Int J Clin Exp Pathol 2016;9(2):2616-2624
www.ijcep.com /ISSN:1936-2625/IJCEP0019418

Original Article

High expression of neurokinin A and its receptor NK2R associate with carcinogenesis and may predict poor survival in colorectal cancer

Ying-Yu Ma[1*], Wei-Jia Fang[2*], Hui-Ju Wang[1], Yi Zheng[2], Xiao-Zhou Mou[1], Xiao-Jun Wang[3], Xiao-Yi Chen[1], Li Li[1], Shi-Bing Wang[1], Ke-Tao Jin[4], Xiang-Lei He[5], Dong-Sheng Huang[1]

[1]Clinical Research Institute, Zhejiang Provincial People's Hospital, Hangzhou, China; [2]Biotherapy Center, Department of Medical Oncology, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China; [3]School of Medicine, Zhejiang University, Hangzhou, China; [4]Department of Gastrointestinal Surgery, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, Shaoxing, China; [5]Department of Pathology, Zhejiang Provincial People's Hospital, Hangzhou, China. [*]Equal contributors.

Received November 9, 2015; Accepted January 3, 2016; Epub February 1, 2016; Published February 15, 2016

Abstract: Increasing evidence has highlighted the impact of inflammation in colorectal cancer (CRC), but the effects of the inflammatory process on CRC remain largely unknown. Neurokinin A (NKA) presents in the tumor microenvironment and could induce neurogenic inflammation by intracellular signaling through NK2R. However, the precise significance of NKA and its receptor NK2R in CRC need to be fully defined. We performed Immunohistochemistry staining on tissue microarrays (TMA) containing 90 pairs of CRC and adjacent normal tissues to evaluate the clinical significance of NKA or NK2R in CRC, and the potential correlation between NKA and NK2R in CRC development was also explored. The expressions of NKA and NK2R were overexpressed in CRC (P<0.001). High expression of NKA in CRC was significantly associated with lymph node metastasis (P=0.008) and TNM stage (P=0.021). Meanwhile, high expression of NK2R in CRC was significantly related to tumor diameter (P=0.025), pathology grading (P=0.032) and lymph node metastasis (P=0.046). A high correlation between NKA and NK2R expression was also observed (r=0.229, P=0.030). Survival analysis showed that CRC patients with high expression of NKA or NK2R have a poorer survival than those with low NKA or NK2R expression (Log rank test, P<0.05). Multivariate analysis using Cox regression model showed that survival was independently correlated with age, distant metastasis, TNM stage and NK2R expression (P<0.05). In conclusion, high expression of NKA-NK2R may play pro-inflammatory role in CRC carcinogenesis and progression, which may also be used as predictors for prognosis of CRC.

Keywords: NKA, NK2R, colorectal cancer

Introduction

Colorectal cancer (CRC) is the fourth most common form of cancer in the world [1]. Especially in China, CRC has become the second and fourth leading cause of cancer death in women and men, respectively [2]. Most of the deaths are caused by the progression of the tumor to metastatic disease, for example, liver metastasis post operation easily cause relapse [3]. Although CRC is one of the most effectively studied subjects in recent years, the pathogenesis of CRC is still not fully identified. Initially it was thought that genetic mutations play a key role in CRC pathogenesis, and many studies have been performed to confirm this hypothesis [4-6]. However, genetic basis for the carcinogenesis of CRC does not allow us to introduce new, effective CRC treatment modalities. Recently, many studies have highlighted the impact of inflammation in CRC, and the effects of the inflammatory process on neoplasia development were demonstrated in endometrial, cervical, ovarian, breast, prostate and colon tumors [7]. The inflammatory process most likely plays a key role in the pathogenesis of CRC, especially in its promotion, which is an important factor in cancer staging and prognosis.

Neurogenic inflammation is orchestrated by a large number of neuropeptides mainly including tachykinins, which are present in the tumor microenvironment and can act on different stages of carcinogenesis [8]. Beside substance

P (SP), tachykinins includes the classical members as neurokinin A (NKA), neurokinin B (NKB), and the biological activity of neurokinins depends on their interaction with three specific NKs receptors, NK1R (specific for SP), NK2R (specific for NKA) and NK3R (specific for NKB) receptors [9-11]. Such as SP, the endogenous ligand for NK1R, activates responses to promote cell growth and inhibit apoptosis in human breast, colon, and prostate cancer cell lines [12]. NKA is a ten amino acid peptide translated from the pre-protachykinin gene with broncho-constrictive properties [13]. Dobson *et al.* reported that NKA levels are considered to play an important role in evaluating carcinoid heart disease and treatment responses [14]. Meanwhile, NKA is also a good predictor of survival in patients with small bowel neuroendocrine neoplasms as the endogenous ligand for NK2R [15].

NK2R is expressed in a variety of organs, including the gastrointestinal tract, while functional data suggest that *NK2R* could be significant in mediating the NK-evoked smooth muscle contraction of organs [16]. Our previous study revealed that *NK2R* polymorphism alone or combination with *NK1R* may be a promising prognostic marker of lymph node metastasis in CRC patients [17]. Studies also suggested that numerous tumors that express NKRs can misuse the NK-induced signaling of normal cells, which leads to promote the proliferation and survival of cancer cells, releasing cytokines and soluble mediators that conducive to tumor cell growth [18]. However, the precise involvement and significance of NKA and its receptor NK2R in CRC pathologies remains to be fully defined.

To better understand the involvement of inflammatory process in human CRC, we evaluate the clinical significance of NKA and NK2R in the progression and prognosis of CRC, and to explore the potential association between NKA and NK2R in CRC progression. We expected that understanding the mechanisms of inflammatory processes in CRC combining it with any other genetic background should allow for a reduction in the incidence of CRC and for effective treatment.

Materials and methods

CRC patients of tissue microarray

Immunohistochemical staining was performed to evaluate the expression level of NKA and NK2R with tissue microarrays (TMA), which were purchased from Shanghai Biochip Co.,Ltd. (Shanghai, China). The TMAs containing a total of 90 formalin-fixed, paraffin-embedded archival samples from a total of 90 CRC patients, as well as 90 corresponding controls derived from adjacent normal tissues. The patient cohort consisted of 47 males and 43 females, with a median age of 70-year (range 24-90) at the time of surgery. All patients had follow-up records for >5 years. The survival time was calculated from the date of surgery to the follow-up deadline or date of death.

Immunohistochemistry analysis

TMA sections were then used for the subsequent immunohistochemical staining. Briefly, TMA sections were deparaffinized and dehydrated according to standard procedures using xylene and graded alcohol. Antigen retrieval was then carried out by autoclaving in 0.01 M citrate buffer (pH 6.0) for 3 min. They were then blocked with 3% (v/v) H_2O_2 for 10 min to quench endogenous peroxidase activity, followed by incubation with 10% normal goat serum to reduce background non-specific binding at room temperature. Then TMA sections were incubated with a rabbit anti-human primary polyclonal antibody against NKA (1:2000, orb13610, Biorbyt, Cambridge, UK) or a goat anti-human polyclonal antibody against NK2R (1:75, SC14121, Santa Cruz, USA) at 4°C overnight. Negative controls were also performed by replacement of the primary antibody with PBS. Subsequently, the sections were incubated with biotin labeled secondary antibody (Invitrogen, Carlsbad, CA, USA) at room temperature for 20 min, rinsed with PBS (phosphate buffered saline), and incubated with streptavidin-biotinylated horseradish peroxidase conjugated antibody (Invitrogen, Carlsbad, CA, USA) for another 20 min at room temperature. Finally, the slides were stained with 3,3-diaminobenzidine (DAB) and counterstained with hematoxylin, dehydrated and mounted.

Evaluation of immunohistochemical staining

Immunohistochemical staining showed that NKA and NK2R positive staining were mainly located in the cytoplasm. The degree of immunostaining was reviewed under a light microscope by two expert pathologists without knowledge of the clinical data and scored independently. The NKA and NK2R expression level was based on the intensity of cellular staining and the proportion of stained tumor cells.

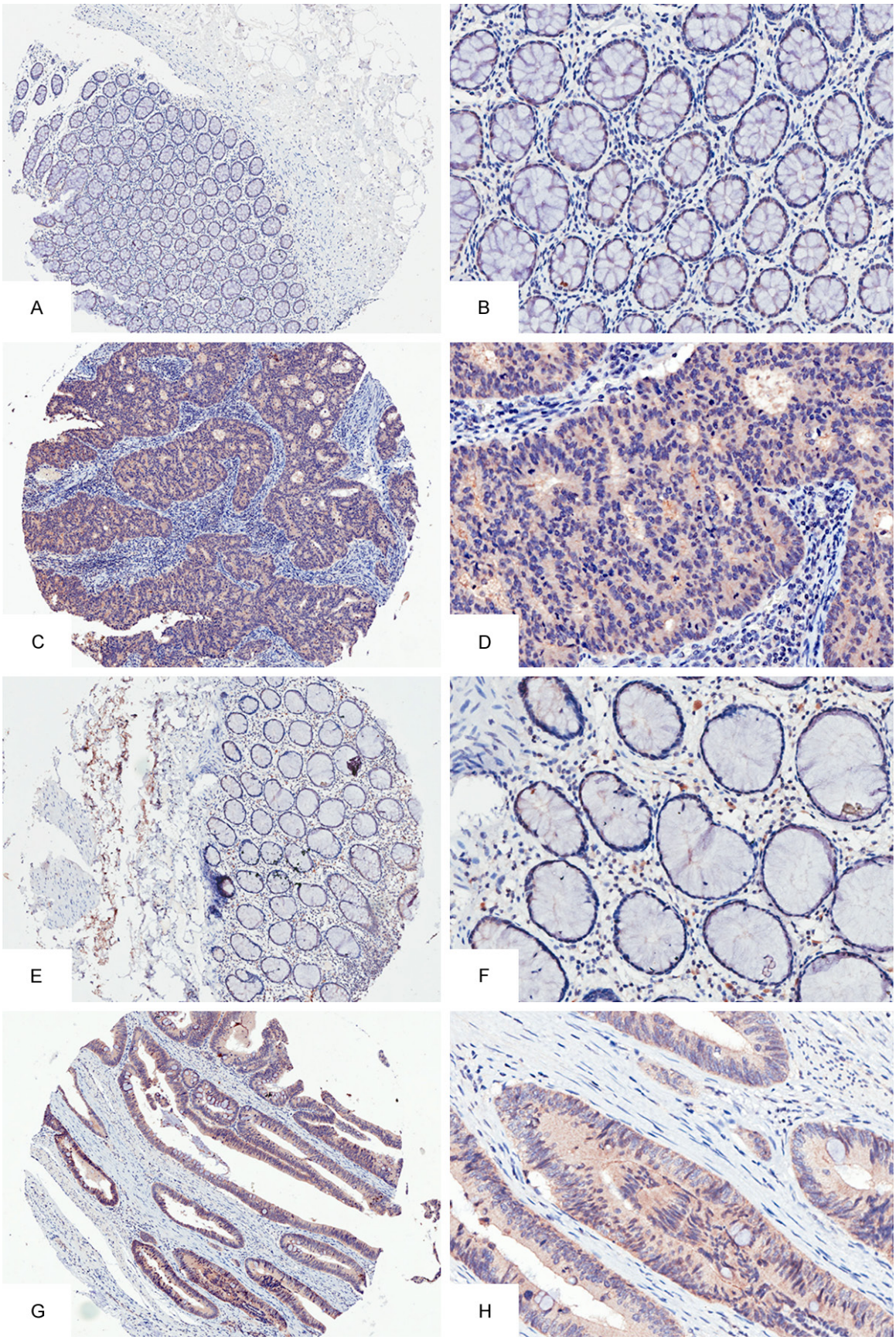
A
B
C
D
E
F
G
H

Figure 1. Immunohistochemical staining of NKA and NK2R in CRC and normal colorectal tissue. A. Low expression of NKA in normal colon tissue; C. High expression of NKA in CRC, and positive staining was mainly in the cytoplasm; E. Low expression of NK2R in normal colorectal tissue; G. High expression of NK2R in CRC, and positive staining was mainly in the cytoplasm. A, C, E, G. Original magnification 100 ×; B, D, F, H. Original magnification 600 ×.

Table 1. Relationship between NKA expression and clinicopathological features of CRC

Clinical parameters	NKA expression		x^2	*P* value
	Low (%)	High (%)		
Gender			1.663	0.197
Male	18 (38.3%)	29 (61.7%)		
Female	11 (25.6%)	32 (74.4%)		
Age (yrs)			0.254	0.766
<60	4 (26.7%)	11 (73.3%)		
≥60	25 (33.3)	50 (66.7%)		
Tumor diameter			0.778	0.378
<20 cm	14 (36.8%)	24 (63.2%)		
≥20 cm	14 (28.0%)	36 (72.0%)		
Pathology grading			2.764	0.262
I	3 (60.0%)	2 (40%)		
II	17 (34.7%)	32 (65.3%)		
III	9 (25.0%)	27 (75.0%)		
TNM stage			5.315	0.021
I+II	24 (42.1%)	33 (58.9%)		
III+IV	5 (15.2%)	28 (82.4%)		
Lymph node metastasis			6.953	0.008
No	23 (41.1%)	33 (57.9%)		
Yes	6 (17.6%)	28 (84.8%)		
Distant metastasis			0.296	1.000
No	28 (31.8%)	60 (68.2%)		
Yes	1 (50.0%)	1 (50.0%)		

Staining intensity was scored according to the following criteria: 0 (no staining), 1 (weak staining, *light yellow*), 2 (moderate staining, *yellow brown*), and 3 (strong staining, *brown*). Proportion of stained tumor cells were scored according to the proportion of positively staining tumor cells as follows: 0 for <5% positive tumor cells; 1 for 6-25% positive tumor cells; 2 for 26-50% positive tumor cells; and 3 for >51% positive tumor cells. On the basis of these data, the immunoreactive score was calculated by Proportion of stained tumor cells × staining intensity score. For further evaluation, staining index score of ≤4 was used to define tumors with NKA or NK2R low expression, and staining index score of >5 was regarded as NKA or NK2R high expression. In cases of discrepancy, a consensus score was chosen for evaluation.

Statistical analysis

All statistical analyses were performed using the Statistical Package of Social Sciences (SPSS, version 13.0; SPSS Inc, Chicago, IL, USA). To assess the relationships between the expression of NKA and NK2R and the clinicopathological parameters of the patients with CRC, the χ^2 test or Fisher's exact test was used. Univariate survival analysis was performed using the Kaplan-Meier method, accompanying log-rank test to calculate differences between the curves. Cox proportional hazards regression model was used to perform multivariate survival analysis to assess predictors related to prognosis. Variables that were significant in the univariate analysis were included in the model with the Backward Wald method. Additionally, correlation between NKA, NK2R protein expression and clinicopathological features were estimated using Spearman correlation method. All *P* values were two-sided and a *P* value <0.05 was considered as statistically significant.

Results

Expression of NKA and NK2R in CRC and noncancerous colorectal mucosa

The immunostaining for NKA was mainly located in the cytoplasms of the cells (**Figure 1**). High expression level of NKA was detected in 61 (67.8%) of the 90 patients with CRC, which was significantly higher than that in the noncancerous colorectal mucosae (2.2%, 2/90, *P*<0.001). The immunostaining for NK2R was dominantly distributed in the cytoplasms (**Figure 1**). High expression level of NK2R was

Table 2. Relationship between NK2R expression and clinicopathological features of CRC

Clinical parameters	NK2R expression		x^2	*P* value
	Low (%)	High (%)		
Gender			1.386	0.239
Male	15 (31.9%)	32 (68.1%)		
Female	9 (20.9%)	34 (79.1%)		
Age (yrs)			0.409	0.533
<60	5 (33.3%)	10 (66.7%)		
≥60	19 (25.3%)	56 (74.7)		
Tumor diameter			5.019	0.025
<20 cm	15 (39.5%)	23 (60.5%)		
≥20 cm	9 (18.0%)	41 (82.0%)		
Pathology grading			6.710	0.032
I	3 (60.0%)	2 (40.0%)		
II	16 (32.7%)	33 (67.3%)		
III	5 (13.9)	31 (86.1%)		
TNM stage			1.918	0.166
I+II	18 (31.6%)	39 (68.4%)		
III+IV	6 (18.2%)	27 (81.8%)		
Lymph node metastasis			3.997	0.046
No	19 (33.9%)	37 (66.1%)		
Yes	5 (14.7%)	29 (85.3%)		
Distant metastasis			0.569	1.000
No	23 (26.1%)	65 (73.9%)		
Yes	1 (50.0%)	1 (50.0%)		

detected in 66 (73.3%) of the 90 patients with CRC, which was significantly higher than that in the non-cancerous colorectal mucosae (10.0%, 9/90, *P*<0.001).

Correlation between expression of NKA and NK2R in CRC

Our present study showed that a high correlation between NKA and NK2R expression was observed in CRC. Of the 61 patients with high expression of NKA, 49 (80.3%) also had a high expression of NK2R. The correlation between the expression of NKA and NK2R in patients with CRC was statistically significant (*r*=0.229, *P*=0.030, Spearman's ρ-test).

Relationship of NKA and NK2R expression with clinicopathological features of CRC

High expression of NKA in CRC has no significant association with age, gender, tumor diameter, pathology grading and distance metastasis, but was significantly associated with lymph node metastasis and TNM stage (**Table 1**). The detection rate of NKA high expression was 84.8% (28/34) in CRC patients with lymph node metastasis, which was higher than that without lymph node metastasis (57.9%, 33/56, x^2=6.953, *P*=0.008). High expression of NKA was detected in 82.4% (28/33) CRC patients with TNM stage III+IV, which was higher than that with TNM stage I+II (58.9%, 33/57, x^2=5.315, *P*=0.021). The Spearman correlation coefficient of NKA high expression with lymph node metastasis and TNM stage were 0.243 and 0.279 (*P*<0.05), respectively.

Similarly, high expression of NK2R in CRC has no significant association with age, gender, TNM stage and distance metastasis, but was significantly related to tumor diameter, pathology grading and lymph node metastasis (**Table 2**). High expression of NK2R was detected in 82.0% (41/50) CRC cases with tumor diameter ≥20 cm, which was higher than that with tumor diameter <20 cm (60.5%, 23/38, x^2=5.019, *P*=0.025). The detection rates of NK2R high expression were 40.0% (2/5), 67.3% (33/49), 86.1% (31/36) in CRC patients with pathology grading I, II, III respectively, which revealed a significant difference (x^2=6.710, *P*=0.032). Meanwhile, high expression of NK2R was detected in 85.3% (29/34) CRC patients with lymph node metastasis, which was higher than that without lymph node metastasis (66.1%, 37/56, x^2=3.997, *P*=0.046). The Spearman correlation coefficient of NK2R high expression with tumor diameter, pathology grading and lymph node metastasis were 0.239, 0.264 and 0.211 (*P*<0.05), respectively.

Correlation of NKA and NK2R expression with prognosis

Survival analysis revealed the 3- and 5-year cumulative survival rates were 69.2% and 61.5% for patients with low NKA expression, and 59.6% and 43.9% for those with high NKA expression, respectively. The mean survival time for patients of CRC with high NKA expression was 50.07±4.12 months, and 63.42±5.55

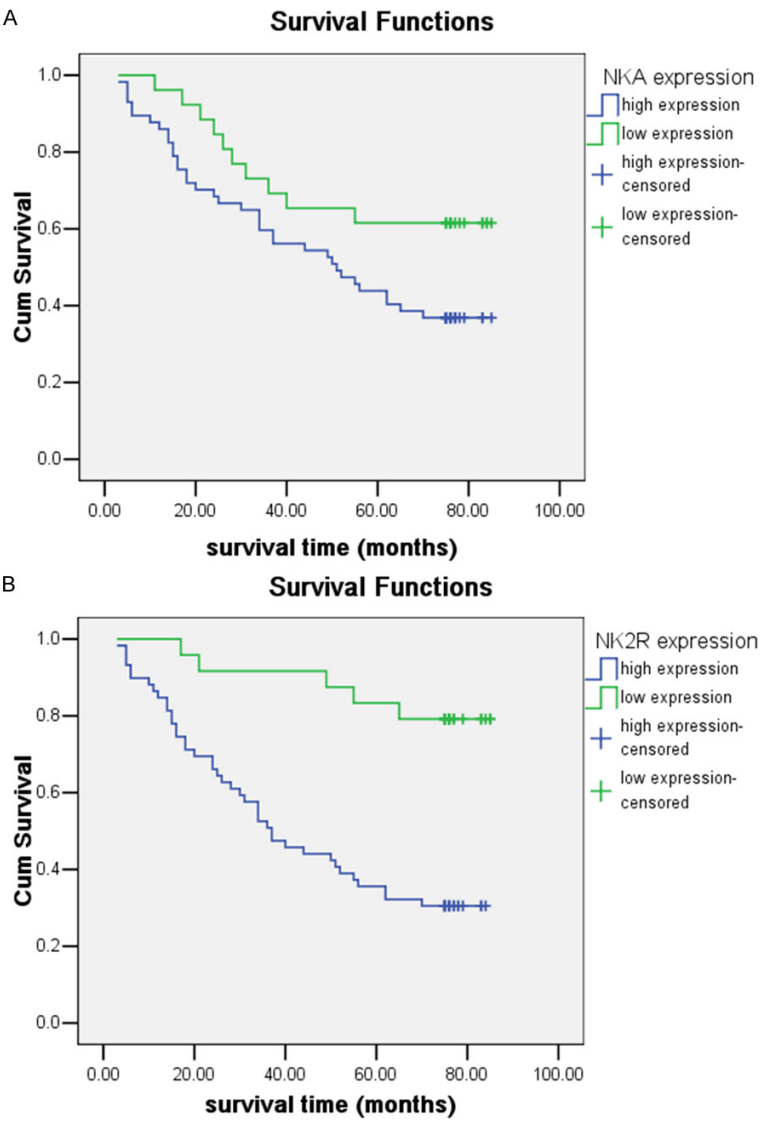

Figure 2. Kaplan-Meier survival curve analysis in patients with positive and negative NKA or NK2R expression. It shows that patients with NKA (A) or NK2R (B) high expression level have long-term survival time than those with NKA or NK2R low expression level.

months for those with low expression of NKA. Clearly, CRC patients with high expression of NKA have a poorer prognosis than those with low NKA expression (Log rank test, x^2=3.890, *P*=0.049, **Figure 2**). Likewise, the 3- and 5-year cumulative survival rates were 91.7% and 71.2% for patients with low NK2R expression, and 50.8% and 35.6% for those with high NK2R expression, respectively. The mean survival time for patients of CRC with high NKA expression was 45.14±3.88 months, and 75.92±4.03 months for those with low expression of NKA. Therefore, CRC patients with high expression of NK2R have a poorer prognosis than those with low NK2R expression (Log rank test, x^2=15.073, *P*<0.001, **Figure 2**). Multivariate analysis using Cox regression model showed that survival was independently correlated with age, distant metastasis, TNM stage and NK2R expression (**Table 3**).

Discussion

Innate immune system cells are included in the microenvironment of tumors, and these cells secrete proinflammatory cytokines, chemokines, growth factors and reactive oxygen species that could cause DNA damage [19, 20]. Inflammatory processes play an important role in the development and progression of CRC, which additionally are the basis by which we can determine their progression and outcome [21].

Neuropeptides mainly including tachykinins (SP, NKA and NKB) are traditionally viewed as with well-defined functions as neurotransmitters, which also have an impact on the function of the immune system [22]. After exposure to allergens, inflammatory cell derived tachykinins are a major second source of these proinflammatory mediators [23], which can alter the function of the immune system [22]. Tachykinins may induce the so called neurogenic inflammation by recruitment and activation of the inflammatory cells [23]. Neuropeptides were reported to have modulatory effects on immune cells, especially on T-helper (Th)1/Th2 balance [24]. In this context, NKA have been recognized as key mediators of neuro-immune interactions in some autoimmune diseases by intracellular signaling through NK2R [25]. Recent articles reported that NK2R expression was observed in neurons [26], murine macrophages [27], alveolar mac-

Table 3. Multivariate Analysis of the Correlation between Clinicopathological Parameters and Prognosis of Patients with CRC

Covariates	Coefficient	Standard Error	HR	95% CI for HR	*P* value
Age	1.164	0.496	3.202	1.212-8.457	0.019
Distant metastasis	3.147	1.075	23.264	2.830-191.268	0.003
TNM stage	0.596	0.268	1.815	1.072-3.071	0.026
NK2R expression	1.779	0.550	5.921	2.015-17.399	0.001

rophages from patients with COPD [28] and tissue from chronic pancreatitis [29]. Kitamura et al suggested that the NKA-NK2R cascade would be a promising target in chronic inflammation caused by excessive type 1-dominant immunity [30]. However, the function of NKA-NK2R signaling in the process of inflammatory induced pathogenesis of CRC is less-well defined.

In this retrospective study, we evaluated the expression NKA and NK2R in CRC and its prognostic implications. We found that the expression of NKA was upregulated in CRC as compared with that in adjacent non-cancerous colonrectal mucosae, and elevated NKA expression was significantly associated with lymph node metastasis and TNM stage. The findings presented by Sun et al suggest that NKA, as an important mediator of neuroimmunomodulatory activity and that macrophages and proinflammatory chemokines, may be implicated in NKA mediated inflammatory and immunological conditions [27]. Study by Zhi et al shows that NKA stimulation of macrophages leads to activation of NF-kB, a transcription factor that has a crucial modulatory role in inflammation, immunity, cell proliferation and apoptosis [31]. Consistent with the above study, our present results indicate that NKA may have a pro-inflammatory role that is linked to CRC carcinogenesis and development.

We also found in our study that patients with high expression of NKA also had a high rate of upregulated expression of NK2R in CRC, and the expression of NKA positively correlated with that of NK2R. Furthermore, the expression of NK2R was significantly correlated with tumor diameter, pathology grading and lymph node metastasis. It showed that NK2R may play important role in tumorigenesis and progression of CRC. Extensive reviews have also described the neuropeptide receptors as targets for the treatment and diagnosis of cancer [18]. Our present study revealed a link between inflammation and cancer, which contributing to a microenvironment favouring cancer progression. This mentioned results indicated that when NKA-NK2R increased, they can be used as a markers predicting the presence of cancer invasiveness and metastases.

Epithelial cells are now seen as important players in the gut immune response because they have the ability to secrete a range of pro-inflammatory factors and other mediators that modulate the function of the immune cells [32]. Therefore, the inflammatory process most likely plays a key role in the pathogenesis of CRC, which is an important factor in cancer staging and prognosis. It has been suggested that NKA levels may be an independent indicator of neuroendocrine tumor prognosis; >50 pmol/l associated with decreased 3 year survival rates [33]. Diebold *et al.* found that patients with midgut neuroendocrine neoplasms who have serial plasma NKA levels <50 pg/mL have an excellent short-term prognosis, while patients with plasma NKA levels >50 pg/mL have a poor short-term prognosis [15]. Our present study also showed that CRC patients with high expression of NKA or NK2R have a poorer prognosis than those with low expression, which revealed that NKA-NK2R expression may be used as predictors for prognosis of CRC.

In conclusion, upregulated expression of NKA-NK2R may play pro-inflammatory role in CRC carcinogenesis and progression, meanwhile, NKA-NK2R expression may also be used as predictors for prognosis of CRC. Based on the present findings, one novel therapeutic strategy for CRC in the future may be the inhibition of the potential signaling pathways associated with NKA and NK2R. Therefore, key signaling molecules involved to control the conditions should be further elucidated.

Acknowledgements

This work was supported by the National Science Foundation of China (Nos. 81472210, 81374014, and 81201783), Zhejiang Provinci-

al Natural Science Foundation of China (Nos. LY13H080005, LY15H160051 and LY14H160041), Funds of Science Technology Department of Zhejiang Province (No. 2014C37101, 2015C37035, 2015C37031), Zhejiang Province Bureau of Health (Nos. 2015ZA009), Open Fund of Zhejiang Provincial Top Key Discipline of Biology.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Dong-Sheng Huang, Clinical Research Institute, Zhejiang Provincial People's Hospital, Hangzhou 310014, China. Tel: 0086-571-85893000; Fax: 0086-571-85131448; E-mail: dshuang@zju.edu.cn

References

[1] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-2917.

[2] Chen W, Zheng R, Zhang S, Zhao P, Li G, Wu L, He J. Report of incidence and mortality in China cancer registries, 2009. Chin J Cancer Res 2013; 25: 10-21.

[3] van Gijn W, Krijnen P, Lemmens VE, den Dulk M, Putter H, van de Velde CJ. Quality assurance in rectal cancer treatment in the Netherlands: a catch up compared to colon cancer treatment. Eur J Surg Oncol 2010; 36: 340-344.

[4] Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Meyerhardt JA, Loda M, Giovannucci EL, Fuchs CS. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut 2009; 58: 90-96.

[5] Wangefjord S, Sundstrom M, Zendehrokh N, Lindquist KE, Nodin B, Jirstrom K, Eberhard J. Sex differences in the prognostic significance of KRAS codons 12 and 13, and BRAF mutations in colorectal cancer: a cohort study. Biol Sex Differ 2013; 4: 17.

[6] Yokota T, Ura T, Shibata N, Takahari D, Shitara K, Nomura M, Kondo C, Mizota A, Utsunomiya S, Muro K, Yatabe Y. BRAF mutation is a powerful prognostic factor in advanced and recurrent colorectal cancer. Br J Cancer 2011; 104: 856-862.

[7] Morrison WB. Inflammation and cancer: a comparative view. J Vet Intern Med 2012; 26: 18-31.

[8] Palma C. Tachykinins and their receptors in human malignancies. Curr Drug Targets 2006; 7: 1043-1052.

[9] Almeida TA, Rojo J, Nieto PM, Pinto FM, Hernandez M, Martin JD, Candenas ML. Tachykinins and tachykinin receptors: structure and activity relationships. Curr Med Chem 2004; 11: 2045-2081.

[10] Maggi CA. The troubled story of tachykinins and neurokinins. Trends Pharmacol Sci 2000; 21: 173-175.

[11] Ramalho R, Soares R, Couto N, Moreira A. Tachykinin receptors antagonism for asthma: a systematic review. BMC Pulm Med 2011; 11: 41.

[12] Mayordomo C, Garcia-Recio S, Ametller E, Fernandez-Nogueira P, Pastor-Arroyo EM, Vinyals L, Casas I, Gascon P, Almendro V. Targeting of substance P induces cancer cell death and decreases the steady state of EGFR and Her2. J Cell Physiol 2012; 227: 1358-1366.

[13] Joos GF, Vincken W, Louis R, Schelfhout VJ, Wang JH, Shaw MJ, Cioppa GD, Pauwels RA. Dual tachykinin NK1/NK2 antagonist DNK333 inhibits neurokinin A-induced bronchoconstriction in asthma patients. Eur Respir J 2004; 23: 76-81.

[14] Dobson R, Burgess MI, Banks M, Pritchard DM, Vora J, Valle JW, Wong C, Chadwick C, George K, Keevil B, Adaway J, Ardill JE, Anthoney A, Hofmann U, Poston GJ, Cuthbertson DJ. The association of a panel of biomarkers with the presence and severity of carcinoid heart disease: a cross-sectional study. PLoS One 2013; 8: e73679.

[15] Diebold AE, Boudreaux JP, Wang YZ, Anthony LB, Uhlhorn AP, Ryan P, Mamikunian P, Mamikunian G, Woltering EA. Neurokinin A levels predict survival in patients with stage IV well differentiated small bowel neuroendocrine neoplasms. Surgery 2012; 152: 1172-1176.

[16] Lecci A, Santicioli P, Maggi CA. Pharmacology of transmission to gastrointestinal muscle. Curr Opin Pharmacol 2002; 2: 630-641.

[17] Fang W, Fu C, Chen X, Mou X, Liu F, Qian J, Zhao P, Zheng Y, Zheng YI, Deng J, Ye P, Wang Y, Zheng S. Neurokinin-2 receptor polymorphism predicts lymph node metastasis in colorectal cancer patients. Oncol Lett 2015; 9: 2003-2006.

[18] Reubi JC. Peptide receptors as molecular targets for cancer diagnosis and therapy. Endocr Rev 2003; 24: 389-427.

[19] Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420: 860-867.

[20] Goswami B, Rajappa M, Sharma M, Sharma A. Inflammation: its role and interplay in the development of cancer, with special focus on gynecological malignancies. Int J Gynecol Cancer 2008; 18: 591-599.

[21] McLean MH, Murray GI, Stewart KN, Norrie G, Mayer C, Hold GL, Thomson J, Fyfe N, Hope M, Mowat NA, Drew JE, El-Omar EM. The inflammatory microenvironment in colorectal neoplasia. PLoS One 2011; 6: e15366.
[22] Zhang Y, Berger A, Milne CD, Paige CJ. Tachykinins in the immune system. Curr Drug Targets 2006; 7: 1011-1020.
[23] Micera A, Lambiase A, Bonini S. The role of neuromediators in ocular allergy. Curr Opin Allergy Clin Immunol 2008; 8: 466-471.
[24] Cohly HH, Panja A. Immunological findings in autism. Int Rev Neurobiol 2005; 71: 317-341.
[25] Veres TZ, Rochlitzer S, Braun A. The role of neuro-immune cross-talk in the regulation of inflammation and remodelling in asthma. Pharmacol Ther 2009; 122: 203-214.
[26] Sculptoreanu A, Aura Kullmann F, de Groat WC. Neurokinin 2 receptor-mediated activation of protein kinase C modulates capsaicin responses in DRG neurons from adult rats. Eur J Neurosci 2008; 27: 3171-3181.
[27] Sun J, Ramnath RD, Tamizhselvi R, Bhatia M. Neurokinin A engages neurokinin-1 receptor to induce NF-kappaB-dependent gene expression in murine macrophages: implications of ERK1/2 and PI 3-kinase/Akt pathways. Am J Physiol Cell Physiol 2008; 295: C679-691.
[28] Miotto D, Boschetto P, Cavallesco G, Zeni E, Querzoli P, Pedriali M, Chiarelli S, Fabbri LM, Mapp CE. Increased neurokinin-2 receptor expression in alveolar macrophages of smokers with COPD. Histopathology 2007; 51: 128-131.
[29] Michalski CW, Shi X, Reiser C, Fachinger P, Zimmermann A, Buchler MW, Di Sebastiano P, Friess H. Neurokinin-2 receptor levels correlate with intensity, frequency, and duration of pain in chronic pancreatitis. Ann Surg 2007; 246: 786-793.
[30] Kitamura H, Kobayashi M, Wakita D, Nishimura T. Neuropeptide signaling activates dendritic cell-mediated type 1 immune responses through neurokinin-2 receptor. J Immunol 2012; 188: 4200-4208.
[31] Zhi L, Ang AD, Zhang H, Moore PK, Bhatia M. Hydrogen sulfide induces the synthesis of pro-inflammatory cytokines in human monocyte cell line U937 via the ERK-NF-kappaB pathway. J Leukoc Biol 2007; 81: 1322-1332.
[32] Mladenova D, Kohonen-Corish MR. Review: Mouse models of inflammatory bowel disease--insights into the mechanisms of inflammation-associated colorectal cancer. In Vivo 2012; 26: 627-646.
[33] Turner GB, Johnston BT, McCance DR, McGinty A, Watson RG, Patterson CC, Ardill JE. Circulating markers of prognosis and response to treatment in patients with midgut carcinoid tumours. Gut 2006; 55: 1586-1591.