

Original Article

Correlation of NGX6 expression with clinicopathologic features and prognosis in colon cancer

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Abstract: Objective: The aim was to explore the correlation of NGX6 expression with clinicopathological features and prognosis in colon cancer. Methods: Clinicopathological feature of 145 patients with colon cancer were analyzed. NGX6 expression was measured using immunohistochemistry methods. The correlation of NGX6 expression with clinicopathological features and prognosis were assessed. Results: Among 145 cases of colon cancer, NGX6 positive expression were found in 76 (52.4%) cases and NGX6 negative expression were found in 69 (47.6%) cases. The expression of NGX6 was closely associated with size tumor, lymph node metastasis and TNM stage ($P=0.002$, 0.012 , and 0.039 , respectively). Kaplan-Meier analysis showed that NGX6 negative expression was associated with shorter disease-free survival (DFS) ($P=0.029$) and overall survival (OS) ($P=0.015$). Multivariate survival analysis demonstrated that NGX6 expression was the important independent prognostic factor for colon cancer ($P=0.022$). Conclusion: NGX6 is involved in the invasion and metastasis activity of colon cancer. NGX6 could may be applied as a novel and promising prognostic marker for colon cancer.

Keywords: Colon cancer, nasopharyngeal carcinoma associated gene 6 (NGX6), prognosis, metastasis, pathology

Introduction

Colon cancer is one of the most common cancers and a major cause of morbidity and mortality worldwide. Gene mutations and epigenetic alterations contribute to colon cancer formation through the activation of oncogenic pathways and the inactivation of tumor suppressor genes [1, 2]. NGX6 is a newly discovered tumor suppressor gene (GenBank accession no. AF188239). It contains one epidermal growth factor (EGF)-like domain. Research has shown that protein with contain (EGF)-like domain structure can affect a variety of biological actions of tumor [3-5]. However, little is known about the influence of NGX6 expression in colon cancer. We attempted to verify the correlation of NGX6 expression with clinicopathologic features and prognosis in order to yield clinically useful information for colon cancer.

Materials and methods

Patients

This study was approved by the ethics committee of Third Xiangya Hospital. Between June 1,

2008 and January 1, 2012, a total of 145 patients scheduled for surgery were confirmed to be colon cancer with pathological examination. There were 87 males (60.0%) and 58 females (40.0%). The mean age was 53.0 years, ranging from 28 to 76 years. The clinicopathologic information of the study subjects and primary tumor samples were recorded. All patients received standard post-operative chemotherapy according to the National Comprehensive Cancer Network guidelines. None of the patients had preoperative chemotherapy or preoperative radiotherapy. The staging of tumors was determined according to the American Joint Committee on Cancer (AJCC) TNM staging system [6]. Each tumor was pathologically classified according to the World Health Organization classification criteria. All the subjects signed the informed consent.

Immunohistochemistry (IHC)

The 4 μ m-thick sections cut from formalin-fixed, paraffin-embedded tissue specimens were deparaffinized with xylene and rehydrated with graded ethanol. Antigen retrieval was per-

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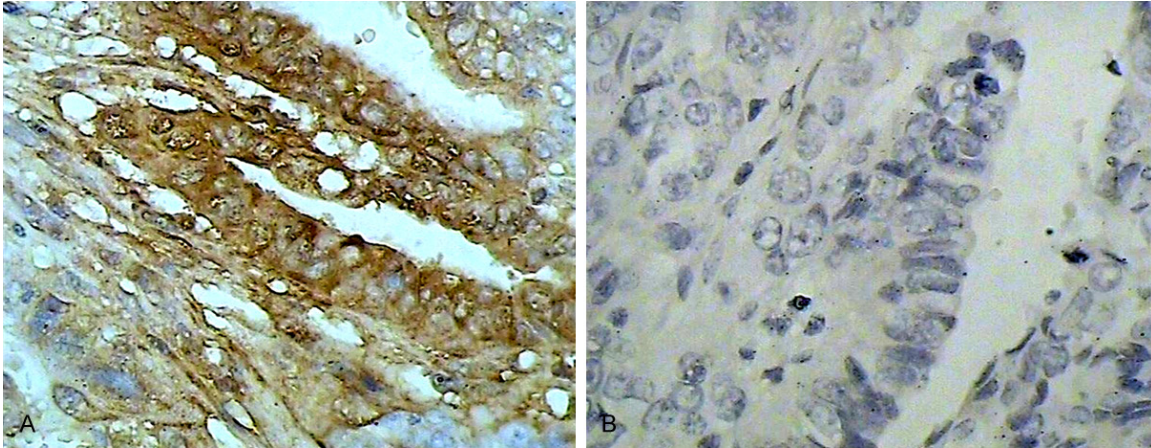


Figure 1. Representative immunohistochemical staining of NGX6 expression in colon cancer tissues. A. Positive expression of NGX6; B. Negative expression of NGX6. Representative images are shown at $\times 400$ magnifications.

Table 1. Correlation of NGX6 expression with clinicopathological features in colon cancer

| Clinicopathologic features | n | NGX6 | | χ^2 | P |
|----------------------------|----|------|-----|----------|-------|
| | | (+) | (-) | | |
| Gender | | | | | |
| Male | 87 | 47 | 40 | 0.635 | 0.226 |
| Female | 58 | 29 | 29 | | |
| Age | | | | | |
| <60 years | 69 | 37 | 32 | 0.781 | 0.077 |
| ≥ 60 years | 76 | 39 | 37 | | |
| Tumor size | | | | | |
| <5 cm | 70 | 46 | 24 | 9.599 | 0.002 |
| ≥ 5 cm | 75 | 30 | 45 | | |
| Lymph node metastasis | | | | | |
| Negative | 62 | 40 | 22 | 6.361 | 0.012 |
| Positive | 83 | 36 | 47 | | |
| Depth of tumor invasion | | | | | |
| T1-T2 | 56 | 32 | 24 | 0.818 | 0.366 |
| T3-T4 | 89 | 44 | 45 | | |
| TNM stage | | | | | |
| I-II | 48 | 31 | 17 | 4.260 | 0.039 |
| III-IV | 97 | 45 | 52 | | |
| Histological grade | | | | | |
| Well/moderately | 63 | 35 | 28 | 0.441 | 0.507 |
| Poorly | 82 | 41 | 41 | | |

formed in a 10 mmol/L sodium citrate (pH 6.0) for 5 min with a high pressure. The tissue sections were immersed in 3% H_2O_2 for 10 min to inactivate endogenous peroxidase. 10% goat serum was added to the tissue sections and incubated for 30 min at 37°C. The sections

were incubated with Rabbit anti-NGX6 monoclonal antibodies (1:200 dilution, Abcam, USA) overnight at 4°C, and then incubated at 37°C for 30 min with a secondary antibody against rabbit and mouse immunoglobulins (EnVision, DAKO, Denmark). Afterwards, the sections were stained with DAB for 5 min. Classification is done according to the strength of cells staining and the proportion of the positive cell [7-9]. Tissue sections confirmed high expression of the target molecules served as positive control, while those incubated with the primary antibody diluent instead of the primary antibody were used as the negative control.

Statistical analysis

Data processing and statistical analysis were performed using SPSS13.0 statistical analysis package, the measurement data used variance test, counting information using chi-square test. The Kaplan-Meier method was used to estimate the survival outcomes; groups were compared using the log-rank test. The Cox proportional hazards model was used for multivariate analysis. Significance level was set at $P < 0.05$ (both sides).

Results

NGX6 expression in colon cancer

Among 145 cases of colon cancer, positive expression was found in 76 (52.4%) cases (**Figure 1A**) and negative expression was found in 69 (47.6%) cases (**Figure 1B**).

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Table 2. Univariate Cox regression analysis of potential prognostic parameters associated with DFS and OS in colon cancer patients

| Variable | DFS | | OS | |
|----------------------------|---------------------|-------|---------------------|-------|
| | HR (95% CI) | P | HR (95% CI) | P |
| Ages, years | | | | |
| ≥60 years vs. <60 years | 1.872 (0.985-2.773) | 0.334 | 0.982 (0.716-1.235) | 0.286 |
| Family history | | | | |
| Yes vs. No | 1.105 (0.753-1.475) | 0.392 | 1.828 (0.886-2.912) | 0.238 |
| Gender | | | | |
| Male vs. Female | 1.247 (0.693-1.694) | 0.183 | 2.739 (1.283-4.243) | 0.421 |
| Tumor size | | | | |
| ≥5 cm vs. <5 cm | 3.146 (1.425-4.869) | 0.029 | 2.454 (1.714-3.332) | 0.031 |
| Depth of tumor invasion | | | | |
| T1-T2 vs. T3-T4 | 2.245 (1.187-3.262) | 0.326 | 1.774 (1.134-2.458) | 0.378 |
| NGX6 status | | | | |
| Positive vs. Negative | 1.982 (1.186-2.981) | 0.048 | 2.855 (1.752-3.764) | 0.028 |
| Lmph node metastasis | | | | |
| Positive vs. Negative | 3.687 (2.362-4.984) | 0.036 | 2.726 (1.843-3.581) | 0.023 |
| Histological grade | | | | |
| Well/moderately vs. Poorly | 4.487 (2.765-6.123) | 0.079 | 1.056 (0.861-1.273) | 0.082 |
| TNM stage | | | | |
| I-II vs. III-IV | 2.671 (1.465-4.183) | 0.032 | 3.743 (2.658-4.913) | 0.021 |

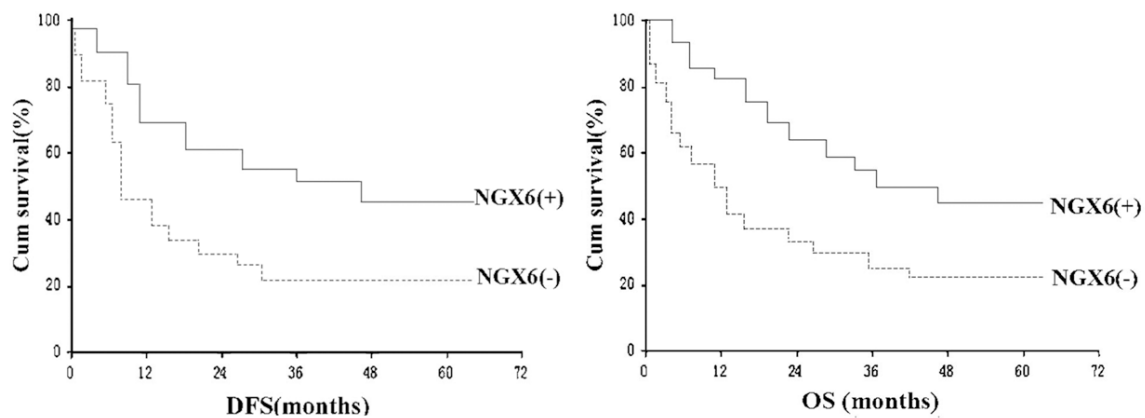


Figure 2. Kaplan-Meier analysis of DFS, OS in patients with colon cancer, according to the status of NGX6 expression (positive or negative). n=145. It's showing the correlation between of NGX6 positive expression and higher DFS or higher OS in patients with colon cancer. NGX6 (+): NGX6 expression positive group; NGX6 (-): NGX6 expression negative group.

Correlation of NGX6 expression with clinicopathological features in patients with colon cancer

Occurrence rate of large size tumor (≥5 cm), lymph node metastasis and high TNM stage (III-IV) in NGX6 negative expression group were higher than NGX6 positive expression group in colon cancer, NGX6 expression was associated

with tumor size, lymph node metastasis and TNM stage. Gender, age, depth of tumor invasion and histological grade were not associated with NGX6 expression, which were shown in **Table 1.**

Survival analysis

In the **Table 2**, univariate Cox regression analysis revealed that tumor size, NGX6 expression,

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Table 3. Multivariate Cox regression analysis of DFS and OS in colon cancer patients

| Survival | Influencing factors | HR (95% CI) | P |
|----------|-----------------------|---------------------|-------|
| DFS | Tumor size | 2.776 (1.785-3.827) | 0.048 |
| | Lymph node metastasis | 3.313 (1.254-5.239) | 0.031 |
| | Histological grade | 2.304 (1.562-3.298) | 0.155 |
| | NGX6 status | 4.365 (2.479-6.125) | 0.028 |
| | TNM stage | 2.327 (1.396-3.385) | 0.036 |
| OS | Tumor size | 2.497 (1.382-3.569) | 0.029 |
| | Lymph node metastasis | 2.691 (1.892-3.387) | 0.037 |
| | Histological grade | 1.528 (1.146-2.013) | 0.083 |
| | NGX6 status | 2.869 (1.498-4.179) | 0.022 |
| | TNM stage | 5.438 (2.731-8.894) | 0.031 |

lymph nodal status and TNM stage were significantly associated with the DFS and OS.

Kaplan-Meier analysis of DFS and OS in a subgroup of colon cancer, according to the status of NGX6 expression, and Kaplan-Meier curves of survival are shown below. This reveals the correlation between NGX6 positive expression and higher DFS ($P=0.029$, log rank test) or higher OS ($P=0.015$, log rank test) (Figure 2).

Multivariate Cox regression analysis of DFS and OS in colon cancer patients, tumor size, NGX6 expression, lymph node metastasis and TNM stage was the independent prognostic factors for DFS and OS. As shown in Table 3.

Discussion

In this study, occurrence rate of large size tumor (≥ 5 cm), lymph node metastasis, high TNM stage (III-IV) and poorly histological grade in NGX6 negative expression group were higher than NGX6 positive expression group in colon cancer, NGX6 expression was related with clinicopathologic features of colon cancer. Lian P [10] found tumor size and microvessel density in NGX6 transfection group was obviously less than non-transfection group. Guo Q [11] reported that NGX6 inhibits cell invasion and adhesion through suppression of Wnt/ β -catenin signal pathway in colon cancer. Liu M [12] found that NGX6 gene mediated by promoter methylation as a potential molecular marker in colorectal cancer. All these imply that NGX6 was involved in the invasion and metastasis activity and play an important role in colon cancer patients.

Kaplan-Meier curves showed NGX6 positive group had higher DFS or higher OS than that of negative group. In univariate and multivariate survival analysis of colon cancer patients, the data showed NGX6 expression was significantly associated with DFS and OS. NGX6 is an important prognostic factor for DFS and OS. We believe that our report is the first to confirm the relationship between NGX6 and clinicopathologic features and prognosis in patients with colon cancer. NGX6 expression was demonstrated to be independently associated with outcomes, and NGX6 may be employed

as promising prognostic factor and useful therapeutic targets to improve the survival of colon cancer patients.

It is well known that tumor size, TNM and lymph node metastasis are prognostic factors for colon cancer and are responsible, at least in part, for the mortality from colon cancer patients. Our results were well in accordance with previously researches [9, 13-17]. Many other factors including gender, age and histological grade were not identified as prognostic factor in our statistical analysis. The major limitations of our study are its small size and its retrospective nature, making these factors difficult to reach statistical significance.

In conclusion, NGX6 was involved in invasion and metastasis activity and play an important role in colon cancer. NGX6 expression was the important independent prognostic factor for colon cancer. NGX6 expression and clinicopathologic findings may complement each other, and can provide important information for prognosis in colon cancer patients.

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Disclosure of conflict of interest

None.

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