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Editorial board member of *World Journal of Gastroenterology*, Tamara Vorobjova, DA, PhD, Academic Research, Department of Immunology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu 51014, Estonia

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Basic Study

Clinical correlation of B7-H3 and B3GALT4 with the prognosis of colorectal cancer

Ting Zhang, Fang Wang, Jing-Yi Wu, Zhi-Chao Qiu, Yan Wang, Fen Liu, Xiao-Song Ge, Xiao-Wei Qi, Yong Mao, Dong Hua

Ting Zhang, Fen Liu, Dong Hua, Institute of Cancer, Affiliated Hospital of Jiangnan University, Wuxi 214062, Jiangsu Province, China

Fang Wang, Jing-Yi Wu, Zhi-Chao Qiu, Yan Wang, Xiao-Song Ge, Yong Mao, Dong Hua, Department of Oncology, Affiliated Hospital of Jiangnan University, Wuxi 214062, Jiangsu Province, China

Fang Wang, Jing-Yi Wu, Zhi-Chao Qiu, Yan Wang, Wuxi School of Medicine, Jiangnan University, Wuxi 214122, Jiangsu Province, China

Xiao-Wei Qi, Department of Pathology, Affiliated Hospital of Jiangnan University, Wuxi 214062, Jiangsu Province, China

ORCID number: Ting Zhang (0000-0001-6859-6400); Fang Wang (0000-0002-8821-6695); Jing-Yi Wu (0000-0002-9219-2382); Zhi-Chao Qiu (0000-0003-0837-6144); Yan Wang (0000-0002-6310-0987); Fen Liu (0000-0003-1891-6992); Xiao-Song Ge (0000-0002-4180-5900); Xiao-Wei Qi (0000-0001-7761-5095); Yong Mao (0000-0001-5507-8292); Dong Hua (0000-0001-5569-1708).

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Correspondence to: Dong Hua, MD, PhD, Chief Doctor, Professor, Department of Oncology, Affiliated Hospital of Jiangnan University, 200 Huihe Road, Wuxi 214062, Jiangsu Province, China. wx89211@163.com
Telephone: +86-510-88682109
Fax: +86-510-85808820

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Abstract

AIM

To investigate the expression and clinical significance of B7 homolog 3 (B7-H3) and β -1,3-galactosyltransferase-4 (B3GALT4) in colorectal cancer (CRC) patients.

METHODS

Using tissue microarray, we identified the expression of B7-H3 and B3GALT4 in 223 CRC patient samples by immunohistochemistry and evaluated the possible correlation between B7-H3 and B3GALT4 and clinical

outcomes. Further, the mRNA and protein expression were identified to establish the regulatory relationship of B7-H3 with B3GALT4 *in vitro*.

RESULTS

A significant positive correlation between B7-H3 and B3GALT4 was observed in CRC specimens ($r = 0.219$, $P = 0.001$). High expression of B7-H3 was identified as a significant independent predictor of poor overall survival (OS) [hazard ratio (HR) = 1.781; 95%CI: 1.027-3.089; $P = 0.040$]. Moreover, high expression of B3GALT4 was also recognized as an independent predictor of inferior OS (HR = 1.597; 95%CI: 1.007-2.533; $P = 0.047$). Additionally, CRC patients expressing both high B7-H3 and high B3GALT4 contributed to a significant decrease in OS (HR = 2.283; 95%CI: 1.289-4.042; $P = 0.005$). In CRC cell lines with stable expression of high B7-H3, the mRNA and protein expressions of B3GALT4 were significantly upregulated. Similarly, the expression of B3GALT4 was significantly reduced when expression of B7-H3 was knocked down.

CONCLUSION

The expression of B3GALT4 in CRC is positively correlated with B7-H3 expression *in vitro*. B7-H3/B3GALT4 may be used as dual prognostic biomarkers for CRC.

Key words: B7 homolog 3; β -1,3-galactosyltransferase-4; Colorectal cancer; Prognosis; Correlation

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Core tip: The present study for the first time revealed the expression of β -1,3-galactosyltransferase-4 (B3GALT4) in colorectal cancer (CRC) and its correlation with B7 homolog 3 (B7-H3) *in vitro*. Overall, the findings of the present study suggest that B7-H3 and B3GALT4 are novel prognostic biomarkers for CRC and highlight the significance of both B7-H3 and B3GALT4 as promising therapeutic targets for CRC. Thus, here we present our preliminary work on the relationship of the immune function and glycosylation of tumor-associated protein in CRC.

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INTRODUCTION

Colorectal cancer (CRC) is the most prevalent gastrointestinal tract malignancy worldwide among both men and women. Overall CRC incidence and mortality rates

have been declining over the recent decades, yet CRC ranks third overall among other malignancies in men and women^[1]. In China, there were approximately 376300 new cases and 191000 potential deaths for CRC in 2015^[2]. Despite several therapeutic advancements including surgical resection, neoadjuvant chemoradiotherapy, and targeted therapy, the prognosis of CRC patients remains relatively poor. With unprecedented survival benefits in selected patients, immunotherapy has become a promising treatment strategy and continuous progress has been made with immunomodulatory agents that target immune system checkpoints such as anti-programmed death 1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4)^[3].

B7 homolog 3 (B7-H3), also known as CD276, is a type I transmembrane glycoprotein that belongs to the B7/CD28 immunoglobulin superfamily. B7-H3 mRNA is widely expressed on many tissues and cell types. However, B7-H3 protein is not constitutively expressed on T-cells, natural killer cells and antigen-presenting cells, and its expression can be induced on immune cells. While limited expression has been noticed on normal tissues, overexpression is observed in a majority of human malignancies including CRC^[4] and has been correlated with the poor prognosis of patients^[5]. Moreover, B7-H3 has been suggested to promote tumor growth, migration, invasion, and metastasis. Thus, it may be a potential therapeutic target for active immunotherapy.

The β -1,3-galactosyltransferase-4 (B3GALT4) gene belongs to the β -1,3-galactosyltransferase (β 3GalT) gene family, which encodes type II membrane-bound glycoproteins and is located in the centromeric segment of the human MHC class II region^[6]. This gene is abundantly expressed in human organs and tissues, predominantly in the brain and involved in GM1/GD1 ganglioside synthesis^[7]. The β 3GalT family plays an essential role in the O-glycosylation process. The surface of cancer cells express glycoproteins, which are rich in O-glycosylation domains^[8]. Thus, the family may be closely related to the tumor. Besides, Seko *et al*^[9] had confirmed that B3GALT4 could be used as a novel biomarker for the diagnosis of gynecological cancers. However, there are insufficient reports about the correlation between B3GALT4 and CRC.

Therefore, the present study aimed to investigate the clinical correlation of B7-H3 and B3GALT4 with CRC. Further, correlation between the expression of B7-H3 and B3GALT4 was evaluated to determine their prognostic significance in CRC.

MATERIALS AND METHODS

Patients and tissue samples

The medical records of patients who received a histopathological diagnosis and underwent surgery for CRC at Affiliated Hospital of Jiangnan University between June 2008 and December 2011 were retrieved. A total

of 223 formalin-fixed paraffin-embedded CRC tissue samples were included in the study. These patients did not receive radiotherapy or chemotherapy before the surgery. Only histologically confirmed cases were included in the study. However, patients who received chemo- or radiotherapy before surgery and cases with incomplete clinical data were excluded from the study. The study was approved by the Medical Ethics Committee of Affiliated Hospital of Jiangnan University, and written informed consent was obtained from all patients. All the patients were followed up by telephone up to October 31, 2017, to obtain the survival data. The median follow-up was 79 mo (range, 6-114 mo).

Tissue microarray preparation

Two experienced pathologists examined the section stained with hematoxylin and eosin and marked the carcinoma sites in the corresponding paraffin block. Using a manual tissue microarrayer (Quick-Ray, UNITMA, Seoul, Korea), tissue cylinders with a 1.0 mm diameter were punched from representative tissue areas of each donor tissue block and implanted into the hole of the premade recipient paraffin block (UNITMA, Seoul, Korea). The tissue microarray paraffin block was then constructed and cut into 4 μ m thick continuous sections, which were attached to anti-dewaxing slides and stored at room temperature.

Immunohistochemistry

The tissue paraffin blocks were serially sectioned into a 4 μ m thickness, dewaxed in xylene, and hydrated in an ethanol gradient. Antigen retrieval was performed by heating the tissue sections at 100 °C for 30 min in citrate buffer. Moreover, endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide for 10 min. Subsequently, the sections were incubated in 5% bovine serum albumin at room temperature for 30 min. Primary antibodies, mouse anti-human B7-H3 monoclonal antibody (1:200, Santa Cruz, Dallas, TX, United States) and rabbit anti-human B3GALT4 monoclonal antibody (1:50, Abcam, Cambridge, MA, United States) respectively were added drop-wise followed by overnight incubation at 4 °C. The slides were washed and incubated with a horseradish peroxidase-conjugated secondary antibody for 30 min. The immunostaining was carried out by staining with 3,3'-diaminobenzidine tetrahydrochloride solution (GTVisionII Immunohistochemistry Detection Kit for Rabbit/Mouse, Gene Tech, Shanghai, China), counter-stained with hematoxylin, dehydrated, and mounted. Sections were examined under a microscope.

Evaluation of immunohistochemistry staining

Two independent pathologists performed a blinded manner review of the sections. Both the intensity and extent of immunological staining were analyzed semi-quantitatively. According to the percentage of positively stained cells, the sections were graded by five levels: 0 (\leq 5%), 1 (6%-25%), 2 (26%-50%), 3 (51%-75%) and 4 ($>$ 76%). The staining intensity was scored

similarly, with 0 used for negative staining, 1 for weakly positive, 2 for moderately positive and 3 for strongly positive. The scores for the percentage of positive cells and the staining intensity were multiplied to generate an immune-reactive score for each specimen (0-12). Based on the total score, scores of 0-3 were considered as the low expression group, and scores of 4-12 were defined as the high expression group.

Cell lines and culture

The two human CRC cell lines, SW480 and Caco-2, which exhibited different expression levels of B7-H3 were maintained in our lab. We constructed SW480 cells that expressed a high level of B7-H3 (SW480-B7-H3) by transfection with an overexpression plasmid, and Caco-2 cells were stably transfected with a B7-H3 shRNA (Caco-2-shB7-H3). Cells transfected with a mock vector were used as negative controls (SW480-NC and Caco-2-shNC). SW480-NC and SW480-B7-H3 were cultured in L-15 medium (HyClone GE Healthcare Life Sciences, South Logan, UT, United States). Caco-2-NC and Caco-2-shB7-H3 were maintained in RPMI-1640 medium (the same as above). All the media were supplemented with 10% fetal bovine serum (Clark Bioscience, Houston, TX, United States). The cells were incubated at 37 °C in a humidified atmosphere with 5% CO₂.

RNA extraction and cDNA synthesis

Total RNA was extracted from 1×10^6 cells using TRIzol (Invitrogen, Carlsbad, CA, United States), according to the manufacturer's protocol. A NanoDrop 2000 Spectrophotometer (Thermo Scientific, Waltham, MA, United States) was used to estimate the purity and concentration of total RNA by measuring the optical density. The first strand cDNA was generated from 1 μ g RNA using the HiScript II 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China) following the manufacturer's instructions.

Real-time quantitative polymerase chain reaction

The expression levels of B7-H3 and B3GALT4 were detected using the QuantiNova™ SYBR Green PCR Kit (QIAGEN, Hilden, Germany) on ViiA™ 7 Real-Time PCR System (Applied Biosystems, Foster City, CA, United States). β -actin was used as an internal control for normalization of the expression data. The first strand cDNA was amplified in a total 20 μ L PCR reaction mixture: 10 μ L 2 x QuantiNova SYBR Green PCR Master Mix, 0.1 μ L QN ROX Reference Dye, 0.4 μ L of each specific primer set, 2 μ L cDNA and ddH₂O added to 20 μ L. The sequences of primers were as follows: β -actin 5'-CATGTACGTTGCTATCCAGGC-3' (sense), 5'-CTCCTTAATGTCACGCACGAT-3' (antisense); B7-H3 5'-AGCACTGTGGTTCTGCCTCACA-3' (sense), 5'-CACCAGCTGTTTGGTATCTGTCTAG-3' (antisense); B3GALT4 5'-ACTCCTACCGCAACCTCA-3' (sense), 5'-CACATCATCGTCCGTCTT-3' (antisense). Each sample and internal control gene were run in triplicate, using an amplification condition of denaturation at 95 °C for

2 min, followed by 40 cycles of 95 °C for 5 s and 60 °C for 10 s. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression level of the target genes.

Western blotting

Total proteins were extracted from 1×10^6 cells by using ice-cold RIPA lysis buffer containing a cocktail of protease inhibitors (KeyGEN BioTECH, China) for 30 min. The concentrations of whole cell proteins were determined using a BCA Protein Assay Kit (CWBio, Beijing, China) and adjusted to the same concentration. For western blotting assay, the proteins of equal quantity were separated by 10% SDS-PAGE and then transferred onto a PVDF membrane (Merck Millipore, Germany). After blocking with 5% nonfat dry milk for 1 h at room temperature, the membranes were incubated with the primary antibodies at a concentration of 1:1000 at 4 °C overnight. The antibodies included rabbit anti-human B7-H3 monoclonal antibody (Abcam, Cambridge, MA, United States), rabbit anti-human B3GALT4 monoclonal antibody (Abcam, Cambridge, MA, United States) and mouse anti-human β -actin monoclonal antibody (Beyotime, Nantong, China). After washing with TBST (TBS with 0.1% Tween), the membranes were incubated with a corresponding goat-anti-mouse or goat-anti-rabbit IgG-horseradish peroxidase secondary antibody (1:1000, Beyotime, Nantong, China) for 1 h at room temperature. The membranes were finally treated with enhanced chemiluminescence (ECL) assay reagents (absin, Shanghai, China) and the immunoreactive bands were visualized using the ChemiDoc™ XRS+ system with Image Lab™ Software (Bio-Rad, Hercules, CA, United States). Quantitative analysis was carried out with Quantity One (Bio-Rad, Hercules, CA, United States).

Statistical analysis

Statistical analysis of clinical data was performed using the software package SPSS 19.0 software (IBM, Chicago, IL, United States). Chi-square test analyzed the association between B7-H3 or B3GALT4 and the clinicopathological data. The non-parametric Spearman test evaluated the correlation of B7-H3 and B3GALT4 expression. Overall survival (OS) was plotted using the Kaplan-Meier method and was compared using the log-rank test. Multivariate survival analysis was performed using the Cox proportional hazard model. All the *in vitro* experiments were performed in triplicate. A non-paired *t*-test analyzed differences in mean values between groups. All data were analyzed using GraphPad Prism 6.0 (GraphPad Software Inc., La Jolla, CA, United States). A *P*-value less than 0.05 indicated a statistically significant difference.

RESULTS

Expression of B7-H3 and B3GALT4 and their relationship with clinicopathological features of CRC

The expression of B7-H3 and B3GALT4 was analyzed

using immunohistochemistry in CRC tissue. Positive staining of B7-H3 was predominantly detected in the membrane and cytoplasm (Figure 1C), and the location of B3GALT4 expression was identified in the cytoplasm (Figure 1D). The positive rates of the B7-H3 and B3GALT4 expressions were 70.4% (157/223) and 54.7% (122/223), respectively. Further, a highly significant correlation between the positive expression of B7-H3 with the depth of tumor invasion ($P = 0.049$), distant metastasis ($P = 0.034$) and differentiation ($P = 0.017$) was observed. However, the expression of B3GALT4 had no significant correlation with clinicopathological parameters (Table 1).

Correlation between B7-H3 and B3GALT4 expression in CRC

Of the 223 CRC cases, 97 (43.5%) patients exhibited higher expression of both B7-H3 and B3GALT4, and 41 (18.4%) showed lower expression of both. Also, there were 85 (38.1%) cases with either B7-H3 low or B3GALT4 low. Spearman's Correlation was used to examine the association of B7-H3 expression with B3GALT4 expression in CRC. The result showed that there was a highly significant positive correlation between B7-H3 and B3GALT4 expression ($r = 0.219$, $P = 0.001$, Table 2).

High expression of B7-H3 and B3GALT4 in patients with CRC is associated with poor OS

Notably, patients with high expression of B7-H3 had a significantly worse OS as compared to patients with low expression ($P = 0.037$) (Figure 2A). Similarly, the OS in the high B3GALT4 expression group was significantly inferior to that in the low expression group ($P = 0.044$) (Figure 2B). Moreover, a subgroup of patients with high expression of B7-H3 and high B3GALT4 exhibited a significantly worse prognosis as compared to patients with a low expression of B3GALT4 ($P = 0.016$) (Figure 2C). However, in a subgroup of patients with low expression of both B7-H3 and B3GALT4 prognosis of patients with CRC was unaffected ($P = 0.061$) (Figure 2D).

Furthermore, the univariate Cox proportional hazard model analysis was performed to evaluate the risk factors related to the prognosis of patients with CRC. As shown in Table 3, high expression of B7-H3, high expression of B3GALT4, high expression of both B7-H3 and B3GALT4, depth of tumor invasion (T3/4), lymph node metastasis (N1/2), distant metastasis, TNM stage (III/IV), neural invasion, and vascular invasion were correlated with the OS of patients with CRC. However, there was no correlation of OS with gender, age, tumor location, colon cancer site, mucinous adenocarcinoma, differentiation, and B7-H3 low expression group (Table 3). Besides, the multivariate analysis revealed that distant metastasis, TNM stage (III/IV), and vascular invasion were significant independent prognostic factors for OS of patients with CRC (Table 3).

Table 1 Expression of B7 homolog 3 and β -1,3-galactosyltransferase-4 in colorectal cancer patients and their correlation with clinicopathological parameters

Clinicopathological parameter	Case (n)	B7-H3 expression		P value	B3GALT4 expression		P value
		Low	High		Low	High	
Gender				0.616			0.213
Male	124	35	89		63	61	
Female	99	31	68		42	57	
Age (yr)				0.259			0.791
< 60	87	22	65		40	47	
\geq 60	136	44	92		65	71	
Tumor location				0.361			0.905
Colon	88	23	65		41	47	
Rectum	135	43	92		64	71	
Colon cancer site				0.119			0.352
Right-sided	37	7	30		20	17	
Left-sided	186	59	127		85	101	
Depth of tumor invasion				0.049			0.527
T1/2	64	25	39		28	36	
T3/4	159	41	118		77	82	
Lymph node metastasis				0.433			0.145
N0	116	37	79		60	56	
N1/2	107	29	78		45	62	
Distant metastasis				0.034			0.425
Yes	16	1	15		6	10	
No	207	65	142		99	108	
TNM stage				0.297			0.068
I / II	113	37	76		60	53	
III / IV	110	29	81		45	65	
Neural invasion				0.146			0.683
Yes	32	6	26		14	18	
No	191	60	131		91	100	
Vascular invasion				0.079			0.585
Yes	35	6	29		15	20	
No	188	60	128		90	98	
Mucinous adenocarcinoma				0.579			0.506
Yes	20	7	13		8	12	
No	203	59	144		97	106	
Differentiation				0.017			0.186
Poor	73	14	59		39	34	
Moderate/well	150	52	98		66	84	

B7-H3: B7 homolog 3; B3GALT4: β -1,3-galactosyltransferase-4.**Table 2 Correlation between B7 homolog 3 and β -1,3-galactosyltransferase-4 expression in colorectal cancer**

B3GALT4 expression	B7-H3 expression		r	P value
	Low	High		
Low	41	60	0.219	0.001
High	25	97		

B7-H3: B7 homolog 3; B3GALT4: β -1,3-galactosyltransferase-4.**Correlation between B7-H3 and B3GALT4 in CRC cell lines**

We further investigated the relationship between expression of B7-H3 and B3GALT4 *in vitro*. The mRNA and protein expression was detected in the CRC cell lines with different B7-H3 expression levels, which were maintained in our lab. Both the mRNA and protein expression of B7-H3 and B3GALT4 were significantly upregulated in SW480-B7-H3 cell line expressing a high level of B7-H3 as compared to SW480-NC cell line. Similarly, as compared to Caco-2-shNC cell line,

Caco-2-shB7-H3 cell line with low expression of B7-H3 significantly downregulated the expression of B3GALT4. Therefore, B7-H3 and B3GALT4 had a positive correlation both in mRNA and protein expression *in vitro* (Figure 3).

DISCUSSION

CRC continues to be a major cause of cancer-related morbidity and mortality with over 1200000 cases diagnosed every year, and more than 600000 patients

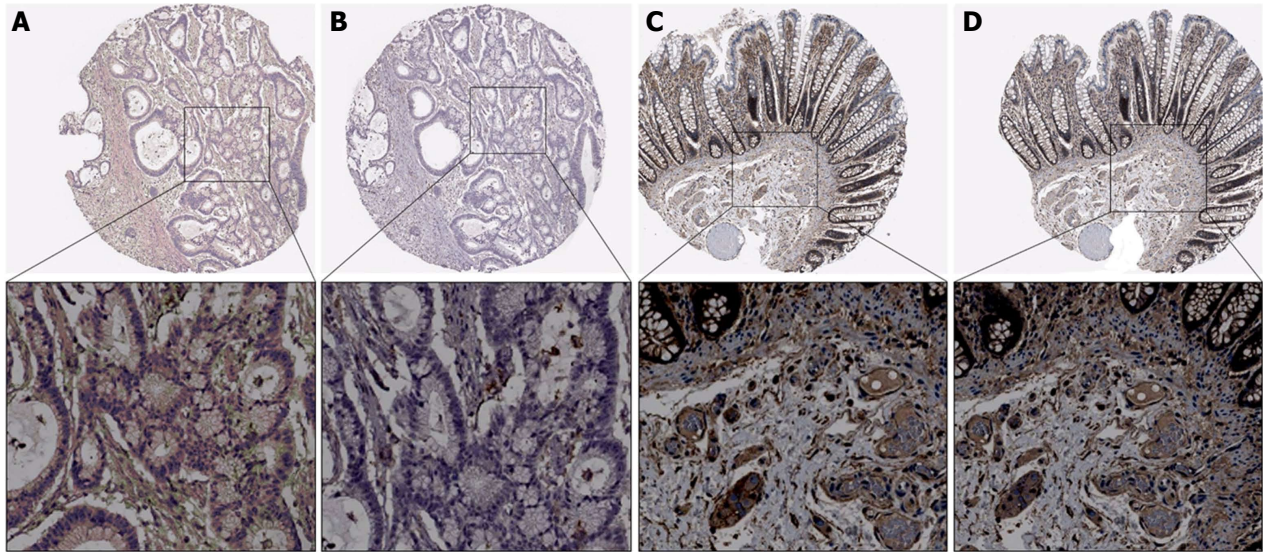


Figure 1 The representative immunohistochemical images of B7 homolog 3 and β -1,3-galactosyltransferase-4 expression in colorectal cancer tissues. A: Low expression of B7 homolog 3 (B7-H3); B: Low expression of β -1,3-galactosyltransferase-4 (B3GALT4); C: High expression of B7-H3; D: High expression of B3GALT4 (100 \times). The images below show the magnification of each zone (400 \times). A and B are from one patient; C and D are from another patient.

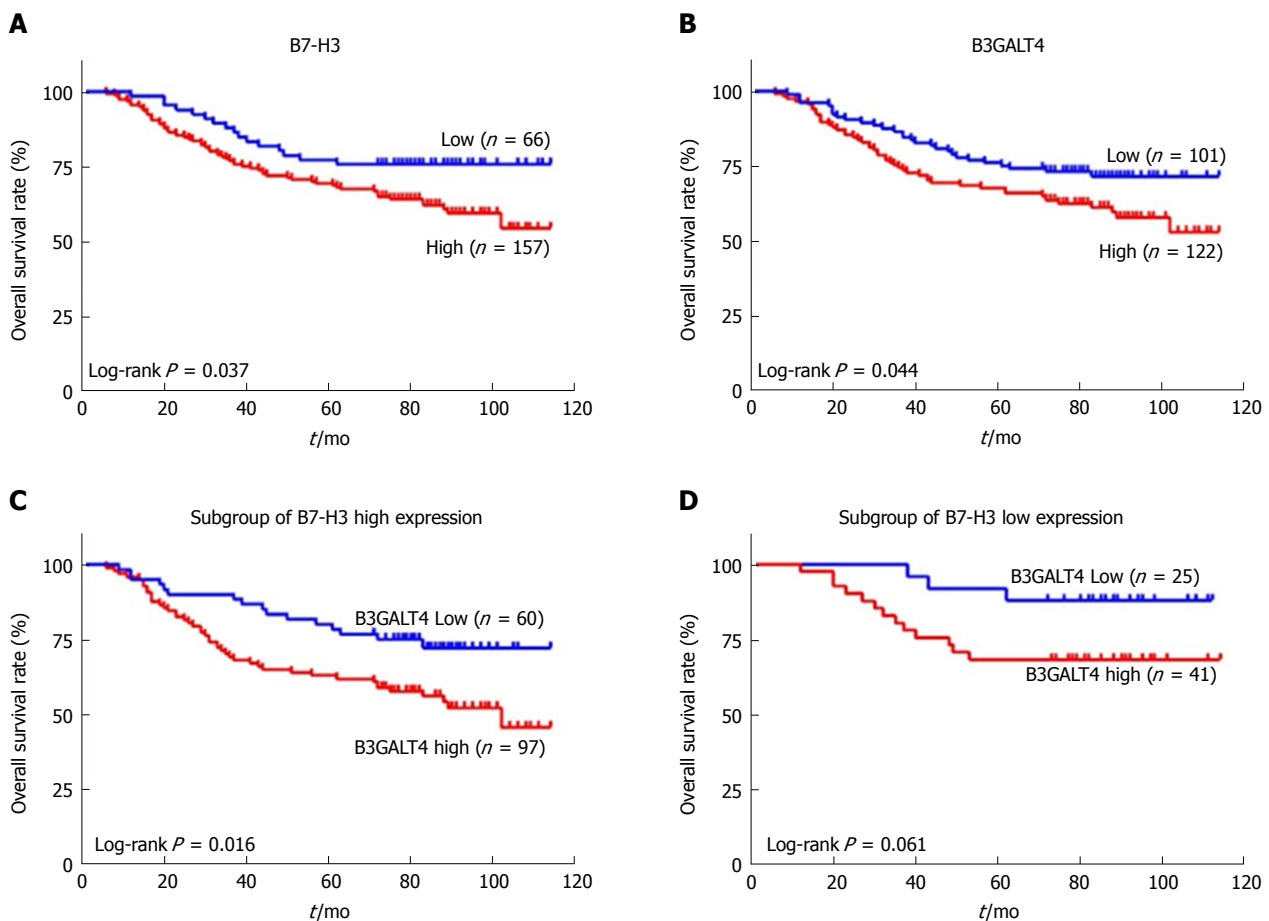


Figure 2 Kaplan-Meier survival curves of the 223 patients with colorectal cancer expressing B7 homolog 3 and β -1,3-galactosyltransferase-4. A: Patients with low expression of B7 homolog 3 (B7-H3) vs high expression of B7-H3; B: Patients with low expression of β -1,3-galactosyltransferase-4 (B3GALT4) vs high expression of B3GALT4; C: Subgroup of patients with high expression of B7-H3 and low expression of B3GALT4 vs high expression of B3GALT4; D: Subgroup of patients with low expression of B7-H3 and high expression of B3GALT4 vs low expression of B3GALT4.

succumb to the disease worldwide. Moreover, five-year survival rates for stage I CRC was more than 90%

where as for stage IV was 10%^[10]. Given the poor prognosis of CRC, the search for novel diagnostic and

Table 3 Univariate and multivariate Cox proportional hazards analyses of overall survival in patients with colorectal cancer

Clinical parameter	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
Gender (male <i>vs</i> female)	0.999 (0.637-1.566)	0.995		
Age (≥ 60 <i>vs</i> < 60) (yr)	0.951 (0.603-1.500)	0.829		
Tumor location (rectum <i>vs</i> colon)	1.351 (0.863-2.117)	0.188		
Colon cancer site (left-sided <i>vs</i> right-sided)	0.970 (0.534-1.760)	0.919		
Depth of tumor invasion (T3/4 <i>vs</i> T1/2)	2.522 (1.362-4.670)	0.003	1.807 (0.949-3.443)	0.072
Lymph node metastasis (N1/2 <i>vs</i> N0)	2.922 (1.801-4.739)	0.000	0.322 (0.077-1.341)	0.119
Distant metastasis (yes <i>vs</i> no)	9.353 (5.206-16.804)	0.000	4.635 (2.224-9.660)	0.000
TNM stage (III/IV <i>vs</i> I/II)	3.497 (2.114-5.783)	0.000	7.490 (1.636-34.280)	0.009
Neural invasion (yes <i>vs</i> no)	2.240 (1.320-3.802)	0.003	0.800 (0.410-1.561)	0.514
Vascular invasion (yes <i>vs</i> no)	2.531 (1.529-4.188)	0.000	1.962 (1.082-3.558)	0.026
Mucinous adenocarcinoma (yes <i>vs</i> no)	1.063 (0.488-2.314)	0.877		
Differentiation (poor <i>vs</i> moderate/well)	0.799 (0.504-1.267)	0.340		
B7-H3 (high <i>vs</i> low)	1.781 (1.027-3.089)	0.040	1.289 (0.721-2.307)	0.392
B3GALT4 (high <i>vs</i> low)	1.597 (1.007-2.533)	0.047	1.209 (0.721-2.307)	0.392
B7-H3 and B3GALT4 (high <i>vs</i> low)	0.737 (0.552-0.983)	0.038	1.153 (0.845-1.572)	0.369
B7-H3 high (B3GALT4 high <i>vs</i> low)	2.283 (1.289-4.042)	0.005		
B7-H3 low (B3GALT4 high <i>vs</i> low)	0.321 (0.091-1.127)	0.076		

B7-H3: B7 homolog 3; B3GALT4: β -1,3-galactosyltransferase-4.

prognostic biomarkers is highly desirable to prevent CRC-related deaths.

Immune checkpoint signaling pathways are most frequently modulated in cancer to inhibit the nascent anti-tumor immune response. Checkpoint antibody inhibitors, such as CTLA-4 and PD-1/PD-L1 are the most extensively studied novel class of inhibitors. Currently, drugs inhibiting these pathways are employed for a wide variety of malignancies and have demonstrated durable anti-tumor activities in a subset of cancer patients^[11,12]. Furthermore, investigations on new inhibitory pathways are also undergoing, and drugs are being investigated including those targeting B7-H3^[13]. As an immune checkpoint protein, B7-H3 has been recognized as an immunoregulatory molecule for T cell activation, proliferation, and cytokine production^[14]. Thus far, overexpression of B7-H3 has been reported in several different cancer types and the expression level of B7-H3 correlated with tumor growth, invasion, metastasis, malignant stage, and recurrence rate^[15]. Furthermore, several groups have generated anti-B7-H3 antibodies and reported overall suppression of tumor growth *in vitro* and *in vivo*^[16]. Therefore, it is expected that B7-H3 plays a crucial role in cancer diagnosis and treatment. The present study revealed that B7-H3 was highly expressed in CRC tissue (70.4%) and significantly associated with the depth of tumor invasion ($P = 0.049$), distant metastasis ($P = 0.034$) and differentiation ($P = 0.017$). These results were consistent with previous findings^[17,18].

Furthermore, B7-H3 is a type I transmembrane glycoprotein and plays a role in immunosuppression. Chen *et al.*^[19] demonstrated that glycoprotein B7-H3 was involved in the pathological process and tumor growth of oral squamous cell carcinoma, and the glycans of B7-H3 from oral cancer cells comprised of terminal α -galactoses and more diverse N-glycan structures with

higher fucosylation than that of healthy cells. In addition, the presence of a carbohydrate-lectin receptor interaction between the tumor-associated B7-H3 from oral cancer cells and immune cells was detected^[19]. B3GALT4, encoding a UDP-galactose: β -N-acetylgalactosamine β -1,3-galactosyltransferase activity, belongs to the human β -3-galactosyltransferase gene family^[7]. Galactosyltransferase (GT) of the glycosyltransferase family, is the most common Golgi "marker" enzyme, catalyzes the transfer of galactose to glycoprotein-bound acetylglucosamine. The enzyme is provided with one N-linked oligosaccharide and palmitate residues and plays a vital role in the process of O-glycosylation. Moreover, in patients suffering from ovarian and breast cancer, increased expression of GT enzyme activity has been reported^[20]. Enzymatic activity of B3GALT4 and T5 in ovarian cancer tissues, and significant overexpression of B3GALT4/T5 mainly in stage I uterine corpus cancers, indicated that B3GALT4/T5 is a novel tumor marker for uterine corpus cancer and other gynecological cancers^[9]. Consequently, B7-H3 and B3GALT4 were both correlated with glycosylation and tumor progression. However, there was a paucity of literature on the expression and association of B7-H3 and B3GALT4 in CRC. Therefore, the association of B7-H3 and B3GALT4 expression in CRC tissues and cell lines was investigated.

The present study revealed that high expression of B7-H3 or B3GALT4 reduced the OS rate of CRC patients independently ($P = 0.037$, $P = 0.044$, respectively). Furthermore, overexpression of both B7-H3 and B3GALT4 in CRC resulted in poorer prognosis ($P = 0.016$). Besides, there was a significant positive correlation between B7-H3 and B3GALT4 ($r = 0.219$, $P = 0.001$). Taken together, B7-H3 and B3GALT4 could serve as a novel, reliable prognostic marker for CRC. Furthermore, we detected that the expression of B3GALT4 was positively altered following B7-H3 expression *in vitro*.

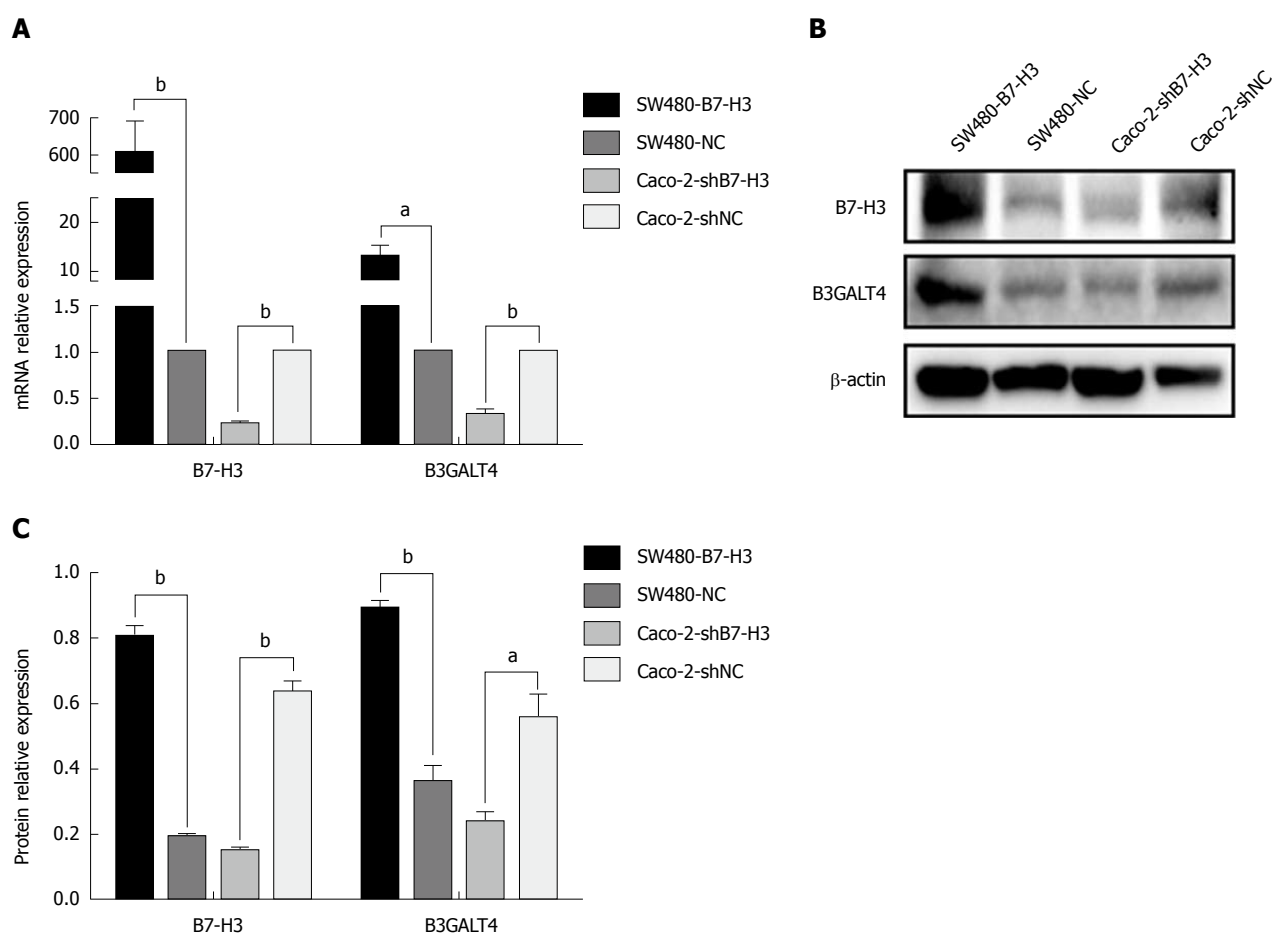


Figure 3 The expression of B7 homolog 3 and β-1,3-galactosyltransferase-4 in colorectal cancer cell lines. A: Real-time quantitative polymerase chain reaction results of relative mRNA expression; B: Western blotting results for protein expression; C: Statistical analysis of protein expression ($^aP < 0.05$, $^bP < 0.01$).

Combined with the results of clinical samples, this study indicated that B7-H3 could positively regulate B3GALT4 at the mRNA as well as the protein level. However, the underlying mechanism of regulation of B7-H3 and B3GALT4 in CRC warrants further investigation.

In summary, the present study for the first time revealed the expression of B3GALT4 in CRC and its correlation with B7-H3 *in vitro*. Overall, the findings of the present study suggested that B7-H3 and B3GALT4 are novel prognostic biomarkers for CRC and highlight the significance of both B7-H3 and B3GALT4 as promising therapeutic targets for CRC. Thus, here we present our preliminary work on the relationship of the immune function and glycosylation of tumor-associated protein in CRC.

ARTICLE HIGHLIGHTS

Research background

Colorectal cancer (CRC) is the most prevalent gastrointestinal tract malignancy worldwide. The prognosis of CRC patients remains relatively poor. B7 homolog 3 (B7-H3) mRNA is widely expressed on many tissues and cell types. However, B7-H3 protein is not constitutively expressed on T-cells, natural killer cells and antigen-presenting cells. The β-1,3-galactosyltransferase-4 (B3GALT4), which belongs to β-1,3-galactosyltransferase (β3GalT) gene family is abundantly expressed in human organs and tissues, predominantly in brain and involved in

GM1/GD1 ganglioside synthesis. The β3GalT family may be closely related to the tumor.

Research motivation

There are insufficient reports about the correlation between B3GALT4 and CRC.

Research objectives

The aim of the present study is to investigate the clinical correlation of B7-H3 and B3GALT4 with CRC, and the correlation between the expression of B7-H3 and B3GALT4 was evaluated to determine their prognostic significance in CRC.

Research methods

The authors identified the expression of B7-H3 and B3GALT4 in 223 CRC patient samples by immunohistochemistry and evaluated the possible correlation between B7-H3 and B3GALT4 and clinical outcomes. The mRNA and protein expression were also identified to establish the regulatory relationship of B7-H3 with B3GALT4 *in vitro*.

Research results

A significant positive correlation between B7-H3 and B3GALT4 was observed in CRC specimens. High expression of B7-H3 was identified as a significant independent predictor of poor overall survival (OS). High expression of B3GALT4 was also recognized as an independent predictor of inferior OS. In CRC cell lines with the stable expression of high B7-H3, the mRNA and protein expressions of B3GALT4 were significantly upregulated. The expression of B3GALT4 was significantly reduced when expression of B7-H3 was knocked down.

Research conclusions

The expression of B3GALT4 in CRC and its positive correlation with B7-H3 *in vitro* was revealed, as well as B7-H3/B3GALT4 as dual prognostic biomarkers for CRC.

Research perspectives

The present study suggested that B7-H3 and B3GALT4 are novel prognostic biomarkers for CRC, and the significance of both B7-H3 and B3GALT4 as promising therapeutic targets for CRC are highlighted.

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