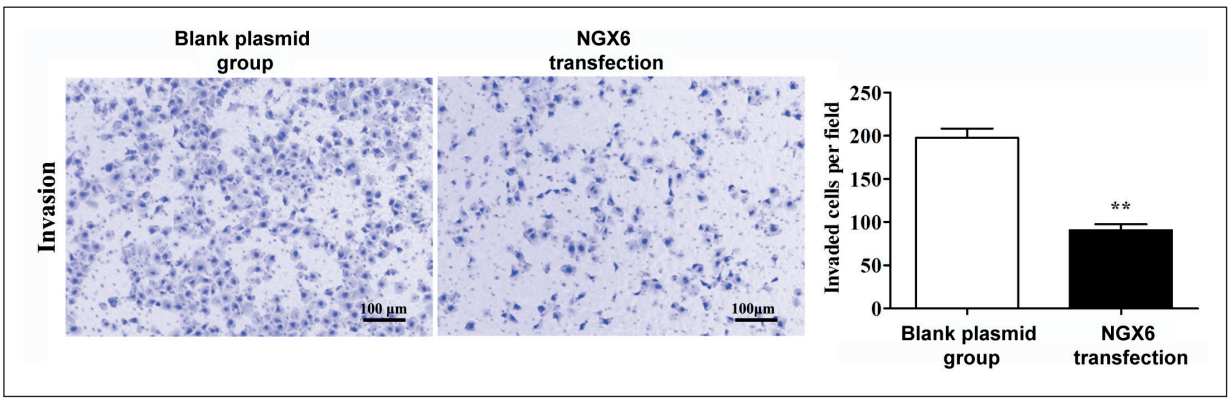

Figure 5. Detection of effect of NGX6 overexpression on HONE1 invasion via Transwell assay. The number of invasive HONE1 transfected with pcDNA3-NGX6 plasmid is significantly reduced. Compared with blank plasmid group, $^{**}p<0.01$.

Table II. NGX6 expression in nasopharyngeal carcinoma tissues.

Group	n	NGX6 expression		Positive expression rate
		Negative	Positive	
Nasopharyngeal carcinoma tissue	50	39	11	22.00%
Non-neoplastic nasopharyngeal carcinoma tissue	20	2	18	90.00%

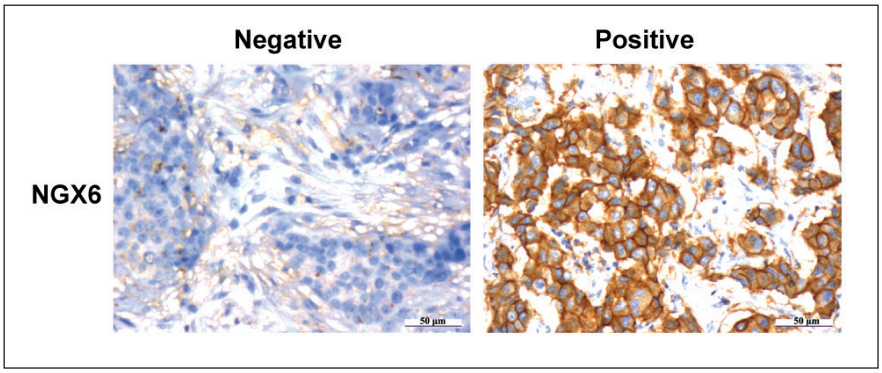

Figure 6. Immunohistochemical detection of NGX6 protein expression in pathological tissues (400×).

Correlation of NGX6 Expression With Survival and Prognosis of Patients With Nasopharyngeal Carcinoma

The follow-up results revealed that the 5-year survival rate of 50 patients with nasopharyngeal carcinoma was 72.00% (34/50), among which the 5-year survival rate was 69.23% (24/39) in NGX6 negative-expression group and 81.82% (10/11) in NGX6 positive-expression group, and the difference in statistical analysis was statistically significant ($p<0.05$). From the survival curve, it could be seen that the survival and prognosis of patients with positive NGX6 expression were better (Figure 7).

Discussion

Studies[11-13] have found that the incidence rate of nasopharyngeal carcinoma is higher in South-East Asia and in Southern China, but it is rare in other places in the world, mainly because the occurrence of nasopharyngeal carcinoma is due to the combined effects of heredity, environment, diet, etc. NGX6 is a novel candidate tumor suppressor gene associated with nasopharyngeal carcinoma. It has been confirmed that the abnormal expression of NGX6 can block the EGFR/ Ras/Mek/MAPK signaling pathway, thus inhibiting the proliferation, metastasis and invasion of nasopharyngeal carcinoma cells[14]. Studies have found that the metastasis of solid tumors requires the formation of new blood vessels, and NGX6 protein contains the EGF-like specific domain, so it can affect the formation of tumor blood vessels through regulating various vascular factors. The lower the NGX6 protein expression is, the higher the VEGF protein will be. The down-regulation of NGX6 expression results in the high expression of VEGF, leading to the invasion and metastasis of nasopharyngeal carcinoma cells[15]. After the up-regulation of NGX6 expression in nasopharyngeal carcinoma 5-8F cells via transfection,

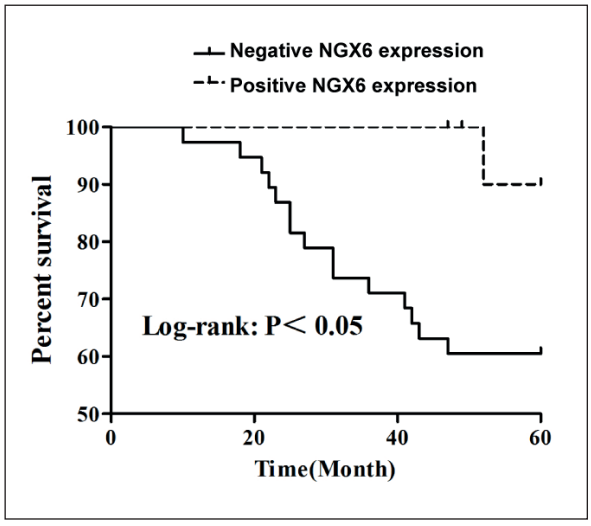

Figure 7. Correlation of NGX6 expression with survival and prognosis of patients with nasopharyngeal carcinoma. Compared with patients with negative NGX6 expression, patients with positive NGX6 expression had a better prognosis.

the expressions of angiogenin Tie2 and EphB4 in cells will be down-regulated. So, NGX6 may inhibit the angiogenesis through down-regulating the expressions of Tie2 and EphB4, thereby inhibiting the invasion and metastasis of nasopharyngeal carcinoma cells[16,17]. The study on correlation of NGX6 expression with metastasis and prognosis of nasopharyngeal carcinoma showed that NGX6 mRNA can be used as a molecular marker for predicting the lymph node metastasis of nasopharyngeal carcinoma[18]. Some scholars[19] argued that after nasopharyngeal carcinoma cells were transfected with NGX6, the number of nasopharyngeal carcinoma cells with NGX6 overexpression in the G0/G1 phase was increased and that in S/M phase was decreased significantly, but the cell apoptosis rate had no obviously change. In our investigation, the mRNA and protein expressions of NGX6 in HONE1 and NP69 were detected via qRT-PCR and Western blot. The results showed that the mRNA and protein expressions of NGX6 in HONE1 were significantly lower than those in NP69. In order to further verify the roles of NGX6 in the proliferation and invasion of nasopharyngeal carcinoma, the expression of NGX6 in HONE1 was up-regulated using the gene transfection technique. The results revealed that the proliferative and invasive capacities of HONE1 were significantly reduced after NGX6 overexpression. At the same time, the protein expression of NGX6 in nasopharyngeal carcinoma tissues was also detected using the immunohistochemical method, and it was found that the positive expression rate of NGX6 protein was 22.00% in nasopharyngeal carcinoma tissues and 90.00% in non-neoplastic nasopharyngeal carcinoma tissues. To study the correlation of NGX6 expression with the survival and prognosis, patients with nasopharyngeal carcinoma were followed up for 5 years. The results revealed that the 5-year survival rate of 50 patients with nasopharyngeal carcinoma was 72.00% (34/50), among which the 5-year survival rate was 69.23% (24/39) in NGX6 negative-expression group and 81.82% (10/11) in NGX6 positive-expression group. From the survival curve, it could be seen that the survival and prognosis of patients with positive NGX6 expression were better.

Conclusions

NGX6 is lowly expressed in nasopharyngeal carcinoma, and it can inhibit the proliferation and invasion of nasopharyngeal carcinoma cells, whose expression is positively correlated with the survival and prognosis of patients with nasopharyngeal carcinoma. Therefore, NGX6 may serve as a biomarker to diagnose and predict the prognosis of patients with nasopharyngeal carcinoma.

Conflict of Interest

The authors declared no conflict of interest.

References

1) Parkin DM, Laara E, Muir CS. Estimates of the worldwide frequency of sixteen major cancers in 1980. Int J Cancer 1988; 41: 184-197.
2) Xu T, Yuan Y, Xiao DJ. The clinical relationship between the slug-mediated Puma/p53 signaling pathway and radiotherapy resistance in nasopharyngeal carcinoma. Eur Rev Med Pharmacol Sci 2017; 21: 953-958.
3) Choi PH, Suen MW, Huang DP, Lo KW, Lee JC. Nasopharyngeal carcinoma: genetic changes, Epstein-Barr virus infection, or both. A clinical and molecular study of 36 patients. Cancer 1993; 72: 2873-2878.
4) Shen ZH, Zhao KM, Du T. HOXA10 promotes nasopharyngeal carcinoma cell proliferation and invasion via inducing the expression of ZIC2. Eur Rev Med Pharmacol Sci 2017; 21: 945-952.
5) Song LB, Liao WT, Mai HQ, Zhang HZ, Zhang L, Li MZ, Hou JH, Fu LW, Huang WL, Zeng YX, Zeng MS. The clinical significance of twist expression in nasopharyngeal carcinoma. Cancer Lett 2006; 242: 258-265.
6) Ma J, Li J, Zhou J, Li XL, Tang K, Zhou M, Yang JB, Yan Q, Shen SR, Hu GX, Li GY. Profiling genes differentially expressed in NGX6 overexpressed nasopharyngeal carcinoma cells by cDNA array. J Cancer Res Clin Oncol 2002; 128: 683-690.
7) Peng SP, Li XL, Wang L, Ou-Yang J, Ma J, Wang LL, Liu HY, Zhou M, Tang YL, Li WS, Luo XM, Cao L, Tang K, Shen SR, Li GY. The role of NGX6 and its deletion mutants in the proliferation, adhesion and migration of nasopharyngeal carcinoma 5-8F cells. Oncology 2006; 71: 273-281.
8) Eskandari-Nasab E, Hashemi M, Rezaei H, Fazaeli A, Mashhadi MA, Moghaddam SS, Arbabi F, Jahantigh M, Taheri M. Evaluation of UDP-glucuronosyltransferase 2B17 (UGT2B17) and dihydrofolate reductase (DHFR) genes deletion and the expression level of NGX6 mRNA in breast cancer. Mol Biol Rep 2012; 39: 10531-10539.
9) Guo Q, Shen S, Liao M, Lian P, Wang X. NGX6 inhibits cell invasion and adhesion through suppression of Wnt/beta-catenin signal pathway in colon cancer. Acta Biochim Biophys Sin (Shanghai) 2010; 42: 450-456.
10) Xiao J, Zhou Y, Liu B. Correlation of NGX6 expression with clinicopathologic features and prognosis in colon cancer. Int J Clin Exp Med 2015; 8: 13644-13648.

11) Jeannel D, Bouvier G, Hubert A. Nasopharyngeal carcinoma: an epidemiological approach to carcinogenesis. Cancer Surveys 1999; 33: 125-155.
12) Wei WI, Sham JS. Nasopharyngeal carcinoma. Lancet 2005; 365: 2041-2054.
13) Sun LM, Epplein M, Li CI, Vaughan TL, Weiss NS. Trends in the incidence rates of nasopharyngeal carcinoma among Chinese Americans living in Los Angeles County and the San Francisco metropolitan area, 1992-2002. Am J Epidemiol 2005; 162: 1174-1178.
14) Wang L, Ma J, Li J, Li X, Zhang Q, Peng S, Peng C, Zhou M, Xiong W, Yang J, Zhou J, Fan S, Tan C, Yan Q, Shen S, Li G. NGX6 gene inhibits cell proliferation and plays a negative role in EGFR pathway in nasopharyngeal carcinoma cells. J Cell Biochem 2005; 95: 64-73.
15) Chew MM, Gan SY, Khoo AS, Tan EL. Interleukins, laminin and Epstein-Barr virus latent membrane protein 1 (EBV LMP1) promote metastatic phenotype in nasopharyngeal carcinoma. BMC Cancer 2010; 10: 574.
16) Mammoto T, Jiang A, Jiang E, Mammoto A. Platelet rich plasma extract promotes angiogenesis through the angiopoietin1-Tie2 pathway. Microvasc Res 2013; 89: 15-24.
17) Martiny-Baron G, Korff T, Schaffner F, Esser N, Eggstein S, Marme D, Augustin HG. Inhibition of tumor growth and angiogenesis by soluble EphB4. Neoplasia 2004; 6: 248-257.
18) Li J, Tan C, Xiang Q, Zhang X, Ma J, Wang JR, Yang J, Li W, Shen SR, Liang S, Li G. Proteomic detection of changes in protein synthesis induced by NGX6 transfected in human nasopharyngeal carcinoma cells. J Protein Chem 2001; 20: 265-271.
19) Ma J, Zhou J, Fan S, Wang L, Li X, Yan Q, Zhou M, Liu H, Zhang Q, Zhou H, Gan K, Li Z, Peng C, Xiong W, Tan C, Shen S, Yang J, Li J, Li G. Role of a novel EGF-like domain-containing gene NGX6 in cell adhesion modulation in nasopharyngeal carcinoma cells. Carcinogenesis 2005; 26: 281-291.