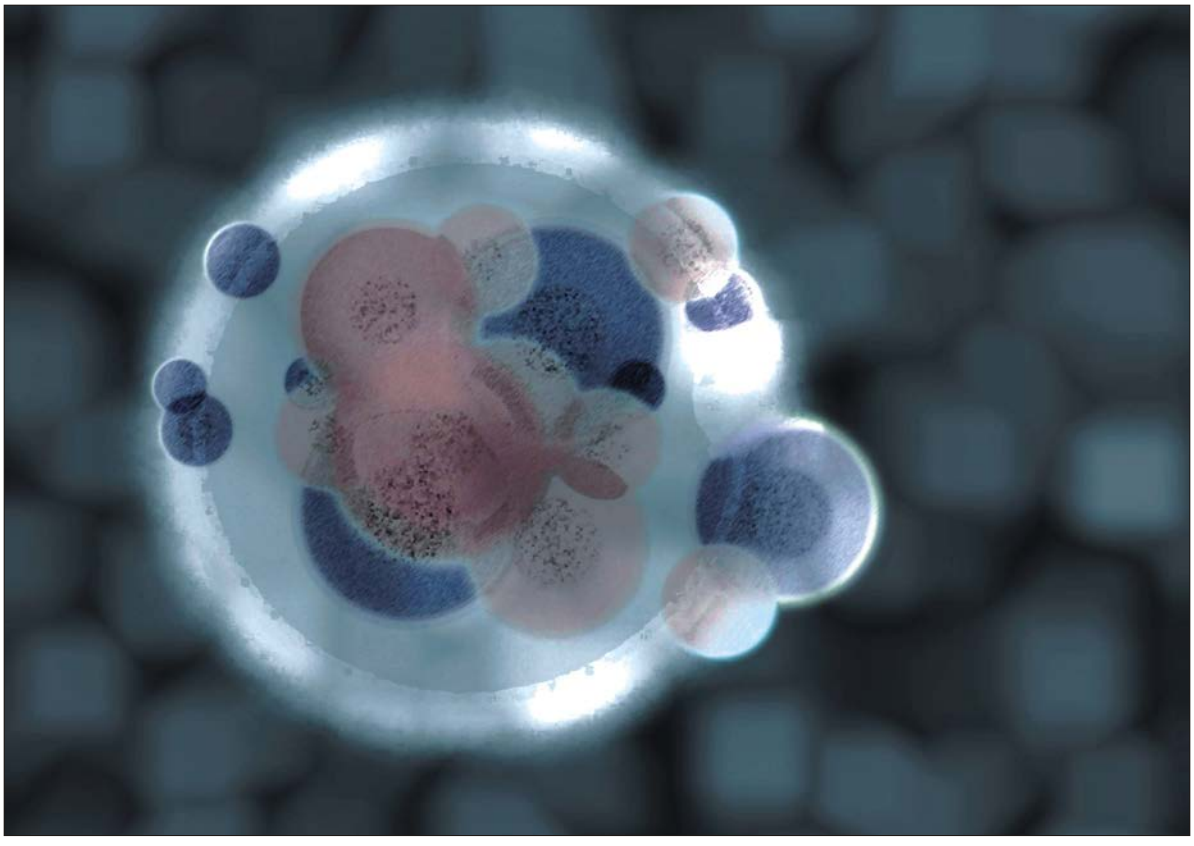

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Prevalence of p53 and p21 Expression in Colorectal Cancer: A Histopathologic Study from Iran

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Abstract: Disruption or inactivation of p53 and p21 proteins' expression may lead to colorectal adenocarcinoma. In addition, prognosis of the patients and their response to chemotherapy depends on these proteins' expression. Therefore, in this study, we attempted to evaluate the prevalence of these proteins' expression in colorectal adenocarcinoma and their correlation with pathological parameters. We have studied immunostained-preserved tissues of 70 patients with colorectal adenocarcinoma who have tolerated colectomy from 2003 to 2010. Specimens were evaluated for the expression of p53 and p21 proteins. Over-expression of p53 and p21 proteins were seen in 52.9 and 47.1% of the patients, respectively. There was no correlation between over-expression of p53 and p21 with the pathological parameters. A significant association was found between p21 and tumor differentiation ($p<0.05$). High expression of p21 was also found in well-differentiated tumors. Also, the absence of correlation between p21 expression and p53 status was found ($p<0.05$). The results of our study indicate that p53 protein over-expression plays an important role in progression of colorectal cancer. p21 may also play an important role in differentiation of tumor cells. It also seems that p21 expression induction occurs in a p53-independent pathway in colorectal cancer.

Key words: p53, p21, colorectal cancer, immunohistochemistry

INTRODUCTION

Colorectal Cancer (CRC) is considered to be the third most common cancer and the second leading cause of death from cancer in western countries (Haggar and Boushey, 2009). Using biomarkers became one of the methods of choice for the detection and diagnosis of neoplastic diseases (Azarhoush *et al*., 2006; Galal *et al*., 2009; Ghaleb and Alkaladi, 2011; Rmali *et al*., 2006).

Genetic mutations are the molecular cause of colorectal cancers (3). Chromosome 17 is involved in most cases of CRC (Golmohammadi *et al*., 2005) and p53 gene is located on the short arm of chromosome 17. Derangement or inactivation of the p53 protein may lead to cancer (Azarhoush *et al*., 2006; Golmohammadi *et al*., 2007). The Waf1/Cip1 gene, located on chromosome 6, codes the p21 protein. In colorectal cancer, there is an inverse correlation between the prevalence of p21 protein and some properties of the tumor, including the stage of the tumor, the depth of invasion and lymph node metastasis. The survival and prognosis of patients in the presence of this protein is favorable (Pasz-Walczak *et al*., 2001; Zirbes *et al*., 2000). Simultaneous presence of p21 and normal p53 proteins is associated with increased sensitivity of the tumor to chemotherapy and radiotherapy (Girlando *et al*., 1999).

The prevalence of p53 and p21 proteins is very different among patients with colorectal cancer from country to country. Prevalence of p53 protein ranges from 43% in China (Zhao *et al*., 2005) to 47% in Switzerland (Bouzourene *et al*., 2000), from 70% in Poland (Paluszkiewicz *et al*., 2004; Pasz-Walczak *et al*., 2001) to 82.1% in Turkey (Erhan *et al*., 2002) and from 34-52% in studies of Iranian patients (Ghavam-Nasiri *et al*., 2007; Golmohammadi *et al*., 2007). Prevalence of p21 is also differs between 26 and 39% among various studies (Noske *et al*., 2009; Pasz-Walczak *et al*., 2001; Schwandner *et al*., 2002).

The purpose of this study is to survey the prevalence of p21 and p53 proteins among patients with colorectal adenocarcinoma in the city of Zahedan, Iran, between 2003 and 2010 and to correlate the prevalence of those proteins with the pathologic properties of the cancers.

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MATERIALS AND METHODS

This study examined tumor tissue specimens from 70 patients with colorectal adenocarcinoma who tolerated colectomy surgery. The specimens were collected from the pathology department archives of hospitals in Zahedan, Iran, from 2003 to 2010. Our study adhered to codes 17 and 20 adopted by the Iranian ethics committee for medical research.

Demographic data collected from the patients' files, archived in the pathology departments, included the patient's age, sex, tumor site; (1) Proximal region (cecum, ascending colon or transverse colon) and (2) Distal region (descending colon, sigmoid colon or rectum) and pathologic properties including the type, differentiation grade and stage of the tumor. The specimens divided into three groups based on differentiation grade (well, moderate and poor) and into four groups based on the Duck staging criteria (A, B, C and D).

Our inclusion criteria were: (1) Patients with colorectal cancer diagnosis between years 2003-2010 who tolerated colectomy surgery, (2) Availability of pathology report with definite diagnosis of colorectal adenocarcinoma and (3) Availability of paraffin blocks archive in pathology department.

The exclusion criteria were as follows: (1) Final pathologic report revealed diagnosis other than colorectal adenocarcinoma and (2) Lack of availability of paraffin blocks archive in pathology department.

The specimens were stained using Immunohistochemistry (IHC) techniques. After staining the specimens, we identified the immunoreactivity with the estimated percentage of tumor cell nuclei positive for p53 and p21 proteins. The specimens were considered "positive" if = 5% of the cells were stained; if <5% were stained, the specimen considered "Negative".

After microscopic examination of the specimens, the status of p53 and p21 proteins were recorded on the information form.

Data analysis: Data were analyzed statistically using SPSS.16 software. In correlating the prevalence of p53 and p21 proteins with a tumor's differentiation grade and its Duck stage, as well as the correlation between expression of the p53 and p21 proteins, we applied the Mann-Whitney test using a significance level of $p<0.05$.

RESULTS

IHC staining was applied to 70 specimens of colorectal adenocarcinoma acquired from October 2003 through October 2010. The patients' ages ranged from 11 to 86 years. Of the 70 patients, 37 (52.9%) were male and 33 (47.1%) were female; 15 (21.4%) of the patients were under 40 years old and 55 (78.6%) of them were 40 years old or older (Table 1).

Table 2 shows the correlation of over-expressed p53 and p21 proteins with the clinical and pathological properties of the tumors. Although, there was no statistically significant correlation between p53 and p21 staining and the patients' age, sex, site and type of tumor ($p>0.05$), p53 and p21 proteins were more frequently identified in distal areas of the colon (Table 2). Somewhat more than two-thirds of the specimens (67.1%) were the non-mucinous type of tumor and nearly one-third of the specimens (32.9%) were the mucinous type.

Table 2 shows that 38 specimens were well differentiated, 26 were moderately differentiated and 6 were poorly differentiated. Most of the specimens (64.1%) were in Duck stage B. p53 protein expression was seen in 66.7% of the specimens with poor differentiation and most of the p21-positive specimens were from well-differentiated tumors but there was no statistically significant correlation between these two protein expressions and the differentiation grade ($p>0.05$). There was also no significant correlation between the Duck stage of the tumor and expression prevalence of p21 and p53 proteins ($p>0.05$) (Table 2). Further, we found no significant correlation between the prevalence of p53 and p21 proteins ($p>0.05$).

Table 1: Distribution of p21 and p53 proteins in patients with colorectal adenocarcinoma patients for age and sex

Parameter	Case	p21 -ve	p21 +ve	p53 -ve	p53 +ve	p-value
Male	37 (52.9)	21 (56.8)	16 (48.5)	16 (48.5)	21 (56.8)	>0.05
Female	33 (47.1)	16 (43.2)	17 (51.5)	17 (51.5)	16 (43.2)	>0.05
Age (year)						
<40	15 (21.4)	8 (21.6)	7 (21.2)	8 (24.2)	7 (18.9)	>0.05
>40	55 (78.6)	29 (78.4)	26 (78.8)	25 (75.8)	30 (81.1)	>0.05

Percentage is given in parenthesis, data is considered significant at the level of $p<0.05$

Table 2: Distribution of p21 and p53 proteins in patients with colorectal adenocarcinoma according to location, type and degree of tumor differentiation and Duke's staging

Parameter	Case	p53 +ve	p53 -ve	p21 +ve	p21 -ve	p-value
Tumor site						
Proximal	31 (44.3)	13 (35.1)	18 (54.5)	15 (45.5)	16 (43.2)	>0.05
Distal	39 (55.7)	24 (64.9)	15 (45.5)	18 (54.5)	21 (56.8)	>0.05
Tumor type						
Mucinous	23 (32.9)	13 (35.1)	10 (30.3)	11 (33.3)	12 (34.2)	>0.05
Non-Mucinous	49 (67.1)	24 (69.4)	23 (69.7)	22 (66.7)	25 (67.6)	>0.05
Tumor differentiation						
Well	38 (54.3)	23 (62.2)	15 (45.5)	23 (69.7)	15 (40.5)	>0.05
Moderate	26 (37.1)	10 (27.0)	16 (48.5)	8 (24.2)	18 (48.6)	>0.05
Poor	6 (8.6)	4 (10.8)	2 (6.1)	2 (6.1)	4 (10.8)	>0.05
Duck stage						
A	1 (1.4)	1 (2.7)	0 (0.0)	1 (3)	0 (0)	>0.05
B	43 (61.4)	21 (56.8)	22 (66.7)	21 (63.6)	22 (59.5)	>0.05
C	19 (27.1)	12 (32.4)	7 (21.2)	8 (24.2)	11 (29.7)	>0.05
D	7 (10.0)	3 (8.1)	4 (12.1)	3 (9.1)	4 (10.8)	>0.05
Total	70 (100.0)	37 (100.0)	33 (100.0)	33 (100.0)	37 (100.0)	>0.05

Percentage is given in parenthesis, data is considered significant at the level of $p<0.05$

DISCUSSION

Present study revealed that expression of p53 and p21 proteins was positive in 52.9 and 47.1% of colorectal adenocarcinoma specimens, respectively. There was no significant correlation between p53 protein prevalence and tumor differentiation grade ($p>0.05$). Also, we found no significant correlation between p53 protein expression and the patient's age, sex and site of the tumor ($p>0.05$).

In this study, the prevalence of p53 protein in patients with colorectal adenocarcinoma agrees with studies done in Iran, China and Switzerland. The prevalence of p53 was 43% in China (Zhao *et al*., 2005) and 47% in Switzerland (Bouzourene *et al*., 2000); in Iran, the prevalence ranged from 34 to 75.9% (Bidgoli *et al*., 2007; Erhan *et al*., 2002; Ghavam-Nasiri *et al*., 2007; Paluszkiewicz *et al*., 2004) but it was 70% in Poland (Pasz-Walczak *et al*., 2001) and 82.1% in Turkey (Erhan *et al*., 2002).

In previous studies, the prevalence of p21 protein varied from 26-39% (Noske *et al*., 2009; Pasz-Walczak *et al*., 2001; Schwandner *et al*., 2002). Variations in the staining techniques, the antibodies used, the grading systems applied to cell nucleus tonality and the study populations are the most probable causes of differences found in the prevalence of p21 and p53 proteins (Ghavam-Nasiri *et al*., 2007).

Other studies have found that p53 protein expression is more prevalent in higher Duck stages (C and D) for poorly differentiated tumors (Han *et al*., 2006) but we did not find this correlation in our study. Also we found that most of the cases were in Stage B and cancer is more prevalent in colon than rectum which is in agreement with other studies (Bidgoli *et al*., 2005, 2007).

A review of the literature about p53 protein in colorectal cancer showed that expression of this protein is more prevalent in the distal areas of colon and rectum in comparison to the proximal areas (Iacopetta, 2003).

In this study, the correlation between the site of the tumor and the expression of p53 was not statistically significant ($p>0.05$). Although, we found that p53 positive cell nucleus were more prevalent in the distal areas than in the proximal areas of the colon (64.9 and 35.1%, respectively) but Mahdavinia and colleagues found that p53 protein was significantly more prevalent in the distal areas (Mahdavinia *et al*., 2008). This finding was in agreement with findings of Bidgoli *et al*. (2007) study in which p53 positive cancer cells were more prevalent in colon than in rectum of the patients (Bidgoli *et al*., 2007). In contrast to our findings Mohammadi *et al*. (2011) found that colorectal cancer was more prevalent in rectum in comparison to other sites. However, they confirm that cancer was more prevalent in distal than in proximal of colon.

There is still little knowledge about the correlation of p21 protein and colorectal adenocarcinoma. Our study is the first in this regard in Iran. We found that there is no significant correlation between the prevalence of p21 and the Duck stage.

In a 1999 study of 191 colorectal cancer specimens by Viale *et al*. (1999), 51% prevalence of p21 had a reverse correlation with the tumor stage In a 1997 study by Yasui *et al*. (1997) on 377 patients with colorectal adenoma and adenocarcinoma, 55% of adenomas and 66% of adenocarcinomas were p21-positive. The tonality of cell nuclei in stages 0, 1 and 2 was greater than in stages 3 and 4 (based on the TNM staging system). Also the prevalence of p21 decreased incrementally with the depth of the tumors invasion and there was a significant strong correlation between the decrease in prevalence of p21 and the increase in lymph-node metastasis.

In the study by Pasz-Walczak *et al*. (2001) of 122 colorectal cancer specimens, 39% of the specimens were p21-positive and there was no significant correlation between tumor grade and the prevalence of p21. But, the expression of p2 protein decreased simultaneously with the increment of tumor stage, depth of invasion and metastasis to lymph nodes.

Similar to our study results, Zirbes *et al*. (2000) studied 294 colorectal cancer specimens and found no significant correlations between the prevalence of p21 and a patient's age, sex, tumor site, type and stage.

Present study revealed that there is no correlation between the prevalence of p21 and p53 proteins; this result agrees with some studies that indicate that expression of p21 protein in colorectal cancer may be related to a pathway independent of p53 expression (Noske *et al*., 2009; Yasui *et al*., 1997). There is another study, however which found a significant reverse correlation between expressions of these two proteins, supporting the theory that expression of p21 protein depends on expression of p53 protein (Han *et al*., 2006).

CONCLUSION

p53 protein overexpression in more than half of the patients in our study with CRC indicates the important role this protein plays in the formation and progress of this cancer. Differences in the prevalence of p53 protein expression, even differences in the types of gene mutations in the distal and proximal areas of the colon may show differences in the molecular etiology of cancer

formation in different areas of the gastrointestinal (GI) tract. This study also highlights the great need for further studies of p53 protein expression in regions of the GI system other than the colorectal area.

This study was the first to examine p21 protein expression in colorectal adenocarcinoma in Iran. Based on our results, p53 protein expression may be used to determine recurrence of colorectal cancer in patients who have tolerated surgical treatment of a p53-positive tumor.

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