Original Article

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Role of p53 Mutations in Colorectal Cancer

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Abstract

Introduction: The p53 tumor suppressor gene undergoes mutations in many diverse human cancers. It is commonly mutated in colorectal adenocarcinomas and can be studied by mutational analysis or by immunohistochemical methods. It has been correlated with poor prognosis and treatment outcomes in colorectal cancers. *Aims and Objectives:* To perform and interpret p53 immunostaining on all colorectal lesions and infer the rate of p53 positivity and to correlate it with the type of lesion and grade of tumor. *Materials and Methods:* This was a retrospective hospital based study done in two years (October 2009 to October 2011) at MGM Hospital, Warangalin collaboration with Biogene Quest, a research laboratory based in Hyderabad. Tissue from colorectal lesions from 65 patients was studied for histopathology and for p53 by immunohistochemistry (IHC). *Observations and Results:* p53 IHC was done on all the cases. 25/65(38.4%) cases were positive for p53 immunostaining. In these 25 cases, 21 (84%) were colorectal adenocarcinomas,2 (8%) were adenomatous polyps,1 (4%) were hyperplastic polyps and 1(4%) were ulcerative colitis.2/4(50%)adenomatous polyps were positive. Among these positive cases one was adenomatous polyp with highgrade dysplasia and other was tubulo-villous polyp. 21/35 (60%) adenocarcinomas including mucinous adenocarcinoma were positive.61%(13/21) well differentiated adenocarcinoma, 55%(5/9)moderately differentiated adenocarcinomas, 66%(2/3) poorly differentiated adenocarcinomas showed overexpression of p53. 40% (8/21) cases were located in right colon, 30% (6/20) cases were located in left colon, and 30% (6/20) positive colorectal adenocarcinomas were located in rectum. *Conclusion:* In our study, p53 overexpression was seen in 60% of colorectal adenocarcinomas and in 50% of adenomas. As p53 mutations are known to have a role in adenoma-carcinoma sequence, we recommend testing of all colorectal adenocarcinomas and adenomatous adenomas for p53 mutation.

Keywords: P53; Colorectal Lesions; Adenocarcinomas; Histopathology; Immunohistochemistry.

Introduction

The p53 suppressor gene, located on the short arm of chromosome 17 [1] encodes a 53-kd nuclear phosphoproteinthat regulates the cell cycle [2]. p53,by way of different mechanisms, prevents neoplastic transformation of cells such as activation of temporary cell cycle arrest (quiescence), induction of permanent cell cycle arrest (senescence), and by triggering programmed cell death (apoptosis). p53 helps in the repair of deoxyribonucleic acid (DNA) by arresting the cells in G1 phase and by inducing DNA-repair genes. The cells that are damaged beyond repair undergo apoptosis due to p53. When p53 becomes mutated, its normal function is lost, thereby causing accumulation of damaged DNA and persistence of mutations which in turn lead to malignancy.

The two common modalities of cancer treatment are irradiation and chemotherapy which act by causing damage to the DNA and subsequent apoptosis of such cells. Such therapy is more effective for tumors that retain normal p53 and are more likely to show good

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response than the tumors that have mutated p53. Lung cancers and colorectal cancers, which frequently carry p53 mutations, are relatively resistant to chemotherapy and irradiation [3].

Aims and Objectives

1. To perform and interpret p53 immunostaining on all diagnosed colorectal lesions.
2. Infer the rate of p53 positivity and to correlate it with the type of lesion and grade of tumor.

Materials and Methods

This was aretrospective study done for a period of two years from October 2009 to October 2011 in MGM Hospital,Warangal in collaboration with Biogene Quest, a research laboratory, based in Hyderabad.

Inclusion Criteria

All the colorectal biopsies and resectionspecimens with definite histopathological diagnosis were considered. Only representative areas in the biopsies were included for the study.

Exclusion Criteria

Intestinal obstructions, volvulus, gangrene and congenital lesions like Hirschprung'sdisease were excluded. Inadequate samples were excluded.

Specimen Handling

Colorectal biopsies and the resection specimens were fixed in 10%formalin and then sent for routine histopathological processing. After a histopathological diagnosis of the lesion was made, the paraffin blocks of the samples containing representative material were selected and p53 immunohistochemistry was performed on the sections.

p53 Immunostaining using p53 Antibody (Dako)

1. Sections assessed on histology to select blocks without necrotic and hemorrhagic areas.
2. Consecutive 3-4μm sections were taken on poly-lysine coated slides.The sections were deparaffinized and Antigen-retrieval procedure was performed by trilogy solution using microwave method. Sections were thoroughly washed with wash buffer in between every step.
3. Endogenous peroxidase blocking was done by horse radish peroxidase. Then, monoclonal antibody against p53 protein (clone DO-7; Dako), was applied to the sections and incubated for 30 minutes at room temperature.

Then, secondary antibody was added and incubated for 20 minutes.

5. Freshly prepared diaminobenzidine (DAB)was added to the sections for 10 minutes and counterstainedlightly with hematoxylin.
6. Slides were dehydrated, cleared, mounted and examined.

Interpretation

Immunoreactivity for p53 was evaluated semi-quantitatively according to the percentage of positive tumor nuclei and scored as below-

None (<5%)

Weak (+, 5 – 25%),

Moderate (++, 25 – 75%),

Intense (+++, >75%)

All tumors showing p53 immunoreactivity (at least +) were considered to be positive [4].

Observations and Results

On routine histopathology, there were 20 cases of non-neoplastic colorectal lesions including solitary

Table 1: Types of p53 positive colorectal lesions

Histopathology	Total no. of cases	No. of positive p53 cases (%)
Non-neoplastic lesions	20	-
Adenomatous polyp	4	2 (8%)
Hyperplastic polyp	3	1 (4%)
Ulcerative colitis	3	1 (4%)
Well differentiated adenocarcinoma	21	13 (61%)
Moderately differentiated adenocarcinoma	9	5 (55%)
Poorly differentiated adenocarcinoma	3	2 (66%)
Mucinous adenocarcinoma	2	1 (4%)
Total	65	25 (38.4%)

rectal ulcer, tuberculosis, Crohn's disease and non-specific colitis. None of them showed p53 overexpression. There were 35 cases of adenocarcinomas, 21 (60 %) were well- differentiated, 9 (25.7 %) were moderately differentiated, 3 (8.5 %) were poorly differentiated and 2 cases (5.7 %) were mucinous carcinomas.

IHC for p53 was Done in all 65 Cases

Out of 65 cases, 25 (38.4%) were positive.

All the non-neoplastic lesions except one case of ulcerative colitis were negative for p53 IHC.

In the 25 positive cases,21 (84%) were colorectal adenocarcinomas,2 (8%) were adenomatous polyps,1 case (4%) each was of hyperplastic polyp and ulcerative colitis.

2/4(50%)adenomatous polypswere positive. Among these positive cases one was adenomatous polyp with highgrade dysplasia and the other was tubulo-villous type polyp.

Out of total 35 adenocarcinomas, 21cases (60%) were positive.

p53 Positivity and Grade of Tumor

66%(14/21) well differentiated adenocarcinomas, 55%(5/9)moderately differentiated adenocarcinomas, 66%(2/3)poorly differentiated adenocarcinomas and 50% (1/2) mucinous adenocarcinoma showed overexpression of p53.

p53 Positivity and Site of Carcinoma

38% (8/21) cases were located in right colon, 33%(7/21) cases were located in left colon and, 28%(6/21)positive colorectal adenocarcinomas were located in rectum.

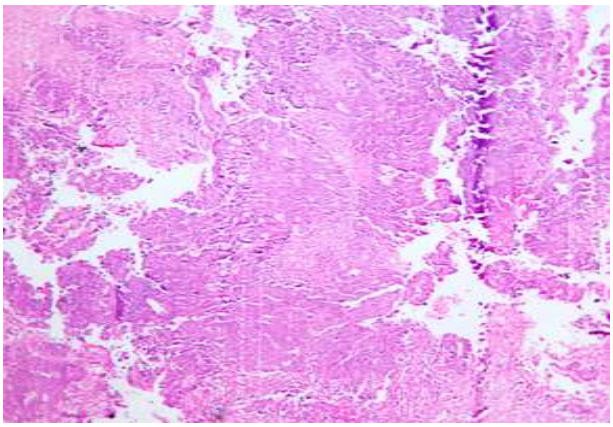

Fig. 1: Moderately differentiated colonic adenocarcinoma (H&E stain, 100X)

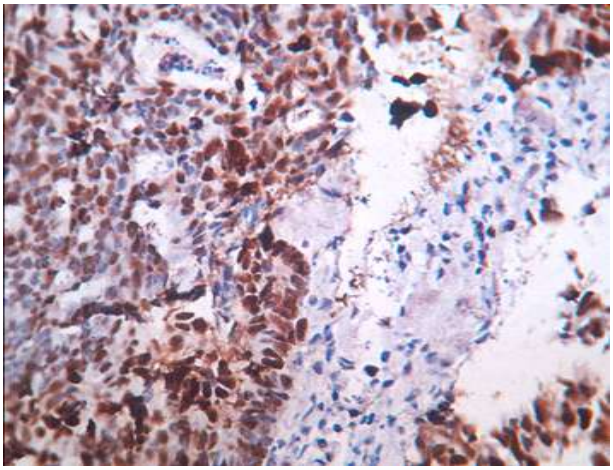

Fig. 2: Strong p53 positivity in moderately differentiated colonic adenocarcinoma on IHC

Discussion

Role of p53 in Colorectal Carcinogenesis

Mutations in p53 gene are encountered in many cancers in humans [5]. They occur late in the adenoma-carcinoma sequence in colorectal cancers (CRC) [6]. It has been reported by various studies that p53 mutations seem to be associated with poor prognosis in colorectal cancers [7,8,9]. However, contradictory results have also been observed [10].

The prevalence of p53 overexpression in CRC has been reported as ranging from 52.5% to 61.4% using IHC methods [11]. In a great majority of studies, p53 protein overexpression detected on IHC has been used as a surrogate marker for p53 mutations. But such an assumption is not entirely correct. As the IHC methods are more practical and economic they are often preferred over the molecular methods. Studies have shown that immunohistochemistry results do not always match with mutation results [12].

In some studies, p53 protein overexpression correlated with poor survival of patients, whereas, other authors have not observed any such correlation [13]. Mutations in the p53 tumor suppressor gene are found in almost half of CRC. Mutations in different domains of the gene lead to a variable prognosis [14,15].

Various studies have reported p53 mutations to be more common in the distal CRC [7,8]. Proximal tumors with mutated p53 were more likely to exhibit lymphatic invasion [15].

Individuals with wild type p53 have a superior survival rate with 5-FU therapy in rectal cancer [16].

At present there is no strong data to support the

role of p53 as a prognostic or predictive marker in CRC [17].

Laboratory analysis of p53 gene status may be presently achieved by three different approaches [4].

Sequencing of p53 gene: Done after amplifying DNA samples obtained from tumor specimens using the polymerase chain reaction (PCR) technique.

Direct observation of intracellular p53 protein stained by immunohistochemical (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.

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 [18].

Table 2: Overexpression of p53 by IHC and mutation detection in colorectal cancers in various studies

Study	No of patients	p53 positivity %(n)	Method employed
Morrin et al[19]	52	62%	Immunohistochemistry with p53 antibody
Pan[20] et al	97(rectal cancers)	62.9%(61)	Immunohistochemistry with p53 antibody
		52.6%(51)	PCR-SSCP
Tortola et al[21]	140	50%(66)	PCR-SSCP
Akkiprik et al[22]	43	32.5%(14)	PCR-SSCP followed by sequencing
Nasiri et al[4]	100	59%(59)	Immunohistochemistry with p53 antibody
Present study	35	60%(21)	Immunohistochemistry with p53 antibody

In the study by Rambau et al, p53 protein expression was seen in 56% of colorectal carcinomas [11]. Our results are comparable with the above studies that have used IHC for overexpression of p53. But the incidence of p53 mutations as detected by molecular methods is little lower than that detected by IHC. This discrepancy may be due to the combination of wild type p53 proteins with viral oncoproteins or cellular oncoproteinswhich can prolong its half-life, leading to accumulation of p53 protein in the cells. In such cells, IHC staining will be positive even without p53 gene mutations. Furthermore, about 10% p53 gene mutations occur outside of exons 5-8. The PCR-SSCP test targeting only exons 5-8 will miss such mutations and give lower positive results than that of IHC [23].

Table 2: Clinicopathological variables and frequency of p53 overexpression

Variables	Part of colon	Morrin et al [19] (62%)		Present Study (60%)	
		Cases	No of p53 positive	Cases	No of p53 positive
Location	Right colon	11	6(55%)	14	8(57%)
	Left colon	25	13(52%)	13	7(46%)
	Rectum	15	11(73%)	8	6(75%)
Differentiation	Well	27	17(63%)	21	14 (66%)
	Moderate	21	12(57%)	9	5(55%)
	Poor	4	2(50%)	3	2(66%)
Type	Mucinous	-	-	2	1 (50%)

Comparison of p53 Overexpression in Colorectal Adenocarcinomas with other Study

Morrrin et al [19] studied p53 expression by immunostaining in 52 cases of colorectal cancers and correlated thep53 overexpression with the survival rates.62% cases were positive. Most of the rectal carcinomas showed p53 overexpression. Sixty three per cent of the well differentiated tumors were positive for p53 overexpression, 57% of the moderately and 50% of the poorly differentiated tumors showed overexpression. They found no statistical significance in correlation to p53 status and survival rates.

In our study, 66% (14/21),55%(5/9),66%(2/3) of well,moderately and poorly differentiated adenocarcinomas were positive for p53 overexpression respectively.Our results are comparable with the results of above study. Russo et al observed p53 mutations in 34% cases of proximal and 45% of the distal colon and rectal tumors respectively.

p53 Mutation in Ulcerative Colitis in Various Studies

Kim et al [24] studied p53 mutations in 6 patients with ulcerative colitis who had variable duration of colitis by using p53 IHC and PCR-SSCP for exon 4-8. Among 16 patients, 2 patients showed dysplasia on routine histopathology. p53 mutations were detected in 4 cases(two dysplasia and two normal looking mucosa) on both PCR-SSCP and IHC. They concluded that p53 mutations may be an early molecular event of cancerous change in ulcerative colitis.

In our study, we did p53 immunostaining on three cases of ulcerative colitis and found one case(33%)positive for p53 overexpression. This case also revealed mild dysplasia on histopathology.

p53 Overexpression in Colorectal Adenomas in other Studies.

Study	No of cases	Positive for overexpression of p53
Saigusa et al[25]	35	8(22.9%)
Watson et al[26]	19	6(31.6%)
Present study	4	2(50%)

In our study, two out of four adenomas (50%) were positive for p53 overexpression, one case was a highgrade dysplasia and the other was a tubulo-villous polyp. The positivity rate of p53 is high in comparison to others. This may be attributed to the low sample numbers (i.e only four cases).

One hyperplastic polyp (33%) in our study showed p53 overexpression in focal areas.

Conclusion

In our study, p53 overexpression was seen in 60% of colorectal adenocarcinomas and in 50% of adenomas. At present there is no definite data to support the role of p53 as a prognostic or predictive marker in colorectal carcinomas. As p53 mutations are known to have a role in adenoma-carcinoma sequence, we recommend to includeit in the testing of colorectal adenocarcinomas and adenomatous adenomas along with other established biomarkers in future studies.

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