Expression and significance of autonomic nerves and α9 nicotinic acetylcholine receptor in colorectal cancer

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Abstract. The present study evaluated the distribution of sympathetic and parasympathetic nerves and the expression of the α9 nicotinic acetylcholine receptor (α9nAChR) and investigated their potential association with colorectal cancer (CRC) development. The distribution of autonomic nerves and α9nAChR in CRC was detected by immunohistochemistry, which was then used to analyze their association with clinicopathological parameters and prognosis. Sympathetic fibers were primarily observed in the stroma adjacent to cancer cells, whereas parasympathetic fibers were primarily observed in the stroma away from cancer cells. Patients with samples positive for sympathetic nerve fibers had less lymph node invasion and a better prognosis compared with patients with samples negative for sympathetic nerve fibers. The expression of parasympathetic nerves in patients >60 years old was increased compared with patients ≤60 years old. The expression of parasympathetic nerves in patients with lymph node invasion was increased compared with patients without lymph node invasion. The detection of parasympathetic nerves gradually increased as CRC (T stage) advanced. Patients with parasympathetic negative samples had better prognoses compared with patients with parasympathetic positive samples. The expression of α9nAChR was principally localized in cellular membranes and the cytoplasm of CRC tissues and it was revealed to have a positive association with the number of parasympathetic nerves. Increased α9nAChR expression was observed in patients >60 years old compared with patients <60 years old. The detection rate of α9nAChR in tissues from patients with lymph node invasion was increased compared with patients without lymph node invasion. The detection of α9nAChR gradually increased as the CRC stage advanced. The prognoses for patients with α9nAChR negative tissue were improved compared with the prognoses for patients with α9nAChR positive tissue. Sympathetic nerves were primarily detected in the early phases of CRC and indicated a good prognosis. Parasympathetic nerves and α9nAChR were principally observed in the late phases of cancer and indicated a poor prognosis. The present study revealed that parasympathetic nerves may promote the progression of CRC through α9nAChR.

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Abbreviations: CRC, colorectal cancer; α9nAChR, α9 nicotinic acetylcholine receptor; Ach, acetylcholine; AChR, acetylcholine receptor; nAChR, N type cholinergic receptor; TNM, tumor, lymph node, metastasis; TH, Tyrosine hydroxylase; VAChT, vesicular acetylcholine transporter

Key words: colorectal cancer, sympathetic nervous, parasympathetic nervous, α9 nicotinic acetylcholine receptor

Introduction

Colorectal cancer (CRC) is the fourth most commonly diagnosed malignant disease worldwide, after lung, stomach and liver cancer (1). It is estimated that CRC ranks as the fourth and fifth most common cause of cancer death among women and men in China. The prevalence of CRC is rapidly increasing owing to changes in people's lifestyle, such as smoking, obesity, and red meat consumption (2). Despite advances in traditional treatments, such as chemotherapy, radiotherapy, targeted therapy and surgery, there has been no significant breakthrough in the overall curative therapy. The five-year survival rate after diagnosis remains less than 60% (3,4). Like other cancers, CRC exhibits resistance to cell death, the ability of replication, angiogenesis, tissue invasion and metastasis, and immune escape (5). According to a medical view considering Darwinian evolution, the tumor may be a 'new species' in the body that has an increased ability to proliferate and metastasize (6). Tumors may have highly evolved and conserved information transmission systems, so that large numbers of tumor cells can coordinately proliferate, hibernate and metastasize. Steven Paget's theory of 'seed and soil' was considered a milestone in

tumor research. It proposed that a suitable microenvironment is the primary requirement for malignant cells to survive in other non-primary focal areas and to continue to grow and form metastases (7). In 1971, Folkman *et al* proposed the concept of 'angiogenesis' in tumors (8). In 2001, Seifert *et al* proposed a phenomenon similar to angiogenesis, called neurogenesis (9). Subsequent studies confirmed that neurogenesis in bladder, breast, colorectal and pancreatic cancer was closely correlated with tumor progression, as well as prognosis (10-14). Albo *et al* revealed that neurogenesis was a marker for tumor invasion and prognosis, and the five-year survival rate of patients with neurogenesis was reduced by 50% compared with those lacking neurogenesis (13). Tumors cells secrete many cytokines, such as nerve growth factor, insulin-like growth factor and brain-derived nerve growth factor, which not only promote neurogenesis within the tumor, but also play an important role in accelerating tumor development (15,16). Moreover, nerve and tumor cells may interact in an autocrine, paracrine or endocrine manner via the neuro-neoplastic synapse (17).

The autonomic nervous system consists of sympathetic and parasympathetic nerves. Magnon *et al* (18) revealed that in prostate cancer, sympathetic nerves are mainly distributed in normal tissue around the tumor, and parasympathetic nerves are mainly found between the tumor cells. Both types of nerves show complementary progressive effects in tumor occurrence and development. Sympathetic nerves were observed in the early stages of prostate cancer and were associated with occurrence of disease. Parasympathetic nerves were mainly expressed in the late stage, associated with poor progression and metastasis (18). Cutting parasympathetic nerves or local injection of neurotoxic drugs significantly reduced tumor appearance and development, but the effects were limited to regions of innervations (19). Considering this point, parasympathetic nerves were more closely associated with the progression of tumors than the sympathetic nerves. Disrupted cholinergic signals may inhibit Wnt signaling pathways and stem cell proliferation. Blocking or knockdown of the choline M3 receptor inhibited gastric cancer (20). However, to date, the clinical significance of sympathetic and parasympathetic nerves in human CRC has not been reported.

There are multiple neurotransmitter receptors on tumor cells that may affect tumor development (21). Parasympathetic neurotransmission involves acetylcholine (ACh) and two types of cholinergic receptor (acetylcholine receptor, AChR), the toadstool alkali (M) and nicotinic (N) type receptors. The M type receptor has five subtypes: those found in the central nervous system are M1, M3 and M4, and those in peripheral nerves are M1, M2 and M3. The N type cholinergic receptor (nAChR) is a pentameric gate control ion channel composed of different subunits (α2-α10, β2-β4) (22). Because cancer cells express many kinds of AChR (23-26), cholinergic neurotransmitters or other agonists can affect tumor progression via their corresponding receptors. Currently, α7nAChR is widely studied because it has a role in tumor development, such as pancreatic and lung cancer, by regulating signaling pathways such as PI3K-AKT, NF-κB and STAT (27-29). Another cholinergic receptor, α9nAChR, was overexpressed in malignant tumors, especially in breast cancer (30,31), and promoted breast cancer metastasis by activating vimentin and fibronectin (32,33). The expression and significance of α9nAChR in CRC remains unknown. In this study, we examined the expression of autonomic nerves and α9nAChR in CRC by immunohistochemical methods, and then analyzed their relationship with clinical stages, lymph node metastasis and prognosis.

Materials and methods

Patients and specimens. During January 2008 to January 2011, tissue samples were collected (by the same surgeon) from 90 patients with CRC at the Second Department of Surgery of the Forth Hospital of Hebei Medical University. All patients were undergoing their first CRC surgery. Before surgery, no patients received radiation or chemotherapy, and all CRC was confirmed by pathology for adenocarcinoma. CRC tissues were stock in the paraffin-embeded form after fixation in formalin and dehydration with increasing-concentration alcohol. Collection and use of specimens were approved with the patients' informed consent and by the ethics committee of the Forth Hospital of Hebei Medical University. Total survival time was defined from diagnosis to time of patient's death or the last follow-up visit. Postoperative TNM stage of CRC followed the 8th Edition of the American Joint Committee on Cancer grading system (34). Tumor, lymph node, metastasis (TNM) system are considered as standardized classification system for evaluating cancer at a population level in terms of the extent of disease, determined by cancer biology and habitual nature as well as predicting cancer outcome and response to treatment.

Histological analysis. Immunohistochemistry detected the expression of autonomic nerves and α9nAChR in a tumor microenvironment. Tyrosine hydroxylase (TH) and vesicular acetylcholine transporter (VAChT) were used as specific markers for sympathetic and parasympathetic nerves, respectively. Tissue samples were fixed in 4% buffered formaldehyde, decalcified, paraffin-embedded and sectioned to 3-5 μm. Paraffin sections were dewaxed, rehydrated and treated for standard antigen retrieval, and incubated with anti-TH antibody (rabbit anti-human monoclonal antibody, ab6211, Massachusetts, USA, 1:500) and anti-VAChT antibody (rabbit anti-human monoclonal antibody, ab68984, Massachusetts, USA, 1:100). An antibody for a9nAChR (rabbit anti-human monoclonal antibody, ab177119, Massachusetts, USA, 1:100) was used to detect expression of α9nAChR. Goat anti-rabbit IgG-HRP antibody (Aorui Dongyuan Biotechnology, Wuxi, China) was used as secondary antibody and specific binding detected using DAB (Zhongshan Jinqiao Biotechnology, Beijing, China). An OLYMPUS BX61 universal microscope was used to analyze slide images, with nerve fibers stained brown or yellow designed as positive. For α9nAChR detection, the presence of brown-yellow or brown particles in the cell membrane or in the plasma was defined as a positive image. According to the proportion of positive cells and the staining strength of positive cells, the experimental results of α9nAChR were determined. A: The proportion of positive cells <1/3 was scored as 1 point; the proportion of positive cells 1/3 ~ 2/3 was scored as 2 point; the proportion of positive cells >2/3 was scored as 3 point. B: According to the staining of the cells, the non-positive cells was scored as 0 point; the light yellow was scored as 1 point; the claybank was scored as 2 point; and the tan was scored as 3 point. The integral is

Table I. Clinicopathological features and prognosis of 73 patients with colorectal cancer.

	5-year survival rate			OS		
Characteristic	Total no.	Sur no.	Sur R (%)	HR	95% CI	P-value
Gender						
Male	43	34	-79.10	1	-	-
Female	47	37	-78.70	0.968	0.393-2.384	0.944
Location						
Rectum	55	43	-78.20	1	-	-
Colon	35	28	-80.00	0.904	0.356-2.298	0.833
T stage						
T1	2	1	-50.00	1	-	-
T2	20	18	-90.00	0.235	0.021-2.598	0.238
T3	29	22	-75.90	0.641	0.057-4.995	0.648
T4	39	30	-76.90	0.534	0.068-4.218	0.552
LN						
(+)	42	29	-69.00	1	-	-
(-)	48	42	-87.50	0.357	0.135-0.939	0.037[a]
TNM						
I+II	50	45	-90.00	1	-	-
III	40	26	-65.00	4.273	1.536-11.888	0.005[a]
PN						
(+)	45	29	-64.40	1	-	-
(-)	45	42	-93.30	0.161	0.047-0.554	0.005[a]
SN						
(+)	48	42	-87.50	1	-	-
(-)	42	29	-69.00	2.822	1.070-7.444	0.036[a]
α9						
(+)	40	26	-56.00	1	-	-
(-)	50	45	-90.00	0.228	0.082-0.634	0.005[a]

Cox regression analyses were used to analyze survival status. [a]P<0.05. LN, lymph nodes; TNM, tumor, lymph node, metastasis; PN, parasympathetic nerve; SN, sympathetic nerve; α9, α9nAChR; Sur R, survival rate.

equal to A x B. A x B=0 was classified as (-); A x B=1 ~ 2 was classified as (+); A x B=3 ~ 4 was classified as (+ +); A x B=6 ~ 9 was classified as (+ + +).

Statistical analysis. Relationships between the presence of autonomic nerves or α9nAChR and the clinical pathology were assessed using chi-square and correlation tests. Cox proportional risk regression analysis was used for single variable and multivariate analysis to examine the underlying prognostic factors of overall survival. Cox regression analyses were used to analyze survival status. Survival curve differences were analyzed using the log-rank test. Statistical analysis was done using SPSS22.0 statistical analysis software. Two-side tests were used to compare statistical differences, and P<0.05 was regarded as significant.

Results

Clinicopathological features and prognosis of patients with CRC. The clinicopathologic features and prognosis of patients with CRC in this trial are shown in Table I. A return visit for 90 patients was completed via telephone. Seventy-three cases provided survival data, and 19 cases died before the return visit, all of whom died of postoperative tumor distant metastasis (pulmonary, bone and multiple metastases in 11, 4 and 5 cases, respectively). The overall survival rate was 73.97%. The prognosis was not significantly associated with gender, location and stage of cancer (P>0.05). The prognosis of lymph node metastatic negative patients was greater than that of lymph node metastasis positive patients (P<0.05). Considering the few cases of CRC in stage I, we combined the cases in stages I and II, and compared them with stage III cases. Patients with stages I+II CRC had a better prognosis than those with stage III CRC (P<0.05).

Expression of autonomic nerves and α9nAChR in CRC. Expression of autonomic nerves and α9nAChR in CRC is shown in Fig. 1. Most of the sympathetic fibers were seen in the stroma adjacent to cancer cells (Fig. 1A). Most of

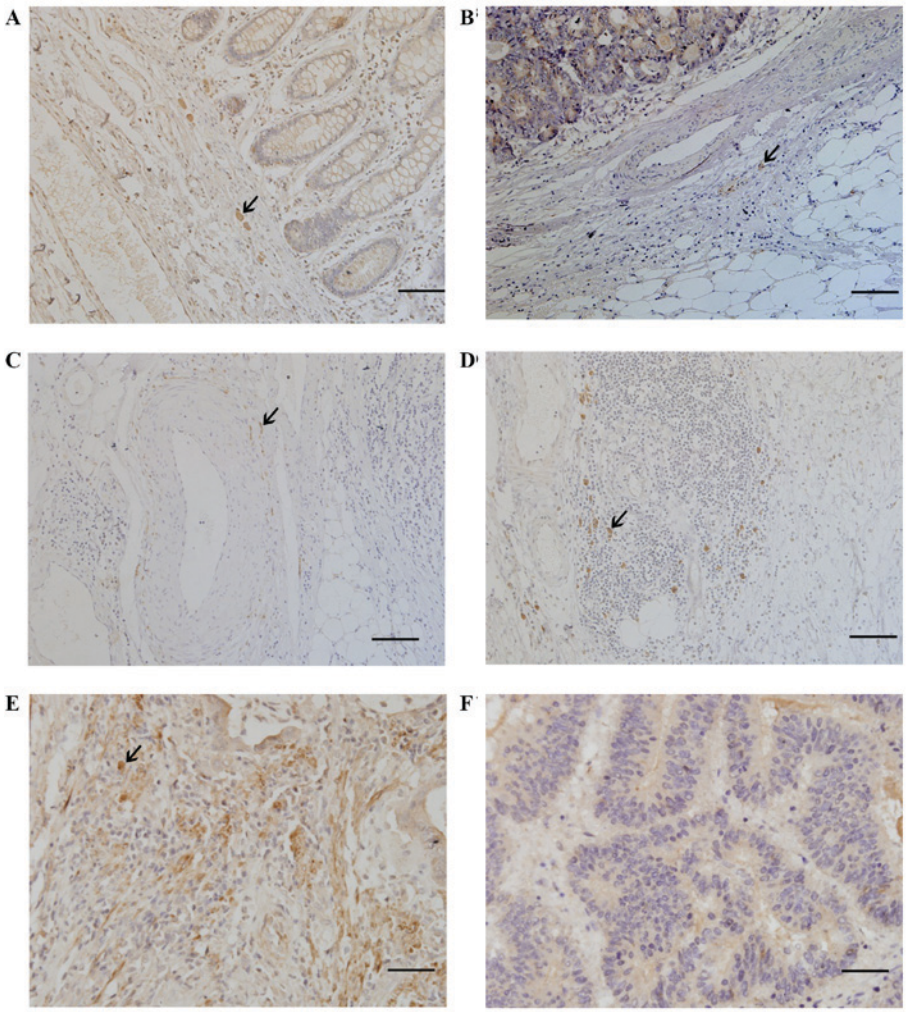

Figure 1. Expression of autonomic nerves and α9nAChR in CRC. (A) Expression of sympathetic fibers in the stroma adjacent to cancer cells. (B) Expression of parasympathetic fibers in the stroma away from cancer cells. (C) Expression of parasympathetic fibers surrounding the blood vessels. (D) Expression of parasympathetic fibers located in tertiary lymphoid tissue. (E) Expression of α9nAChR in CRC tissue. (F) Expression of α9nAChR in normal rectal tissue. Magnification, x400 (scale bar, 20 μm).

the parasympathetic fibers were seen in the stroma away from cancer cells (Fig. 1B). Some parasympathetic fibers surrounded the blood vessels (Fig. 1C) and were sporadically located in tertiary lymphoid tissue (Fig. 1D). The α9nAChR was expressed in CRC tissues (Fig. 1E), and was not detected in normal rectal tissues (Fig. 1F).

Relationship between autonomic nerves as well as α9nAChR and the survival rate of patients with CRC. Relationships between autonomic nerves as well as α9nAChR and the survival rate of patients with CRC are shown in Fig. 2. Comparison of survival rates of patients with CRC showed that patients with sympathetic nerve positive CRC tissue had a better prognosis compared with those having sympathetic nerve negative tissue. There was a significant difference (P<0.05) between the survival rates of these two groups (Fig. 2A). Patients with parasympathetic nerve positive tissue had a worse prognosis compared with patients that had parasympathetic nerve negative tissue, shown by the significant difference (P<0.05) between the survival rates of these two groups (Fig. 2B). Patients with α9nAChR positive CRC tissue had a worse prognosis compared with patients that had α9nAChR negative tissue, shown by the significant difference (P < 0.05) between the survival rates of these two groups (Fig. 2C).

Relationship between autonomic nerves as well as α9nAChR and the clinical pathology of CRC. The relationships between sympathetic nerves and clinical pathological features are shown in Table II. The presence of sympathetic nerves had no significant correlation with age, tumor location, gender, and cancer stages (T and TNM criteria). The lymph node invasion rate of patients with sympathetic nerve positive tissue was significantly lower than that of patients with sympathetic nerve negative tissue (41.7 vs. 66.7%, P=0.018), suggesting that sympathetic nerves are inversely related to the lymph node invasion.

The relationships between parasympathetic nerves and clinical pathological characteristics are shown in Table III. The presence of parasympathetic nerves was not significantly associated with tumor location and gender (P>0.05). Patients >60 years of age had a higher incidence of parasympathetic nerve expression compared with those ≤60 years of age (62.5 vs. 40.0%, P=0.034). Patients with lymph node invasion had a higher incidence of parasympathetic nerve expression than those without lymph nodes invasion (62.5 vs. 35.7%, P=0.011).

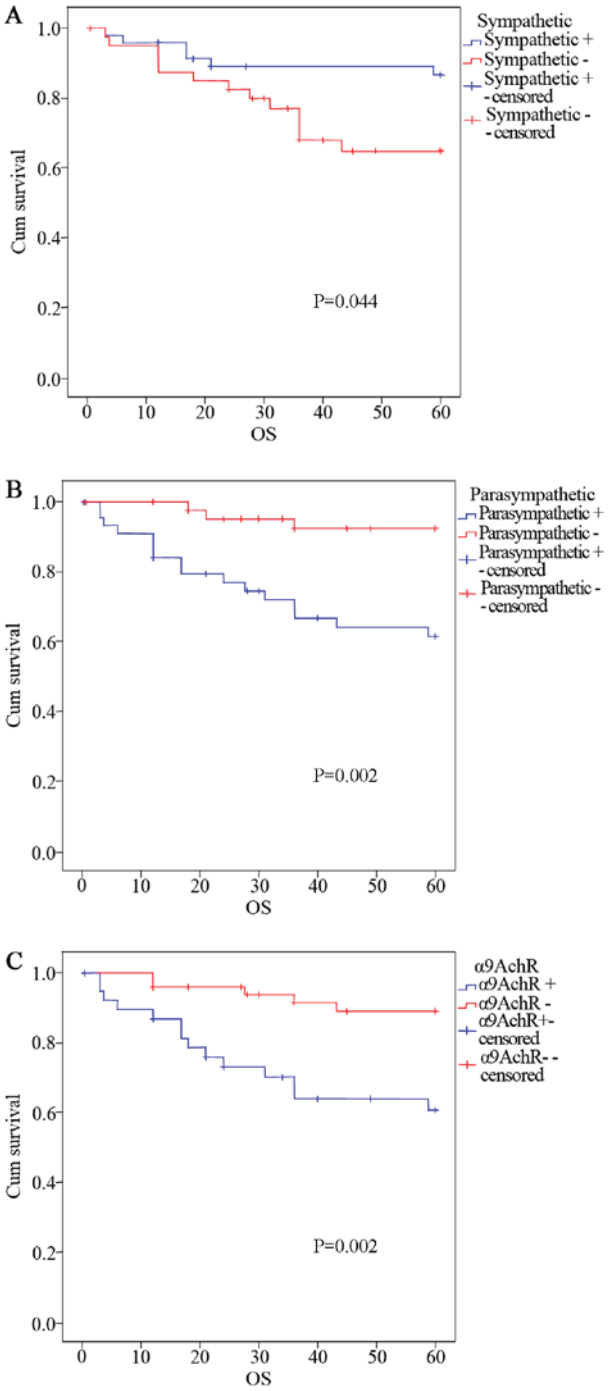

Figure 2. Relationship between autonomic nerves as well as α9nAChR and the survival rates of colorectal cancer patients. (A) Patients with sympathetic nerve positive tissue had a better prognosis than patients with sympathetic nerve negative tissue (P=0.044). (B) Patients with parasympathetic nerve positive tissue had a worse prognosis than patients with parasympathetic nerve negative tissue (P=0.002). (C) Patients with α9nAChR positive expression had a worse prognosis than patients with α9nAChR negative expression (P=0.002).

As the cancer (T stage) advanced, parasympathetic nerve positive rates gradually increased (T1 50.0 T2 25.0 T3 48.3 and T4 64.1%, P=0.043), suggesting that the presence of parasympathetic nerves was positively correlated with age, lymph node invasion and cancer T stage.

The relationships between α9nAChR and clinical pathological features are shown in Table IV. The expression of α9nAChR was not associated with gender or tumor location of CRC patients (P>0.05). The expression of α9nAChR in patients >60 years of age was higher than that of patients ≤60 years of age (57.5 vs. 34.0%, P=0.026). The expression of α9nAChR in patients with lymph node invasion was significantly higher than that of patients without lymph nodes invasion (66.7 vs. 42.9%, P=0.023). As the cancer T stage advanced, the expression of α9nAChR gradually increased (0 T1, 20.0 T2, 44.8 T3, 59.0% T4, P=0.021), suggesting that α9nAChR expression was positively correlated with age, cancer T stage and lymph node invasion.

Discussion

In the present study, we revealed that there are sympathetic and parasympathetic nerves in the human CRC microenvironment. Sympathetic fibers were mainly found in the stroma adjacent to cancer cells. Patients with sympathetic nerves detected in CRC tissue have less lymph node invasion compared with patients exhibiting tissue with no detectable sympathetic nerves. The presence of sympathetic nerves in CRC had no significant correlation with age, tumor location, gender, and cancer (T and TNM) stage. The prognosis of patients with sympathetic nerve positive CRC was better than patients with sympathetic negative tissue. Parasympathetic fibers were mainly detected in the stroma away from cancer cells, and some parasympathetic fibers were observed around the blood vessels. The expression of α9nAChR was mainly located in cellular membrane and cytoplasm of CRC tissues. The detection of parasympathetic nerves and α9nAChR was positively related to cancer T stage, lymph node invasion and age; as expression of parasympathetic nerves and α9nAChR increased in CRC tissue, the prognosis of patients became poor, suggesting that parasympathetic nerves may participate in the late development of tumors via α9nAChR.

Tumors are not an isolated structure, and are associated with the microenvironment of the host tissues (35). Recently, researchers reported that neurogenesis was present in colorectal tumors (36). Our study also revealed that there were sympathetic and parasympathetic nerves in CRC. Parasympathetic nerves were closely associated with the development of tumors. Acetylcholine is a neurotransmitter in parasympathetic nerves, which function via the AChR (37,38). The AChR is also expressed in some non-neuronal cells, such as lung cancer and CRC cells (39). Nicotine, as well as its structural analogues nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and N'-nitrosonornicotine, can activate a variety of nAChR subtypes found in the parasympathetic nervous system, resulting in a various biological responses (40). Nicotine can induce the proliferation of a variety of cancer cells, such as small cell lung cancer, non-small cell lung cancer, pancreatic and colon cancer cells, in receptor-dependent manner (41,42). In human bronchial epithelial cells and lung cancer cells, the α7nAChR inhibitors mecamylamine reduced cell proliferation mediated by nicotine (43). Moreover, the nAChR antagonist d-tubocurarine reduced the proliferation of MSTO-211 mesothelioma cells *in vitro* (44). Another type of AChR was found in mammalian ear vestibular hair cells type II, called α9nAChR (45). The α9nAChR was closely associated with the development of breast cancer (46,47). To date, there have

Table II. Association between sympathetic nerves and clinical pathological features.

	Sympathetic nerve				
Characteristic/group	-	+	N	Expression rate (%)	P-value
Gender					0.382
Male	18	25	43	58.1	
Female	24	23	47	48.9	
Age					0.571
≤60	22	28	50	56.0	
>60	20	20	40	50.0	
Location					0.58
Rectum	21	14	35	40.0	
Colon	27	28	55	50.9	
T					0.555
T1	0	2	2	100.0	
T2	9	11	20	55.0	
T3	15	14	29	48.3	
T4	19	20	39	51.3	
N					0.018[a]
N0	14	28	42	66.7	
N+	28	20	48	41.7	

Cox proportional risk regression analysis was used for single variable and multivariate analysis to examine the underlying prognostic factors of overall survival. [a]P<0.05. T, tumor; N, lymph node.

Table III. Association between parasympathetic nerves and clinical pathological features.

	Sympathetic nerve				
Characteristic/group	-	+	N	Expression rate (%)	P-value
Gender					0.291
Male	19	24	43	55.80	
Female	26	21	47	44.70	
Age					0.034[a]
≤60	30	20	50	40.00	
>60	15	25	40	62.50	
Location					0.052
Rectum	22	13	35	37.10	
Colon	23	32	55	58.20	
T					0.043[a]
T1	1	1	2	50.00	
T2	15	5	20	25.00	
T3	15	14	29	48.30	
T4	14	25	39	64.10	
N					0.011[a]
N0	27	15	42	35.70	
N+	18	30	48	62.50	

Cox proportional risk regression analysis was used for single variable and multivariate analysis to examine the underlying prognostic factors of overall survival. [a]P<0.05. T, tumor; N, lymph node.

Table IV. Association between α9nAChR and clinical pathological features.

	A9nachr				
Characteristic/group	-	+	N	Expression rate (%)	P-value
Gender					0.962
Male	24	19	43	44.20	
Female	26	21	47	44.70	
Age					0.026[a]
≤60	33	17	50	34.00	
>60	17	23	40	57.50	
Location					0.134
Rectum	19	16	35	45.70	
Colon	21	34	55	61.80	
T					0.021[a]
T1	2	0	2	0	
T2	16	4	20	20.00	
T3	16	13	29	44.80	
T4	16	23	39	59.00	
N					0.023[a]
N0	24	18	42	42.90	
N+	16	32	48	66.70	

Cox proportional risk regression analysis was used for single variable and multivariate analysis to examine the underlying prognostic factors of overall survival. [a]P<0.05. T, tumor; N, lymph node.

been no reports regarding the role of α9nAChR in CRC. In the current study, α9nAChR was mainly expressed in the cellular cytoplasm and membranes of CRC tissue, and was not detected in normal colorectal tissues and tumor stroma, which suggested that α9nAChR may not be essential for normal neurotransmitter signaling. We propose that α9nAChR may be induced by some factors found in the tumor, and then plays a role in promoting tumor development by downstream signaling events.

In addition, we found that parasympathetic nerve fibers also exist in tumor tertiary lymphoid tissue, which suggested that parasympathetic nerves are closely associated with immune cells. Immune cells, such as T and B cells, and monocytes, express all five kinds of toadstool alkali AChR (mAChR M1-M5) and different types of nAChR, such as α3, α5, α7, α9 and α10, which provide the structural basis for parasympathetic nerves to regulate the immune system (48). Borovikova *et al* (49) proposed the concept of the 'cholinergic anti-inflammatory pathway', in which ACh released by parasympathetic nerves acted upon α7nAChR in macrophages, which inhibited immune function by suppressing a variety of inflammatory factors (such as TNF-α, IL-1β, IL-6 and high mobility group protein (49,50). The electrical stimulation of parasympathetic nerves or α7nAChR-specific agonists both reduced local or systemic inflammatory responses (51). These findings have been applied to the study of Alzheimer's disease (52), rheumatoid arthritis (53), and endotoxin blood disease (54). Furthermore, nicotine increased the expression of phosphorylated STAT5 by activating α7nAChR on regulatory T cells, ultimately resulting in elevated activity of regulatory T cells, which restrained T cell immunosuppression (55). Therefore, parasympathetic nerves may directly act on tumor cells, as well as inhibit immune cells in the tumor microenvironment, with combined AChR receptor responses promoting tumor progression. The current study may ultimately provide a new avenue for the treatment of CRC.

We found that the expression of parasympathetic nerves in CRC was positively related to the age of the patient. It is well known that the prevalence of both cancer and Alzheimer's disease increases gradually with age (56). Musicco *et al* reported that cancer rates in patients with Alzheimer's disease were reduced by 50% compared with normal people of the same age. However, the rates of Alzheimer's disease in cancer patients were reduced by 35% compared with healthy people of the same age (57). Another epidemiological study showed a reduction of malignant tumor risk was associated with Alzheimer's disease, and the risk of malignant tumors was further reduced using stringent criteria for the diagnosis of dementia (58), which excluded the point that the incidence of malignant tumors affected cognitive impairment. Elderly cognitive impairment may involve chronic inflammation, together with reduced excitability of parasympathetic nerves or the reduction of ACh synthesis and release. The tumor is usually associated with abnormal activation of parasympathetic nerves, which may inhibit immune and inflammatory responses and promote tumor progression. Hence, parasympathetic nerves may provide a 'bridge' connecting cognitive disorders with tumors. Ongoing investigations into the use of

parasympathetic treatments for these two diseases will have to consider the complications that may arise from multiple AChR actions. Regulating the excitability of parasympathetic nerves to maintain an 'appropriate' state is likely to be an important subject of geriatric medicine in the future.

In conclusion, sympathetic and parasympathetic nerves were found in human CRC. Sympathetic nerves were mostly detected in early phases of cancer associated with good prognosis, whereas parasympathetic nerves and α9nAChR were mostly observed in late phases of cancer associated with bad prognosis. The expression of parasympathetic nerves and α9nAChR were positively correlated. These results suggest that parasympathetic nerves may promote the progression of CRC through α9nAChR. The exact role and mechanism of autonomic nervous system actions in CRC deserve further study.

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