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High NUSAP1 expression predicts poor prognosis in colon cancer

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ABSTRACT

Background and aim: Nucleolar and spindle-associated protein 1 (NUSAP1) is an indispensable mitotic regulator. Aberrant NUSAP1 expression is associated with perturbed mitosis and tumorigenesis. In this study, we investigated the clinical significance of NUSAP1 expression in colon cancer.

Methods and materials: Immunohistochemical staining was performed to determine NUSAP1 protein levels in paraffin colon tumor specimens. Real-time fluorescent quantitative polymerase chain reaction (RT-qPCR) was conducted to detect NUSAP1 mRNA levels in colon tumor samples. The association between NUSAP1 protein expression and clinicopathological characteristics of patients with colon cancer was assessed. A Kaplan-Meier analysis was performed to determine the prognostic significance of NUSAP1 in colon cancer. A Cox proportional hazards model was used to calculate univariate and multivariate hazard ratios for the NUSAP1 and other clinicopathological variables.

Result: NUSAP1 protein and mRNA levels were significantly higher in colon tumor tissues than in paired non-cancerous adjacent tissues ($P < 0.001$, respectively). NUSAP1 protein expression was significantly correlated with histopathological grading ($P < 0.001$), depth of invasion ($P = 0.001$), lymph node metastasis ($P < 0.001$) and TNM stage ($P < 0.001$). The overall survival rate of patients with high NUSAP1 expression was significantly lower than for patients with low NUSAP1 expression (log-rank test, $P < 0.001$). A multivariate Cox model demonstrated that NUSAP1 is an independent risk factor for overall survival ($P = 0.025$).

Conclusion: NUSAP1 is overexpressed in colon cancer and high expression of NUSAP1 acts as an independent predictive factor for poor prognosis in colon cancer.

1. Introduction

Colorectal cancer (CRC) represents a significant global health challenge. It ranks as the second leading cause of cancer-related deaths in Western countries and the fourth leading cause of cancer-related death in China [1,2]. The interplay between genetic and environmental factors is involved in colorectal tumorigenesis and progression [3]. Despite recent advances in surgical techniques and chemoradiotherapy, the mortality rate of CRC remains high, especially for advanced cases. The five-year survival rate is estimated at approximately 85% for patients with early stage disease, while the survival rate drops precipitously to 12.5% for patients with distant metastasis [4]. Significant effort has been made to identify reliable biomarkers for CRC, with limited success. Therefore, robust diagnostic and prognostic biomarkers are urgently needed for early diagnosis and treatment, guiding individualized therapy regimens, predicting or monitoring cancer recurrence and improving survival.

Unperturbed mitosis is a fundamental cellular process. The entire process must be carried out with high fidelity to ensure that the duplicated chromosomes are evenly distributed, which requires the participation of numerous proteins [5]. Nucleolar and spindle-associated protein 1 (NUSAP1) is a recently identified 55 kDa vertebrate protein located on chromosome 15q15.1 that is selectively expressed in proliferating cells [6]. Overexpression of NUSAP1 results in excessive bundling and lengthening of spindle microtubules, inhibiting normal spindle assembly [7,8]. Depletion of NUSAP1 leads to G2-M arrest,

Abbreviations: AJCC, American Joint Committee on Cancer; CI, confidential interval; CRC, colorectal cancer; DFS, disease free survival; E2F1, E2F transcription factor 1; FAM101B, family with sequence similarity 101 member B; HR, hazard ratio; MPE, molecular pathological epidemiology; NUSAP1, nucleolar and spindle-associated protein 1; OS, overall survival; PCR, polymerase chain reaction; RB1, retinoblastoma-associated protein 1; TGF-β1, transforming growth factor-β1; TNM, tumor-node-metastasis

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disorganized mitotic spindle assembly, chromosome misalignment and aberrant segregation, and defective cytokinesis [9]. Furthermore, NUSAP1 deficiency in knockout murine embryos leads to early embryonic lethality [10]. Together, these findings show that NUSAP1, as a microtubule-associated protein, plays an important role in mitosis.

Dysregulated mitosis is recognized as a hallmark of malignant tumor cells [5]. NUSAP1 has been shown to be involved in carcinogenesis in several cancers. Most previous studies have shown that upregulated NUSAP1 is positively associated with increased malignancy. NUSAP1 is overexpressed in hepatocellular carcinoma tissue as compared to noncancerous liver tissue [11]. Additionally, the *NUSAP1* gene is overexpressed in glioblastomas as compared to benign pilocytic astrocytomas [12]. Overexpression of NUSAP1 has also been found to be correlated with worse clinical outcomes in melanoma [13], breast cancer [14] and prostate cancer [15]. Although the exact mechanisms remain unclear, these findings suggest that NUSAP1 is involved in cancer progression and metastasis.

Although several studies have documented an association between NUSAP1 expression and clinical outcomes in multiple types of cancer, little is known about NUSAP1 expression and its association with colon cancer. In the current study, we examined the expression of NUSAP1 in human colonic tissue and para-carcinoma normal tissue by immunohistochemistry and PCR method. Additionally, we investigated the correlation between NUSAP1 expression and clinicopathological characteristics and its prognostic value in predicting survival outcomes in colon cancer.

2. Materials and methods

2.1. Tissues and patients

Carcinoma tissues and paired non-cancerous adjacent tissues were harvested from 90 patients with colon cancer. All specimens were formaldehyde fixed, paraffin embedded and sectioned. A pathological diagnosis was performed for all enrolled patients. Patients underwent surgery in the Department of General Surgery (the Affiliated Hospital of Nantong University, Jiangsu, China) between June 2010 and June 2011. The study included 45 males with a mean age of 67.70 ± 9.48 years old for all patients (range, 47–90 years). Patients with a preoperative history of chemotherapy, radiotherapy and/or immunotherapy as well as patients with multiple synchronous colon tumors were excluded. Patients were followed up from the time of primary surgery until death or May 2017. The median follow-up time was 55.24 ± 28.72 months (range, 2–83 months). Additionally, pairs of cancer tissues and non-cancerous adjacent tissue were collected and stored at −80 °C from 14 patients with colon cancer undergoing resection in the Affiliated Hospital of Nantong University in 2017.

Pathological grades were as follows: (1) well-differentiated, including papillary adenocarcinoma and well-differentiated tubular adenocarcinoma; (2) moderately differentiated, including highly to moderately differentiated tubular adenocarcinoma; and (3) poorly differentiated, including poorly differentiated adenocarcinoma, signet-ring cell carcinoma, mucinous adenocarcinoma and undifferentiated carcinoma [16]. Among the 90 cases, 17 cases were well-differentiated, 54 cases were moderately differentiated and 19 cases were poorly differentiated carcinoma. The tumor-node-metastasis (TNM) stages of all patients were assessed according to the 7th Edition American Joint Committee on Cancer (AJCC) staging system [17]. There were 9 cases at stage I, 44 cases at stage II, 36 cases at stage III and 1 case at stage IV. All of the study protocols were approved by the Clinical Research Ethics Committee of the Affiliated Hospital of Nantong University. Written informed consent was obtained from all the participants or the next of kin. Permission for using patient medical information for research purposes and protection of personal privacy was obtained from the Affiliated Hospital of Nantong University.

2.2. Immunohistochemistry

NUSAP1 expression in the 90 pairs of human colonic tissue samples was examined using immunohistochemistry. The formaldehyde-fixed, paraffin-embedded sections (4-μm thick) were heated at 60 °C for 2 h, deparaffinized in xylene for 20 min twice and rehydrated in graded ethanol. The antigen retrieval process was performed in citrate buffer (pH 6.0) by microwave, followed by blocking with 3% H_2O_2 for 15 min. After washing with phosphate buffered saline, sections were incubated with 5% bovine serum to block nonspecific binding, and incubated with anti-NUSAP1 antibody (1:100 dilution; Proteintech, Rosemont, IL) in a moist chamber overnight at 4 °C. After washing, the tissue sections were treated with horseradish peroxidase-conjugated secondary antibody (Changdao, Shanghai, China) for 30 min at room temperature, according to the manufacturer's instructions. Finally, the sections were immersed in 3,3′-diaminobenzidine for 2 min, nuclear counterstained with hematoxylin, dehydrated, and mounted under coverslips. Stained sections were examined and judged by two independent, experienced pathologists who were blinded to the clinical outcome of patients.

The expression of NUSAP1 was semi-quantitatively determined by assessing the staining intensity and percentage of positively stained colon cancer cells. Staining intensity was rated as follows: negative staining (score = 0); weak staining (score = 1); moderate staining (score = 2); strong staining (score = 3). The percentage of positive cells was categorized as follows: 0%–10% (score = 0), 10%–25% (score = 1), 25%–50% (score = 2), 50%–100% (score = 3). The staining index = intensity score + distribution score, and an overall score ranging from 0 to 6 was assigned. An overall score between 0 and 3 was defined as low expression, and an overall score between 4 and 6 was defined as high expression.

2.3. Quantitative RT-PCR

Briefly, total RNA was extracted from the fresh cancer tissues and the adjacent normal colon mucosa by using Trizol reagent (Invitrogen, USA). To generate cDNA, 1 μg total RNA was reverse transcribed using PrimeScript First Strand cDNA Synthesis kit (Takara, DRR047A, Japan) in a total reaction volume of 20 μl according to the manufacturer's instructions. RT-PCR was performed using the CFX 96 Real-time RCR Detection System (Bio-Rad, California, USA) by using genespecific primers with SYBR Premix ExTaq kit (Takara, Japan). NUSAP1:forward, 5′-TCATTTCCTTTTCTTGCCTCA-3′, reverse, 5′-CCCT CAAGTACAGTGACCTGC-3′; β-actin: forward, 5′-GGACTTCGAGCAAG AGATGG-3′, reverse, 5′-AGGAAGGAAGGCTGGAAGA-3′.

2.4. Statistical analysis

Data were analyzed using SPSS software (SPSS Inc., Chicago, IL, USA). McNemar's test was used to compare the NUSAP1 expression in carcinoma tissues with that in the paired non-cancerous mucosal tissues. The association between NUSAP1 expression and clinicopathologic characteristics was estimated by Pearson's chi-square test and Fisher's exact test. Survival curves were plotted using the Kaplan-Meier method and compared hazards method. Cox proportional hazards analysis was used for univariate and multivariate analyses to investigate the effects of NUSAP1 expression and clinicopathologic variables on survival outcome. In multivariate analysis, we adopted the "Enter Method". There were two principles of variable selection for multivariable analyses. Firstly, we selected the variables based on epidemiological evidence. Secondly, we selected the variables of strong associations stemming from the specific dataset of interest ($p < 0.05$ at univariate analysis). $P < 0.05$ indicated that differences were statistically significant.