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Original Article

Expression of p53 in Colorectal Carcinoma: Correlation with Clinicopathologic Features

Mohammad-Reza Ghavam-Nasiri MD*, Ezzatollah Rezaei MD**, Kamran Ghafarzadegan MD***, Mehdi Seilanian-Toosi MD•*, Hamideh Malekifard MD***

Background: The p53 gene mutation is closely related to carcinogenesis in most malignant diseases. The main function of wild p53 protein is to maintain the integrity of genes by detecting mutations and preventing the division of cells with damaged DNA. The mutated form of p53 protein is overexpressed due to an extended half-life and can be easily detected by immunohistochemistry.

Objective: To estimate the frequency of p53 protein overexpression in colorectal carcinoma and its correlation with some clinicopathologic variables.

Methods: One hundred paraffin-preserved colorectal carcinoma samples were collected randomly from patients undergoing tumor resection from April 1995 through April 2001 in Omid Hospital, affiliated to Mashhad University of Medical Sciences, Mashhad, Iran. The overexpression of p53 protein was studied using a monoclonal antibody (clone DO-7; Dako). The number of cells stained were classified semiquantitatively as (–): <5% positive cells, (+): 5 – 25% positive cells, (++): 25 – 75% positive cells, and (+++): >75% positive cells. Clinicopathologic data including gender, age, tumor location, histologic type, and stage (Astler-Coller) were collected from the files maintained at the Department of Pathology. The correlation between p53 protein overexpression and each variable was evaluated using Chi-square analysis.

Results: p53 staining was positive in 59 of 100 specimens. Out of these 100 specimens, 16 were weekly (+), 16 moderately (++), and 27 intensely (+++) positive for p53 protein overexpression. There was no significant correlation between p53 staining and gender ($P = 0.34$), age (< 40 vs. ≥ 40 yr; $P = 0.74$), site of tumor (right vs. left colon and rectum; $P = 0.26$), pathologic type (mucinous vs. nonmucinous; $P = 0.63$), and stage of the disease ($P = 0.12$).

Conclusion: Considering the p53 protein overexpression in a relatively high percentage of patients, it seems that p53 mutation plays an important role in development of colorectal carcinoma. There was no significant association between p53 protein expression and some common clinicopathologic variables such as age, gender, site of tumor, pathologic type, and stage of the disease.

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Keywords: Colorectal carcinoma • immunohistochemistry • p53 gene • protein expression

Introduction

Globally, nearly 800,000 new colorectal cancer cases are believed to occur each year, which account for approximately 10% of all incident cancers.[1] Therefore, considerable interest has focused on the identification of novel tumor-based markers that can more accurately predict the course of this malignancy, as well as determination of optimal adjuvant therapy approaches.

The etiology of colorectal cancer is complex, involving interplay of environmental and genetic factors. Colorectal carcinoma develops through a multistep process as characterized by histopathologic precursor lesions and molecular genetic alterations including adenomatous polyposis coli (APC), K-ras, and p53.[2]

Authors' affiliations: *Department of Radiotherapy Oncology, **Department of Surgery, ***Department of Pathology, Omid Hospital, Mashhad University of Medical Sciences, Mashhad, Iran.
•Corresponding author and reprints: Mehdi Seilanian -Toosi MD, Department of Radiotherapy Oncology, Omid Hospital, Mashhad University of Medical Sciences, Mashhad, Iran.
Cell phone: +98-915-113-4298, Tel: +98-511-842-8621,
E- mail: toosi45@i8group.net.
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The increasing number of studies about the molecular biology aspects of colorectal cancer has raised great interest about p53 gene and respective protein function. Actually, mutation in p53 is the most frequently detected genetic alteration in human cancers.[3] The p53 gene (also known as TP53) is a tumor-suppressor gene located on the short arm of chromosome 17p13.1, which plays an important role in controlling cell growth.[4]

There are two types of p53 proteins: 1) normal or wild type and 2) mutant type. The wild-type p53 is believed to play a role in the regulation of cell proliferation and acts as a tumor suppressor by the following mechanism. When DNA is damaged by irradiation, UV light, or mutagenic chemicals, the wild p53 protein levels increase in the cell. The accumulated wild p53 binds to DNA, stimulates transcription of several genes, and mediates two major effects on the cell; one effect is to arrest the cell cycle in G1 phase, which allows the damaged DNA to be repaired. If the DNA repair has occurred successfully, the level of wild p53 then decreases with the help of MDM2 gene products. The cell begins to form new cells without any defects. The other effect occurs when the DNA damage cannot be successfully repaired. Under such situation, p53 initiates the apoptosis, and cells die with the help of cell death genes BAX and IgF-BP3.[5]

Laboratory analysis of p53 gene status may be presently achieved by three main different approaches: 1) Sequencing of p53 gene after amplifying DNA samples obtained from tumor specimens using the polymerase chain reaction (PCR) technique. 2) Direct observation of intracellular p53 protein stained by immunohisto-chemical (IHC) techniques. This method is based on the fact that p53 mutant protein has a prolonged half-life and can accumulate and be overexpressed in nuclei. 3) Detection of serum p53 antibody in peripheral blood samples. This approach is based on the assumption that presence of mutated p53 protein is associated with appearance of specific antibodies in circulating blood.[6] The result of a recent study has been promising for early detection of colon cancer in future. In this study, a part of isolated exfoliated colonocytes was subjected to DNA analysis of the APC, K-ras, and p53 genes using direct sequence analysis and was also subjected to microsatellite instability (MSI) analysis. In the DNA analysis, the overall sensitivity and specificity were 71% for patients with colorectal cancer and 88% for healthy volunteers.[7]

The objective of the current study was to estimate the frequency of p53 protein over-expression with IHC method in colorectal carcinoma specimens, as well as evaluation of its association with some clinicopathologic features such as age, gender, anatomic site of tumor, histologic type, and stage of the disease.

Materials and Methods

One hundred paraffin-embedded blocks of samples were collected randomly from patients proven to have colorectal carcinomas who underwent tumor resection from April 1995 through April 2001 at the Division of Surgical Oncology, Omid Hospital, affiliated to Mashhad University of Medical Sciences, Mashhad, Iran. Detailed clinicopathologic information of all these patients including gender, age, site of tumor (right and left colon by using middle of the transverse colon as partition), histologic type (mucinous vs. nonmucinous), and stage of the disease were obtained from the files maintained at the Department of Pathology. The tumors were staged according to the modified Astler-Coller classification system.

Consecutive 4-μm sections were cut from the paraffin blocks. Sections underwent histologic evaluation to select blocks without necrotic and hemorrhagic areas. At the next stage, sections were deparaffinized and treated with 3% H_2O_2 in methanol to block endogenous peroxidase activity. Antigen-retrieval procedure was performed by trilogy solution (Cell Marque). Then, monoclonal antibody against p53 protein (clone DO-7; Dako), was applied to the sections and incubated for 30 minutes at room temperature. The antigen-antibody complex was visualized using biotin-streptavidin-peroxidase staining technique. The color was developed with diaminobenzidine (DAB) solution and the sections were lightly counterstained with hematoxylin. Slides were then dehydrated and mounted.

Immunoreactivity for p53 was evaluated semi-quantitatively by two observers and, according to the percentage of positive tumor nuclei, scored as follows: none (<5%), weak (+, 5 – 25%), moderate (++, 25 – 75%), and intense (+++, >75%). All tumors showing p53 immunoreactivity (at least +) were considered to be positive. The pathologists were not aware of the report of each other. After primary evaluation of the results, cases with major

incompatibility in reports were rechecked by both pathologists and after reaching an agreement, a single report was submitted.

The frequency of p53-positive tumors with each variable was compared using χ^2 analysis. *P* values <0.05 were considered statistically significant.

Results

Forty-eight patients were males and 52 were females. The mean age of patients was 57 (range: 28 – 82) years. The tumor location was the right colon in 28, left colon in 25, and rectum in 47 patients. The stage was Duke B1 in 6, B2 in 47, C1 in 1, C2 in 37, and D in 9 patients. We did not have any case with stage Duke A.

P53 staining was positive in 59 of 100 specimens. Out of these 100 specimens, 16 were weekly (+), 16 moderately (++), and 27 intensely (+++) positive for P53 protein overexpression.

The relationship between p53 protein overexpression and several clinicopathologic variables are summarized in Table 1. There were no significant correlations between p53 staining and gender ($P = 0.34$), age (< 40 vs. ≥ 40 yr; $P = 0.74$), site of tumor (right colon vs. left colon and rectum; $P = 0.26$), pathologic type (mucinous vs. nonmucinous; $P = 0.63$), and stage of the disease ($P = 0.12$). The percentage of patients with positive p53 immunoreactivity was relatively higher in patients with advanced stages of disease; eight out of 9 patients with Duke D were positive for p53 overexpression. However, due to low number of metastatic cases, the difference between various stages was not statistically significant.

Discussion

P53 is described as the "guardian of the genome" as it prevents proliferation of cells bearing damaged DNA.[8] In our study, from 100 colorectal carcinoma samples, 59 displayed p53 protein overexpression. The prevalence of p53 mutations in colorectal cancer is highly variable among different series and may be estimated from 40% to 60% of patients.[9, 10] This discrepancy in the results could be due to the choice of methods, sensitivity and specificity of different antibodies used, and various interpretations of the results.

It soon became apparent that nuclear p53 overexpression could sometimes occur in the absence of mutation detected by PCR and vice versa. The reason proposed for identifying a p53-positive phenotype by IHC without detection of TP53 mutation included alterations in genes others than p53 in its pathway (such as MDM2)[11 – 13] leading to accumulation of wild-type p53 protein, since DO-7 recognizes both mutant and wild-type p53 forms. The explanation described for the presence of mutation in the absence of p53 overexpression is the deletion mutations or nonsense mutations which result in stop codons or production of truncated proteins that are not detectable by IHC.[12– 14] Concordance between the IHC and PCR techniques for the detection of p53 alteration is reported to be in the range of 65 – 75%.[10, 15, 16] In one study, mutations were detected in 99 samples (52%) from 189 patients with colorectal carcinoma by cDNA sequencing. Overexpression of p53 protein was demonstrated in tumors taken from 92 (48%) of the 190 patients. In the 188 cases in

Table 1. P53 protein overexpression and its relation to common clinicopathologic variables.

Clinicopathologic variables	No.	p53 positive *n* (%)	*P* value
All cases	100	59 (59%)	–
Age			
<40	18	10 (55.5%)	0.74
≥40	82	49 (59.7%)	
Gender			
Male	48	26 (54.1%)	0.34
Female	52	33 (63.4%)	
Tumor location			
Left	72	40 (55.5%)	0.26
Right	28	19 (67.8%)	
Pathologic type:			
Nonmucinous	78	47 (60.2%)	0.63
Mucinous	22	12 (54.5%)	
Stage (Duke)			
B	53	28 (52.8%)	0.12
C	38	23 (63.5%)	
D	9	8 (88.8%)	

whom a comparison was possible, there was a concordance in the results of the cDNA sequencing and the IHC analysis in 140 tumors (74%).[17] However, in comparison with DNA sequencing, IHC methods are cheaper, easier, and more familiar to pathologist as a standard procedure in everyday diagnosis.

We had no patients with stage Duke A, which is partly resulted from the lack of screening for colorectal carcinoma in Iran. Seventy-two percent of our patients developed carcinoma in left the colon, which seems comparatively higher than western countries.[18] Mucinous type comprised 22% of the pathologic samples, which is relatively higher than the international findings.[19] In our study, the frequency of mucinous type was not significantly different between right (5 cases, 17.8%), and left colon (17 cases, 23.6%; $P = 0.5$). Distinct molecular genetic characteristics, specially higher frequency of MSI, have been observed in mucinous carcinomas of colorectum.[20] Considering the relatively high frequency of mucinous colorectal carcinoma in our series, investigation on other genetic characteristics of colorectal carcinoma in Iran is warranted.

In our study, no significant correlation was found between p53 expression and gender, age, pathologic type, tumor site, and stage. We found higher percentage of p53-positive test in higher stages, especially in stage Duke D (88% of cases). However, the difference did not reach statistical significance, probably due to low number of cases with stage Duke D. Various studies have evaluated the correlation between different clinicopathologic features and p53 mutation. Some studies have found significantly higher rates of p53 mutation in left colorectal cancer when compared with those occurred in right side.[21 – 23] These findings may suggest a different molecular path to carcinogenesis between right and left sided colorectal cancer. In some studies, higher frequency of p53-positive test has been shown for nonmucinous than mucinous tumors.[19, 24] Meanwhile, in most series, no significant correlation has been found between p53 mutation and gender, age, tumor grade, and stage of the disease.[10, 24, 25]

It seems that adjuvant therapy relies on a normal p53 function to trigger apoptosis, so that cells damaged by chemo- or radiotherapy can be destroyed for therapeutic purposes. Several studies have shown that tumor cells with impaired p53 function have poor response to adjuvant or neo-adjuvant therapy.[9, 26, 27]

Several studies with conflicting results assessed the hypothesis that p53 mutation in colorectal cancer may be an independent prognostic factor. While some studies found p53 mutation a significant negative prognostic factor;[21, 28, 29, 30] other studies did not support such hypothesis.[22, 31] However, a recent systematic review of 168 reports including 18,766 patients showed that patients with colorectal carcinoma and abnormal p53 were at increased risk of death (relative risk with IHC 1.32: $CI_{95\%}$ 1.23 – 1.42; and with mutation analysis 1.31: $CI_{95\%}$ 1.19 – 1.45).[32] It is recommended to design a study to elucidate the effect of p53 gene mutation on both the survival rates and the response to treatment in Iran.

In conclusion, p53 protein mutation seems to have an important role in the carcinogenesis of colorectal cancer. Considering the acceptable reliability and feasibility of IHC method for detection of p53 mutation, this technique may be expected to serve as a new genetic marker for predicting recurrence and response to chemotherapy in patients with colorectal cancer.

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