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NAT2 knockdown inhibits the development of colorectal cancer and its clinical significance

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Abstract. – OBJECTIVE: This study aims to explore the correlation between N-acetyltransferase 2 (NAT2) expression in colorectal cancer (CRC) tissues and the progression and prognosis of CRC. Through *in vitro* and *in vivo* experiments, the biological functions of NAT2 in the occurrence and development of CRC were explored.

PATIENTS AND METHODS: Immunohistochemical (IHC) staining, reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) and Western blot were used to detect the difference of NAT2 expression in CRC tissues and normal tissues. The role of NAT2 in the cell proliferation, apoptosis, migration, invasion, and tumorigenesis and development of CRC was analyzed by cell counting kit-8 (CCK-8), colony formation, flow cytometry, transwell cell invasion, wound-healing assays and construction of nude mouse xenograft model. The correlation between the expression level of NAT2, and the overall survival and clinicopathological characteristics of CRC patients were statistically analyzed to preliminarily determine the clinical significance of NAT2 in the diagnosis and prognosis of CRC.

RESULTS: The expression level of NAT2 was notably upregulated in CRC. NAT2 knockdown inhibited the proliferation, migration, invasion and in vivo tumor formation of CRC cells, and promoted cell apoptosis. High NAT2 expression was associated with TNM stage, differentiation degree, tumor size, distant metastasis, lymph node metastasis and poor prognosis in CRC patients.

CONCLUSIONS: This study showed that the expression level of NAT2 in CRC tissues was increased and closely related to the metastasis and prognosis of CRC. In addition, NAT2 can be used as a new prognostic biomarker and therapeutic target for CRC.

Key Words:
NAT2, Development, Colorectal cancer, Clinical significance.

Introduction

Colorectal cancer (CRC) is a common malignant tumor, with the third highest incidence and mortality in the world[1]. Patients with CRC have no evident symptoms in the early stage, and most of them are usually diagnosed in the middle or advanced stage, with poor therapeutic effect and prognosis[2]. At present, the treatment of CRC is still dominated by surgery, supplemented by chemoradiotherapy, but nearly 40% of patients have tumor recurrence or metastasis within 3 years after surgery[3,4]. In malignant tumors such as CRC, the invasion and metastasis of tumors are important factors that aggravate the disease and treatment difficulty and affect the prognosis[5]. Therefore, finding potential markers and therapeutic targets to predict the postoperative progression of CRC patients has become an important clinical scientific issue.

N-acetyltransferase 2 (NAT2) is one of the metabolic enzymes that have attracted much attention in recent years, and its coding gene is located in 8P22[6]. NAT2 catalyzes the acetylation process of acetyl groups from acetyl-Coenzyme A (acetyl-Coa) to heterocyclic amines and plays an important role in the metabolism of some drugs and the inactivation or activation of carcinogens[7]. Chronic acetylation metabolism reduces the metabolic capacity of pathogenic factors in the environment, easily leading to the occurrence of various tumors. As an important phase II metabolic enzyme, NAT2 has evident genetic polymorphism and is thought to be related to the occurrence of multiple cancers, including breast cancer (BC), acute lymphoblastic leukemia and CRC[8-11]. However, there are few studies on its function and mechanism in cancer. Yang et al[12] reported that in CCL4-induced liver injury, NAT2-targeted miR-217 inhibits cell proliferation and promotes apoptosis and autophagy. In CRC, NAT2 expression and its mechanism, as well as its relationship with clinicopathological features and prognosis, are still unclear. Hence, IHC technology was used to evaluate the difference of NAT2 expression in CRC tissues, and the

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correlation between the NAT2 expression and the metastasis and prognosis of CRC patients was explored. The expression level of NAT2 was upregulated in CRC cells to investigate the effect of its expression changes on the biological function of tumor cells. The purpose of this study was to investigate the possible role of NAT2 in the development and progression of CRC. It may provide novel biomarkers and therapeutic targets for clinical treatment of CRC.

Patients and Methods

Tissues Specimens

Colorectal cancer (CRC) tissues and corresponding normal intestinal mucosal epithelial tissues (NC) more than 10 cm from the edge of the tumor were collected from 60 patients admitted to the colorectal surgery department of our hospital from February 2014 to January 2015. There were 40 males and 20 females with an average age of (67.4±11.4) years. Inclusion criteria: (1) Primary colon cancer. (2) Confirmed colon cancer by preoperative biopsy and pathological examination. (3) No serious complications affecting postoperative chemotherapy occurred during the perioperative period. (4) Complete clinical and pathological data. Exclusion criteria: (1) Preoperative neoadjuvant chemotherapy, immunotherapy, etc. (2) Other colonic diseases, such as inflammatory bowel disease, colonic tuberculosis, etc. (3) Multisite lesions in the colon. (4) Incomplete follow-up information. (5) Combined with other malignant tumors and important organ diseases. (6) Adjuvant chemotherapy should be given according to the pathological results, but the patient refused. This study was reviewed and approved by the Ethics Committee of our hospital. All patients provided written informed consent. This study was conducted in accordance with the Declaration of Helsinki.

Cell Culture and Transfection

The NCM460 (human normal colonic epithelial cell) and HCT116, HT-29, SW480, SW620 (CRC cell lines) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured with Roswell Park Memorial Institute-1640 (RPMI-1640) medium containing 10% fetal bovine serum (FBS, Gibco, Rockville, MD, USA), streptomycin (100 μg/mL) and penicillin (100 UI/mL) in an incubator at 37°C, 5% CO_2 and saturated humidity.

The NAT2-shRNAs packed lentivirus (sh-NAT2) and its negative control (sh-NC) were obtained by GeneChem (Shanghai, China). Cells were incubated and transfected with lentivirus and polybrene. The stable cell clones were screened by puromycin (1.5 μg/mL). The expression efficiency was verified by RT-qPCR and Western blot analysis.

CCK-8 Assay

Cells from each group at logarithmic growth stage were inoculated into 96-well plates with 4×10^3 cells per well. At 24 h, 48 h, 72 h and 96 h of culture at 37 °C and 5% CO_2, 10 μL CCK-8 solution was added to each well, respectively. The absorbance value (OD) at 450 nm of each well was detected by a microplate reader (BioTek, Winooski, VT, USA) after culturing 2 h in the 37°C incubator, and the growth curve was plotted.

Colony Formation Assay

Cells were inoculated into 6-well plates with 500 cells per well. The culture was continued for 10-14 days in the incubator. When visible clones appeared, the culture was terminated. 1 mL methanol was added to each well to fix the cells at room temperature for 20 min. After that, 0.2% crystal violet staining solution was added to each well for dyeing 30 min. The number of clones with more than 50 cells was counted under the microscope (Olympus, Tokyo, Japan).

Cell Apoptosis was Analyzed by Flow Cytometry

CRC cells were digested with 0.25% trypsin and 2×10^5 cells were collected. The supernatant was centrifuged and discarded. After washing, 500 μL of binding buffer suspension cells were added. 5 μL membrane coupling protein V-FITC was added and reacted in darkness at 4°C for 30 min, followed by 5 μL propidium iodide (PI) was gently mixed and incubated at 37°C for 5min at room temperature. Apoptosis was detected by apoptosis kit and flow cytometry.

Wound-Healing Assay

Transfected cells were placed into a 6-well plate in a CO_2 incubator for 24 h. A straight line was gently drawn in the dish perpendicular to the cell surface by the tip of the 10 μL pipette tips after the cells were completely adherent to the wall. The non-adherent cells were washed off. ImageJ software was used to measure the scratch width.

Cell scratch width was analyzed, and the scratch healing rate was calculated. Scratch healing rate = (scratch width at 0 h - scratch width at 48 h)/ scratch width at 0 h×100%. The experiment was repeated three times.

Transwell Assay

Cell invasion was detected by transwell assay. After 48 h of transfection, the cells were digested by 0.25% trypsin. After centrifugation, the cells were resuspended, and inoculated in the small chamber with 2×10^5/mL. The lower compartment was full of 600 μL medium containing 10% FBS and the upper compartment coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) was added with 200 μL cell suspension. The conditions were as follows: 37°C, 5% CO_2 incubator, 24-48 h. The culture medium was discarded, and the unperforated cells were wiped off with a cotton wiper. The cells were fixed with methanol and stained with crystal violet. Under the microscope, 5 fields were selected to calculate the number of cells penetrating the membrane.

qRT-PCR

48 h after transfection, the total RNA was extracted by TRIzol regents (TaKaRa, Otsu Shiga, Japan). The total RNA was converted to cDNA according to the instructions of the PrimeScript RT Reagent Kit (TaKaRa, Otsu, Shiga, Japan). The synthesized cDNA was amplified using SYBR Premix Ex Taq II kit (TaKaRa, Otsu, Shiga, Japan) by ABI 7900 system (Applied Biosystems, Foster City, CA, USA), and the expression of NAT2 was detected using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the internal reference. The relative expression of the gene was analyzed using $2^{-\Delta\Delta Ct}$ method. The experiment was repeated three times. The primer sequences are shown in Table I.

Western Blot Assay

Radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) was used to extract the cell lysis products. Bicinchoninic acid (BCA) protein determination kit (Beyotime, Shanghai, China) was used to determine the protein concentration. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed after high temperature denaturation. After electrophoresis, the protein was transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA), sealed with 5% skim milk powder for 1 h, and then incubated with primary antibody (NAT2, Bcl-2, Bcl-xl, Bax, cleaved-caspase3 and GAPDH, 1:2500, Abcam, Cambridge, UK) at 4°C overnight. The PVDF membrane was washed by TBST 3 times, 5 min each. Then, the incubation of horseradish peroxidase (HRP)-coupled secondary antibody (1:5000, Abcam, Cambridge, UK) was performed in a shaking table at room temperature for 2 h, and the membrane was washed with TBST for 3 times, 5 min each. Finally, a chemiluminescence reagent kit (Millipore, Billerica, MA, USA) was used to detect protein signals.

Table I. Primer sequences for real-time fluorescence quantification PCR.

Gene name	Primer sequences (5′-3′)
GAPDH	F ACGCTGCATGTGTCCTTAG
	R GAGCCTCTTATAGCTGTTTG
NAT2	F TTGGAAGCAAGAGGATTGCAT
	R GATCTGGTGCTCAAGAATGTCAGT

Immunohistochemical (IHC) Staining

The tissue specimens were fixed with 4% paraformaldehyde, embedded with paraffin, and prepared into 4 m thick tissue slices. The slices were baked at 60°C for 2 h and soaked in xylene solution for 2×15 min to dewax. Ethanol with different concentrations (100%, 95%, 90%, 75% and 70%) was used for hydration for 5 min, and sodium citrate (10 mmol/L, pH=6.0) was used for antigen repair for 20 min. Then slices were placed at room temperature and cooled for 30 min. Slices were treated for 20 min in 3% hydrogen peroxide prepared with methanol to block endogenous peroxidase. After blocking with 5% bovine serum protein for 2 h, NAT2 monoclonal antibody (1:500, Abcam, Cambridge, UK) was used for incubation at 4°C overnight. The next day, a secondary antibody labeled with HRP (Dako, Carpinteria, CA) was added and incubated at room temperature for 30 min. 3,3′-diaminobenzidine (DAB) color was developed, and the reaction time was controlled under a microscope. Hematoxylin was used to redye the nucleus. Finally, the slices were dehydrated with anhydrous ethanol, sealed with neutral gum, and examined under Olympus optical microscope.

IHC Results Score

The whole section was observed under a light microscope, and 5 fields of high magnification were randomly selected for the result determination in each section. The appearance of brownish

yellow or brown granules was considered positive for staining. The observation was conducted by two experienced pathologists using a double-blind method, referring to the second-level scoring method: (1) According to the number of positive cells: ≤5%, counted as 0 points, counted as 1 point between 6% and 25%, counted as 2 points between 26% and 50%, and counted as 3 points between 51% and 75%, 4 points for >75%; (2) According to the staining depth: 0 points for non-positive cells, 1 point for yellow, 2 points for brownish, and 3 points for tan. Multiply the two scores: 0 points are (-), 1~2 points are (+), 3~4 points are (++), 5~12 points are (+++). 2 was set as high expression, ≤2 as low expression. The difference of positive expression of NAT2 in CRC patients with different clinical characteristics was compared, and the prognostic factors of CRC patients were analyzed.

In Vivo Tumorigenic Assay

10 nude mice were randomly divided into two groups, numbered and kept in a specific-pathogen-free (SPF) animal house. After trypsin digestion, logarithmic growth cells were collected, and the cell density was adjusted to 1×10^7 cells/ml. The skin on the right axillary back of nude mice was disinfected and 0.2 ml cell suspension was inoculated in each nude mouse. The needle moved along the subcutaneous oblique path, and the formation of a pimple with a diameter of 0.5-0.8 cm could be seen after injection, indicating successful inoculation. When visible tumor nodules were formed at the inoculation site, the longest diameter (a) and the short diameter (b) perpendicular to the tumor were measured with Vernier caliper every 7 days, and the final tumor volume was calculated according to the formula $V=1/2ab^2$. Four weeks after inoculation, nude mice were sacrificed by cervical vertebra removal. Subcutaneous tumors were removed, and the tumor weight was weighed after taking photos. All animal procedures were approved by the Ethics Committee of the Weifang People's Hospital.

Statistical Analysis

SPSS 17.0 (Chicago, IL, USA) software was used for statistical analysis of the experimental data. Groups of data are represented by mean ± SD (standard deviation). An independent sample *t*-test was used to compare mean values between the two groups. The overall survival rate was calculated by the Kaplan-Meier method and the log-rank test. $p < 0.05$ was considered statistically significant.

Results

NAT2 is Upregulated in CRC Specimen and Cell Lines

We collected 60 fresh CRC tissues and NC tissues, and detected the expression of NAT2 mRNA in CRC tissues and NC tissues by RT-qPCR. The results showed that NAT2 mRNA expression was significantly increased in CRC tissues compared with NC tissues (Figure 1A). At the same time, Western blot analysis of 8 pairs of tissues showed that NAT2 protein expression was notably enhanced in CRC tissues compared with NC tissues (Figure 1B). In addition, GEPIA database analysis showed that the NAT2 mRNA expression in colon adenocarcinoma (COAD) tissues (275 cases) were significantly higher than that in normal tissues (349 cases) (Figure 1C). Moreover, we detected the expression of NAT2 in CRC cells through Western blot analysis. The results showed that NAT2 protein expressions were significantly increased in CRC cells compared with NCM460 (Figure 1D).

NAT2 Knockdown Suppresses Proliferation of CRC Cells

CRC cell lines with stable expression of sh-NAT2 (SW480) were constructed by lentivirus infection, and total mRNA and protein of the cells were extracted, respectively. The transfection efficiency of lentivirus was verified by RT-qPCR and Western blot experiments. The results showed that NAT2 mRNA and protein expression in the sh-NAT2 were significantly downregulated compared with the sh-NC (Figure 2A). The effect of NAT2 on the proliferation ability of CRC cells was tested by CCK-8 and clone formation experiment. CCK-8 results showed that the absorbance value of cells in the sh-NAT2 was markedly lower than that in the sh-NC (Figure 2B). The colony formation experiment results showed that the colony formation ability of cells in the sh-NAT2 was significantly decreased versus the sh-NC (Figure 2C). Apoptosis results showed that the apoptosis rate of SW480 cells was significantly increased after NAT2 knockdown (Figure 2D). Meanwhile, Western blot results showed that anti-apoptotic proteins (Bcl-2, Bcl-xl) in sh-NAT2 were significantly down-regulated compared

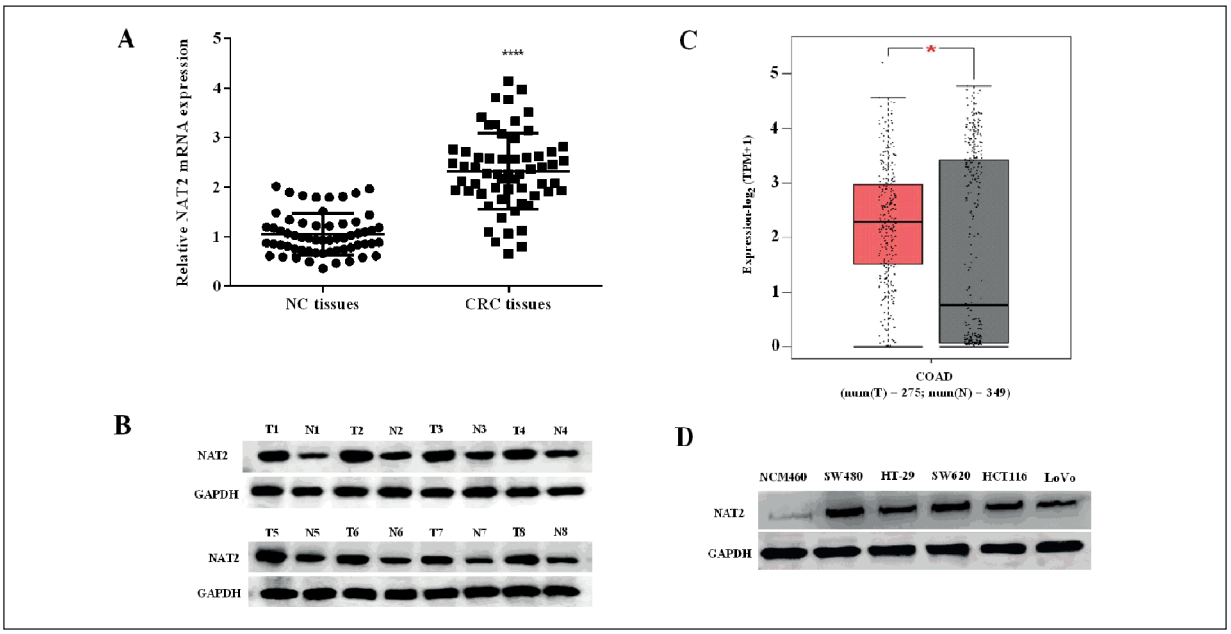

Figure 1. NAT2 is upregulated in CRC. **A**, Expression of NAT2 gene in NC tissue and CRC tissue. **B**, Analysis of NAT2 protein expression in CRC tissue by Western blot. **C**, Bioinformatics analysis was performed to test NAT2 expression patterns in CRC tissue and normal tissue by GEPIA databank. **D**, NAT2 expression was evaluated in CRC cells. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, compared with normal tissues or NCM460 group.

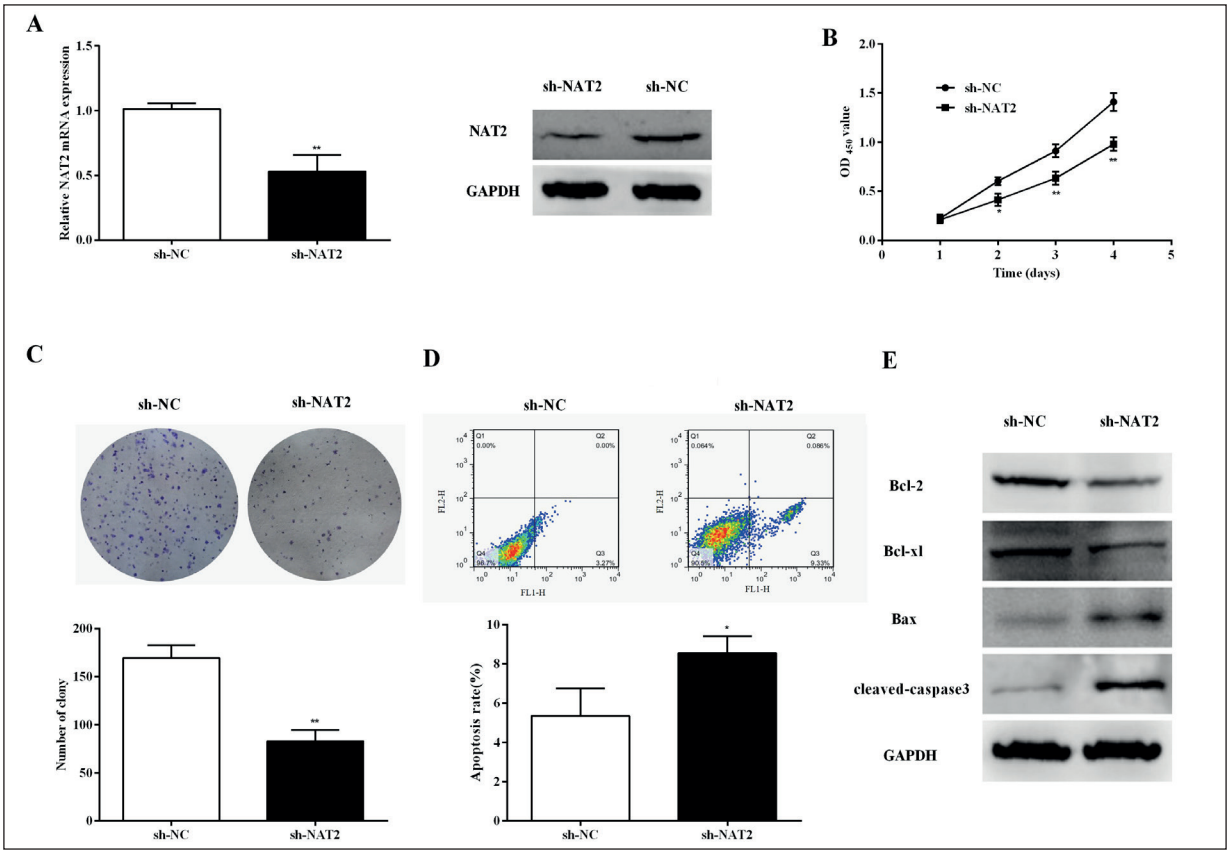

Figure 2. NAT2 knockdown suppresses proliferation and promotes apoptosis of CRC cells. **A**, Analysis of NAT2 expression in NAT2 knockdown CRC cells by qRT-PCR and Western blot. **B**, Cell proliferation was inhibited of NAT2 knockdown CRC cells. **C**, Clone formation ability was reduced of CRC cells after NAT2 knockdown. **D**, Cell apoptosis of CRC cells was promoted by sh-NAT2. **E**, Expression of apoptosis-related proteins. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with sh-NC group.

with sh-NC, while pro-apoptotic proteins (Bax, cleaved-caspase3) were significantly up-regulated (Figure 2E). The above results indicated that NAT2 knockdown inhibited the proliferation of CRC cells.

NAT2 Knockdown Hampers CRC Cell Migration and Invasion

Wound-healing and transwell assays were used to detect the effect of NAT2 on the migration and invasion ability of CRC cells respectively. Wound-healing results showed that compared with sh-NC, the scratch healing rate in sh-NAT2 was dramatically reduced (Figure 3A). The results of transwell invasion experiment showed that compared with sh-NC, the number of transmembrane cells in sh-NAT2 was also signally reduced (Figure 3B). Therefore, all the above results showed that NAT2 knockdown inhibited the migration and invasion ability of CRC cells.

NAT2 Knockdown Reduces Tumor Growth In Vivo

Finally, we evaluated the effect of NAT2 on the tumorigenic ability of CRC cells using *in vivo* tumorigenic experiments in mice. Compared with the control group, the tumorigenic ability of SW480 cells stably expressed by sh-NAT2 was significantly inhibited (Figure 4). Therefore, we suggested that NAT2 knockdown could reduce the tumorigenic ability of CRC cells.

Association of NAT2 Expression in CRC with Clinicopathological Characteristics

Immunohistochemistry was used to analyze the protein expression level of NAT2 in CRC tissues. The expression of NAT2 protein was mainly localized in the cytoplasm (Figure 5A), and the positive expression of NAT2 was brown or tan in CRC tissues. The results showed that the high expression rate of NAT2 in CRC tissues was 63.3% (38/60), and the low expression rate was 36.7% (22/60). More importantly, the upregulation of NAT2 protein was significantly correlated with the TNM stage, differentiation degree, tumor size, distant metastasis and lymph node metastasis of CRC, but not statistically correlated with other clinicopathological parameters, such as age and sex (Table II). The results suggested that the occurrence and metastasis of CRC may closely relate to the expression level of NAT2.

In addition, Kaplan-Meier method was used to analyze the correlation between NAT2 expression level and patients' overall survival. The results

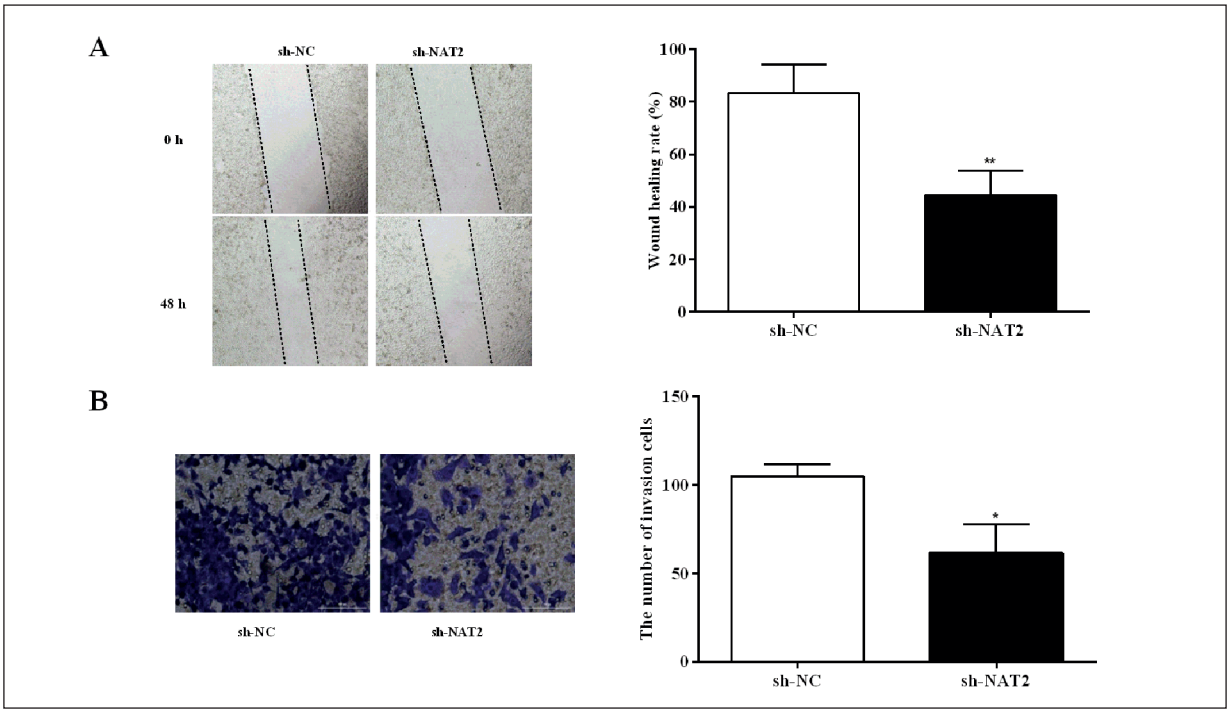

Figure 3. NAT2 knockdown suppresses migration and invasion of CRC cells. **A**, The cell migration ability of CRC cells with NAT2 knockdown was detected by a Wound-healing assay. **B**, The number of invasion cells after NAT2 knockdown was measured by transwell assay. *p <0.05, **p < 0.01, compared with sh-NC group.

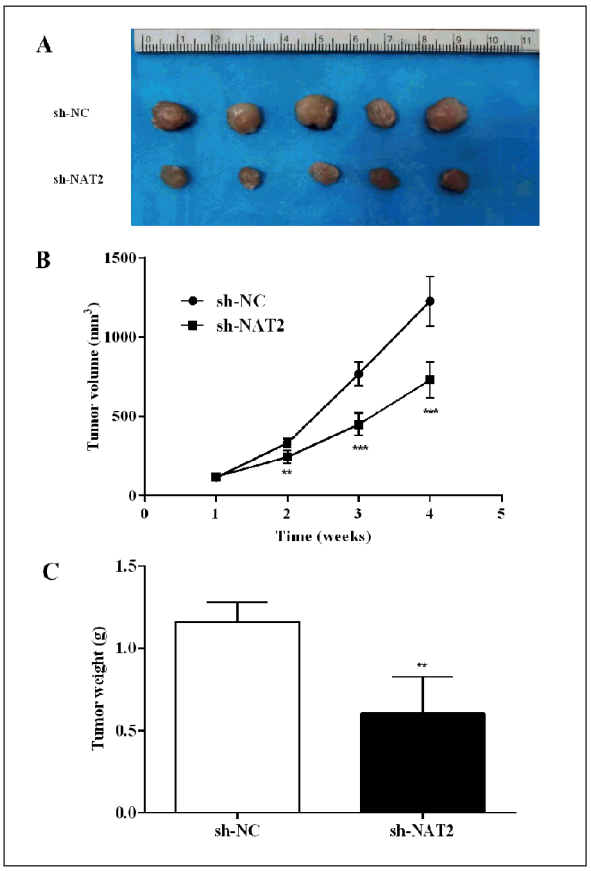

Figure 4. NAT2 knockdown reduces tumor growth *in vivo*. **A**, The size of transplanted tumor in sh-NAT2 group was obviously smaller than that in the sh-NC group. **B**, The volume of the transplanted tumor in sh-NC and sh-NAT2 groups. **C**, The weight of transplanted tumor was notably reduced in sh-NAT2 group *vs.* sh-NC group. $^{**}p < 0.01$, $^{***}p < 0.001$, compared with sh-NC group.

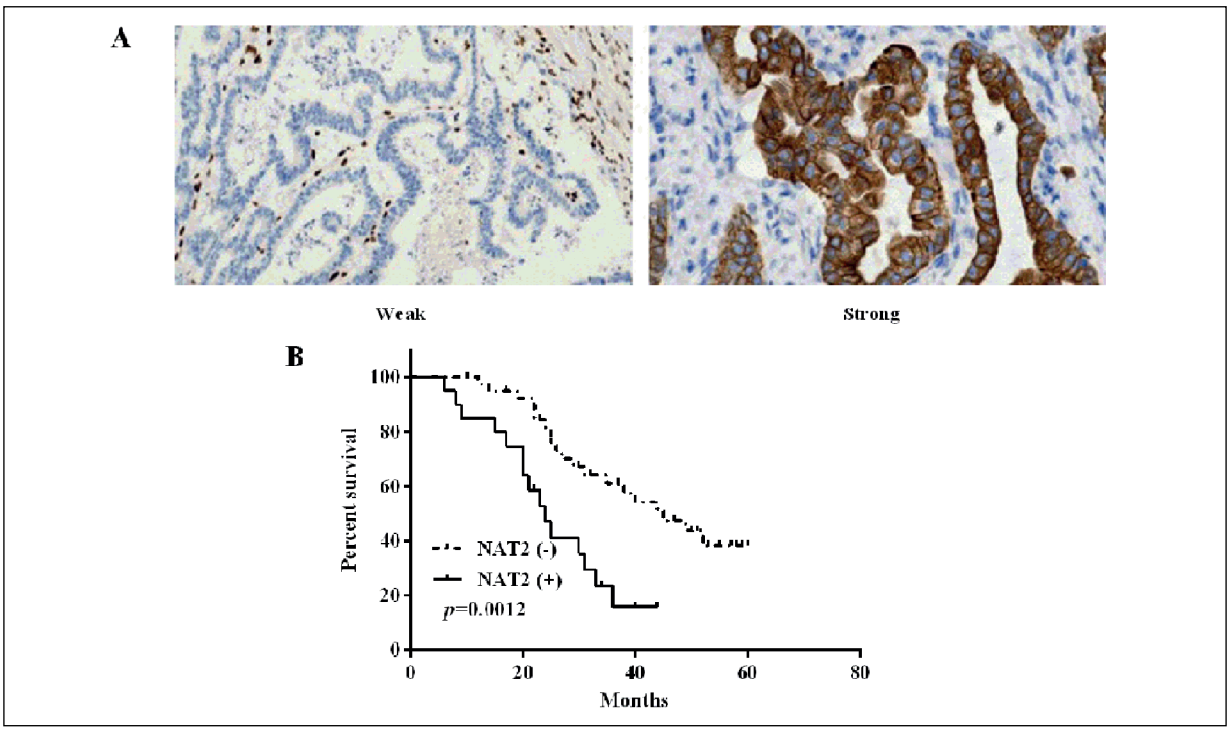

Figure 5. Association of NAT2 expression with clinical indexes. **A**, The expression of NAT2 protein in CRC tissues was analyzed by IHC (×200). **B**, Kaplan-Meier Plotter database analyzed the survival time of CRC patients basing on NAT2 expression.

Table II. Correlation between the clinicopathologic characteristics and expression of NAT2 in CRC.

Clinicopathological features	Cases	NAT2 expression		p
		High	Low	
Age (years)				0.464
> 60	31	21	10	
≤ 60	29	17	12	
Gender				0.428
Male	34	23	11	
Female	26	15	11	
Tumor size (cm)				0.012*
≥ 4.0	37	28	9	
< 4.0	23	10	13	
Differentiation degree				0.001*
High/middle differentiation	29	12	17	
Low differentiation	31	26	5	
TNM stage				0.049*
I-II	23	11	12	
III	37	27	10	
Lymphnode metastasis				0.006*
Yes	27	12	15	
No	33	26	7	
Distant metastasis				0.013*
Yes	29	23	6	
No	31	15	16	

*Statistically significant.

showed that survival rate in CRC patients was directly correlated with NAT2 expression levels. Among them, patients with low NAT2 expression (NAT2 (-)) had significantly higher survival rate than those with high NAT2 expression (NAT2 (+)) (Figure 5B). At the same time, Cox regression analysis showed that NAT2 expression could be an independent risk factor affecting the prognosis of CRC patients (Table III). These results suggested that CRC patients' prognosis is closely related to NAT2 expression.

Discussion

Although the diagnosis and treatment of cancer have made some progress, the early stage of CRC is easy to be ignored, leading to a gradual increase in mortality in China[13]. Nowadays, the treatment of CRC mainly relies on surgery and chemotherapy, and the subsequent recurrence and metastasis only result in a total survival time of about 24 months[14]. Compared with traditional chemotherapy drugs, molecular targeted

Table III. Univariate and multivariate analysis of OS in CRC patients.

Clinicopathological features	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p	HR (95% CI)	p
Age (> 60 *vs.* ≤ 60 years)	1.125 (0.580, 2.184)	0.728	0.737 (0.324, 1.681)	0.469
Gender (male *vs.* female)	0.916 (0.461, 1.823)	0.803	0.880 (0.417, 1.859)	0.738
Tumor size (≥ 4.0 < 4.0 cm)	0.744 (0.364, 1.520)	0.418	1.054 (0.481, 2.309)	0.895
Differentiation (high/middle *vs.* low)	0.381 (0.191, 0.757)	0.006*	0.412 (0.186, 0.912)	0.029*
TNM stage (I-II vs. III)	1.097 (0.557, 2.160)	0.788	1.069 (0.480, 2.380)	0.870
Lymphnode metastasis (yes *vs.* no)	2.766 (1.333, 5.741)	0.006*	2.547 (1.150, 5.640)	0.021*
Distant metastasis (yes *vs.* no)	2.644 (1.286, 5.436)	0.008*	1.617 (0.662, 3.952)	0.292
NAT2 expression (high *vs.* low)	3.152 (1.559, 6.370)	0.001*	2.342 (1.065, 5.150)	0.034*

HR, hazard ratio; 95% CI: 95% Confidence Interval. *Statistically significant.

therapy has stronger specificity and fewer side effects, while effective molecular targeted drugs for CRC are still lacking[15,16]. Therefore, the search for new molecular targets can provide directions for the diagnosis and follow-up treatment of CRC.

As an important two-phase metabolic enzyme in human body, NAT2 has attracted much attention in the world in recent years. NAT2 mRNA was found in liver, gastrointestinal tissues, etc., and NAT2 mRNA was mainly located in epithelial cells of different tissues[17-20]. The distribution of NAT2 enzyme was dominant in liver and intestinal epithelial cells[21]. Revealing the relationship between NATZ gene polymorphism and tumor susceptibility is of great significance for the early detection of cancer, gene therapy, screening of high-risk population and early prevention. Since Lang et al[22] first reported the association between NAT2 acetylation and CRC, many studies on the relationship between human NAT2 gene polymorphism and CRC genetic susceptibility have been published [11,23-25]. However, the function of NAT2 gene in CRC remains to be further studied.

In this study, NAT2 was found to be highly expressed in CRC tissues, and high-expressed CRC cell lines were screened out. Subsequently, RT-qPCR and Western blot confirmed that plasmid transfection could effectively decrease the expression of NAT2. In terms of cell function, CCK-8 and clone formation experiments verified that the proliferation ability of cells after NAT2 inhibition was reduced. Flow cytometry showed that knockdown of NAT2 promoted apoptosis. The transwell experiment proved that the knockdown of NAT2 would lead to the reduction of cell migration and invasion ability. The tumorigenic ability of CRC cells was inhibited by NAT2 knockdown. In summary, NAT2 inhibition can inhibit the proliferation, migration and invasion of CRC cells and promote cell apoptosis. This study also found that NAT2 expression level was related to TNM stage, differentiation degree, tumor size, distant metastasis, lymph node metastasis and survival prognosis of CRC, and was an independent factor of these clinical parameters. It suggests that NAT2 plays the role of tumor promoter gene in the occurrence and development of CRC, providing a new target for the diagnosis and treatment of CRC. However, how NAT2 promotes the development of CRC is not yet clear, which may be the direction of further research in the future.

Conclusions

In summary, our study for the first time confirms that high expression of NAT2 predicts poor prognosis in patients with CRC and is involved in the occurrence and development of CRC. Innovatively, the role of NAT2 in regulating tumor cell proliferation, apoptosis, invasion and metastasis and related molecular mechanisms in CRC was further elucidated. Therefore, detection of NAT2 expression may provide an important reference value for the clinical diagnosis, treatment and prognosis assessment of CRC. Briefly, NAT2 may be a novel potential target for future cancer treatment, providing new ideas for the treatment of CRC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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