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Original Article

Expression of NIBP and its clinical significance in human early colorectal cancer

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Abstract: Aim: To investigate the expression of NIBP and its clinical significance in early colorectal cancer. Patients and Methods: With immunohistochemistry, the expression of NIBP was detected in 23 patients of early colorectal cancer tissues, 102 patients of invasive colorectal cancer tissues, 32 patients of adenoma and 20 patients of normal tissues. The relationship between NIBP expression and clinicopathological characteristic of colorectal cancer were also analyzed. Result: We found that the positive rates of NIBP was higher in early colorectal cancer tissues (82.6%, 19/23) than those in adenomas and normal tissues (x^2=29.07, P<0.05), but not significant than those in invasive colorectal cancer (x^2=1.79, P>0.05). Positivity for T1N0M0, T2N0M0, II, III and IV was 82.6% (19/23), 80.0% (4/5), 78.0% (32/41), 63.6% (21/33), 56.5% (13/23), respectively. With the increase in TNM stage, the positive rate of NIBP decreased, the positive rate of T1N0M0 is highest than other TNM stages, but no statistically significant (P>0.05). Conclusion: These results suggested that NIBP is highly expressed in human early colorectal cancer tissues. NIBP might involve in the tumorigenesis and probably serve as a new marker for human early colorectal cancer.

Keywords: NIBP (NIK-and IKK2-binding protein), early colorectal cancer, immunohistochemistry

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide in males and the second in females [1]. Early colorectal cancer is defined by the Japanese rule as being limited to the mucosa or invading only to the submucosa, regardless of the presence or absence of lymph node metastases [2]. Patients with early CRC were often asymptomatic, therefore the detection rate was low even in nowadays. However, Five-year survival could be up to 90% if the CRC is diagnosed and treated early and suitably [3]. Then early detection becomes the most promising approach to improving long-term survival of patients with CRC [3-7]. In addition, it takes a certain time for a single cell to transform into cancer, proliferate through frequent mitoses, and gradually form a mass [2], which offers a period of time to detect the tumor in an early stage and to interfere with the natural course of the disease [8]. However, markers of colorectal cancer are always detected in advanced CRC but rarely occur in early stages, then to find marker for early CRC is urgent [9-11].

NIBP, also known as TRAPPC9 (trafficking protein particle complex 9) [12], is a novel cellular protein originally identified in a yeast two-hybrid assay of a human brain cDNA library, as a binding partner of NIK. Although most studies about NIBP always showed its association of intellectual disability [12-17]. Recent years, some studies showed that NIBP is associated with a variety of diseases and malignancies, but the studies were limited. Shu Liu and Hong Wang reported that NIBP highly expressed in human gastrointestinal cancer cells, also was significantly increased in invasive colon carcinoma compared with non-cancer colon tissues [18]. There was less report about the expression of NIBP in patients with early colorectal

Table 1. Characteristics of the 177 patients

Characteristics	Value
Age, (years)	
Median (mean ±SD)	56.2 (58.0±15.4)
Gender	
Male	98 (55.4%)
Female	79 (44.6%)
Localisation	
Ascending colon	34 (19.2%)
Transverse colon	9 (5.1%)
Descending colon	14 (7.9%)
Sigmoid colon	45 (25.4%)
Rectum	75 (42.4%)
Size	
Nomal colorectal	(0.2±0.0) cm
Colorectal adenoma	(2.1±2.1) cm
Early CRCs	(2.6±1.0) cm
Invasive CRCs	(5.0±2.0) cm
TMN	
I	
T1N0M0	23 (18.4%)
T2N0M0	5 (4.0%)
II	41 (32.8%)
III	33 (26.4%)
IV	23 (18.4%)
Specimen come from	
ESD or EMR	44 (24.9%)
Surgical treatment	110 (62.1%)
Endoscopic biopsy	23 (13.0%)
Pathology	
Well differentiation	21 (11.9%)
Moderate differentiation	91 (51.4%)
Poor differentiation	13 (7.3%)
Serrated adenomas	5 (2.8%)
Tubular adenomas	5 (2.8%)
Tubulavillous adenomas	7 (4.0%)
Tubulavillous with high-grade intraepithelial neoplasia	15 (8.5%)
Normal tissue	20 (11.3%)

cancer, So in this study, we investigated the potential association with NIBP and early colorectal cancer and figured out whether NIBP might be a probable new marker for early colorectal cancer.

Materials and methods

Patients and tissues

177 patients underwent ESD, EMR, surgical treatment or endoscopic biopsy in our department from June 2011 to December 2014 were included in this study. All the patients were divide into four groups by pathology, 23 early colorectal cancer, 102 invasive colorectal cancer, 32 colorectal adenoma and 20 normal patient. In our study, there were no lymph node metastases in early CRC group. Patient characteristics are shown in **Table 1**. Written informed consent was obtained from each patient on the day of procedure. Institutional review board approval was obtained by The Third Affiliated Hospital of Southern medical University (Guangzhou, China).

Immunohistochemistry

All samples from the 177 patients were reviewed histologically by H&E staining, and representative areas were paraffin-embedded, which tissues were subjected to routine sectioning of 4 mm thickness. Two step immunohistochemical staining was used for NIBP detection. Then put in an incubator chamber at 60°C for 20 minutes, Dimethyl benzene dewaxed, Rehydrated through graded alcohol to PBS, After washed with PBS, the sections were subjected to antigen retrieval in boiling sodium citrite buffer (0.01 M, pH 6.0) for 10 min (microwave 450 W). After cooled at room temperature for 30 min, and washed with PBS and distilledwater sequentially, then inmmersed in 3% hydrogen peroxide at room temperature for 10 min to block endogeneous peroxidase activity. The anti-NIBP rabbit antibody (Abcam, UK) diluted 1:80 in primary antibody diluents (Beyotime, Jiangsu, China) was then added, Then the slides were incubated 1 hour in a humidified chamber in 37°C. After washed with PBS, each of the sections was incubated at 37°C for

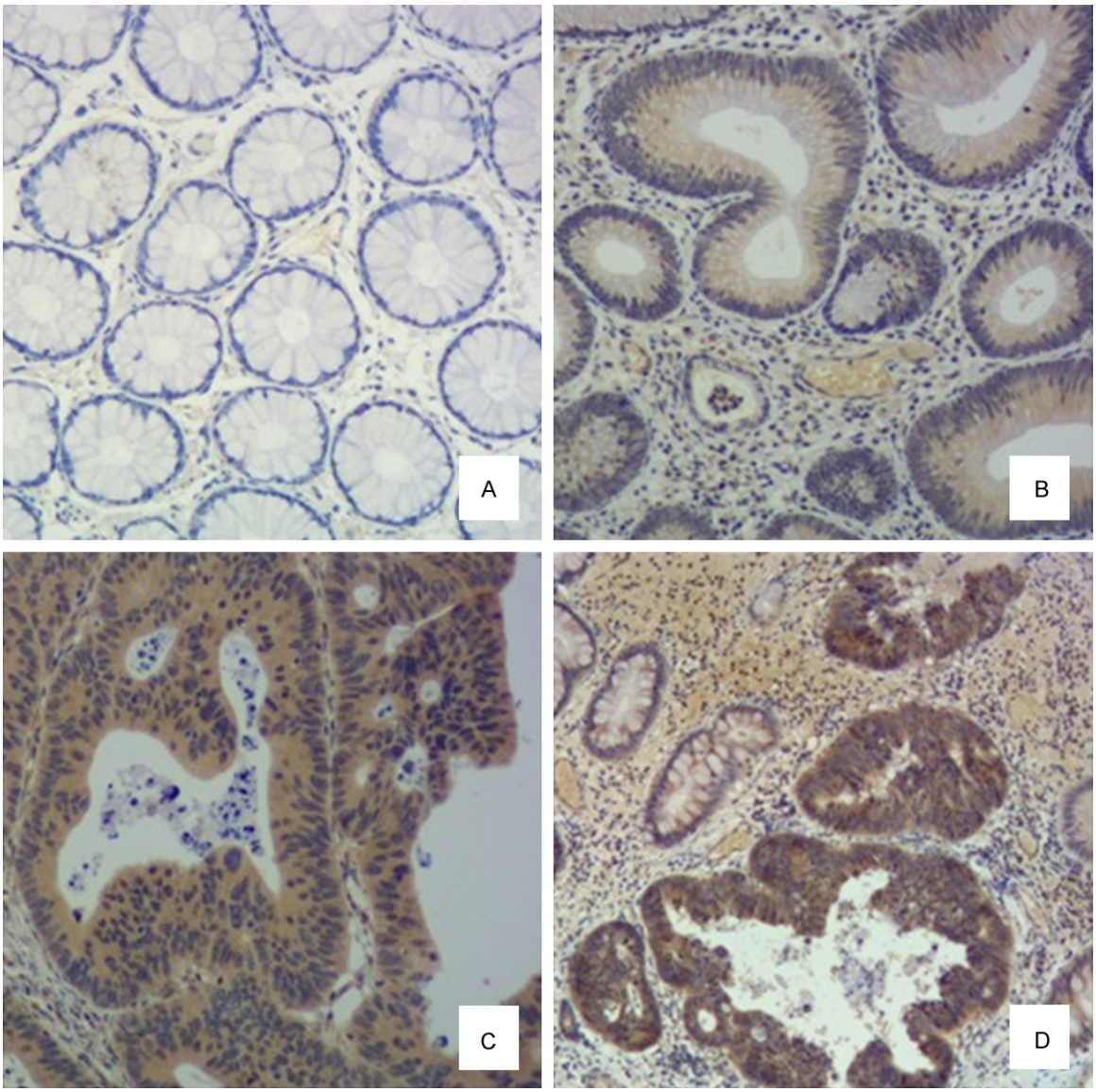

Figure 1. Representative immunohistochemical (IHC) staining for NIBP expression (×100). A: Normal colorectal mucosa: no staining; B: Colorectal adenoma tissues: Weak staining or part membranous staining; C: Invasive colorectal cancer: dark yellow staining; D: Early colorectal cancer: brown staining.

Table 2. Expression of NIBP in each group

GROUPS	N	NIBP		
		Expression level	Positive number	Positive rate
Normal	20	0.20±0.29	2	10.0%
Adenoma	32	0.41±0.53	7	21.9%*
Early CRC	23	1.45±0.90	19	82.6%**
Invasive CRC	102	1.25±0.98	70	68.6%***

*Fisher's Exact Test, P=0.454; **x^2=29.07, P=0.000; *** x^2=1.79, P=0.181.

1 hour with 50 ml of GTVision I detection system/mouse or rabbit from Gene Tech Company Limited, Shanghai, China. After washed with PBS again, the sections were subjected to sequential DAB substrate chromogen system (Dako, Danish) for immune complex visualization and then counterstained with haemotoxylin for 30 seconds. Formalin-fixed and paraffin-embedded sections of human colon carcinoma with strong staining served as positive control whereas PBS instead of antibody as negative control. The NIBP staining was blindly reviewed by two histopathologists. Microscopically, the slides with no staining in negative control and specific dark yellow staining of cytoplasm and neuclear in positive control were eligible for fur-

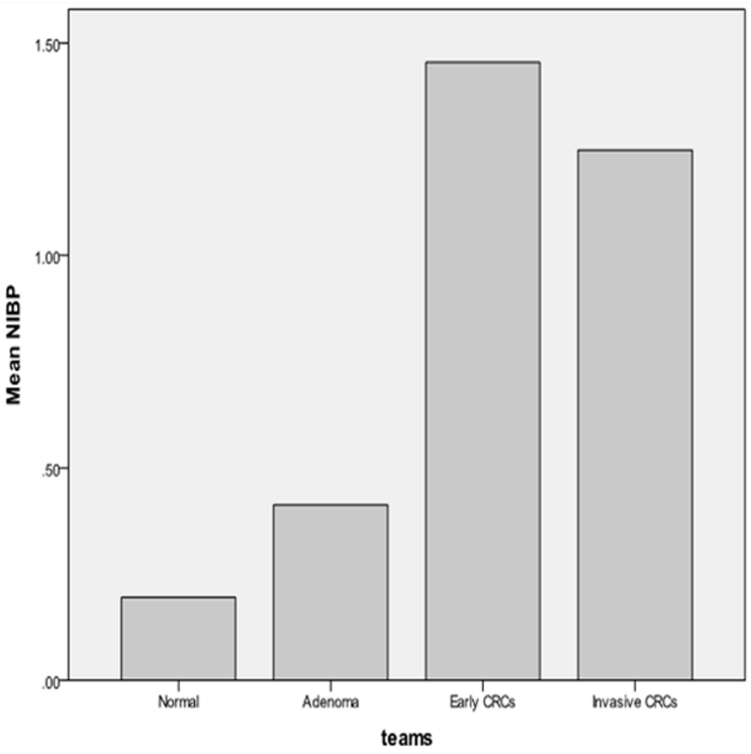

Figure 2. NIBP expression leaver in each team. A Mean NIBP scores in normal mucosa (n=20), adenoma (n=32), early colorectal cancers (CRC) (n=23) and Invasive colorectal cancers (n=102).

Table 3. NIBP expression in CRC with TNM (P>0.05)

TNM	N	NIBP	
		Positive number	Positive rate
I			
T1N0M0	23	19	82.6%
T2N0M0	5	4	80.0%
II	41	32	78.0%
III	33	21	63.6%
IV	23	13	56.5%

ther analysis. The immunostaining was evaluated according to the following standards, staining intensity was classified as 0 (lack of staining), 1 (weak yellow), 2 (dark yellow) or 3 (brown staining). For each section, the semiquantitative score was calculated by multiplying the staining intensity score and the percentage of staining, the threshold for positivity was 0.7.

Statistical analysis

Statistical analyses were conducted by IBM SPSS Statistical 19 (SPSS, USA). Data are presented as mean ± standard deviation (SD). The Relationships between NIBP expression and antibody response with clinicopathological parameters were tested by x^2 test or Fisher's exact test. Continuous variables were analyzed with t test and P<0.05 was considered significant.

Results

Expression of NIBP

NIBP expression was examined in 23 early CRC, 102 invasive CRC, 32 colorectal adenomas and 20 normal colorectal tissues by IHC. It was mostly localized to the cytoplasm of colorectal carcinoma cells. No or a weak staining of NIBP was observed in normal tissue (**Figure 1A**), NIBP expression was relatively weak in adenoma (**Figure 1B**) but strong in the invasive CRC tumor cells with dark yellow or brown staining (**Figure 1C**), NIBP expression was much stronger in the early CRC tumor cells with dark yellow or brown staining (**Figure 1D**). The positive rate and expression level are shown in **Table 2** and **Figure 2**. The positive rates of NIBP was higher in early colorectal cancer tissues (82.6%, 19/23) than those in invasive colorectal cancer tissues (68.6%, 70/102), adenoma tissues (21.9%, 7/32) and normal tissues (10.0%, 2/20). The positive rate of NIBP in early colorectal cancer tissues was significantly higher than those in adenoma tissues and normal tissues (x^2=29.07, P=0.000, P<0.05), but not significant compared with those in invasive colorectal cancer (x^2=1.79, P=0.181, P> 0.05). There is not statistics significantly between adenoma tissues and normal tissues either (P=0.454, P>0.05). Positivity for T1N0M0, T2N0M0, II, III and IV was 82.6% (19/23), 80.0% (4/5), 78.0% (32/41), 63.6% (21/33), 56.5% (13/23), respectively. With the increase in TNM stage, the positive rate of NIBP decreased, the positive rate of T1N0M0 is highest than other TNM stages, but no statistically significant (P>0.05) (**Table 3**).

Table 4. Relationship between NIBP expression and clinicopathological factors of colorectal cancer

Item		N	Positive		Negative		P
			N	Rate	N	Rate	
Gender	Male	64	47	73.4%	17	26.6%	0.571
	Female	61	42	68.9%	19	31.1%	
Age	≥60	63	49	77.8%	14	22.2%	0.102
	<60	62	40	64.5%	22	35.5%	
Size	≥3 cm	102	73	71.6%	29	28.4%	0.848
	<3 cm	23	16	69.6%	7	30.4%	
Location	Rectum	54	35	64.8%	19	35.2%	0.169
	Colon	71	54	76.1%	17	23.9%	
Depth of invasion	Mucosa and submucosa	23	19	82.6%	4	17.4%	0.181
	Over submucosa	102	70	68.6%	32	31.4%	
Differentiation	Well	21	16	76.2%	5	23.8%	0.580
	Moderately and poorly	104	73	70.2%	31	29.8%	
Lymph node metastasis	Yes	50	31	62.0%	19	38.0%	0.064
	No	75	58	77.3%	17	22.7%	

Relationship between NIBP expression and clinicopathological characteristic of colorectal cancer

The study failed to find the correlation between NIBP expression in tumor and characteristic such as gender and age of the patients, size, location, depth of invasion, differentiation and lymph node metastasis of tumors (**Table 4**).

Discussion

Colorectal cancer (CRC) is one of the most frequent cancers and a major cause of cancer mortality. Early detection and treatment of CRC could always improve long-term survival for those patients. NIBP was first founded in 2005 by Hu, and expressed at high levels in muscles and kidney and lower levels in brain, heart and immune organs [12]. As if Mir et al. reported Human NIBP is a 1246-amino acid peptide (139.4 kDa), which is found on chromosome 8q24.3, contains 23 exons [19]. It localizes to the cytoplasm and the Golgi apparatus, as well as to the endoplasmic reticulum (ER) [20]. Previous studies about NIBP were always showed its association with intellectual disability, while closely correlate with mental retardation by genetic mutations [12-16, 21]. However, Barrowman et al. reported that NIBP is a key member of trafficking particle protein complex II which is essential in trans-Golgi networking (TGN) [22]. Additionally, Hu et al. reported NIBP functions as an NF-κB activator, specifically by promoting increased phosphorylation of IKK proteins. Both NF-κB and TGN are critical in inflammation-linked tumorigenesis and cancer metastasis [23-25], then the relationship between NIBP and cancer arouses interests for researchers, and recent study showed that NIBP is associated with malignancies [26], Zhang et al. reported NIBP is expressed in most tumor tissues, particularly in breast and colon cancer, even so, the studies were limited. The expression of NIBP in patients with early colorectal cancer is unclear.

In our study, the results showed the prevalence of NIBP overexpression in human early CRC tissues by immunohistochemistry (IHC). As for IHC staining, NIBP was mostly localized to the cytoplasm of colorectal carcinoma cells, and it expression was much stronger in the early CRC tumor cells with deeper staining. We found the positive ratio of 82.6% in the early CRC group was significantly higher than that of the adenoma group and the normal control group ($P<0.05$), but no significantly than those in invasive colorectal cancer group. The mean expression lever of NIBP is (1.45±0.90) in early CRC, which is the highest above all the groups. In addition, with the increase in TNM stage, the positive rate of NIBP decreased, the positive rate of T1N0M0 is highest than other TNM stages, although no statistically significant ($P>0.05$), which is in accord with Wang's research [18], however, these dates showed a tendency that the higher NIBP positive rate indicated the earlier CRC stages.

NIBP is a novel NIK and IKKβ-binding protein that enhances NF-κB activation, Both IKKβ and NF-κB are play an important role in regulating tumorigeness [23, 27-29]. NIBP promotes tumorigenesis via NFκB signaling in colon cancer cells, knockdown of NIBP inhibited the proliferation, migration and colony formation of colon cancer cell lines in vitro as well as the tumor formation in vivo [15]. According to the theory that most CRC have evolved through the adenoma-cancer sequence [30], our study was on the patient who with early colorectal cancer, and the result showed that NIBP was rarely expressed in adenomas but highly in early CRC. In combination with Wang's report, NIBP mRNA was highly expressed in early colorectal cancer [18], it could indicated that NIBP might be involved in early tumorigenesis.

Our results indicate that NIBP has potential value as a new marker for the detection of early CRC, however more studies are needed for NIBP to be a accurate marker. Since the early CRC was unevenly distributed in the tissues, frozen tumor biopsies were difficult to collected, and we had to take all of those tissues for pathological examination, then further studies of NIBP mRNA and protein in fresh human early CRC tissues are needed. Due to the limited quantity of the included studies, the results suggest that further and larger-scale randomized double-blind contrast trials are needed. NIBP was high expressed and might serve as a new marker for the early CRC, however, it was unclear whether NIBP could be a serologic biomarkers, which might provided a relatively noninvasive and economically advantageous method for the detection of early CRC.

In conclusion, NIBP is highly expressed in human early colorectal cancer tissues, with the development in TNM stage, the positive rate of NIBP decreased, and we could speculate NIBP may serve probably as a new marker for the early colorectal cancer.

Disclosure of conflict of interest

None.

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