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BCL2 and P53 immunoexpression in colorectal carcinoma

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Tenya Tariq Abdulhameed * Salah Abubakir Ali*

Abstract

Background and objective: The products of BCL2 and P53 genes are involved in the regulation of proliferation and apoptosis and have been associated with prognosis in several malignancies, including colorectal carcinoma. This study aimed to investigate the expression of antiapoptotic BCL2 protein in colorectal carcinoma and to examine its association with one of the important mediators of apoptosis (P53 protein) and with clinicopathological factors, and to assess the prognostic value of the combined BCL2 /P53 phenotypes by studying their relation with the two most important prognostic factors of grading and staging.

Methods: A retrospective and prospective study was carried out from the period of August 2010 to June 2011. Sixty eight formalin-fixed, paraffin-embedded blocks of colorectal carcinoma cases were evaluated regarding BCL2 expression, P53 nuclear accumulation and their concomitant expression using immunohistochemistry by Dako Cytomation.LSAB + System-HRP.

Results: The study shows that 29.41% of the colorectal tumors were positive for BCL2 protein expression and associated with earlier stage tumors (P = 0.021), while 72.06% were positive for P53 protein expression and associated with later stage tumors (P = 0.006) and male gender (P = 0.005). There was a trend toward an inverse correlation between BCL2 and P53 expression (P = 0.0013). Tumors that did not express detectable levels of BCL2 but exhibited nuclear accumulation of P53 were most common and associated with later stage tumors (P = 0.003).

Conclusion: Concomitant assessment of both BCL2 and P53 status may be valuable in predicting the aggressiveness of tumors in patients with colorectal carcinomas.

Keywords: Colorectal carcinoma, BCL2, P53.

Introduction

Colorectal cancer (CRC) is the commonest malignancy in the gastrointestinal tract,[1] accounts for about 10% of all cancers,[2] and it is the fourth leading cause of cancer death in the world.[1] BCL2 was first identified as an antiapoptotic proto-oncogene in non-Hodgkin's follicular lymphoma cells,[3] and is located at chromosome t(14;18). BCL2 as a key regulator of apoptosis, promotes cell survival by inhibiting factors needed for the activation of the caspases.[4] P53 mutations are a hallmark in CRC progression, leading to stabilization of protein and elevated immunohistochemical staining. The ability of P53 to serve as a prognostic biomarker has been extensively studied in CRC, with most studies focusing on increased immunohistochemical staining.[5]

Methods

Sixty eight formalin-fixed, paraffin-embedded blocks of colorectal carcinoma cases were collected during the period from August 2010 to June 2011. The anatomic subsets were categorized according to International Classification of Diseases for Oncology, third edition (ICD-0 -3) topography codes and were grouped as noted by Wu et al.[6] Tumor typing was performed according to recommendation of

* Department of Pathology, College of medicine, Hawler Medical University, Erbil, Iraq.

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WHO as mucinous and non mucinous adenocarcinoma,[7] according to the recommendations of CAP, well and moderately differentiated conventional adenocarcinomas were grouped together and called low grade malignancy, while poorly differentiated and undifferentiated conventional adenocarcinomas and non mucinous adenocarcinomas were called high grade malignancy.[8] Stage grouping were made according to 7th Edition of AJCC Cancer Staging Manual as stage I, II, and III[9] without evaluations of stage IV tumors, because we were unable to asses pathological metastasis (pM). Hence, our cases regarding staging were divided into three groups, group one (G1): tumors invading muscularis propria without lymph node metastasis, group two (GII): tumors invading beyond muscularis propria without lymph node metastasis, group three (GIII): tumors with one or more lymph node metastasis at any level of invasion. Dako Cytomation LSAB + System –HRP Staining protocol was used for immunostaining to detect BCL2 and P53 expression and was applied to formalin- fixed, paraffin embedded tissues, with each batch of stain positive and negative control sections were incubated. Negative controls were obtained by omitting the primary antibody and by using N-Universal negative control. Positive controls tissues specimens for P53 were prepared using invasive ductal carcinoma of the breast already stained for P53 and contained more than 50% tumor cells. For BCL2, positive controls were prepared using thymus gland. Lymphocytes in lamina propria of the tumors and cells in base of the crypts were used as internal positive control for BCL2. Positive expression of BCL2 give cytoplasmic staining while that of P53 give nuclear staining of brown color. Positive cells were determined by counting 1000 tumor cells, all significantly stained cells were considered positive and divided by 10 to decide whether the case was positive. At least 10 HPF were examined (with 400x) in each case for the purpose of scoring. The extent of BCL2 and P53 immunostaining was assessed as follows:

* Positive: when BCL2 and P53 indices were $\geq$ 5%.
* Negative: when BCL2 and P53 indices were < 5%.

Statistical Analysis was done by using statistical package for social sciences (SPSS, version 18) computer software. Cross tables and associations between different variables were measured by using Chi-square and Fisher's exact tests. $P < 0.05$ was considered as the level of significance (S), $P < 0.001$ was considered as the level of highly significance (HS) and any value more than 0.05 was considered to be non significant (NS).

Results

The age of the patients ranged from 22 to 83 years. The mean age at surgery (±SD) was 52.04 (±15.19) years, 46 cases were males with a male to female ratio of 2.1:1. Most of the tumors were located in the distal colon and rectum, of non mucinous adenocarcinomas type and of low grade malignancy. The clinicopathological characteristics of the studied cases are shown in Table 1. Brown cytoplasmic reaction product was detected in 20 out of 68 cases of CRC (29.41%), 49 out of 68 cases showed P53 immunreactivity (72.06%), giving brown nuclear staining pattern. Staining was confined to malignant nuclei and was never found in adjacent normal mucosa. There were no statistical significant correlation between age, tumor location, tumor type and tumor grade with neither BCL2 nor P53 protein expression. The only statistical significant correlation was that between BCL2 expression and tumor staging. The extent of BCL2 expression by tumor cells decreased in a statistically significant way ($P = 0.021$). There was a statistical significant correlation between P53 expression and gender distribution ($P = 0.005$). There was also a statistical significant correlation between P53 expression and tumor staging, (p value=0.006). As shown in Table 2, there was a trend toward an inverse correlation

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between BCL2 and P53 proteins by that out of the 20 cases which were positive for BCL2, 11 cases were negative for P53 and 9 cases were positive for P53, while out of 48 cases which were negative for BCL2, the majority (40 cases) were positive for P53 and only 8 cases were negative for P53. The correlation between BCL2 and P53 was statistically significant (P = 0.0013). The correlation between BCL2 and P53 immunoreactivity gave four possible immunophenotypes as shown in Figure 1.

Table 1: Numbers and percentages of different clinicopathological variables of the studied cases.

Clinicopathological variables		No.	%
Gender	Female	22	32.35
	Male	46	67.64
Age	<40 Y	17	25
	40-60 Y	32	47.05
	>60Y	19	27.94
Site	Proximal colon	25	36.76
	Distal colorectal	43	63.23
Histological type	Mucinous	13	19.11
	Non mucinous	55	80.88
Grading	Low	50	73.52
	High	18	26.47
Staging	I	17	25
	II	13	19.11
	III	38	55.88

Table 2: Correlation between BCL2 and P53 expression.

P53		BCL2 positive (20 cases) No. (%)	negative (48 cases) No. (%)	p value
Positive (49 cases)	No. (%)	9 (13.23)	40 (58.82)	0.0013
Negative (19 cases)	No. (%)	11 (16.10)	8 (11.76)	

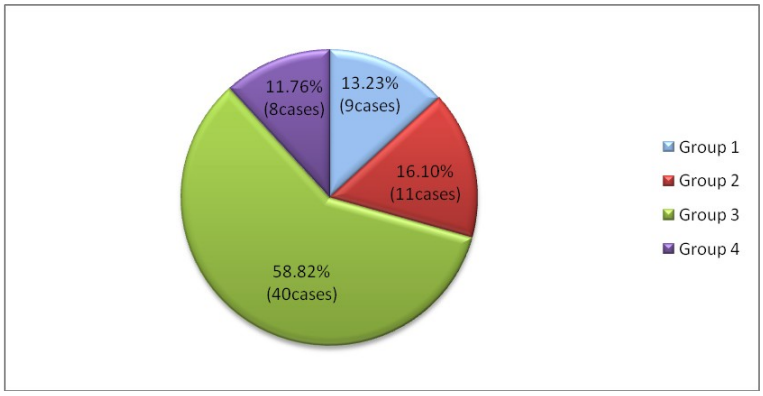

Figure 1: BCL2 / P53 combinations immunophenotypes.

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There were no statistical significant correlations between the groups and grading of cases, (P = 0.17, >0.99, 0.14, >0.99, respectively). Table 3 shows the correlation between group three immunophenotype with staging of the studied cases. The correlation was statistically significant (P = 0.003).

Table 3: Correlation between group three immunophenotype and staging.

Staging	Total No.	BCL2 negative / P53 positive		p value
		No.	%	
I	17	7	41.17	0.003
II	13	4	30.76	
III	38	29	76.31	

Discussion

Studies indicate that tumor stage is the most important prognostic variable in CRC, despite this fact, considerable stage-independent variability in clinical outcome is observed that may be a consequence of altered rates of apoptosis and cell proliferation.[10] In this study, the immunohistochemical expressions of BCL2 and P53 was investigated separately and together in the colorectal tumors. The results were correlated with the two most important prognostic factors of grading and staging. There was a clear correlation in our study between BCL2 expression and staging of the tumors in that most of positive BCL2 cases were in stage I and II. Reduced BCL2 immunoreactivity on progression to invasive carcinomas are in accordance with the results of previous studies,[11,12,14] which can be explained by that BCL2 overexpression probably occurs as an early event of colonic carcinogenesis and gradually diminishes during the evolution to CRC[12]. These findings may explain our data in which low levels of BCL2 were associated with an advanced stage than BCL2 positive tumors. BCL2 can inhibit the transition of cells from resting (G0) to a cycling phase and in that way reduce their proliferation and turnover, possibly owing to an anti-proliferative domain, distinct from the domains required for its antiapoptotic activity.[13,14] In our study, the vast majority of cases in stage III stained positive for P53 and the relation was significant, suggesting that P53 mutation may be a late event in the pathogenesis of CRC and that increased incidence of mutation of P53 gene occurring during colorectal carcinogenesis[15]. The relation between BCL2 and P53 have been demonstrated by many investigators in that mutant P53 can down-regulate (silence) BCL2 gene expression[16] and that BCL2 overexpression appears to occur before the development of P53 genetic alterations.[17] It has been postulated that BCL2 expression increases cell life span, increasing the risk of acquiring other alterations, such as chromosomal abnormalities and viral infections, and resulting in malignant transformation or tumor progression.[18] In addition when cell differentiation was correlated with P53, no statistical significance was found. Among the present study, the immunohistochemical expression of BCL2 was not significantly correlated with the degree of cell differentiation, and this has also been shown in most other studies.[11,14] Despite these results, a clear difference was observed between the P53 and BCL2 expressions, in comparison with tumor expression. The majority of our cases showed BCL2 negative/P53 positive immunophenotype, this pattern showed a significant correlation with staging, while when we evaluated the combined effects of these related cell cycle proteins in regard to grading, as for each antigen alone, none of the combined phenotypes showed significant relation to grading. It appears logical that combined analysis of P53 with BCL2 should prove greater than the sum of its parts as P53 is a mediator of apoptosis via the BCL2/Bax pathway; it promotes apoptosis either by down-regulation of BCL2 itself (the counteracting twin of Bax) or by concomitant up-regulation of the

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apoptosis-promoting protein Bax.[11,19] In this study we have found tumors that express a combination of negative cytoplasmic BCL2 expression and positive nuclear P53 expression in CRC were in the advanced stage, implying that these tumors form an entity with a more aggressive neoplastic transformation pathway and defines a population of patients with a clinically more aggressive phenotype and a subset of patients for whom more aggressive adjuvant treatment is indicated.

Conclusion

Concomitant assessment of both BCL2 and P53 status may be valuable in predicting the aggressiveness of tumors in patients with colorectal carcinomas.

Conflicts of interest

The authors report no conflicts of interest.

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