PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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• BRIEF REPORTS •

Comparison of nuclear matrix proteins between gastric cancer and normal gastric tissue

Qin-Xian Zhang, Yi Ding, Zhuo Li, Xiao-Ping Le, Wei Zhang, Ling Sun, Hui-Rong Shi

Qin-Xian Zhang, Yi Ding, Xiao-Ping Le, Wei Zhang, Molecular Cell Biology Research Center, Medical College of Zhengzhou University, Zhengzhou 450052, Henan Province, China
Zhuo Li, Ling Sun, Hui-Rong Shi, Henan Key Laboratory of Molecular Medicine, Zhengzhou 450052, Henan Province, China
Supported by the National Natural Science Foundation of China, No. 39170440
Correspondence to: Professor. Qin-Xian Zhang, Molecular Cell Biology Research Center, Medical College of Zhengzhou University, 40 Daxue Lu, Zhengzhou 450052, Henan Province, China. qxz53@zzu.edu.cn
Telephone: +86-371-6977002
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Abstract

AIM: To study the alteration of nuclear matrix proteins (NMPs) in gastric cancer.

METHODS: The NMPs extracted from 22 cases of gastric cancer and normal gastric tissues were investigated by SDS-PAGE technique and the data were analyzed using Genetools analysis software.

RESULTS: Compared with normal gastric tissue, the expression of 30 ku and 28 ku NMPs in gastric cancer decreased significantly (P=0.002, P=0.001, P<0.05). No significant difference was found in the expression of the two NMPs between the various differentiated grades (P=0.947, P=0.356) and clinical stages of gastric cancer (P=0.920, P=0.243, P>0.05).

CONCLUSION: The results suggested that the alteration of NMPs in gastric cancer occurred at the early stage of gastric cancer development.

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INTRODUCTION

Nuclear matrix (NM) is the structural framework of the nucleus comprising the peripheral lamins and pore complexes, an internal ribonucleic protein outside nucleoli[1]. Nuclear matrix proteins (NMPs) are important for a variety of cell functions, including nuclear assembly, replication, transcription, and nuclear integrity[2]. Specific changes of NMPs are associated with many cancers[3-6]. However, the alteration of NMPs in gastric cancer has not been reported. In this paper, the NMPs in gastric cancer and normal gastric tissue were studied by SDS-PAGE and Genetools quantitative analysis software.

MATERIALS AND METHODS

Materials

Twenty-two cases of gastric cancer specimens (with no history of radio- or chemotherapy preoperatively) and normal gastric mucosa were collected from the First Affiliated Hospital of Medical College of Zhengzhou University and the People's Hospital of Henan Province. All the specimens were diagnosed pathologically (well and moderately differentiated cancers in 10 cases, poorly differentiated cancers in 12 cases). According to the PTNM of International Alliance of Anticancer in 1987, the specimens were divided into 4 clinical stages, Nine were at stage I and II, and 13 at stage III and IV.

Preparation of nuclei

The gastric cancers and normal gastric tissues were minced and mixed with STM (0.25 mol/L sucrose, 10 mmol/L Tris-HCl pH 7.4, 5 mmol/L $MgCl_2$) and homogenized with a homogenizer. Nuclei were initially separated by low speed centrifugation at 750 r/min for 10 min. The pellet was suspended with STM containing 5 g/L Triton X-100 for 10 min and centrifuged at 750 r/min for 10 min. The crude nuclei were resuspended with 2 mol/L sucrose and centrifuged at 12 000 *g* for 10 min. The pellet containing purified nuclei was washed with STM.

Extraction of NMPs

The purified nuclei were digested with DNase I (200 U/mL) at room temperature for 45 min prior to low salt (LS) buffer (10 mmol/L Tris-HCl pH 7.4, 0.2 mmol/L $MgCl_2$) extraction and centrifuged at 1 000 r/min for 15 min. The pellet was extracted twice with high salt (HS) buffer (10 mmol/L Tris-HCl pH 7.4, 2 mol/L NaCl, 0.2 mmol/L $MgCl_2$) and centrifuged at 6 000 r/min for 15 min. Washed with LS buffer, the pellet was resuspended with 2×loading buffer and detected for the concentration of proteins in the sample.

SDS-PAGE

A 100 μg proteins were loaded in each well of 100 g/L SDS-polyacrylamide gel. The proteins were electrophoresed at constant voltage of 200 V. The gel was stained with Coomassic brilliant blue R-250 over 4 h. The protein bands were photoed with gel photography system and quantitatively analyzed with Genetools software.

Statistic analysis

Data were analyzed using nonparametric statistics with SPSS 10.0 statistic software and P<0.05 was considered statistically significant.

RESULTS

On the gel stained with Coomassic brilliant blue R-250, many bands were exhibited in both gastric cancer and normal gastric tissue, which suggested that NMPs were abundant in these tissues. The bands of 30 ku and 28 ku NMPs in the gastric cancer were stained more lightly than those in the normal gastric tissue (Figure 1). Analyzed with Genetools quantitative software, the expression of 30 ku and 28 ku NMPs in normal gastric tissues was significantly higher than those in gastric cancer (P<0.05). The difference of the expression of 30 ku and 28 ku NMPs between well and moderately differentiated and

poorly differentiated gastric cancers was not significant (P>0.05). There was no significant difference in the expression of the two NMPs between stageI, II and stage III, IV (P>0.05) (Table 1).

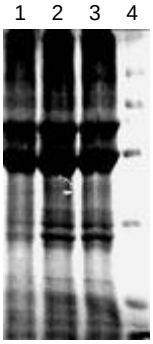

Figure 1 SDS-PAGE of nuclear matrix 1 gastric carcinoma tissue; 2 adjacent cancer tissue; 3 normal tissue; 4 marker.

Table 1 Comparison of the 30 ku, 28 ku bands between gastric cancer tissues and normal tissues

Group	30 ku			28 ku	
	n	*T*	*Z*	*T*	*Z*
Normal tissue group[a]	22	7.25	-3.165	4.33	-3.263
Gastric cancer group	22	12.44		14.19	
Well and moderately differentiated	10	11.60	-0.066	12.90	-0.923
Poorly differentiated	12	11.42		10.33	
Stage I, II	9	11.33	-0.100	13.44	-1.169
Stag III, IV	13	11.62		10.15	

T: mean of rank sum, *Z*: *z* value [a]P<0.05 *vs* gastric cancer.

DISCUSSION

NM is the structural framework of the nucleus[7], and is involved in a variety of cell functions, including DNA replication[8], RNA transcription[9], architecture of chromatin[10], carcinogenesis[11] and apoptosis[12]. The study on the relationship between NMPs and carcinogenesis has been carried out for a few years. In the experiment of Spencer *et al*.[13], specific changes in NMPs of breast cell line were identified by two-dimensional gel electrophoresis. NMP66 was evaluated as a potential biomarker for early breast cancer in large-scale clinical trials[11]. The extent of chromosomal rearrangements correlates positively with the level of expression of the nuclear matrix high mobility group (HMG) proteins HMG I (Y) when tested in three human prostate cancer cell lines (PC-3>DU145>LNCaP)[14]. Using both one-dimensional and high-resolution two-dimensional immunoblot analyses, Leman *et al*.[15] found that, in the transgenic adenocarcinoma of mouse prostate (TRAMP) model, HMG I (Y) was an NMP expressed as two protein bands with a molecular mass of 22-24 ku and HMG I (Y) expression was correlated with neoplastic and malignant properties in late stage of prostate tumor TRAMP model. In 26 pairs of human prostate cancer and normal tissue, Ishiguro *et al*.[16] identified a specific upregulated gene encoding a 55 ku nuclear matrix protein (nmt55) by RT-PCR and real time quantitative PCR. nmt55 gene expression in human prostate cancer tissue was higher (20/26) than that in normal prostate tissue.

NMP22 has been identified as a tumor marker for transitional cell carcinoma of urinary tract[17] and bladder cancer[18-21]. Eissa *et al*.[22] evaluated the diagnostic efficacy of NMP22, fibronectin and urinary bladder cancer antigen (UBC) in comparison with voided urine cytology on the detection of bladder cancer. They found that NMP22 and fibronectin had superior sensitivities compared to UBC and voided urine cytology, while NMP22 and voided urine cytology had the highest specificities. Xu[23] reported that the examination of NMP22 in urine was a rapid and effective way to detect the recurrence of bladder cancer. The urinary NMP22 levels were significantly higher in the renal cell carcinoma group than in the control group. The urinary NMP22 might be used in the evaluation of patients at risk of renal cell carcinoma[24]. Konety *et al*.[25] reported that the BLCA-4 was a very sensitive and specific marker for bladder cancer.

NMPs alterations were also associated with the cancer of digestive tract. Chen *et al*.[26] found that the interaction between HPV-16 E6 and nuclear matrix might contribute to virus induced carcinogenesis in esophageal carcinoma. Brunagel *et al*.[27] analyzed the NMPs expression by high-resolution two-dimensional gel electrophoresis, and found that the NMP composition was able to differentiate liver metastases from normal liver tissue and normal hepatocytes. In 2003, they identified an NMP, calreticulin, which was expressed much more strongly in colon cancer compared to adjacent and normal colon tissue[28]. In our study, we found that the expression of 30 ku, 28 ku NMPs was significantly reduced in gastric cancer when compared with that in the normal gastric tissue (P<0.05). There were no significant differences in the expression of these two proteins between the various differentiation grades and clinical stages of the gastric cancer. The results suggested that the changes of NMPs in gastric cancer might occur at early stage of the tumor development.

Matrix attachment regions (MARs) are postulated to anchor chromatin onto the NM, thereby organizing genomic DNA into topologically distinct loop domains that are important in replication and transcription[29]. The p300-SAF-A interactions at MAR elements of nontranscribed genes might poise these genes for transcription[30]. NM was a key locus for CK2 signaling in the nucleus[31]. Expression of p16 gene was significantly reduced in gastric cancer. The down- regulated expression of 30 ku, 28 ku NMPs in gastric cancer might be related to the down-regulated expression of p16 gene. In our previous study, we found the hypermethylation, mutation and microsatellite instability of p16 gene in gastric cancer. The binding of NMPs to the upstream of p16 gene and its relation to the down regulated expression of p16 gene in gastric cancer will be studied further.

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