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The Prognostic Roles of PYCR2 and ZBTB18 Expression in Tissues of Colorectal Carcinoma and Non-Neoplastic Tissues: An Immunohistochemical Study

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Abstract

Background It is important to detect novel biomarkers responsible for the progression and spread of colorectal cancer (CRC) to better evaluate the prognosis of the patients, provide better management, and foster the development of therapeutic targets. In humans, pyrroline-5-carboxylate reductase 2 (PYCR2) is encoded on chromosome *1q42.12*, and its metabolic activity has been linked to oncogenesis in many cancers. Zinc finger and broad-complex, tramtrack, and bric-à-brac (BTB) domain-containing protein 18 (ZBTB18), a zinc finger transcriptional repressor, has been found to have a tumor-suppressor role and to be methylated in CRCs. To date, the prognostic roles of PYCR2 and ZBTB18 in CRC patients have not been thoroughly studied.

Objective To evaluate the tissue protein expression of PYCR2 and ZBTB18 in CRC and adjacent non-neoplastic intestinal tissues, to detect their roles in CRC carcinogenesis, progression and metastases.

Patients and methods After applying the inclusion criteria, 60 CRC patients were included in the study. Tissue samples from the tumor and the adjacent non-neoplastic tissues were stained with PYCR2 and ZBTB18. The patients were followed up for about 30 months (range: 10 to 36 months). We performed a correlation regarding the expression of the markers, and clinicopathological and prognostic parameters.

Keywords
- colorectal cancer
- PYCR2
- ZBTB18
- immunohisto chemistry
- prognosis

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Results Upregulation of PYCR2 and downregulation of ZBTB18 were found to be higher in CRC tissue than in the adjacent non-neoplastic colonic mucosa ($p = 0.026$ and $p < 0.001$ respectively). High expression of PYCR2 and low expression of ZBTB18 were positively correlated with large tumor size, higher tumor grade, advanced tumor stage, presence of spread to lymph nodes, and presence of distant metastases ($p < 0.001$). High PYCR2 and low ZBTB18 expressions were significantly associated with poor response to therapy ($p = 0.008$ and 0.0.17 respectively), as well as high incidence of progression and recurrence ($p = 0.005$), and unfavorable overall survival (OS) rates ($p = 0.001$).

Conclusion High expression of PYCR2 and low expression of ZBTB18 were independent predictors of CRC, progression, poor prognosis and unfavorable patient OS and progression-free survival (PFS) rates.

Introduction

Colorectal cancer (CRC) is considered the third most common cancer and the second most common cause of cancer-related death worldwide. The prognosis of CRC markedly depends on its stage at the initial diagnosis.[1,2]

Distant metastases and cancer progression are the most important causes of death in CRC patients.[3] It is critical to detect novel biomarkers responsible for the progression and spread of CRC to better evaluate the prognosis of the patients, provide better management, and foster the development of therapeutic targets.

In humans, pyrroline-5-carboxylate reductase 2 (PYCR2) is encoded on chromosome 1q42.12, considered a key housekeeping protein which consumes nicotinamide adenine dinucleotide phosphate (NAD[P]H as a coenzyme for the production of proline.[4] The metabolic activity of PYCR2 has been linked to oncogenesis in many cancers;[5] it is a regulator of amino acid metabolism in hepatocellular carcinoma that has a prognostic role.[6] However, its role in CRC prognosis and metastases needed to be evaluated.[7]

Moreover, CRC progression and the development of metastases are multistep processes which have been observed to result from the accumulation of genetic and epigenetic defects which lead to the transformation of normal colonic epithelial cells into metastatic CRC.[8] Histone modifications in CRC, such as acetylation and methylation, could alter gene expression, which drives the oncogenesis of colon cancer. There are many epigenetic events responsible for CRC progression and spread that have been insufficiently studied.[9] Zinc finger and broad-complex, tramtrack, and bric-à-brac (BTB) domain-containing protein 18 (ZBTB18), a zinc finger transcriptional repressor, has been found to have a tumor-suppressor role and to be methylated in CRCs.[10] To date, a comprehensive assessment of the prognostic roles of PYCR2 and ZBTB18 in CRC patients has not been sufficiently studied.

In the current study, we aim to evaluate the expression of the PYCR2 and ZBTB18 proteins in CRC and adjacent non-neoplastic intestinal tissues to detect their roles in the carcinogenesis, progression, and metastases of conventional colorectal adenocarcinoma.

Patients and Methods

The present study was performed in the Faculty of Medicine, Zagazig University Hospitals, and Benha University, where we collected samples from 60 CRC patients in between January 2017 and May 2020. The colonoscopic diagnosis of CRC and biopsies were performed and collected from the Gastrointestinal (GI) Endoscopy Unit of the Internal Medicine and Tropical Medicine Departments. The samples were delivered to the Pathology Department, where processing and initial diagnosis were performed, and the operable patients were admitted to the General Surgery Department, where surgical excision was performed according to the site of the cancer. Surgical samples were delivered to the Pathology Department, where processing, diagnosis, grading and staging were performed. The patients were sent to the Medical Oncology, Clinical Oncology, and Nuclear Medicine Departments for to undergo the chemotherapy and/or radiotherapy treatment protocols according to the stage.

Treatment protocols: postoperative systemic adjuvant chemotherapy is traditionally performed for pathological stage (pStage) III and high-risk pStage II colon cancer at our institute. In the present study, 5-fluorouracil-based regimens were commonly used in pStage-II patients, while oxaliplatin-based and 5-fluorouracil-based regimens were most commonly used in pStage-III patients.

Early rectal cancer patients received neoadjuvant concurrent chemoradiotherapy (long-course radiation therapy at doses ranging from 45 to 50 Gray (Gy) in 25 to 28 fractions to the pelvis plus capecitabine) and, subsequently, postoperative chemotherapy. Metastatic CRC patients received chemotherapy protocols in the form of 5-fluorouracil/leucovorin combined with oxaliplatin (FOLFOX) or 5-fluorouracil/leucovorin combined with irinotecan (FOLFIRI).

Inclusion Criteria

Patients with complete clinical data, diagnosed with conventional adenocarcinoma, who accepted to participate were included in the study.

Exclusion Criteria

Patients with insufficient data, inoperable patients, and patients diagnosed with other histopathological subtypes of CRC were excluded.

After the application of the inclusion criteria, 60 patients were included in the study. We collected tissue samples from tumor and adjacent non-neoplastic tissues, the distance between the tumor and the adjacent non-neoplastic tissues retrieved was after a surgical safety, margins from the malignant tissues about 5 cm to avoid expression of various proteins related to CRC.[7] Written informed consent were obtained from all included patients after approval by the Ethical Committee of the institutional Review Board of Faculty of Medicine, Zagazig University. The pathological staging of CRC patients was performed according to the guidelines of The American Joint Committee on Cancer (AJCC), 7th edition.[11] Patients were followed up for about 30 months (range: 10 to 36 months) for the detection of recurrence, distant metastasis, and to establish the overall survival (OS). Most patients were regularly followed up through clinical examinations, and laboratory and radiological investigations in the outpatient clinic. The follow–up period ended in May 2020.

Immunohistochemical Staining of PYCR2 and ZBTB18

Sections containing tumor tissue and adjacent non-neoplastic tissues of the study sample were taken from paraffin blocks.

Table 1 Clinicopathological features, immunohistochemical markers, and outcomes of 60 patients with colorectal carcinoma

Characteristics	All patients (N = 60)		Characteristics	All patients (N = 60)	
	N	%		N	%
Gender			Distant metastasis		
Male	36	60%	Absent	43	71.7%
Female	24	40%	Present	17	28.3%
Age (years)			Duke stage		
Mean ± standard deviation	58.33	± 12.27	Stage A	14	23.3%
Median (range)	60	(29-80)	Stage B	12	18.3%
Initial site			Stage C	17	30%
Ascending	19	31.7%	Stage D	17	28.3%
Transverse	6	10%	American Joint Committee on Cancer (AJCC) stage		
Descending	5	8.3%	Stage I	14	23.3%
Rectosigmoid	30	50%	Stage II	12	20%
Size			Stage III	17	28.3%
≤ 5 cm	27	45%	Stage IV	17	28.3%
> 5 cm	33	55%	Follow-up (months)		
Pathological type			Mean ± standard deviation	23.11	± 9.98
Conventional	52	86.7%	Median (range)	25	(8-35)
Mucoid	8	13.3%	Relepase	(N = 42)	
Grade			Absent	28	66.7%
			Present	14	33.3%
Grade I	13	21.7%	Death		
Grade II	31	51.7%	Alive	40	66.7%
Grade III	16	26.7%	Died	20	33.3%
T					
T1	9	15%			
T2	14	23.3%			
T3	12	20%			
T4	25	41.7%			
Lymph node metastasis					
Node negative	26	43.3%			
Node positive	34	56.7%			

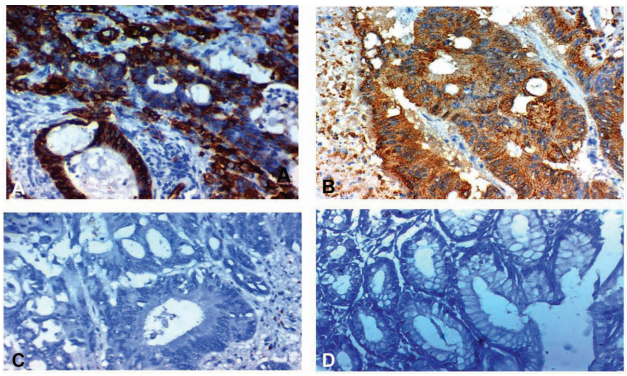

Fig. 1 Cytoplasmic expression of pyrroline-5-carboxylate reductase 2 (PYCR2) in tissues of colorectal carcinoma (CRC) and adjacent non-neoplastic tissues (**A**); high PYCR2 expression in grade-III, stage-III CRC (magnification: x400) (**B**); high PYCR2 expression in grade-II, stage-III CRC (magnification: x400) (**C**) negative PYCR2 expression in grade-I, stage-I CRC (magnification: x400) (**D**); negative PYCR2 expression in non-neoplastic mucosa of the colon (magnification: x400).

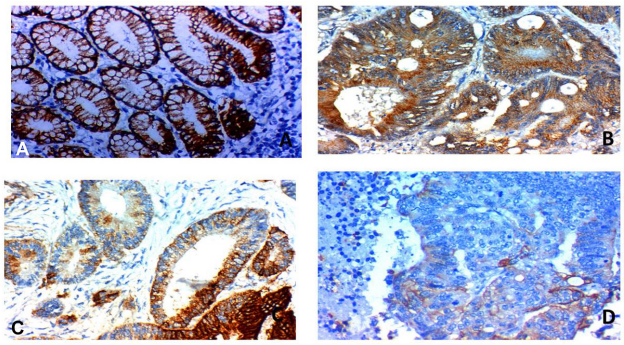

Fig. 2 Cytoplasmic expression of zinc finger and broad-complex, tramtrack, and bric-à-brac (BTB) domain-containing protein 18 (ZBTB18) in in CRC and adjacent non-neoplastic tissues (**A**); high ZBTB18 expression in non-neoplastic colonic mucosa (magnification: x400) (**B**); high ZBTB18 expression in grade-I, stage-I CRC (magnification: x400) (**C**); low ZBTB18 expression in grade-II, stage-II CRC (magnification: x400) (**D**); negative ZBTB18 expression in grade-III, stage-III CRC (magnification: x400).

The sections were deparaffinized in xylene, rehydrated using different ethanol concentrations, and then incubated in 3% hydrogen peroxide for about 10 minutes to block and antagonize the activity of endogenous peroxidase. Heating slides in citrate buffer for about five minutes is needed for antigen retrieval. The tissue slides were incubated with primary monoclonal anti- PYCR2 antibody (1:1000, Proteintech, Rosemont, IL, US) and primary rabbit polyclonal anti-ZBTB18 antibody (1:1000, Biorbyt, Cambridge, UK; cat# orb357631) overnight at 4 °C. Then, the slides were incubated with anti-rabbit immunoglobulin antibody. The tissues were counterstained with hematoxylin.

Evaluation of PYCR2 and ZBTB18 Expression in Stained Tissues

The extent and intensity of the staining were assessed by two pathologists who were blinded to the patients' clinicopathological data.

We evaluated 5 representative fields of each section; then, we assessed the extent of the staining as follows: 0 if $< 5\%$, 1 if $< 30\%$, 2 if between 30% and 70%, and 3 if $> 70\%$. We assessed the intensity of staining as follows: no stain, 0; weak stain, 1; intermediate stain, 2; and strong stain, 3. The extent and intensity scores were multiplied to obtain the final staining scores, which ranged from 0 to 9. To facilitate the statistical analysis, we divided the scores into low staining (0 to 3) and high staining (4 to 9).[12]

Statistical Analysis

For the statistical analyses, we used the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, US), version 23.0. Chi-squared tests were performed to evaluate the associations among PYCR2 and ZBTB18 expression, clinicopathological characteristics, and follow-up data, while the association between the OS and progression-free survival (PFS) rates were assessed through Cox regression analyses. Values of $p < 0.05$ were considered statistically significant.

Results

Patient Data

We evaluated 120 samples from 60 patients with CRC; 60 samples were retrieved from CRC tissue, and 60 samples, from the adjacent non-neoplastic colonic mucosa (►**Table 1**).

Immunohistochemical Results

The expression of PYCR2 and its association with clinicopathological data of CRC patients are shown in ►**Figure 1**, and ►**Tables 1** and **2**. Expression of PYCR2 was observed in 31

Table 2 Correlations between PYCR2 and ZBTB18 expression and histopathological diagnosis

		Colorectal carcinoma N = 60	Non-neoplastic mucosa N = 60	Total N = 120	*p*-value
PYCR2	Low	29 (48.0%)	55 (92.0%)	84 (70%)	0.026
	High	31 (52.0%)	5 (8.0%)	36 (30%)	
ZBTB18	Low	29 (48.0%)	0 (0.0%)	29 (19%)	< **0.001**
	High	31 (52.0%)	60 (100.0%)	91 (81%)	

Abbreviations: PYCR2, pyrroline-5-carboxylate reductase 2; ZBTB18, zinc finger and broad-complex, tramtrack, and bric-à-brac (BTB) domain-containing protein 18.

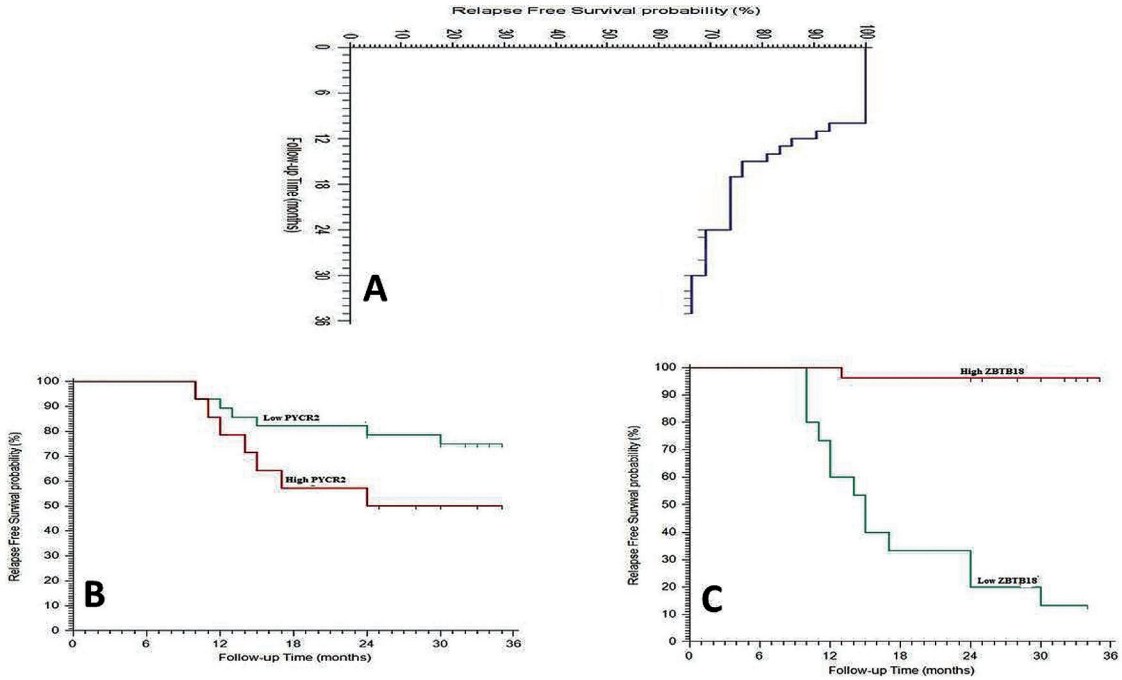

Fig. 3 Kaplan Meir survival curves of the recurrence-free survival (RFS) rate of CRC patients (**A**); RFS rate of all CRC cases (**B**); RFS rate of cases stratified according to PYCR2 expression (**C**); RFS rate of cases stratified according to ZBTB18 expression.

(52.0%) samples of CRC tissue and in 5 (8.0%) samples of non-neoplastic colon mucosa, and it was more upregulated in CRC tissue than in the adjacent non-neoplastic colonic mucosa ($p = 0.026$). Significant expression of PYCR2 was more often observed in cases of cancer in the ascending region than other sites ($p = 0.034$), and PYCR2 expression in CRC was positively correlated with large tumor size, higher grade, advanced tumor stage, and presence of spread to the lymph nodes ($p < 0.001$).

The expression of ZBTB18 and its association with clinicopathological features of CRC patients are shown in ►**Figure 2**, and ►**Tables 1** and **2**. Expression of ZBTB18 was observed in 31 (52.0%) samples of CRC tissue and in 60 (100.0%) samples of non-neoplastic colon mucosa, and it was

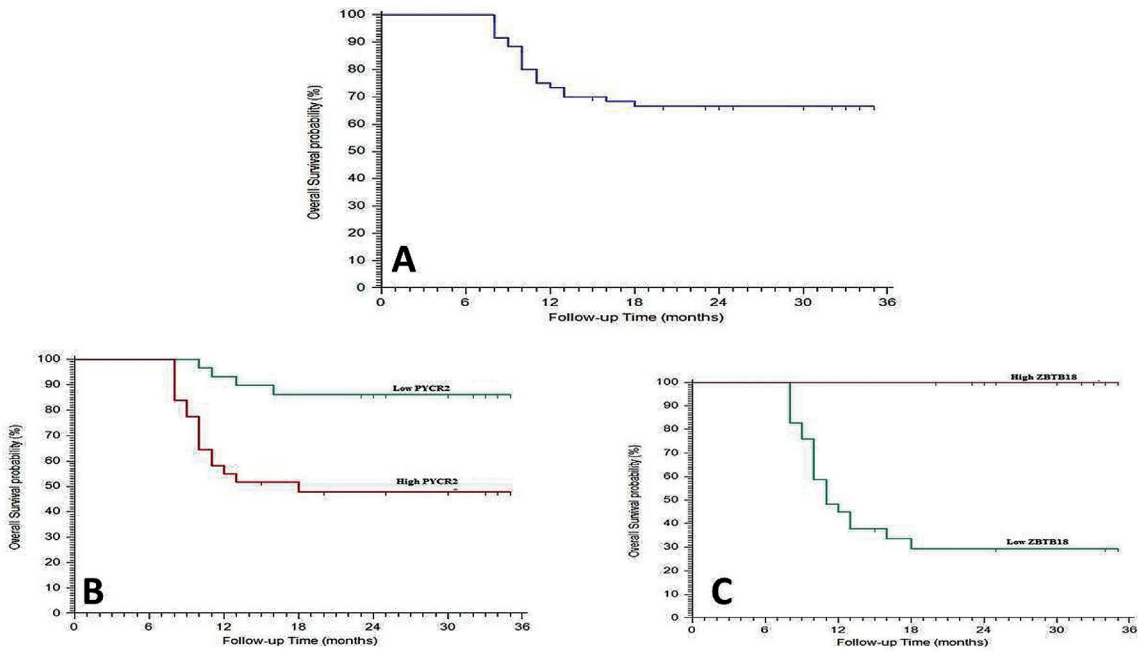

Fig. 4 Kaplan Meir survival curves of the overall survival (OS) rate of CRC patients (**A**); OS rate of all CRC cases (**B**); OS rate of cases stratified according to PYCR2 expression (**C**); OS rate of cases stratified according to ZBTB18 expression.

Table 3 Relationships regarding PYCR2, ZBTB18, immunohistochemistry, and clinicopathological parameters in 60 patients with colorectal carcinoma

	All patients (N = 60)		PYCR2					*p*-value	ZBTB18					*p*-value
			Low (N = 29)			High (N = 31)			Low (N = 29)			High (N = 31)		
	N	(%)	N	(%)		N	(%)		N	(%)		N	(%)	
Gender														
Male	36	(60%)	18	(50%)		18	(50%)	0.752‡	21	(58.3%)		15	(41.7%)	0.058‡
Female	24	(40%)	11	(45.8%)		13	(54.2%)		8	(33.3%)		16	(66.7%)	
Age (years)														
Mean ± standard deviation	58.33	± 12.27	57.10	± 11.66		59.48	± 12.90	0.378•	59.13	± 13.90		57.58	± 10.70	0.627*
Median (range)	60	(29-80)	55	(30-80)		62	(29-75)		60	(29-80)		55	(30-75)	
Initial site														
Ascending	19	(31.7%)	4	(21.1%)		15	(78.9%)	0.034‡	13	(68.4%)		6	(31.6%)	0.017‡
Transverse	6	(10%)	3	(50%)		3	(50%)		5	(83.3%)		1	(16.7%)	
Descending	5	(8.3%)	3	(60%)		2	(40%)		2	(40%)		3	(60%)	
Rectosigmoid	30	(50%)	19	(63.3%)		11	(36.7%)		9	(30%)		21	(70%)	
Size														
≤ 5 cm	27	(45%)	22	(81.5%)		5	(18.5%)	< 0.001‡	5	(18.5%)		22	(81.5%)	< 0.001‡
> 5 cm	33	(55%)	7	(21.2%)		26	(78.8%)		24	(72.7%)		9	(27.3%)	
Pathological type														
Conventional	52	(86.7%)	28	(53.8%)		24	(46.2%)	0.053‡	22	(42.3%)		30	(57.7%)	0.024‡
Mucoid	8	(13.3%)	1	(12.5%)		7	(87.5%)		7	(87.5%)		1	(12.5%)	
Grade														
Grade I	13	(21.7%)	13	(100%)		0	(0%)	< 0.001§	2	(15.4%)		11	(84.6%)	< 0.001§
Grade II	31	(51.7%)	14	(45.2%)		17	(54.8%)		14	(45.2%)		17	(54.8%)	
Grade III	16	(26.7%)	2	(12.5%)		14	(87.5%)		13	(81.3%)		3	(18.8%)	
T														
T1	9	(15%)	9	(100%)		0	(0%)	< 0.001§	0	(0%)		9	(100%)	< 0.001§
T2	14	(23.3%)	10	(71.4%)		4	(28.6%)		4	(28.6%)		10	(71.4%)	
T3	12	(20%)	4	(33.3%)		8	(66.7%)		6	(50%)		6	(50%)	
T4	25	(41.7%)	6	(24%)		19	(76%)		19	(76%)		6	(24%)	
Lymph node metastasis														
Node negative	26	(43.3%)	22	(84.6%)		4	(15.4%)	< 0.001‡	5	(19.2%)		21	(80.8%)	< 0.001‡
Node positive	34	(56.7%)	7	(20.6%)		27	(79.4%)		24	(70.6%)		10	(29.4%)	
Distant metastasis														
Absent	43	(71.7%)	27	(62.8%)		16	(37.2%)	< 0.001‡	15	(34.9%)		28	(65.1%)	0.001‡
Present	17	(28.3%)	2	(11.8%)		15	(88.2%)		14	(82.4%)		3	(17.6%)	
Duke stage														
Stage A	14	(23.3%)	14	(100%)		0	(0%)	< 0.001§	2	(14.3%)		12	(85.7%)	< 0.001§
Stage B	12	(18.3%)	7	(63.6%)		5	(36.4%)		4	(27.3%)		8	(72.7%)	
Stage C	18	(30%)	6	(33.3%)		12	(66.7%)		10	(55.6%)		8	(44.4%)	
Stage D	17	(28.3%)	2	(11.8%)		15	(88.2%)		14	(82.4%)		3	(17.6%)	
AJCC stage														
Stage I	14	(23.3%)	14	(100%)		0	(0%)	< 0.001§	2	(14.3%)		12	(85.7%)	< 0.001§
Stage II	12	(20%)	8	(66.7%)		4	(33.3%)		3	(25%)		9	(75%)	
Stage III	17	(28.3%)	5	(29.4%)		12	(70.6%)		10	(58.8%)		7	(41.2%)	
Stage IV	17	(28.3%)	2	(11.8%)		15	(88.2%)		14	(82.4%)		3	(17.6%)	
PYCR2														
Low	29	(48.3%)							8	(27.6%)		21	(72.4%)	0.002‡
High	31	(51.7%)							21	(67.7%)		10	(32.3%)	
ZBTB18														
Low	29	(48.3%)	8	(27.6%)		21	(72.4%)	0.002‡						

Table 3 (*Continued*)

	All patients (N = 60)		PYCR2				p-value	ZBTB18				p-value
			Low (N = 29)		High (N = 31)			Low (N = 29)		High (N = 31)		
	N	(%)	N	(%)	N	(%)		N	(%)	N	(%)	
High	31	(51.7%)	21	(67.7%)	10	(32.3%)						

Abbreviations: AJCC, American Joint Committee on Cancer; PYCR2, pyrroline-5-carboxylate reductase 2; ZBTB18, zinc finger and broad-complex, tramtrack, and bric-à-brac (BTB) domain-containing protein 18.
Notes: * Independent samples Student t-test; • Mann Whitney U test; ‡ Chi-squared test; § Chi-squared test for trend; values of $p < 0.05$ are statistically significant.

Table 4 Relationships regarding the immunohistochemistry of PYCR2 and ZBTB18 and outcome of treatment of 60 patients with colorectal carcinoma

	All patients		PYCR2				p-value	ZBTB18				p-value
			Low		High			Low		High		
	N	(%)	N	(%)	N	(%)		N	(%)	N	(%)	
Response	(N = 22)		(N = 1)		(N = 21)			(N = 16)		(N = 6)		
PD	8	(36.4%)	1	(100%)	7	(33.3%)	0.008‡	6	(37.5%)	2	(33.3%)	0.017‡
SD	6	(27.3%)	0	(0%)	6	(28.6%)		6	(37.5%)	0	(0%)	
PR	3	(13.6%)	0	(0%)	3	(14.3%)		2	(12.5%)	1	(16.7%)	
CR	5	(22.7%)	0	(0%)	5	(23.8%)		2	(12.5%)	3	(50%)	
Relapse	(N = 42)		(N = 28)		(N = 14)			(N = 15)		(N = 27)		
Absent	28	(66.7%)	21	(75%)	7	(50%)	0.005‡	2	(13.3%)	26	(96.3%)	< 0.001‡
Present	14	(33.3%)	7	(25%)	7	(50%)		13	(86.7%)	1	(3.7%)	
Relapse-free survival	(N = 42)		(N = 28)		(N = 14)			(N = 15)		(N = 27)		
Mean (95%confidence interval)	28.49 (25.52–31.46) months		30.31 (27.03–33.59) months		24.86 (19.30–30.41) months		0.002†	18.13 (13.85–22.42) months		34.19 (32.62–35.75) months		< 0.001†
12 months	85.7%		89.3%		78.6%			60%		100%		
24 months	69.1%		78.6%		50%			20%		96.3%		
30 months	66.4%		74.8%		50%			13.3%		96.3%		
Death	(N = 60)		(N = 29)		(N = 31)			(N = 29)		(N = 31)		
Alive	40	(66.7%)	25	(86.2%)	15	(48.4%)	0.002‡	9	(31%)	31	(100%)	< 0.001‡
Died	20	(33.3%)	4	(13.8%)	16	(51.6%)		20	(69%)	0	(0%)	
Overall survival	(N = 60)		(N = 29)		(N = 31)			(N = 29)		(N = 31)		
Mean (95% confidence interval)	26.85 (23.92–29.79) months		31.90 (29.06–34.74) months		22.12 (17.69–26.54) months		0.001†	17.93 (13.75–22.11) months		35 months		< 0.001†
12 months	73.3%		93.1%		54.8%			44.8%		100%		
24 months	66.5%		86.2%		47.9%			29.5%		100%		
30 months	66.5%		86.2%		47.9%			29.5%		100%		

Abbreviations: PYCR2, pyrroline-5-carboxylate reductase 2; ZBTB18, zinc finger and broad-complex, tramtrack, and bric-à-brac (BTB) domain-containing protein 18.
Notes: ‡ Chi-squared test; † Log-rank test; values of $p < 0.05$ are statistically significant.

more downregulated in CRC tissue than in the adjacent non-neoplastic colonic mucosa ($p < 0.001$). Significant expression of ZBTB18 was more often observed in cancer of the recto-sigmoid region than other sites ($p = 0.017$), and ZBTB18 expression in CRC was associated with smaller size tumor size, lower grade, initial stage of the tumor, absence of spread to the lymph nodes and absence of distant metastases ($p < 0.001$).

No significant correlation was observed regarding the expressions of PYCR2 and ZBTB18 and age or gender (►**Table 2**; ►**Fig. 1**). Follow-up and survival data are shown in ►**Figures 3** and **4**, and ►**Tables 3**–**5**.

High PYCR2 and low ZBTB18 expressions were significantly associated with poor response to therapy ($p = 0.008$ and 0.0.17 respectively), increased incidence of progression and

Table 5 Multivariant analysis of overall and disease-free survival in relation to some studied parameters

Variables		3-year disease-free survival rate (%)	*p*-value	3-year overall survival rate (%)	*p*-value
Age group (years)	< 60	68%	**0.568**	80.8%	**0.120**
	> 60	58.3%		62.2%	
Gender	Male	57.4%	**0.287**	65.6%	**0.180**
	Female	78.6%		83.3%	
Tumor size	< 5 cm	87.8%	< **0.001**	100%	< **0.001**
	> 5 cm	26.7%		43.3%	
Tumor grade	I	90.9%	**0.109**	100%	**0.021**
	II	55%		69%	
	III	50%		50%	
Duke stage	A	91.7%	< **0.001**	100%	< **0.001**
	B	81.8%		100%	
	C	37.5%		58.8%	
	D	0.0%		30.0%	
Lymph node metastasis	No	87.5%	< **0.001**	100%	< **0.001**
	Yes	31.3%		45.6%	
Distant metastasis	No	66.3%	< **0.001**	82.5%	< **0.001**
	Yes	0.0%		30%	
PYCR2	Low	66.3%	< **0.001**	82.5%	< **0.001**
	High	0.0%		30%	
ZBTB18	Low	66.3%	< **0.001**	82.5%	< **0.001**
	High	0.0%		30%	
AJCC stage	Stage I	91.7%	< **0.001**	100%	< **0.001**
	Stage II	83.3%		100%	
	Stage III	33.3%		56.2%	
	Stage IV	0.0%		30.0%	

Abbreviations: AJCC, American Joint Committee on Cancer; PYCR2, pyrroline-5-carboxylate reductase 2; ZBTB18, zinc finger and broad-complex, tramtrack, and bric-à-brac (BTB) domain-containing protein 18.

relapse recurrence ($p = 0.005$), and unfavorable OS rate ($p = 0.001$). There was an inverse association between the expressions of PYCR2 and ZBTB18 ($r = -0.521; p = 0.002$).

In the univariate analyses, high PYCR2 expression, reduced ZBTB18 expression, depth of tumor invasion, lymphatic metastasis, distant metastasis, and tumor, node, metastasis (TNM) stage were significantly related to the OS and recurrence-free survival (RFS) rates. The multivariate analysis revealed that PYCR2 expression ($p < 0.001$), ZBTB18 ($p = 0.001$), distant metastasis ($p < 0.001$), and TNM stage ($p < 0.001$) were independent predictors of the OS rate, and that PYCR2 expression, ZBTB18 expression, and distant metastasis ($p < 0.001$) were predictors of the RFS rate.

Discussion

The detection of predictive and prognostic biomarkers and novel targeting therapies for CRC is important to improve its prognosis.[13]

Proline metabolism and its resulting products are important for many biological processes which occur in normal and oncogenic processes that lead to the development and progression of many cancers.[14–16] A proline family member, PYCR2 has been studied, and was found to have many roles in the progression of cancers of various organs, but its role in the oncogenesis and progression of CRC has not been sufficiently studied.

In the current study, we assessed the expression of PYCR2 in malignant tissues and in the adjacent non-neoplastic colonic mucosa, and found that PYCR2 expression was more upregulated in malignant tissues than normal tissues, which indicates its role in CRC oncogenesis. Additionally, the tissue protein expression of PYCR2 was upregulated in high-grade and advanced-stage CRCs, which indicates its role in cancer progression and in the development of metastases. Furthermore, we showed that increased PYCR2 expression was associated with shortened survival and poor outcome of CRC patients.

Our results were in line with those of Yin et al.,[13] who were the first to investigate the genetic roles of PYCR2 in CRC oncogenesis, progression and shortened OS. Additionally, our results are similar to those of Wang et al.,[7] who assessed the genetic and tissue protein expression of PYCR2 in CRC and other tumor types, and found that levels of PYCR2 are upregulated in CRC tumor tissues at the mRNA and protein levels. PYCR2 was found to have many roles in CRC progression, by controlling apoptosis in cancer cells and mutations in the Wnt/b-catenin and Notch signaling pathway pathways, which are involved in CRC oncogenesis.[7,17] Mutations in the Wnt pathway result in CRC stem cell expansion, in addition to stimulation of the epithelial mesenchymal transition process, which are responsible for metastases and progression of the CRC.[18] Abnormal PYCR2 expression has been associated with abnormalities in the Jagged1/Notch signaling pathway, which have been correlated with CRC development and progression.[19,20]

PYCR2 has been found to be correlated with MYC levels in CRC, and MYC knockdown leads to a reduction in the growth, progression and metastasis of CRC.[21,22] Yin et al.,[13] demonstrated the roles of PYCR2 in apoptosis in CRC as its silencing could induce cell apoptosis by increasing the levels of proapoptotic factors and decreasing the levels of antiapoptotic factors. Another role of PYCR2 has been studied in CRC metastases, by the activation of matrix metalloproteinases (MMPs), which mediate CRC cell invasion and migration through degradation of the extracellular matrix (ECM).[13,23]

All of the aforementioned results and those of current study show that the upregulation of PYCR2 could lead to CRC progression and metastases through several mechanisms that need to be clarified. To clarify role of PYCR2 in CRC, we assessed the levels of another biomarker, ZBTB18, in sections from CRC and adjacent non-neoplastic tissues of the colonic mucosa. To our knowledge, there are few studies assessing its roles in CRC carcinogenesis, and the current study is the first to assess the tissue protein expression of PYCR2 and ZBTB18 in CRC.

The expression of ZBTB18 was high in the non-neoplastic colonic mucosa, and it is downregulated in malignant tissues; additionally, decreased ZBTB18 expression was associated with increased tumor invasion and metastases. We assessed the association of ZBTB18 with patient survival, and found that intense expression was associated with favorable outcomes and longer survival. Our results highlighted the possible tumor-suppressor role of ZBTB18 in CRC patients. Our results were similar to those of Sarah Bazzocco et al.,[10] who were the first to investigate values of tissue protein expression of ZBTB18 in CRC tumorigenesis, and declared that some zinc finger proteins participated in it. ZBTB18 has been found to play an essential role in supporting normal myogenesis[24] and normal brain development,[25] but its role in the normal development of the intestinal epithelium and CRC carcinogenesis have not been sufficiently clarified. The expression of ZBTB18 reduces the proliferation of brain tumor cells and promotes the apoptosis of glioblastoma multiforme (GBM) and medulloblastoma (MB).[26] Additionally, the downregulation of ZBTB18 has been associated with aggressive phenotype, unfavorable survival, and poor prognosis of GBM patients.[26] These results are in line with our results regarding CRC. Previous reports on the roles of ZBTB18 expression in CRC were in line with our results: the downregulation of ZBTB18 in CRC cells is associated with increased growth, proliferation, and invasion, which, in turn are associated with poor prognosis.

Collectively, our results and those of previous studies showed that ZBTB18 is a tumor-suppressor gene that could be used in a novel targeted therapy against CRC. Therefore, strategies aimed at rescuing ZBTB18 expression or downregulating some of the downstream ZBTB18 target genes may offer therapeutic potential in CRC patients.[10,27] In addition, the present study showed that reduced ZBTB18 expression in the tumors was associated with shorter patient survival, which was similar to the results of the study by Bazzocco et al.,[10] who showed that ZBTB18 expression was reduced in the presence of lymph-node metastases in comparison to primary CRC tumors. These findings confirmed the tumor-suppressor role of ZBTB18 in CRC tissues that could detect a subset of CRC patients with poor prognosis who will benefit from the aggressive targeted therapy.

Conclusion

The present is the first study in the expression of PYCR2 and ZBTB18 in CRC tissues and non-neoplastic tissues. We observed an inverse association between the expression of PYCR2 and ZBTB18 in the tissues of CRC and non-neoplastic colonic mucosa: PYCR2 has an oncogenic role, and ZBTB18, an oncosupressor role. High expression of PYCR2 and low expression of ZBTB18 were independent predictors of CRC, progression, poor prognosis, and unfavorable OS and PFS rates.

Recommendation

Further genetic studies including larger samples from multicenter institutions and longer follow-up with genetic evaluation of both PYCR2 and ZBTB18 levels in CRC are needed to assess the roles of PYCR2 and ZBTB18 in controlling CRC proliferation, migration, invasion, and apoptosis, which will help in the establishment of a targeted therapy to improve prognosis.

The limitations of the present study are: the small sample and the fact that we only used immunohistochemistry to evaluate the tissue protein expression of PYCR2 and ZBTB18.

Conflict of Interests

The authors have no conflict of interests to declare.

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