

# World Journal of *Gastroenterology*

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**ABOUT COVER**

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## Retrospective Study

# Insulin receptor substrate 1 may play divergent roles in human colorectal cancer development and progression

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### Institutional review board

**statement:** This study was reviewed and approved by the Ethics Committee of the Medical University of Bialystok, Poland.

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## Abstract

### BACKGROUND

Despite effective prevention and screening methods, the incidence and mortality rates associated with colorectal cancer (CRC) are still high. Insulin receptor substrate 1 (IRS-1), a signaling molecule involved in cell proliferation, survival and metabolic responses has been implicated in carcinogenic processes in various cellular and animal models. However, the role of IRS-1 in CRC biology and its value as a clinical CRC biomarker has not been well defined.

### AIM

To evaluate if and how IRS-1 expression and its associations with the apoptotic and proliferation tumor markers, Bax, Bcl-xL and Ki-67 are related to clinicopathological features in human CRC.

### METHODS

The expression of IRS-1, Bax, Bcl-xL and Ki-67 proteins was assessed in tissue samples obtained from 127 patients with primary CRC using immunohistochemical methods. The assays were performed using specific antibodies against IRS-1, Bax, Bcl-xL, Ki-67. The associations between the expression of IRS-1, Bax, Bcl-xL, Ki-67 were analyzed in relation to clinicopathological parameters, *i.e.*, patient age, sex, primary localization of tumor, histopathological type, grading, staging and lymph node spread. Correlations



**Informed consent statement:**

Patients were not required to give informed consent to the study because the analysis used anonymous data that were obtained after each patient agreed to treatment by written consent.

**Conflict-of-interest statement:**

All the Authors have no conflict of interest related to the manuscript.

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No additional data are available.

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between variables were examined by Spearman rank correlation test and Fisher exact test with a level of significance at  $P < 0.05$ .

**RESULTS**

Immunohistochemical analysis of 127 CRC tissue samples revealed weak cytoplasmatic staining for IRS-1 in 66 CRC sections and strong cytoplasmatic staining in 61 cases. IRS-1 expression at any level in primary CRC was associated with tumor grade (69% in moderately differentiated tumors, G2 *vs* 31% in poorly differentiated tumors, G3) and with histological type (81.9% in adenocarcinoma *vs* 18.1% in adenocarcinoma with mucosal component cases). Strong IRS-1 positivity was observed more frequently in adenocarcinoma cases (95.1%) and in moderately differentiated tumors (85.2%). We also found statistically significant correlations between expression of IRS-1 and both Bax and Bcl-xL in all CRC cases examined. The relationships between studied proteins were related to clinicopathological parameters of CRC. No significant correlation between the expression of IRS-1 and proliferation marker Ki-67, excluding early stage tumors, where the correlation was positive and on a high level ( $P = 0.043$ ,  $r = 0.723$ ).

**CONCLUSION**

This study suggests that IRS-1 is co-expressed with both pro- and antiapoptotic markers and all these proteins are more prevalent in more differentiated CRC than in poorly differentiated CRC.

**Key words:** Colorectal cancer; Insulin receptor substrate-1; Bax protein; Bcl-xL protein; Apoptosis; Antigen Ki-67

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**Core tip:** We analyzed the expressions of Insulin receptor substrate 1 (IRS-1), Bax, Bcl-xL and Ki-67 proteins in primary colorectal cancer (CRC). We found that IRS-1 expression was associated with tumor grade and histological type, and was more prevalent in more differentiated CRC. Interestingly, IRS-1 expression was significantly correlated with Bax and Bcl-xL, but not with Ki-67. We hypothesize that coexpression of IRS-1 and proapoptotic and antiapoptotic markers could result in a complex and diverse interplay characteristic for earlier stages in CRC.

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**INTRODUCTION**

Based on GLOBOCAN 2018 data, colorectal cancer (CRC) is the third most common cancer diagnosed across the world<sup>[1]</sup>. Most of the relevant research is aimed at finding new prognostic factors or therapeutic strategy in order to reduce high CRC-related mortality. In addition to the numerous transcription factors and signaling molecules involved in the development of CRC, the role of insulin receptor substrate 1 (IRS-1) has been the subject of recent intense investigation<sup>[2,3]</sup>. IRS-1 is a member of the IRS family (IRS-1 to IRS-6) and is generally considered to be a substrate of the insulin receptor (IR) and the insulin-like growth factor 1 (IGF-1) receptor (IGF-1R)<sup>[4]</sup>. Activation of insulin signaling pathway is crucial in regulation of cell metabolism, while activation of IGF-1 signaling mediates processes, such as mitogenesis, differentiation and cell survival due to signal transmission in the phosphoinositol-3-kinase pathway and mitogen-activated protein kinase pathways<sup>[5]</sup>. Signaling effectors that bind to IRS-1 include the phosphoinositol-3-kinase pathway, Grb-2, SHP-2, c-Crk, and NCK<sup>[6-9]</sup>. Many of these signaling pathways have been implicated in carcinogenesis and cancer progression, thus IRS-1 has been proposed to play a central

role in determining the response of tumor cells to microenvironmental signals, including growth factors and hormones. Constitutive activation of IRS-1 has been found in various solid tumors<sup>[10]</sup>. For instance, in ER-positive breast cancer cells, overexpression of IRS-1 enhances cell proliferation and reduces estrogen growth dependence<sup>[11,12]</sup>. Also, overexpression of IRS-1 and IRS-2 in the mammary gland of mice was found to cause mammary tumorigenesis and metastasis<sup>[13]</sup>. Intestinal epithelium express both the IR and the IGF-1R, and the levels of these receptors are higher in CRC compared with normal colonic mucosa<sup>[14]</sup>. Accumulating evidence suggested that IRS-1 may be important component of the pathophysiologic mechanisms that underlie the colorectal carcinogenesis and tumor progression. Intestinal epithelial differentiation is regulated by multiple pathways, including  $\beta$ -catenin-dependent WNT signaling<sup>[15]</sup>. Most CRC appear to initiate after inactivating mutations in the adenomatous polyposis coli gene, which results in uncontrolled cell proliferation through constitutive activation of WNT/ $\beta$ -catenin signaling<sup>[16]</sup>. In relevance, IRS-1 is highly upregulated in cells with exogenously-induced or constitutive  $\beta$ -catenin signaling and promotes transformation in cells that ectopically express  $\beta$ -catenin<sup>[17]</sup>. Also, upregulation of IRS-1 by WNT/ $\beta$ -catenin signaling in the mouse hepatocellular carcinoma model was found to play an important role in hepatocarcinogenesis<sup>[18]</sup>. Furthermore, a study in the adenomatous polyposis coli min/+ mouse model showed that the intestinal tumorigenesis is attenuated by IRS-1 knock-out<sup>[3]</sup>. Inhibition of growth of colon cancer cells was also observed as an effect of blocking the IGF-1R signaling by micro Ribonucleic Acid 145<sup>[19]</sup>.

Tumorigenesis is a multistep process, involving not only abnormal proliferation but also avoidance of apoptosis in transformed cells. The impairment of apoptosis is a critical step in tumor development but it also can contribute to therapeutic resistance as induction of apoptosis is a major cytotoxic mechanism of anticancer therapies<sup>[20]</sup>. There are two primary apoptotic pathways: The extrinsic and the intrinsic, also called mitochondrial apoptotic pathway. In this work, we have focused on intrinsic apoptotic pathway and Bcl-2 family proteins that regulate programmed cell death. The Bcl-2 family consists of three subgroups of proteins. The prosurvival subfamily, which includes proteins such as Bcl-2 and Bcl-xL, protects cells from a wide range of cytotoxic insults, whereas two other subfamilies (Bax-like apoptotic subfamily and the BH3-only proteins) promote apoptosis. Tumor cells develop a variety of strategies to avoid apoptosis, including enhanced expression of antiapoptotic Bcl-2 family members, such as Bcl-xL and reduced expression of proapoptotic Bcl-2 family members, such as Bax<sup>[21]</sup>.

Numerous studies have shown that IRS-1 signaling contributes to tumor cell survival. In ER-positive breast cancer cells, suppression of IRS-1 expression accelerates apoptosis and renders cells more vulnerable to tamoxifen-induced cell death<sup>[22]</sup>. Further, study in IRS-1-deficient mice have shown that reduced expression of IRS-1 increases apoptosis of crypt stem or progenitor cells and protects against intestinal tumors development<sup>[3]</sup>. However, other evidence points to proapoptotic IGF-1R/IRS-1 functions<sup>[23]</sup>.

Here, we assessed IRS-1 expression in primary CRC and analyzed associations between the expression of IRS-1 and apoptotic (Bax, Bcl-xL) and proliferation (Ki-67) markers in relation to clinicopathological variables in CRC.

## MATERIALS AND METHODS

### Study design

To assess and compare immunohistochemical expression of IRS-1, Bax, Bcl-xL and Ki-67 tissue material obtained from 127 patients with pathologically confirmed diagnosis of CRC were analyzed. In order to obtain a sample reflecting the general population, the study was designed with wide range of inclusion criteria. The exclusion criteria included: (1) Age < 35 years; and (2) Known genetic predisposition to the development of colon cancer. We analyzed material from patients who underwent radical surgery with lymph node dissection.

Small biopsy specimens were excluded from the study. This study protocol was reviewed and approved by the Local Ethical Committee at the Medical University of Bialystok (Resolution No.: APK.002.105.2020).

### Tissue samples

The postoperative material was fixed in 10% buffered formalin and paraffin-embedded. From paraffin blocks, 5  $\mu$ m sections were cut, deparaffinized, rehydrated

and stained with hematoxylin-eosin. Next, routine histopathological analysis of slides was performed in accordance with recommendation of World Health Organization.

Among 127 tumors, 57 had primary localization in rectum and 69 in colon. Histopathological analysis revealed 104 of adenocarcinoma cases and 23 of adenocarcinoma with mucosal component cases. Tumors were classified, according to their extramural depth of invasion, into two categories: pT1 + pT2 for tumors assessed as pT1 or pT2, which counted 11 cases and pT3 + pT4 for tumors assessed as pT3 or pT4, which counted 116 cases. Furthermore, in agreement with guidelines of World Health Organization, 69 tumors were classified as moderately differentiated (G2) and 87 tumors were classified as poorly differentiated (G3). Presence of metastases to regional lymph nodes was observed in 67 cases.

### **Immunohistochemical staining**

Immunohistochemical assays were performed on formalin-fixed, paraffin-embedded tissue samples using primary antibodies against IRS-1, Bax, Bcl-xL, Ki-67 (Santa Cruz Biotechnology Incorporated, Santa Cruz, California, United States). To improve antigen expression, we applied pretreatment using Heat-induced Epitope Retrieval for 15 min in microwave. Next, the sections were incubated with blocking serum for 10 min to minimize false-positive staining. Tissues with confirmed immunohistochemical expression of IRS-1, Bax, Bcl-xL, Ki-67 served as positive control, while in negative control primary antibodies were substituted with phosphate-buffered saline. To visualize the target-antibody interaction, Dako Envision kit (Dako, Carpinteria, CA, United States) was used and 3,3'-Diaminobenzidine (DAB-kit, Dako Cytomation, Denmark) served as a chromogen.

Evaluation of biomarker expression was performed by two independent pathologists in 10 representative fields in each immunohistochemistry slide (magnification of 200 ×). The percentage of positive cells was scored as follows: 0, less than 10% immunoreactive cells in tumor; (1) 10%-50% immunoreactive malignant cells in tumor; and (2) More than 50% immunoreactive neoplastic cells in tumor.

### **Statistical analysis**

The correlations between expression of IRS-1 and Bax, Bcl-xL, Ki-67 in primary CRC were analyzed in relation to clinicopathological parameters, including: patient age, sex, primary localization of tumor, histopathological type, grading, staging and lymph node spread. Correlations between variables were analyzed by Spearman rank correlation test with a level of significance at  $P < 0.05$ . Additionally, to estimate the strength of the correlation, Guilford's classification method was applied [correlation factor ( $r$ ): 0.0-0.2 (slight); 0.2-0.4 (low); 0.4-0.7 (moderate); 0.7-0.9 (high); 0.9-1.0 (very high)]. Data analysis was conducted using STATISTICA PL v.12.0. software.

## **RESULTS**

### **Expression of IRS-1 in CRC**

Immunohistochemical analysis of CRC sections revealed weak cytoplasmic staining for IRS-1 in 66 CRC sections, while strong cytoplasmic staining for IRS-1 was observed in 61 cases of primary CRC. IRS-1 staining was not detected in negative controls. We observed that IRS-1 expression at any level in CRC was associated with moderately differentiated tumors (G2) (69% in G2 tumors compared with 31% in G3 tumors) and with histological type (81.9% in adenocarcinoma cases compared with 18.1% in adenocarcinoma with mucosal component cases) (Table 1).

### **Correlation of IRS-1 with Bax expression in CRC**

The results demonstrated a moderate positive correlation between IRS-1 and Bax expression is statistically significant in all groups excluding cases of adenocarcinomas with mucosal component (mucinous adenocarcinoma), poorly differentiated tumors (G3) and early stage tumors (Table 2).

### **Correlation of IRS-1 with Bcl-xL expression in CRC**

A positive correlation on a low to high level was found between IRS-1 and Bcl-xL in all cases, except patients characterized with tumor with primary localization in rectum, adenocarcinoma with mucosal component, poorly differentiated tumor (Table 2).



**Table 1 Analysis of correlation between insulin receptor substrate 1 expression and clinicopathologic features, *n* (%)**

Clinicopathological features		IRS-1 expression		P Fisher exact test
		Weak (0), <i>n</i> = 66	Strong (1+, 2+), <i>n</i> = 61	
Age (yr)	≤ 60 ( <i>n</i> = 42)	22 (33.8)	20 (33.3)	0.552
	> 60 ( <i>n</i> = 83)	43 (66.2)	40 (66.7)	
Gender	Male ( <i>n</i> = 70)	37 (56.1)	33 (54.1)	
	Female ( <i>n</i> = 56)	28 (42.4)	28 (45.9)	
Tumor Localization	Rectum ( <i>n</i> = 57)	33 (50.8)	24 (39.3)	0.134
	Colon ( <i>n</i> = 69)	32 (49.2)	37 (60.7)	
Histological Type <sup>a</sup>	A ( <i>n</i> = 104)	46 (69.7)	58 (95.1)	< 0.001
	MA ( <i>n</i> = 23)	20 (30.3)	3 (4.9)	
Histological differentiation <sup>a</sup>	G2 ( <i>n</i> = 87)	35 (53.8)	52 (85.2)	< 0.001
	G3 ( <i>n</i> = 39)	30 (46.2)	9 (14.8)	
Tumor size	pT1 + pT2 ( <i>n</i> = 11)	7 (10.6)	4 (6.6)	0.312
	pT3 + pT4 ( <i>n</i> = 116)	59 (89.4)	57 (93.4)	
Lymph node Involvement	Negative ( <i>n</i> = 60)	29 (43.9)	31 (50.8)	0.438
	Positive ( <i>n</i> = 67)	37 (56.1)	30 (49.2)	

Correlations were analyzed by Fisher exact test.

<sup>a</sup>*P* < 0.05; *n*: Number of cases; *r*: Correlation coefficient; A: Adenocarcinoma; MA: Mucinous adenocarcinoma; G2: Moderately differentiated; G3: Poorly differentiated; N (-): Negative lymph node invasion; N (+): Positive lymph node invasion.

### Correlation of IRS-1 with Ki-67 expression in CRC

No significant correlations were found between IRS-1 and Ki-67, excluding early stage tumors (pT1 + pT2) where the correlation was positive and on a high level (*P* = 0.043, *r* = 0.723) (Table 3). Interestingly, the same group of tumors was negative for coexpression of Bax and Bcl-xL.

### Correlation of Bax with Bcl-xL expression in CRC

We also found statistically significant positive correlations between proapoptotic Bax and antiapoptotic Bcl-xL protein expression in all groups, except early stage tumors (pT1 + pT2) (Table 4).

## DISCUSSION

The present study demonstrates positive correlations between IRS-1 expression and the presence of proapoptotic Bax as well as antiapoptotic Bcl-xL in primary CRC. These associations were prevalent in more differentiated CRC compared with less differentiated CRC. Previously, we found similar relationships in primary breast cancer (positive correlation between IRS-1 and Bax with *P* < 0.001 and *r* = 0.346 as well as IRS-1 and Bcl-xL with *P* < 0.001 and *r* = 0.315) and in lymph node metastases (positive correlation between IRS-1 and Bax with *P* = 0.037 and *r* = 0.356 as well as IRS-1 and Bcl-xL with *P* = 0.004 and *r* = 0.447)<sup>[24]</sup>. Based on this data we assume that influence of IRS-1 on tumor cell survival mechanisms can be diverse and can be related to cancer stage.

In general, cell survival depends on the balance between expressed amounts of proapoptotic and antiapoptotic Bcl-2 family members. Increasing evidence suggested that function of Bcl-2 family proteins could be also regulated by its phosphorylation or dephosphorylation rates<sup>[25]</sup>. Overexpression of IRS-1 was reported to suppress insulin-induced phosphorylation and activation of Bcl-2 protein<sup>[26]</sup>. Another study documented that IRS-1-mediated signals lead to resistance to apoptosis induced by Transforming Growth Factor-β1 in hepatocellular carcinoma cells<sup>[27]</sup>. Also, overexpression of IRS-1 in glioblastoma cells was found to promote cell survival<sup>[28]</sup>. Moreover, a study on brown pre-adipocytes indicated that IGF-1 as well as insulin

**Table 2 Analysis of correlations between Insulin receptor substrate 1, Bax and Bcl-xL expressions in primary colorectal cancer**

Groups of patients		IRS-1-Bax			IRS-1-Bcl-xL		
		<i>n</i>	<i>P</i> value	<i>r</i>	<i>n</i>	<i>P</i> value	<i>r</i>
All		123	< 0.001	0.513 <sup>a</sup>	119	< 0.001	0.354 <sup>a</sup>
Age (yr)	≤ 60	40	< 0.001	0.661 <sup>a</sup>	41	0.001	0.481 <sup>a</sup>
	> 60	81	< 0.001	0.424 <sup>a</sup>	76	0.020	0.267 <sup>a</sup>
Sex	Male	67	< 0.001	0.497 <sup>a</sup>	63	0.011	0.319 <sup>a</sup>
	Female	55	< 0.001	0.537 <sup>a</sup>	55	0.002	0.415 <sup>a</sup>
Localization	Rectum	54	< 0.001	0.460 <sup>a</sup>	51	0.080	0.247 <sup>a</sup>
	Colon	68	< 0.001	0.536 <sup>a</sup>	67	< 0.001	0.446 <sup>a</sup>
HP-type	A	101	< 0.001	0.535 <sup>a</sup>	96	< 0.001	0.535 <sup>a</sup>
	MA	22	0.953	-0.013	23	0.841	-0.044
G	G2	84	< 0.001	0.523 <sup>a</sup>	83	0.006	0.300 <sup>a</sup>
	G3	38	0.263	0.186	35	0.474	0.125
T	pT1 + pT2	10	0.455	0.268	11	0.005	0.772 <sup>a</sup>
	pT3 + pT4	113	< 0.001	0.529 <sup>a</sup>	108	0.001	0.302 <sup>a</sup>
N	pN (-)	58	< 0.001	0.507 <sup>a</sup>	57	0.007	0.351 <sup>a</sup>
	pN (+)	65	< 0.001	0.493 <sup>a</sup>	62	0.007	0.336 <sup>a</sup>

Associations analyzed by Spearman's correlation rank test.

<sup>a</sup>*P* < 0.05; *n*: Number of cases; *r*: Correlation coefficient; A: Adenocarcinoma; MA: Mucinous adenocarcinoma; G2: Moderately differentiated; G3: Poorly differentiated; N (-): Negative lymph node invasion; N (+): Positive lymph node invasion.

exerts antiapoptotic effect, which can be impaired by IRS-1 deletion and restored by its re-expression<sup>[29]</sup>. It was also reported that overexpression of the IGF-1R in human CRC cell line (Hematocrit 116/IGF-1R) results in up-regulation of the antiapoptotic protein Bcl-xL<sup>[30]</sup>.

In contrast, several reports suggested that IRS-1 is either not critical or plays a negative role in cell survival. For example, in 32 D hematopoietic cells lacking IRS-1 expression, IGF-1R activation still protected cells from apoptosis, suggesting that non-IRS-1 pathways were involved in the process<sup>[31]</sup>. Another study in the 32 D model system showed that IRS-1 expression sensitized cells to chemotherapy-induced death, but it did not affect the expression of pro- or antiapoptotic proteins<sup>[32]</sup>. Furthermore, IRS-1 overexpression in transgenic mouse livers enhanced cell proliferation and caused up-regulation of *Fas* receptor but increased cell sensitivity to apoptosis<sup>[33]</sup>. Another study demonstrated that IGF-1 treatment of MG63 osteosarcoma cells stimulated growth and proliferation but also mildly induced apoptosis through caspase-3 activation, annexin-V binding and deoxyribonucleic acid degradation<sup>[23]</sup>. Surprisingly, the same study also showed coactivation of antiapoptotic signals such as Bad phosphorylation at serine 112. Thus, in this model, the increased growth induced by IGF-1 treatment might be balanced by activation of pro-death mechanisms. In line with this suggestion, our results show differential associations between expression of IRS-1 and Bax and Bcl-xL that depend on tumor size. IRS-1 and antiapoptotic Bcl-xL protein were positively correlated in cases of small tumors (pT1 + pT2) while in more extended tumors (pT3 + pT4) IRS-1 expression was positively correlated with Bcl-xL as well as proapoptotic Bax protein. Based on this, we assume that IRS-1 could play a fortifying role in fast growing tumors in which blood supply and nutrients become limited. Under these conditions, coactivation of antiapoptotic and proapoptotic pathways might provide balanced cell turnover and result in tumor progression. The precise mechanisms of how IRS-1 interplays with apoptotic proteins are not yet understood. The abovementioned study<sup>[26]</sup> demonstrated that IRS-1 suppress apoptotic cell death induced by growth factor withdrawal probably through regulating phosphorylation of Bcl-2. The authors have also shown that IRS-1 was able to bind Bcl-xL but not Bax. Bcl-xL is capable of heterodimerize with Bax and counteracting its apoptotic effect. It is quite possible that during early step of tumor development, IRS-1

**Table 3 Analysis of correlations between Insulin receptor substrate 1 and Ki-67 expression in primary colorectal cancer**

Groups of patients		IRS-1 – Ki-67		
		<i>n</i>	<i>P</i> value	<i>r</i>
All		102	0.589	0.054
Age (yr)	≤ 60	32	0.291	-0.193
	> 60	68	0.147	0.178
Sex	Male	55	0.893	0.018
	Female	46	0.517	0.098
Localization	Rectum	45	0.662	-0.067
	Colon	56	0.285	0.145
HP-type	A	85	0.751	0.035
	MA	17	0.239	-0.302
G	G2	71	0.845	0.024
	G3	30	0.714	0.070
T	pT1 + pT2	8	0.043	0.723 <sup>a</sup>
	pT3 + pT4	94	0.973	-0.004
N	pN (-)	49	0.471	0.105
	pN (+)	53	0.999	0.000

Associations were analyzed by Spearman's correlation rank test.

<sup>a</sup>*P* < 0.05; *n*: Number of cases; *r*: Correlation coefficient; A: Adenocarcinoma; MA: Mucinous adenocarcinoma; G2: Moderately differentiated; G3: Poorly differentiated; N (-): Negative lymph node invasion; N (+): Positive lymph node invasion.

exerts synergistic effect with antiapoptotic Bcl-xL protein to enable the escape of tumor cells from death signals and continue abnormal proliferation. The expression of Bax protein in more advanced tumor can be independently regulated for example by the oxidative stress<sup>[34]</sup>.

Role of insulin and IGF-1 signaling in promoting cell growth and proliferation is well established but in present study we did not find evidences for the association between expression of IRS-1 and increased cell proliferation (assessed by Ki-67 positivity), except cases of smaller tumors (pT1 + pT2). Interestingly, in the same groups of patients, IRS-1 expression was positively correlated with antiapoptotic Bcl-xL. Thus, it can be concluded that IRS-1 could promote proliferation and survival signals or activate apoptotic signals in tumor cells, depending on the microenvironmental conditions such as availability of oxygen or nutrients. On the other hand, in our previous study on breast cancer, we found that IRS-1 was positively correlated with Ki-67 in ERα-positive primary tumors and negatively correlated in ERα-negative tumors<sup>[35]</sup>. These data could suggest that IRS-1 can promote enhanced proliferation primarily in steroid-dependent cells.

We also observed decreased IRS-1 expression in poorly differentiated, high-grade colorectal tumors and in adenocarcinoma with mucosal component cases. This observation is consistent with other study showing that IRS-1 is expressed at low levels or absent in undifferentiated and mucinous CRCs<sup>[2]</sup>. A similar downregulation of IRS-1 expression was observed in non-small cell lung cancer where loss of IRS-1 occurred more frequently in stage IB than in IA tumors and was more frequently observed in squamous cell carcinoma<sup>[36]</sup>. The loss of IRS-1 expression at some stage during malignant transformation could suggest that IRS-1-dependent signals play a significant role in the early, but not advanced, stages of tumor development. In line with previous studies, we found that IRS-1 expression is more prevalent in more differentiated tumors, but others have shown that despite this fact IRS-1 expression was also correlated with markers of biological aggressiveness, including Ki-67, p53, and cytoplasmic beta-catenin<sup>[2]</sup>.

The overall goal of this work was to examine the expression of IRS-1 in CRC and analyze its associations with proliferation and apoptotic markers Ki-67, Bax and Bcl-xL in relation to clinicopathologic features. Our data suggest that (1) IRS-1 expression is

**Table 4 Analysis of correlations between Bax and Bcl-xL expression in primary colorectal cancer**

Groups of patients		Bax-Bcl-xL		
		<i>n</i>	<i>P</i> value	<i>r</i>
All		115	< 0.001	0.556 <sup>a</sup>
Age(yr)	≤ 60	39	< 0.001	0.575 <sup>a</sup>
	> 60	74	< 0.001	0.530 <sup>a</sup>
Sex	Male	60	< 0.001	0.453 <sup>a</sup>
	Female	54	< 0.001	0.643 <sup>a</sup>
Localization	Rectum	48	< 0.001	0.490 <sup>a</sup>
	Colon	66	< 0.001	0.594 <sup>a</sup>
HP-type	A	93	< 0.001	0.546 <sup>a</sup>
	MA	22	0.013	0.519 <sup>a</sup>
G	G2	80	< 0.001	0.523 <sup>a</sup>
	G3	34	< 0.001	0.543 <sup>a</sup>
T	pT1 + pT2	10	0.537	0.222
	pT3 + pT4	105	< 0.001	0.577 <sup>a</sup>
N	pN (-)	55	< 0.001	0.509 <sup>a</sup>
	pN (+)	60	< 0.001	0.582 <sup>a</sup>

Associations were analyzed by Spearman's correlation rank test.

<sup>a</sup>*P* < 0.05; *n*: Number of cases; *r*: Correlation coefficient; A: Adenocarcinoma; MA: Mucinous adenocarcinoma; G2: Moderately differentiated; G3: Poorly differentiated; N (-): Negative lymph node invasion; N (+): Positive lymph node invasion.

more prevalent in more differentiated tumors, and (2) IRS-1 expression is correlated with both proapoptotic Bax and antiapoptotic Bcl-xL proteins. The first observation indicates that loss of IRS-1 in CRC may be considered as potential marker for poor differentiation and more aggressive phenotype. The second observation aligns with published evidence suggesting a potential diverse role of -insulin receptor IGF-1R/IRS-1 signaling in regulating apoptotic processes<sup>[23]</sup>.

While this study is not conclusive, it provides a good starting point for discussion regarding interactions and functional dependence between IRS-1, Bax and Bcl-xL in CRC. In the future, the assessment of IRS-1 expression could be used to evaluate individual patient prognosis and might offer new insights into developing more efficient treatment strategies and identify patients who are most likely to respond to targeted therapies, for example IGF-1R inhibition.

## ARTICLE HIGHLIGHTS

### Research background

Insulin receptor substrate 1 (IRS-1), a signaling molecule involved in cell proliferation, survival and metabolic responses has been implicated in carcinogenic processes in various cellular and animal models. However, the role of IRS-1 in human colorectal cancer (CRC) biology and its value as a clinical CRC biomarker has not been well defined.

### Research motivation

CRC is the third most common cancer diagnosed across the world. Despite effective prevention and screening methods CRC represents one of the most common causes of cancer-related deaths. Most of the research is aimed at finding new prognostic factors or therapeutic strategy in order to reduce high CRC-related mortality.

### Research objectives

This study evaluated if and how IRS-1 expression and its associations with the apoptotic and proliferation tumor markers, Bax, Bcl-xL and Ki-67 are related to clinicopathological features in human CRC, *i.e.*, patient age, sex, primary localization of tumor, histopathological type, grading, staging and lymph node spread.

### Research methods

We retrospectively collected data from 127 patients with primary CRC who underwent radical surgery with lymph node dissection. We analyzed the expressions of IRS-1, Bax, Bcl-xL and Ki-67 proteins using immunohistochemical methods. Correlations between variables were examined by Spearman rank correlation test and Fisher exact test with a level of significance at  $P < 0.05$ .

### Research results

Immunohistochemical analysis revealed weak cytoplasmatic staining for IRS-1 in 66 CRC sections and strong cytoplasmatic staining in 61 cases. IRS-1 expression at any level in primary CRC was associated with tumor grade (69% in moderately differentiated tumors, G2 *vs* 31% in poorly differentiated tumors, G3) and with histological type (81.9% in adenocarcinoma *vs* 18.1% in adenocarcinoma with mucosal component cases). Strong IRS-1 positivity was observed more frequently in adenocarcinoma cases (95.1%) and in moderately differentiated tumors (85.2%). We also found different relationships between IRS-1 expression and both Bax and Bcl-xL proteins depended on clinicopathological parameters. Further analysis of the data revealed no significant correlation between expression of IRS-1 and proliferation marker Ki-67, excluding early stage tumors, where the correlation was positive and on a high level ( $P = 0.043$ ,  $r = 0.723$ ).

### Research conclusions

Our study adds to a growing corpus of research showing that (1) IRS-1 expression is more prevalent in more differentiated tumors, and our data indicate that (2) IRS-1 expression is correlated with both proapoptotic Bax and antiapoptotic Bcl-xL proteins.

### Research perspectives

Further research on this topic might extend the knowledge on the interactions and functional dependence between IRS-1 and apoptotic markers in CRC. In the future, the assessment of IRS-1 expression could be used to evaluate individual patient prognosis and might offer new insights into developing more efficient treatment strategies and identify patients who are most likely to respond to targeted therapies, for example the insulin-like growth factor 1 receptor inhibition.

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