Rom J Morphol Embryol 2022, 63(3):521–528 ISSN (print) 1220–0522, ISSN (online) 2066–8279 doi: 10.47162/RJME.63.3.06

ORIGINAL PAPER

RJME Romanian Journal of Morphology & Embryology http://www.rjme.ro/

Bcl-2 and p53 immunophenotypes in colorectal adenocarcinoma in type 2 diabetes mellitus *versus* non-diabetic patients

HORAŢIU-CRISTIAN POPESCU-VÂLCEANU[1)], MIHAI CIPRIAN STOICEA[2)], VALENTIN ENACHE[3)], RĂZVAN MATEI BRATU[4)], PETRONEL MUSTĂŢEA[4)], RAMONA MARIA DRĂGUŢ[1)], EMILIA RUSU[1)], CONSTANTIN IONESCU-TÎRGOVIŞTE[1,5)], GABRIELA RADULIAN[1,5)]

[1)]*Department of Diabetes, Nutrition and Metabolic Diseases, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

[2)]*Department of Pathology, Regina Maria Central Reference Laboratory, Bucharest, Romania*

[3)]*Department of Pathology, Emergency Clinical Hospital, Bucharest, Romania*

[4)]*Department of Surgery, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

[5)]*Department of Diabetes, Nutrition and Metabolic Diseases, Prof. Dr. N. C. Paulescu National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania*

Abstract

We aimed to investigate immunohistochemical expression of the p53 tumor suppressor protein, and the B-cell lymphoma-2 (Bcl-2) apoptotic protein in colorectal adenocarcinoma patients with or without type 2 diabetes mellitus (T2DM). Tissue sections from 95 paraffin-embedded colorectal adenocarcinomas, originating from 52 T2DM and 43 non-diabetic patients, were immunostained for p53 [Ventana mouse monoclonal primary antibody (mAb) *in vitro* diagnostic (IVD) anti-p53, clone Bp53-11] and Bcl-2 (Ventana mAb IVD anti-Bcl-2, clone Bcl-2/124). Immunohistochemistry analysis did not find statistically significant differences between the two groups, but analysis on subgroups of patients in terms of presence or absence of obesity identified overexpression of p53 (>70% of cells) in the T2DM obese patients compared to non-diabetics. Overexpression of p53 was present in 80% of tumor cells coming from T2DM obese patients compared to 37.2% of tumor cells coming from non-diabetics obese and non-obese, and in 36.6% of tumor cells coming from non-diabetic non-obese patients (p=0.024). There was a single non-diabetic obese patient with p53 overexpression. Most cancer cells of T2DM obese patients presented more frequently p53 overexpression by comparison with cancer cells of the T2DM non-obese patients (80% *vs* 40.5%, p=0.028). Bcl-2/p53 co-expression was an infrequent event in T2DM patients' group. The results of this study suggest that patients with colorectal adenocarcinoma that associate T2DM and obesity exhibit higher p53 protein expression in malignant cells. In conclusion, our research highlights that obesity is a potential key factor in the relationship between T2DM and colorectal cancer.

Keywords: p53, Bcl-2, colorectal adenocarcinoma, type 2 diabetes, immunohistochemistry, obesity.

Introduction

Cancer and metabolic diseases, especially diabetes mellitus (DM) and obesity are complex, frequent chronic conditions with increasing incidence and prevalence worldwide. Studies evidenced that people with type 2 DM (T2DM) had a significantly risk for certain cancers, most notably digestive and genital tract: colorectal [1–7], pancreatic [8–13], hepatic [14–17], breast [18–21], endometrial [22, 23] also with a higher cancer mortality rate [24].

Obesity is an important risk factor in the pathophysiology of the DM and colorectal cancer (CRC). Obesity, defined by a body mass index (BMI) ≥30 kg/m^2, is a strong risk factor for T2DM and is positively associated with many cancer types especially the CRC risk [25].

Diabetic patients present an approximately 30% higher risk of developing CRC [1–7] compared to non-diabetics, with an increased risk of complications, cancer recurrence and death [24, 26, 27]. In particular, obesity appears to be a risk factor for the increased incidence of CRC in T2DM [28–33].

The B-cell lymphoma-2 (Bcl-2) antiapoptotic protein (member of Bcl-2 family proteins) and the p53 suppressor protein, encoded by the tumor protein p53 (*TP53*) gene, plays an essential role in regulating apoptosis [34–37] and metabolism [38–42]. Through these various roles, the two proteins can be a potential link between neoplastic disease and metabolic diseases in general, and particularly between cancer and T2DM.

Immunohistochemical (IHC) technique can be used as a method of indirect detection of *p53* gene mutation, commonly used as a screening method for detecting the presence of this mutation [43]. Under normal conditions, the IHC method cannot detect the "wild-type" (WT) p53 protein due to its instability, with a very short lifetime (approximately 5–30 minutes) and extremely low nuclear expression [44]. However, the mutant form of p53 will accumulate at the nuclear level [by inhibiting mouse double minute 2 homolog (MDM2) metabolism] and will

reach an IHC detection threshold due to increased aberrant expression and longer lifespan [43, 44]. Thus, we can assert that the IHC staining pattern of p53 can be considered a surrogate marker for the *p53* gene mutation in cancer [43, 45, 46].

Colorectal adenocarcinoma is the most common form of CRC accounting for >90% of all histological types [47]. Studies evidenced an association between p53 and Bcl-2 in CRC [48–51], positive p53 expression being generally associated with an unfavorable prognosis [48, 52].

We know that Bcl-2 and p53 proteins are involved in both metabolic and cancer pathogenesis: T2DM and obesity (in β-cell regulation) and CRC (in apoptosis regulation), and also know that hyperglycemia could influence the expression of the Bcl-2 and p53 proteins [53–55] and that CRC is more common in T2DM and in obesity compared to the general population. Starting from these facts, we propose to investigate the potential mechanism of pathogenic association between cancer, diabetes, and obesity by comparative quantification of IHC expression of these two proteins. We supposed the existence of a diabetic environment with related pathophysiological changes that lead to excessive signaling in certain pathways which interfere with complex p53 protein activity but also indirectly with Bcl-2 protein activity either *via* p53 or by modifying the ratio of pro-apoptotic and anti-apoptotic Bcl-2 family members.

Aim

The aim of the present research was to investigate the IHC expression of the p53 tumor suppressor protein and the Bcl-2 apoptotic protein in CRC patients with or without T2DM or obesity. This study set out to examine the comparative relationship between the two proteins in these important diseases.

Patients, Materials and Methods

Our research had a retrospective design. The study included 95 patients with CRC, which met the following inclusion criteria: men and women age >18 years; patients diagnosed with primary CRC; without a heredo-collateral CRC history; without oncological treatment prior to CRC diagnosis; patients diagnosed with T2DM prior to CRC diagnosis (for the CRC diabetic group). Eligible patients included 32 women and 63 men, aged 46–92 years (median age of 69.88 years) divided into two groups: 52 T2DM patients and 43 non-diabetes mellitus (non-DM) patients. Our study also included four cases of non-tumoral (non-lesional epithelium) colonic mucosa sample for control, from patients who required surgery for non-neoplastic conditions.

Patients were selected from the database of the Department of Anatomical Pathology, Bucharest Emergency Clinical Hospital, Bucharest, Romania, hospitalized from 01.01.2012 to 30.08.2015 after receiving the agreement of the Ethics Committee, Bucharest Emergency Clinical Hospital (Approval No. 13918/11.06.2015). The cases were identified using the Hipocrate Medical Informatics Program, randomized (in order of selection, respecting inclusion criteria), according to the 10th edition of the *International Statistical Classification of Diseases and Related Health Problems* (ICD-10). Data were extracted from the discharge summary and the pathology reports. Subsequently, for each case, one block of paraffin and the Hematoxylin–Eosin (HE) stained slide were extracted from the Laboratory Archive.

The subsequent blocks and slides were analyzed in the Department of Histopathology of the Synevo Central Laboratory, Bucharest, for IHC assessment and testing in an anonymous manner, with no patient identification data, clinical and paraclinical data. At reception in the Laboratory, each case was assigned a unique identification number. Paraffin blocks were cut using a conventional microtome to obtain 2 μm thick sections. Further, each section was recovered from the water bath on an electrostatically charged slide. The slides were dehydrated at 58°C in a thermostat for one hour.

IHC testing was performed in an automated system using a Ventana BenchMark XT platform. The dehydrated slides were labeled with a barcode associated with the standardized protocol, the case number, and the block number. A dual Bcl-2/p53 IHC protocol was used, including prediluted *in vitro* diagnostic (CE-IVD) antibodies: Ventana mouse monoclonal primary antibody (mAb) immunoglobulin G1 (IgG1) anti-Bcl-2 (clone Bcl-2/124) and Ventana mAb IgG2α anti-p53 (clone Bp53-11). The IHC protocol steps included: dewaxing; antigen retrieval; incubation with the anti-Bcl-2 antibody (16 minutes at 37°C); incubation with the anti-p53 antibody (16 minutes at 37°C); counterstaining. The IHC reactions were visualized using ultraView, with 3,3'-Diaminobenzidine (DAB) chromogen for Bcl-2 and Red chromogen for p53. After automatic post-processing, the glass slides were degreased, dehydrated, and mounted to obtain permanent sealed slides for microscopic examination.

The optical microscopy step was the central point of histopathological (HP) evaluation of tissue sections and was performed independently by two pathologists according to current professional standards. The tissue sections were initially reevaluated in standard HE staining for conventional HP characteristics. Subsequently, the glass slides with dual IHC staining were examined. The intensity (low, medium, or high) of the reactivity to the anti-p53 antibody, marked by red nuclear reaction and to the anti-Bcl-2 antibody marked by brown cytoplasmic reaction was scored for each case as a percentage of total viable tumor cells.

Bcl-2 immunoreactions were considered positive if present in >3% of tumor cells and grades, as follows: low positive reaction in 3–10% tumor cells; high positive in >10% reaction in tumor cells.

Evaluation of p53 immunoreactions was performed using the following algorithm: score 0, reaction in ≤25% of tumor cells, of low small and/or medium intensity; score 1, reaction in 26–70% of tumor cells, of low and/or medium intensity; score 2, reaction in >70% of tumor cells, of predominantly high intensity (overexpression).

Score 2 reactivity indicates the existence of an abnormal p53 protein because of an epigenetic or genetic event in the *TP53* gene. Reactions scored 1 most likely represent non-mutational events, but not excluding post-transcriptional modulation of *TP53* gene expression. Reactions scored 0 are interpreted as negative (non-mutational) except for the complete absence of reactivity (0%) in which there is the suspicion of a truncated *TP53* gene mutation with null immunophenotype.

Based on the immunophenotypic profile (presence or absence of the reactivity to anti-Bcl-2 and anti-p53 antibodies), the 95 tested cases were subdivided into four IHC classes: class I, Bcl-2(-) and p53(-) (score 0); class II, Bcl-2(+) and p53(-) (score 0); Class III, Bcl-2(-) and p53(+) (scores 1 and 2); class IV, Bcl-2(+) and p53(+) (scores 1 and 2). Class division is dependent on the

predominant aspect of the expression (reaction in most cells); cases that contained a definite minority of tumor cells co-expressing Bcl-2 and p53 were classified according to the predominant pattern of reactivity with "class IV clone".

Statistical analysis was performed using Microsoft Office Excel 2013 and Statistical Package for the Social Sciences (SPSS) ver. 15.00. Data were synthesized as percentage and the significance level used in the statistical tests was $p \leq 0.05$. We performed Fisher's exact test and Bonferroni's correction.

Results

Compared with the non-DM group, the T2DM group showed a slightly higher frequency of female sex, prevalence of elderly patients >65 years and an older age, averaging approximately one year at CRC diagnosis. There were no significant differences between the two groups according to the rural/urban area. Regarding tumor location, the T2DM group present tumor located more frequent on colon and rectum, while non-DM exhibit tumors located on sigmoid colon (Table 1).

Table 1 – ***Comparison of demographic data, histological type, and tumor location in T2DM and non-DM groups***

Variables			non-DM (*n*=43)		T2DM (*n*=52)		Total (*n*=95)	
			n	*Percent*	*n*	*Percent*	*n*	*Percent*
Demographic data	Sex	*Male*	30	69.8%	33	63.5%	63	66.3%
		Female	13	30.2%	19	36.5%	32	33.7%
	Age [years]	*≤65*	18	51.4%	17	48.6%	35	36.8%
		>65	26	43.3%	34	56.7%	60	63.2%
	Average age [years]		69.5		70.2		69.8	
	Area	*Urban*	35	82.7%	43	81.3%	78	82.1%
		Rural	8	17.3%	9	18.7%	17	17.9%
Tumor location		*Colon*	22	51.2%	34	65.4%	56	59.0%
		Sigmoid	18	41.9%	13	25%	31	32.6%
		Rectum	3	6.9%	5	9.6%	8	8.4%
Histological type: adenocarcinoma			43		52		95	100%

n: No. of cases; non-DM: Non-diabetes mellitus; T2DM: Type 2 diabetes mellitus.

Immunochemistry showed negative p53 and Bcl-2 protein expression in all four controls (non-tumoral tissue of colonic mucosa) (Figure 1). Positive p53 expression was marked by red nuclei staining and Bcl-2 expression was marked by brown cytoplasm staining (Figures 2–6). Presence of Bcl-2 in stromal lymphocytes was marked by brown cytoplasmic Bcl-2 staining and served as internal positive control. The suppressor p53 and Bcl-2 IHC staining (intensity and extension) showed variable results, p53 positivity rate ranging from 0 to 99% in both T2DM and non-DM groups, Bcl-2 reactivity ranging from 0 to 80% in non-DM group and from 0% to 65% in T2DM groups, also Bcl-2/p53 co-expression varied widely from 0 to 90% in T2DM group and from 0 to 75% in non-DM patients (Figure 2–6). There was a unique case in our investigation, a T2DM patient which associates tumoral clones with mono-expression of Bcl-2 and p53 proteins and clone with Bcl-2/p53 co-expression. We considered p53 reactivity in over 70% of cells with moderate and high intensity as highly suggestive for *TP53* gene mutation.

Although, Bcl-2 expression in tumoral cells was less frequent, it was better represented in non-DM patients than in T2DM patients. The p53 protein was overexpressed in T2DM-CRC group. Generally, Bcl-2/p53 co-expression was also a rare phenomenon in colorectal tumors but was more frequently seen in T2DM patients than in non-DM patients. Thus, the T2DM compared to the non-DM associated more frequently immunophenotypic class III and immunophenotypic class IV and showed a downregulated Bcl-2 expression without statistical significance (Table 2).

Table 2 – ***Comparison of p53 and Bcl-2 expressions between T2DM and non-DM groups***

Protein expression			non-DM (*n*=43)		T2DM (*n*=52)		Total (*n*=95)		*p* value
			n	*Percent*	*n*	*Percent*	*n*	*Percent*	
p53 expression	0 (<25%)		27	62.8%	25	48.1%	52	54.7%	NS*
	1 (26–70%)		1	2.3%	3	5.8%	4	4.2%	
	2 (>70%)		15	34.9%	24	46.2%	39	41.1%	
	0+1		28	65.1%	28	53.8%	56	58.9%	
	2		15	34.9%	24	46.2%	39	41.1%	
Bcl-2 expression	-		36	83.7%	48	92.3%	83	87.4%	
	+ (3–10%)		3	7.0%	1	1.9%	4	4.2%	
	++ (>10%)		4	9.3%	3	5.8%	8	8.4%	
Bcl-2/p53 co-expression	-		40	93%	46	88.5%	86	90.5%	
	+ (3–10%)		1	2.3%	4	7.7%	5	5.3%	
	++ (>10%)		2	4.7%	2	3.8%	4	4.2%	
IHC class	I		19	44.2%	21	40.4%	40	42.1%	
	II		7	16.3%	3	5.8%	10	10.5%	
	III		14	32.6%	22	42.3%	36	37.9%	
	IV	III clone 4	2	4.7%	4	7.7%	6	6.3%	
		IV	1	2.3%	2	3.8%	3	3.2%	
		IV (total)	3	7%	6	11.5%	9	9.6%	

*NS: $p>0.05$. Bcl-2: B-cell lymphoma-2; IHC: Immunohistochemical; *n*: No. of cases; non-DM: Non-diabetes mellitus; NS: Not significant; T2DM: Type 2 diabetes mellitus.

Although the IHC analysis of the two groups did not identify statistically significant differences, the subgroup analysis in terms of the presence or absence of obesity, identified statistically significant differences between over-expression of p53 (>70% of cells) in the cancer cells of T2DM obese patients compared to non-DM or T2DM non-obese patients with colorectal tumors. Overexpression of p53 was present in 80% of cancer cells of T2DM obese patients compared to 37.2% in non-DM (obese and non-obese) tumors, respectively of 36.6% in non-DM non-obese tumors (p=0.024) and in 40.5% of T2DM non-obese tumors (p=0.028). There was a single p53(+) non-DM obese patient (Table 3).

Table 3 – ***Obesity impact on p53 expression in T2DM and non-DM groups***

CRC / Groups		p53 expression				Total		
		Negative (<70%)		*Positive (>70%)*				
		n	*Percent*	*n*	*Percent*	*n*	*Percent*	*n*
non-DM	BMI >30 kg/m^2	1	50%	1	50%	2	100%	43
	BMI <30 kg/m^2	26	63.4%	15	36.6%	41	100%	
T2DM	BMI >30 kg/m^2	3	20%	12	80%	15	100%	52
	BMI <30 kg/m^2	22	59.5%	15	40.5%	37	40.5%	
Total		52	54.7%	43	45.3%	95	100%	95

BMI: Body mass index; CRC: Colorectal cancer; *n*: No. of cases; non-DM: Non-diabetes mellitus; T2DM: Type 2 diabetes mellitus.

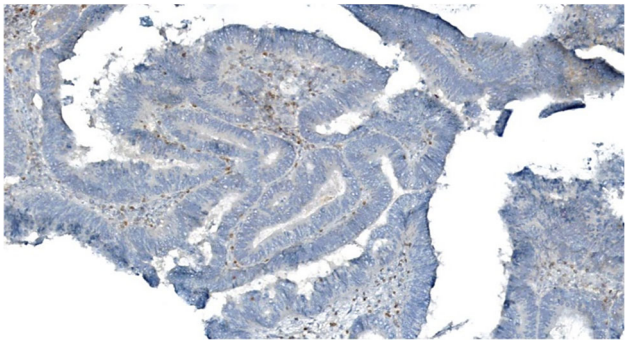

Figure 1 – ***Immunohistochemistry image (160×). Colon adenocarcinoma, moderately differentiated, stage I. Tumor cells without Bcl-2 or p53 expression.***

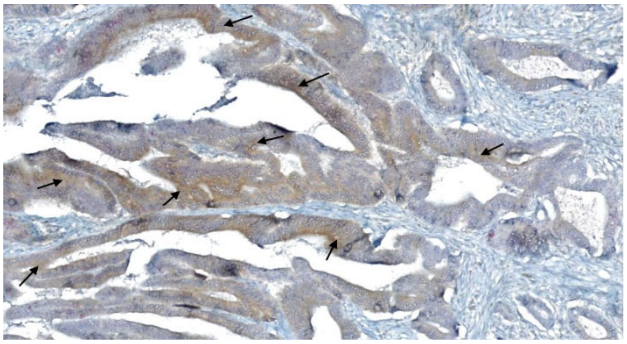

Figure 2 – ***Immunohistochemistry image (110×). Colon adenocarcinoma, moderately differentiated, metastatic stage IV. Bcl-2 reactivity (brown cytoplasm) in 80% of tumor cells, absent p53 reactivity (indicated by arrows).***

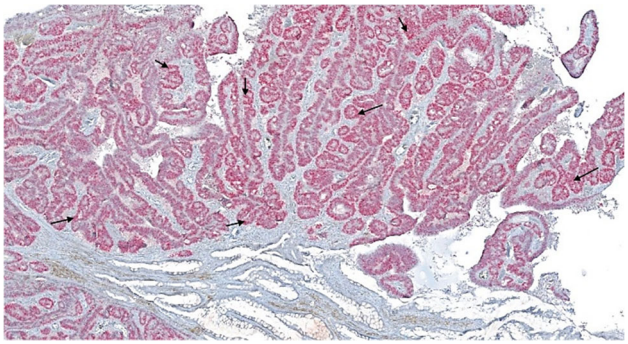

Figure 3 – ***Immunohistochemistry image (90×). Colon adenocarcinoma, moderately differentiated, stage IIA. Co-expression of p53 (red nuclei) and Bcl-2 (brown cytoplasm) is observed in 25% of tumor cells, single p53 expression in 75% of tumor cells (indicated by arrows).***

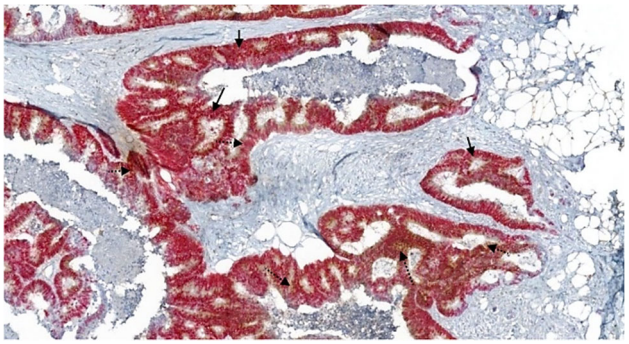

Figure 4 – ***Immunohistochemistry image (90×). Rectal adenocarcinoma, moderately differentiated, stage IIIB. Dual p53/Bcl-2 immunostaining: co-expression of p53 (red nuclei) and Bcl-2 (brown cytoplasm) in 90% of tumor cells, single p53 expression in 10% of tumor cells (indicated by arrows).***

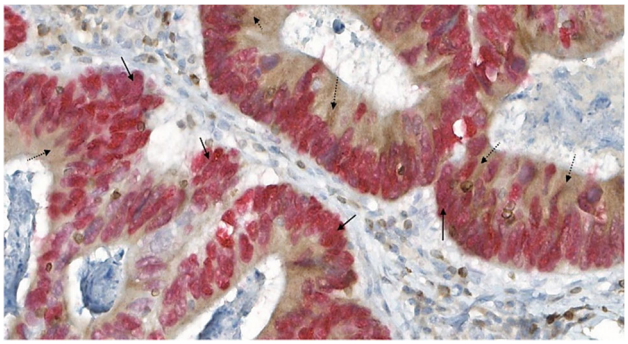

Figure 5 – ***Immunohistochemistry image (400×). Colon adenocarcinoma, moderately differentiated, stage IIA. Co-expression of p53/Bcl-2 visualized by expression of p53 (red nuclei) and Bcl-2 (brown cytoplasm) in 75% of tumor cells, single p53 expression in 25% of tumor cells (indicated by arrows).***

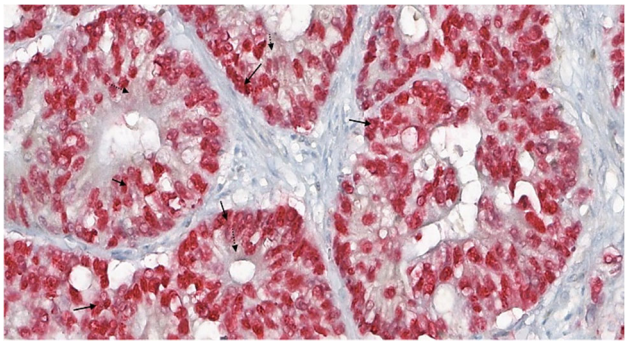

Figure 6 – ***Immunohistochemistry image (400×). Colon adenocarcinoma, moderately differentiated, stage IIA. Co-expression of p53/Bcl-2 visualized by expression of p53 (red nuclei) and Bcl-2 (brown cytoplasm) in 25% of tumor cells, single p53 expression in 75% of tumor cells (indicated by arrows).***

Discussions

A lot of studies [56–60] have indicated an increased variability in IHC expression of Bcl-2 and p53 proteins in CRC. Compared with other studies, the current study revealed a moderate expression of p53, but a lower expression of Bcl-2. This variability can be explained by the higher heterogeneity of the groups (without any specificity in terms of clinical-morphopathological parameters, ethnicity, presence of DM or obesity) and also by increased variability of genetic tumor mutations or of the epigenetic changes.

Single p53 expression or in combination with Bcl-2 presents a reserved prognostic marker [51, 56]. In the current research, the p53 expression score 2 had an increased frequency in tumor cells of T2DM group compared to the non-DM group (p=0.306). Analysis of T2DM patients identified a statistically significant difference in the expression of the p53 protein between the subgroup of T2DM obese patients and DM non-obese patients and even T2DM non-obese patients. Thus, the subgroup of T2DM obese patients exhibited a higher expression of p53(+) score 2 compared to T2DM non-obese (p=0.028) or non-DM (p=0.024) patients.

Based on Bcl-2/p53 immunophenotypic profiles, studies have shown that there are immunophenotypic classes with different aggressiveness in CRC [51, 56, 61–63]. Thus, the lowest prognosis is for p53(+)/Bcl-2(-) immunophenotypes, and the best prognosis for p53(-)/Bcl-2(+) [51, 56, 61–63] with a higher risk of death of 6.38 [56] between the two immunophenotypes. At the same time, there is an inversely correlation between the presence of Bcl-2 and p53, also evidenced in the studied groups [60]. Basically, the risk of death in CRC increases progressively depending on the immunophenotypic profile, from p53(-)/Bcl-2(+) to p53(-)/Bcl-2(-), to p53(+)/Bcl-2(+) (associated chemoresistance) to the highest p53(+)/Bcl-2(-) (associated radioresponsiveness) [56]. In our research, the T2DM tumors presented immunophenotypes associated with poorer prognosis in terms of immunophenotypic profiles. Colorectal tumors in T2DM patients expressed the p53(+)/Bcl-2(-) dominant immunophenotypes, while non-DM tumors expressed more common less aggressively p53(-)/Bcl-2(-) immunophenotypes.

All these changes in the expression of these proteins in the T2DM tumor cells may explain the excessive risk of mortality in CRC attributable to the presence of T2DM and obesity [27, 29, 30, 32, 33, 64]. The presence of obesity is associated with an increased risk of both malignancy and mortality [29, 30, 32, 33, 65]. There is a direct relationship between cancer mortality (regardless of the type of cancer) and glucose levels, based on data from the *Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe* (DECODE) study [66], which highlighted an increase in cancer mortality in those presenting gluco-regulations disorders, rising linearly with increasing glucose concentrations [66]. In a meta-analysis conducted by Barone *et al.* [24], there was an increased risk of long-term mortality of all causes in patients diagnosed with pre-existing cancer and diabetes compared to non-diabetics [24, 66] with higher risk for all types of cancer compared with normoglycemic individuals [24]. In the same study, subtype cancer analysis revealed an increased risk of mortality for CRC diabetic patients [24]. Other meta-analyzes revealed an increased risk of both long-term and short-term mortality in patients with pre-existing CRC and DM [24, 66], but also an increased frequency of therapeutic complications, such as post-chemotherapy but also an increased recurrence of CRC [24]. Maintaining good metabolic control is essential both before and after diagnosis of CRC, good metabolic control being associated with decreased mortality [24, 65].

Two studies of the same group of authors on diabetic murine models indicate an association between the presence of the diabetic environment and changes in p53 expression in oral carcinogenesis [67, 68]. However, the same authors did not identify an association for the Bcl-2 family of proteins (for Bax/Bcl-2 proteins) [68]. In these models, DM is associated with an increased frequency of mutations in the *TP53* gene and there is an increased expression of the altered p53 protein in all stages of carcinogenesis, from the initial stages to the advanced stages [67]. Interestingly, several studies have shown low expression of Bcl-2 in DM-specific complications, such as in glomerular cells from diabetic nephropathy or vascular endothelium from diabetic retinopathy, the presence of Bcl-2 being associated with a decrease in the risk of complications [69, 70].

The presence of p53 protein score 2 expressed in CRC cells of patients associating T2DM and obesity in our study can be explained by multiple mechanisms. The p53 protein appears to be involved in the differentiation processes of adipose tissue both *in vivo* and *in vitro* [71]. Thus, it exerts a positive regulatory effect on the brown adipose tissue but has a suppressive effect on the white adipose tissue on both human and murine mouse cells [71]. Adipocyte hypertrophy by lipid accumulation in adipocytes, activates p53 that maintains homeostatic status by repressing the peroxisome proliferator-activated receptor gamma (PPARγ) key adipogenic factor [71]. The abrogation of p53 function in the skeletal muscle tissue alters its differentiation capacity in brown adipose tissue and induces an abnormal morphology in adult mice [71], a mechanism mediated by the "positive regulatory 16" transcription factor: PR-domain containing 16 (PRDM16) protein [71]. The p53 protein can thus play a dual role depending on the adipogenic differentiation program [71]. This type of direct, cell-dependent regulation reflects an additional way for p53 to maintain homeostatic status at cellular levels [71]. Altering the expression of p53 function can lead to changes in the differentiation process with the increase in the accumulation of white adipose tissue [71]. The *p53* mutation is associated with loss of p53 function and stimulation of Warburg phenotype, which explains the poorer prognosis of T2DM and obese patients with the p53(+)/Bcl-2(-) immunophenotype [72]. All these changes may be a potential link between obesity, metabolic alterations, and cancer.

It is very interesting that besides the more frequent mutational events of p53 in DM, p53 can be inactivated by non-mutational phenomena. Although the p53 expression in the current study was increased, in the general group of T2DM patients compared to non-DM it was not statistically significant (p=0.306). This lack of association of abnormal expression of p53 in the diabetic group can be hypothetically justified by increasing the frequency of non-mutagenic phenomena of p53 tumor suppressor protein from DM-CRC. These phenomena may further explain both the increased risk of CRC mortality and increased mortality in DM. A relative recent cell culture study indicates a non-mutational mechanism of inhibiting WTp53 action based on blood glucose levels [53]. Thus, there is a level of direct proportionality between glycemic value and p53 activity

[53]. An increased level of glucose (hyperglycemic medium) inhibits p53 activity at the specific apoptosis-activating site, and a low level of glucose activates p53 and decreases the pro-oncogenic activity of mutant p53 [53]. Inhibition of p53 activity at Ser46 (Serine) by phosphorylation (p53Ser46) is performed only for WTp53 and not mutant p53 (mutant Ser46D p53) [53]. Blocking p53 function either through mutational or non-mutational mechanisms, explains the increased resistance to treatment encountered in diabetes (hyperglycemic) and may be a molecular mechanism for linking metabolic diseases (diabetes, obesity) and oncogenesis [53].

The findings from this research make several contributions to the current literature. A key strength of the present study was the originality. To our knowledge this is the first research that investigates in parallel, factors involved in both the pathogenesis of DM and CRC (expression of Bcl-2 and p53 proteins) in humans. Until now, these markers have not been studied together as a tandem, but separately for each pathology, either in DM or in various types of cancer. Another important strength of this study was the use of the dual IHC detection technique. This technique is used to identify the concomitant expression of Bcl-2 and p53 protein in the same tumor cell. The dual Bcl-2/p53 IHC staining method has the advantage of correct identification of various tumor immunophenotypes compared to the individual testing of the two markers [56] and has allowed the recognition of several different clonal populations within the same tumor (*e.g.*, clones with p53 overexpression coexisting with clones with Bcl-2/p53 co-expression).

Study limitations

Finally, a few important limitations need to be considered. The findings in this paper are subject to at least two major limitations. First issue of the current study refers to the limited number of patients included in our investigation. Second, another weakness of this study was the paucity of data regarding diabetes. The lack of information includes limited data from the discharge summary and pathology reports, without any information regarding diabetes history, glucose control level prior to diagnosis of colorectal adenocarcinoma, about type of oral hypoglycemic drugs, regimen of insulin therapy or lifestyle factors. A limitation in using of this kind of data precludes the analyses of factors related to T2DM or obesity, our study being unable to analyses these variables.

Conclusions

This project is the first investigation to study the Bcl-2/p53 proteins IHC expression in colorectal tumor cells of T2DM patients and has been one of the first attempts to study the IHC expression of the two proteins in tumors which associate these two conditions. The current research has not been able to establish a relationship between Bcl-2/p53 immunophenotypes in T2DM-CRC cells and non-DM-CRC cells. However, analysis on subgroups of T2DM patients in terms of presence or absence of obesity identified the most significant findings to emerge from this study: the IHC overexpression of p53 protein in colorectal malignant cells of T2DM obese patients. Compared to the other subgroups, the T2DM obese subgroup of patients exhibited the highest p53 protein expression in tumor cells. Further studies need to be carried out to validate the results of our investigation.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] Ma Y, Yang W, Song M, Smith-Warner SA, Yang J, Li Y, Ma W, Hu Y, Ogino S, Hu FB, Wen D, Chan AT, Giovannucci EL, Zhang X. Type 2 diabetes and risk of colorectal cancer in two large U.S. prospective cohorts. Br J Cancer, 2018, 119(11): 1436–1442. https://doi.org/10.1038/s41416-018-0314-4 PMID: 30401889 PMCID: PMC6265303

[2] de Kort S, Masclee AAM, Sanduleanu S, Weijenberg MP, van Herk-Sukel MPP, Oldenhof NJJ, van den Bergh JPW, Haak HR, Janssen-Heijnen ML. Higher risk of colorectal cancer in patients with newly diagnosed diabetes mellitus before the age of colorectal cancer screening initiation. Sci Rep, 2017, 7:46527. https://doi.org/10.1038/srep46527 PMID: 28436468 PMCID: PMC5402260

[3] Guraya SY. Association of type 2 diabetes mellitus and the risk of colorectal cancer: a meta-analysis and systematic review. World J Gastroenterol, 2015, 21(19):6026–6031. https://doi.org/10.3748/wjg.v21.i19.6026 PMID: 26019469 PMCID: PMC4438039

[4] González N, Prieto I, Del Puerto-Nevado L, Portal-Nuñez S, Ardura JA, Corton M, Fernández-Fernández B, Aguilera O, Gomez-Guerrero C, Mas S, Moreno JA, Ruiz-Ortega M, Sanz AB, Sanchez-Niño MD, Rojo F, Vivanco F, Esbrit P, Ayuso C, Alvarez-Llamas G, Egido J, García-Foncillas J, Ortiz A; Diabetes Cancer Connect Consortium. 2017 Update on the relationship between diabetes and colorectal cancer: epidemiology, potential molecular mechanisms and therapeutic implications. Oncotarget, 2017, 8(11):18456–18485. https://doi.org/10.18632/oncotarget.14472 PMID: 28060743 PMCID: PMC5392343

[5] Luo W, Cao Y, Liao C, Gao F. Diabetes mellitus and the incidence and mortality of colorectal cancer: a meta-analysis of 24 cohort studies. Colorectal Dis, 2012, 14(11):1307–1312. https://doi.org/10.1111/j.1463-1318.2012.02875.x PMID: 23046351

[6] Deng L, Gui Z, Zhao L, Wang J, Shen L. Diabetes mellitus and the incidence of colorectal cancer: an updated systematic review and meta-analysis. Dig Dis Sci, 2012, 57(6):1576–1585. https://doi.org/10.1007/s10620-012-2055-1 PMID: 22350783

[7] Krämer HU, Schöttker B, Raum E, Brenner H. Type 2 diabetes mellitus and colorectal cancer: meta-analysis on sex-specific differences. Eur J Cancer, 2012, 48(9):1269–1282. https://doi.org/10.1016/j.ejca.2011.07.010 PMID: 21889332

[8] Ben Q, Xu M, Ning X, Liu J, Hong S, Huang W, Zhang H, Li Z. Diabetes mellitus and risk of pancreatic cancer: a meta-analysis of cohort studies. Eur J Cancer, 2011, 47(13):1928–1937. https://doi.org/10.1016/j.ejca.2011.03.003 PMID: 21458985

[9] Elena JW, Steplowski E, Yu K, Hartge P, Tobias GS, Brotzman MJ, Chanock SJ, Stolzenberg-Solomon RZ, Arslan AA, Bueno-de-Mesquita HB, Helzlsouer K, Jacobs EJ, LaCroix A, Petersen G, Zheng W, Albanes D, Allen NE, Amundadottir L, Bao Y, Boeing H, Boutron-Ruault MC, Buring JE, Gaziano JM, Giovannucci EL, Duell EJ, Hallmans G, Howard BV, Hunter DJ, Hutchinson A, Jacobs KB, Kooperberg C, Kraft P, Mendelsohn JB, Michaud DS, Palli D, Phillips LS, Overvad K, Patel AV, Sansbury L, Shu XO, Simon MS, Slimani N, Trichopoulos D, Visvanathan K, Virtamo J, Wolpin BM, Zeleniuch-Jacquotte A, Fuchs CS, Hoover RN, Gross M. Diabetes and risk of pancreatic cancer: a pooled analysis from the Pancreatic Cancer Cohort Consortium. Cancer Causes Control, 2013, 24(1):13–25. https://doi.org/10.1007/s10552-012-0078-8 PMID: 23112111 PMCID: PMC3529822

[10] Everhart J, Wright D. Diabetes mellitus as a risk factor for pancreatic cancer. A meta-analysis. JAMA, 1995, 273(20): 1605–1609. PMID: 7745774

[11] Huxley R, Ansary-Moghaddam A, Berrington de González A, Barzi F, Woodward M. Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies. Br J Cancer, 2005, 92(11):2076–2083. https://doi.org/10.1038/sj.bjc.6602619 PMID: 15886696 PMCID: PMC2361795

[12] Tan J, You Y, Guo F, Xu J, Dai H, Bie P. Association of elevated risk of pancreatic cancer in diabetic patients: a systematic review and meta-analysis. Oncol Lett, 2017, 13(3):1247–1255. https://doi.org/10.3892/ol.2017.5586 PMID: 28454242 PMCID: PMC5403376

[13] Song S, Wang B, Zhang X, Hao L, Hu X, Li Z, Sun S. Long-term diabetes mellitus is associated with an increased risk of pancreatic cancer: a meta-analysis. PLoS One, 2015, 10(7): e0134321. https://doi.org/10.1371/journal.pone.0134321 PMID: 26222906 PMCID: PMC4519136

[14] Gao S, Yang WS, Gao J, Wang J, Xiang YB. [A meta-analysis of cohort studies on the association between diabetes and the risk of primary liver cancer]. Zhonghua Yu Fang Yi Xue Za Zhi, 2010, 44(8):711–716. PMID: 21055021

[15] Mantovani A, Targher G. Type 2 diabetes mellitus and risk of hepatocellular carcinoma: spotlight on nonalcoholic fatty liver disease. Ann Transl Med, 2017, 5(13):270. https://doi.org/10.21037/atm.2017.04.41 PMID: 28758096 PMCID: PMC5515814

[16] Wang C, Wang X, Gong G, Ben Q, Qiu W, Chen Y, Li G, Wang L. Increased risk of hepatocellular carcinoma in patients with diabetes mellitus: a systematic review and meta-analysis of cohort studies. Int J Cancer, 2012, 130(7):1639–1648. https://doi.org/10.1002/ijc.26165 PMID: 21544812

[17] Wang P, Kang D, Cao W, Wang Y, Liu Z. Diabetes mellitus and risk of hepatocellular carcinoma: a systematic review and meta-analysis. Diabetes Metab Res Rev, 2012, 28(2):109–122. https://doi.org/10.1002/dmrr.1291 PMID: 21898753

[18] Hardefeldt PJ, Edirimanne S, Eslick GD. Diabetes increases the risk of breast cancer: a meta-analysis. Endocr Relat Cancer, 2012, 19(6):793–803. https://doi.org/10.1530/ERC-12-0242 PMID: 23035011

[19] Boyle P, Boniol M, Koechlin A, Robertson C, Valentini F, Coppens K, Fairley LL, Boniol M, Zheng T, Zhang Y, Pasterk M, Smans M, Curado MP, Mullie P, Gandini S, Bota M, Bolli GB, Rosenstock J, Autier P. Diabetes and breast cancer risk: a meta-analysis. Br J Cancer, 2012, 107(9):1608–1617. https://doi.org/10.1038/bjc.2012.414 PMID: 22996614 PMCID: PMC3493760

[20] Peairs KS, Barone BB, Snyder CF, Yeh HC, Stein KB, Derr RL, Brancati FL, Wolff AC. Diabetes mellitus and breast cancer outcomes: a systematic review and meta-analysis. J Clin Oncol, 2011, 29(1):40–46. https://doi.org/10.1200/JCO.2009.27.3011 PMID: 21115865 PMCID: PMC3055858

[21] Zhou Y, Zhang X, Gu C, Xia J. Diabetes mellitus is associated with breast cancer: systematic review, meta-analysis, and *in silico* reproduction. PanMinerva Med, 2015, 57(3):101–108. PMID: 25971328

[22] Friberg E, Orsini N, Mantzoros CS, Wolk A. Diabetes mellitus and risk of endometrial cancer: a meta-analysis. Diabetologia, 2007, 50(7):1365–1374. https://doi.org/10.1007/s00125-007-0681-5 PMID: 17476474

[23] Saed L, Varse F, Baradaran HR, Moradi Y, Khateri S, Friberg E, Khazaei Z, Gharahjeh S, Tehrani S, Sioofy-Khojine AB, Najmi Z. The effect of diabetes on the risk of endometrial cancer: an updated a systematic review and meta-analysis. BMC Cancer, 2019, 19(1):527. https://doi.org/10.1186/s12885-019-5748-4 PMID: 31151429 PMCID: PMC6544993

[24] Barone BB, Yeh HC, Snyder CF, Peairs KS, Stein KB, Derr RL, Wolff AC, Brancati FL. Long-term all-cause mortality in cancer patients with preexisting diabetes mellitus: a systematic review and meta-analysis. JAMA, 2008, 300(23):2754–2764. https://doi.org/10.1001/jama.2008.824 PMID: 19088353 PMCID: PMC3093051

[25] Ma Y, Yang Y, Wang F, Zhang P, Shi C, Zou Y, Qin H. Obesity and risk of colorectal cancer: a systematic review of prospective studies. PLoS One, 2013, 8(1):e53916. https://doi.org/10.1371/journal.pone.0053916 PMID: 23349764 PMCID: PMC3547959

[26] Ranc K, Jørgensen ME, Friis S, Carstensen B. Mortality after cancer among patients with diabetes mellitus: effect of diabetes duration and treatment. Diabetologia, 2014, 57(5):927–934. https://doi.org/10.1007/s00125-014-3186-z PMID: 24633676

[27] Stein KB, Snyder CF, Barone BB, Yeh HC, Peairs KS, Derr RL, Wolff AC, Brancati FL. Colorectal cancer outcomes, recurrence, and complications in persons with and without diabetes mellitus: a systematic review and meta-analysis. Dig Dis Sci, 2010, 55(7):1839–1851. https://doi.org/10.1007/s10620-009-0944-8 PMID: 19731028 PMCID: PMC3093049

[28] Peeters PJHL, Bazelier MT, Leufkens HGM, de Vries F, De Bruin ML. The risk of colorectal cancer in patients with type 2 diabetes: associations with treatment stage and obesity. Diabetes Care, 2015, 38(3):495–502. https://doi.org/10.2337/dc14-1175 PMID: 25552419

[29] Larsson SC, Wolk A. Obesity and colon and rectal cancer risk: a meta-analysis of prospective studies. Am J Clin Nutr, 2007, 86(3):556–565. https://doi.org/10.1093/ajcn/86.3.556 PMID: 17823417

[30] Dobbins M, Decorby K, Choi BCK. The association between obesity and cancer risk: a meta-analysis of observational studies from 1985 to 2011. ISRN Prev Med, 2013, 2013:680536. https://doi.org/10.5402/2013/680536 PMID: 24977095 PMCID: PMC4062857

[31] Lin J, Zhang SM, Cook NR, Rexrode KM, Lee IM, Buring JE. Body mass index and risk of colorectal cancer in women (United States). Cancer Causes Control, 2004, 15(6):581–589. https://doi.org/10.1023/B:CACO.0000036168.23351.f1 PMID: 15280637

[32] Dai Z, Xu YC, Niu L. Obesity and colorectal cancer risk: a meta-analysis of cohort studies. World J Gastroenterol, 2007, 13(31): 4199–4206. https://doi.org/10.3748/wjg.v13.i31.4199 PMID: 17696248 PMCID: PMC4250618

[33] Moghaddam AA, Woodward M, Huxley R. Obesity and risk of colorectal cancer: a meta-analysis of 31 studies with 70,000 events. Cancer Epidemiol Biomarkers Prev, 2007, 16(12):2533–2547. https://doi.org/10.1158/1055-9965.EPI-07-0708 PMID: 18086756

[34] Opferman JT, Kothari A. Anti-apoptotic BCL-2 family members in development. Cell Death Differ, 2018, 25(1):37–45. https://doi.org/10.1038/cdd.2017.170 PMID: 29099482 PMCID: PMC5729530

[35] Warren CFA, Wong-Brown MW, Bowden NA. BCL-2 family isoforms in apoptosis and cancer. Cell Death Dis, 2019, 10(3): 177. https://doi.org/10.1038/s41419-019-1407-6 PMID: 30792387 PMCID: PMC6384907

[36] Fridman JS, Lowe SW. Control of apoptosis by p53. Oncogene, 2003, 22(56):9030–9040. https://doi.org/10.1038/sj.onc.1207116 PMID: 14663481

[37] Haupt S, Berger M, Goldberg Z, Haupt Y. Apoptosis – the p53 network. J Cell Sci, 2003, 116(Pt 20):4077–4085. https://doi.org/10.1242/jcs.00739 PMID: 12972501

[38] Gurzov EN, Eizirik DL. Bcl-2 proteins in diabetes: mitochondrial pathways of β-cell death and dysfunction. Trends Cell Biol, 2011, 21(7):424–431. https://doi.org/10.1016/j.tcb.2011.03.001 PMID: 21481590

[39] Gross A. BCL-2 family proteins as regulators of mitochondria metabolism. Biochim Biophys Acta, 2016, 1857(8):1243–1246. https://doi.org/10.1016/j.bbabio.2016.01.017 PMID: 26827940

[40] Gross A, Katz SG. Non-apoptotic functions of BCL-2 family proteins. Cell Death Differ, 2017, 24(8):1348–1358. https://doi.org/10.1038/cdd.2017.22 PMID: 28234359 PMCID: PMC5520452

[41] Puzio-Kuter AM. The role of p53 in metabolic regulation. Genes Cancer, 2011, 2(4):385–391. https://doi.org/10.1177/1947601911409738 PMID: 21779507 PMCID: PMC3135642

[42] Flöter J, Kaymak I, Schulze A. Regulation of metabolic activity by p53. Metabolites, 2017, 7(2):21. https://doi.org/10.3390/metabo7020021 PMID: 28531108 PMCID: PMC5487992

[43] Yemelyanova A, Vang R, Kshirsagar M, Lu D, Marks MA, Shih IeM, Kurman RJ. Immunohistochemical staining patterns of p53 can serve as a surrogate marker for *TP53* mutations in ovarian carcinoma: an immunohistochemical and nucleotide sequencing analysis. Mod Pathol, 2011, 24(9):1248–1253. https://doi.org/10.1038/modpathol.2011.85 PMID: 21552211

[44] Moll UM, Petrenko O. The MDM2–p53 interaction. Mol Cancer Res, 2003, 1(14):1001–1008. PMID: 14707283

[45] Hwang HJ, Nam SK, Park H, Park Y, Koh J, Na HY, Kwak Y, Kim WH, Lee HS. Prediction of *TP53* mutations by p53 immunohistochemistry and their prognostic significance in gastric cancer. J Pathol Transl Med, 2020, 54(5):378–386. https://doi.org/10.4132/jptm.2020.06.01 PMID: 32601264 PMCID: PMC7483024

[46] Murnyák B, Hortobágyi T. Immunohistochemical correlates of *TP53* somatic mutations in cancer. Oncotarget, 2016, 7(40): 64910–64920. https://doi.org/10.18632/oncotarget.11912 PMID: 27626311 PMCID: PMC5323125

[47] Fleming M, Ravula S, Tatishchev SF, Wang HL. Colorectal carcinoma: pathologic aspects. J Gastrointest Oncol, 2012, 3(3):153–173. https://doi.org/10.3978/j.issn.2078-6891.2012.030 PMID: 22943008 PMCID: PMC3418538

[48] Russo A, Bazan V, Iacopetta B, Kerr D, Soussi T, Gebbia N, TP53–CRC Collaborative Study Group. The *TP53* Colorectal Cancer International Collaborative Study on the prognostic and predictive significance of *p53* mutation: influence of tumor site, type of mutation, and adjuvant treatment. J Clin Oncol, 2005, 23(30):7518–7528. https://doi.org/10.1200/JCO.2005.00.471 PMID: 16172461

[49] Flamini G, Curigliano G, Ratto C, Astone A, Ferretti G, Nucera P, Sofo L, Sgambato A, Boninsegna A, Crucitti F, Cittadini A. Prognostic significance of cytoplasmic p53 over-expression in colorectal cancer. An immunohistochemical analysis. Eur J Cancer, 1996, 32A(5):802–806. https://doi.org/10.1016/0959-8049(95)00625-7 PMID: 9081357

[50] Flohil CC, Janssen PA, Bosman FT. Expression of Bcl-2 protein in hyperplastic polyps, adenomas, and carcinomas of the colon. J Pathol, 1996, 178(4):393–397. https://doi.org/10.1002/(SICI)1096-9896(199604)178:4<393::AID-PATH488>3.0.CO;2-G PMID: 8691316

[51] Zhao DP, Ding XW, Peng JP, Zheng YX, Zhang SZ. Prognostic significance of bcl-2 and p53 expression in colorectal carcinoma. J Zhejiang Univ Sci B, 2005, 6(12):1163–1169. https://doi.org/10.1631/jzus.2005.B1163 PMID: 16358373 PMCID: PMC1390638

[52] Wang P, Liang J, Wang Z, Hou H, Shi L, Zhou Z. The prognostic value of p53 positive in colorectal cancer: a retrospective cohort study. Tumour Biol, 2017, 39(5):1010428317703651. https://doi.org/10.1177/1010428317703651 PMID: 28468582

[53] Garufi A, D'Orazi G. High glucose dephosphorylates serine 46 and inhibits p53 apoptotic activity. J Exp Clin Cancer Res, 2014, 33(1):79. https://doi.org/10.1186/s13046-014-0079-4 PMID: 25260780 PMCID: PMC4181716

[54] Hoshino A, Ariyoshi M, Okawa Y, Kaimoto S, Uchihashi M, Fukai K, Iwai-Kanai E, Ikeda K, Ueyama T, Ogata T, Matoba S. Inhibition of p53 preserves Parkin-mediated mitophagy and pancreatic *β*-cell function in diabetes. Proc Natl Acad Sci U S A, 2014, 111(8):3116–3121. https://doi.org/10.1073/pnas.1318951111 PMID: 24516131 PMCID: PMC3939874

[55] Tinahones FJ, Coín Aragüez L, Murri M, Oliva Olivera W, Mayas Torres MD, Barbarroja N, Gomez Huelgas R, Malagón MM, El Bekay R. Caspase induction and BCL2 inhibition in human adipose tissue: a potential relationship with insulin signaling alteration. Diabetes Care, 36(3):513–521. https://doi.org/10.2337/dc12-0194 PMID: 23193206 PMCID: PMC3579349

[56] Manne U, Myers RB, Moron C, Poczatek RB, Dillard S, Weiss H, Brown D, Srivastava S, Grizzle WE. Prognostic significance of Bcl-2 expression and p53 nuclear accumulation in colorectal adenocarcinoma. Int J Cancer, 1996, 74(3):346–358. https://doi.org/10.1002/(sici)1097-0215(19970620)74:3<346::aid-ijc19>3.0.co;2-9 PMID: 9221816

[57] Kaklamanis L, Savage A, Whitehouse R, Doussis-Anagnostopoulou I, Biddolph S, Tsiotos P, Mortensen N, Gatter KC, Harris AL. Bcl-2 protein expression: association with p53 and prognosis in colorectal cancer. Br J Cancer, 1998, 77(11):1864–1869. https://doi.org/10.1038/bjc.1998.310 PMID: 9667660 PMCID: PMC2150362

[58] Menezes HL, Jucá MJ, Gomes EG, Nunes BL, Costa HO, Matos D. Analysis of the immunohistochemical expressions of p53, bcl-2 and Ki-67 in colorectal adenocarcinoma and their correlations with the prognostic factors. Arq Gastroenterol, 2010, 47(2):141–147. https://doi.org/10.1590/s0004-28032010000200005 PMID: 20721457

[59] Watson AJ, Merritt AJ, Jones LS, Askew JN, Anderson E, Becciolini A, Balzi M, Potten CS, Hickman JA. Evidence of reciprocity of bcl-2 and p53 expression in human colorectal adenomas and carcinomas. Br J Cancer, 1996, 73(8):889–895. https://doi.org/10.1038/bjc.1996.178 PMID: 8611422 PMCID: PMC2075819

[60] Goussia AC, Ioachim E, Agnantis NJ, Mahera M, Tsianos EV. Bcl-2 expression in colorectal tumours. Correlation with p53, mdm-2, Rb proteins and proliferation indices. Histol Histopathol, 2000, 15(3):667–672. https://doi.org/10.14670/HH-15.667 PMID: 10963109

[61] Bosari S, Viale G, Roncalli M, Graziani D, Borsani G, Lee AK, Coggi G. *p53* gene mutations, p53 protein accumulation and compartmentalization in colorectal adenocarcinoma. Am J Pathol, 1995, 147(3):790–798. PMID: 7677190 PMCID: PMC1870957

[62] Zeng ZS, Sarkis AS, Zhang ZF, Klimstra DS, Charytonowicz E, Guillem JG, Cordon-Cardo C, Cohen AM. p53 nuclear over-expression: an independent predictor of survival in lymph node-positive colorectal cancer patients. J clin Oncol, 1994, 12(10):2043–2050. https://doi.org/10.1200/JCO.1994.12.10.2043 PMID: 7931472

[63] Baretton GB, Diebold J, Christoforis G, Vogt M, Müller C, Dopfer K, Schneiderbanger K, Schmidt M, Löhrs U. Apoptosis and immunohistochemical bcl-2 expression in colorectal adenomas and carcinomas. Cancer, 1996, 77(2):255–264. https://doi.org/10.1002/(SICI)1097-0142(19960115)77:2<255::AID-CNCR6>3.0.CO;2-L PMID: 8625232

[64] Mills KT, Bellows CF, Hoffman AE, Kelly TN, Gagliardi G. Diabetes mellitus and colorectal cancer prognosis: a meta-analysis. Dis Colon Rectum, 2013, 56(11):1304–1319. https://doi.org/10.1097/DCR.0b013e3182a479f9 PMID: 24105007 PMCID: PMC3800045

[65] Lee J, Meyerhardt JA, Giovannucci E, Jeon JY. Association between body mass index and prognosis of colorectal cancer: a meta-analysis of prospective cohort studies. PLoS One, 2015, 10(3):e0120706. https://doi.org/10.1371/journal.pone.0120706 PMID: 25811460 PMCID: PMC4374868

[66] Zhou XH, Qiao Q, Zethelius B, Pyörälä K, Söderberg S, Pajak A, Stehouwer CD, Heine RJ, Jousilahti P, Ruotolo G, Nilsson PM, Calori G, Tuomilehto J; DECODE Study Group. Diabetes, prediabetes and cancer mortality. Diabetologia, 2010, 53(9):1867–1876. https://doi.org/10.1007/s00125-010-1796-7 PMID: 20490448

[67] Vairaktaris E, Kalokerinos G, Goutzanis L, Spyridonidou S, Vassiliou S, Derka S, Nkenke E, Yapijakis C, Vylliotis A, Lazaris A, Patsouris E. Diabetes alters expression of p53 and c-myc in different stages of oral oncogenesis. Anticancer Res, 2007, 27(3B):1465–1473. PMID: 17595763

[68] Vairaktaris E, Kalokerinos G, Goutzanis L, Yapijakis C, Derka S, Vassiliou S, Spyridonidou S, Vylliotis A, Nkenke E, Lazaris A, Patsouris E. Diabetes enhances cell proliferation but not Bax/Bcl-2-mediated apoptosis during oral oncogenesis. Int J Oral Maxillofac Surg, 2007, 37(1):60–65. https://doi.org/10.1016/j.ijom.2007.06.012 PMID: 17825529

[69] Jung DS, Lee SH, Kwak SJ, Li JJ, Kim DH, Nam BY, Kang HY, Chang TI, Park JT, Han SH, Yoo TH, Kang SW. Apoptosis occurs differentially according to glomerular size in diabetic kidney disease. Nephrol Dial Transplant, 2012, 27(1):259–266. https://doi.org/10.1093/ndt/gfr301 PMID: 21652547

[70] Kern TS, Du Y, Miller CM, Hatala DA, Levin LA. Overexpression of Bcl-2 in vascular endothelium inhibits the microvascular lesions of diabetic retinopathy. Am J Pathol, 2010, 176(5):2550–2558. https://doi.org/10.2353/ajpath.2010.091062 PMID: 20363911 PMCID: PMC2861119

[71] Molchadsky A, Ezra O, Amendola PG, Krantz D, Kogan-Sakin I, Buganim Y, Rivlin N, Goldfinger N, Folgiero V, Falcioni R, Sarig R, Rotter V. p53 is required for brown adipogenic differentiation and has a protective role against diet-induced obesity. Cell Death Differ, 2013, 20(5):774–783. https://doi.org/10.1038/cdd.2013.9 PMID: 23412343 PMCID: PMC3619245

[72] Lee P, Vousden KH, Cheung EC. TIGAR, TIGAR, burning bright. Cancer Metab, 2014, 2(1):1. https://doi.org/10.1186/2049-3002-2-1 PMID: 24383451 PMCID: PMC3892023

Corresponding author

Emilia Rusu, Associate Professor, MD, PhD, Department of Diabetes, Nutrition and Metabolic Diseases, Carol Davila University of Medicine and Pharmacy, 37 Dionisie Lupu Street, Sector 2, 020021 Bucharest, Romania; Phone +40742–959 946, e-mail: emiliarusumd@yahoo.com

Received: May 11, 2020 *Accepted: December 22, 2022*