

The Interplay between p53 and p21 Tumor Suppressor Proteins in the Transformation of Colorectal Adenoma to Carcinoma

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Abstract: This study was carried out for evaluating the interplay of p53, p21 and Ki-67 proteins along the colorectal adenoma-carcinoma oncogenic transformation sequel. Therefore, Fifty colorectal cancer and 14 adenoma patients were involved. The histopathological expression of p53, p21 and Ki-67 proteins was evaluated by immunohistochemistry assay. The results revealed that remarkable overexpression of p53 protein was seen in the tumorous sections of cancer patients more than that in adenoma patients ($p < 0.05$), while no p53 overexpression was found in the corresponding non-tumorous sections. The positive expression of p21 protein was lower in the tumorous sections of cancer patients than that of adenoma patients ($p < 0.05$) and was higher in the non-tumorous than the corresponding tumorous sections of both CRC and adenoma patients ($p < 0.05$). The expression of p53 and p21 proteins in cancer patients was inversely correlated to each other ($p < 0.05$) and the expression of p21 rather than p53 protein was associated with colorectal cancer staging and grading ($p < 0.05$). Ki-67 was higher in cancer than in adenoma patients and higher in the tumorous tissue sections than the corresponding non-tumorous sections ($p < 0.05$). The overexpression of p53 and downexpression of p21 proteins in colorectal cancer might be responsible largely for triggering the transformational changes in normal mucosa to develop adenoma and trigger also the malignant cascade from adenoma to carcinoma. P53 overexpression was shown to occur due to the mutated dysfunctional p53 gene product which loses its transcriptional activator necessary for p21 expression. The level of p21 protein which is a quantitatively affected by the loss of p53 activation is responsible for the association with cancer staging and grading that serves as a good indicator for the disease progression.

Key words: colorectal cancer, adenoma, p53, p21, Ki-67

INTRODUCTION

Colorectal Cancer (CRC) is the fourth commonest form of cancer occurring worldwide. The number of new cases of colorectal cancer has been increasing rapidly since 1975^[1]. Risks for developing colorectal cancer include having inflammatory bowel disease, a personal or family history of colorectal cancer or colorectal polyps and certain hereditary syndromes. There is strong evidence that most invasive colorectal adenocarcinomas arise in pre-existing adenomatous polyps. The malignant risk with an adenomatous polyp is correlated with 3 interdependent features, polyp size, histology and severity of epithelial dysplasia^[2].

In vast majority, CRC arise through a series of genetic mutations that activates proto-oncogenes and disable tumor suppressor genes resulting in the normal

colonic epithelium to give way to precancerous adenoma development and eventually frank adenocarcinoma^[3]. In last decades it has been confirmed that sporadic CRC originate from colorectal adenoma, through adenoma-carcinoma sequence^[4,5] and colorectal adenomas are fairly common in the general population in that 40% of the western population will have colorectal adenoma^[6,7], but only 5-10% progress to a malignant tumor^[5,8]. And recent work continues to support the adenoma-carcinoma sequence, however there is a paucity of data on the interrelationship between different genetic mutations and on the relationship between molecular and other types of genetic abnormalities^[9]. It was demonstrated that colorectal tumor initiation and progression requires at least seven different somatic changes before a cell can develop into a carcinoma^[10]. Cancers arise from the

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sequential acquisition of genetic alterations in specific genes. The high number of mutations in cancer cells led to the hypothesis that an early step in tumor progression is the generation of a genetic instability^[11]. Missense mutations in the p53 tumor suppressor gene on the remaining chromosome 17 are found in more than 80% of CRC and represent a late event in the adenoma-carcinoma sequence^[12-14]. P53 acts as a checkpoint control protein that determines cellular fate upon DNA damages^[15,16]. It can delay the progression of the cell cycle from G1 to S phase, thus allowing for repair of DNA damage^[5,17]. Alternatively, p53 can trigger apoptosis in response to DNA damage, most probably when the lesions are too extensive and DNA repair fails^[16,18]. Loss of p53 tumor suppressor activity, results in cells tolerating DNA damage that can occur by genotoxic products, which eventually may lead to cancer formation due to inability to eliminate damaged cells by apoptosis^[19].

Another tumor suppressor protein is p21, which was identified as the product of the gene activated by wild-type p53 and it was named also WAF1 (wild-type p53 activated factor)^[20-22]. The normal cell cycle is significantly controlled by cyclins and Cyclin Dependent Kinases (CDK) and The balance between CDK activation and inactivation determines whether cells proceed through G1 into S phase and from G2 to M, through regulatory mechanisms that are conserved in more complex eukaryotes^[23]. However, their influence is only effective in the G1 phase, especially during the transition into the S phase^[24]. This part of the cell cycle is a restriction point, since all other cell cycle compartments cannot be modified by interference with diverse proteins or enzymes. In this context, p21/waf1/cip1 has been shown to act as a CDK inhibitor and is located on chromosome 6 in position p21^[16-20]. P21/waf1/cip1 inhibits the CDK 2, 3, 4 and 6, thus controlling the cell cycle at the G1 phase including the restriction point^[25]. Furthermore, It was found that transfection of p21 cDNA suppresses the growth of some normal tissues and tumor cells^[26].

Cell proliferation is regarded as one of the most important biological mechanisms in oncogenesis. Proliferating cell nuclear antigen (PCNA) and Ki-67 are the most popular methods that have been investigated as prognostic factors in colorectal cancer^[27]. There is strong evidence that Ki-67 expression correlates with cell proliferation as measured by S and G2 fractions^[28]. High proliferation (Ki-67 50%) was proved to be reliable and reproducible index. CRC patients with lesions with higher Ki-67 expression had significantly decreased survival than their counterparts, with lesions expressing lower levels of Ki-67^[29].

Many studies were done on the role of p53, p21 and ki-67 on CRC^[30-36] but no previous study focused in detail on the role of the paradoxical changes in the expression of p53 and p21 proteins by examining both the tumorous and non-tumorous, safe margins, tissue sections in both colorectal adenoma and carcinoma tissue sections and comparing all the studied variables with each other in one study. In this study, we aimed at investigating particularly the interplay between p53 and p21 proteins expression in both tumorous and non-tumorous, safe margin, tissue sections along the progression pathway of colorectal adenoma to carcinoma in order to shed light on, the nature of expression of tumor suppression proteins that predispose to CRC development from adenomas and development of adenoma from normal mucosa, understand the interplay between p53 and p21 proteins and their roles in the progression of the disease, understand the nature of the functional status of p53 responsible for the adenoma-carcinoma transformation process. The carcinogenic proliferative changes driven largely by the changed expression of p53 and p21 proteins are best measured by Ki-67 protein expression. The obtained results could offer a better understanding of the entangled role of the oncogenic, proliferative and tumor suppressor factors in predisposing or causing CRC in human beings.

MATERIALS AND METHODS

Patients involved and samples collected in this study: In the period from January 2007 to February 2008, 50 patients with primary CRC who underwent elective surgical resection of colorectal cancer in Selangor state were involved in this study before application of any chemotherapy. Excisional biopsy was taken postoperatively and 2 sets of tissue sections were made, the tumorous and the adjacent non-tumorous safe margin tissues. In addition, 14 adenoma patients were involved who underwent colonoscopic examination for checkup and then resection of adenomatous polyps and non-tumorous punch biopsies were taken from each one. CRC and adenoma patients were medically reviewed, the histopathological paraffin-embedded blocks were retrieved with complete histopathological and surgical reports. Written consents were obtained from all patients in this study. Histopathological paraffin blocks of excisional biopsies of 50 CRC and 14 adenoma patients were sectioned into 4 um thick on positively charged slides in order to be used later for the Immunohistochemistry assay (IHC).

Immunohistochemistry of p53, p21 and Ki-67 proteins: Evaluation of the expression of p53, p21 and Ki-67 proteins in tumorous and non-tumorous tissues sections of CRC and in adenoma was done by using IHC. The procedure of IHC used was according to the manufacturer instructions (LSAB2 Universal Dakocytomation strepavidin-biotin detection system). Monoclonal antibodies used were, anti-p53 (InnoGenex, USA), working dilution 1:100, anti-p21 (DAKO, Denmark), working dilution 1:50 and anti-Ki-67 (DAKO, Denmark), working dilution 1:100.

At every run of IHC for CRC, adenoma, one negative control tissue section, which is DW placed instead of the primary antibodies, one positive control tissue section, which is already tested as strongly positive.

After baking slides in oven at 65°C overnight, slides were deparaffinized by applying sequential immersion for 5 min in xylene, 95% ethanol, 70% ethanol and in DW respectively. In order to obtain the best results, autoclave-based antigen retrieval was done. Slides were placed in a jar containing antigen retrieval solution (0.1M citrate buffer, pH 6) and left in the autoclave, for 2-4 min under 121°C^[37]. Then, 100 µL of the diluted primary antibody was applied onto the sections and the slides were placed in the humid chamber incubated at 4°C overnight. The next day, slides were rinsed gently with PBS-Tween and placed in fresh PBS-Tween bath for 1 min. One-two drops of the biotinylated secondary goat anti-mouse antibodies (DakoCytomation) were applied onto the sections and slides were placed in the humid chamber and incubated at 37°C for 1 h. After rinsing step, One-two drops of streptavidin-Horseradish peroxidase (HRP) reagent (DakoCytomation) was applied onto the sections, slides were placed in humid chamber and incubated at 37°C for 30 min. The prepared DAB-substrate chromogen solution was applied onto sections, Slides were incubated in dark at room temperature for 20 min. Mayer's hematoxylin stain was used as counterstain, then slides were dehydrated and mounted with DPX mounting fluid.

Staining analysis: Expression of p53 and p21 proteins was assessed according to certain scoring system used by previous studies. Positively stained tumorous glandular cells, adenocarcinoma cells, out of non-tumorous, normal glandular cells, were taken into account rather than stromal cells. The used scoring system is composed of 6 scores (0-5), [Table 1](#). Tissue sections were regarded as p53 overexpression positive when immunoreactivity scores ≥ 4 , while p21

Table 1: The scoring system used for the histopathological expression of both p53 and p21 proteins in CRC and adenoma tissue sections.

	Negative	Low		Positive (overexpression)		
	Score 0	Score 1	Score 2	Score 3	Score 4	Score 5
p53	0%	1-5%	5-15%	16-25%	26-75%	>75%
P21	Negative		Positive			
	0%	1-5%	5-15%	16-25%	26-75%	>75%

immunoreactivity was regarded as positive when percentages of stained cells $\geq 5\%$ ^[38].

Ki-67 index (KI) was used as a comparative measure for the level of Ki-67 expression among different tissue sections. KI estimated as the percentage obtained from the number of Ki-67 positively stained glandular cells over total glandular cells in 7-10 high power fields (total counted cells: about 1000)^[39].

RESULTS

Demographic and histopathological features of CRC and adenoma patients: The CRC patients pursued in this study were 27 men and 23 women with a mean age of 57.08 years (range between 43 and 76 years). Adenoma patients were 7 men and 7 women with a mean age of 50.1 years (range between 37 and 70 years). All CRC cases were of adenocarcinoma, 32 cases were left-sided, 12 right-sided and 6 at transverse colon. It was found that 6% of CRC patients were presented at B1 stage, 10% at B2 stage, 10% at C1 stage, 14% at C2 stage and 60% at D stage. Furthermore, it was found that 60% of histopathological sections of CRC patients were poorly differentiated and 40% were mild-moderate differentiated. For adenoma, it was found that 6 patients 42.9% were of villous type, the most aggressive type of colon adenoma, size >2 cm, five patients (35.7%) were of tubulovillous type that is moderately aggressive, size 0.8-1.5 cm, while only 3 patients (21.4%) were of tubular adenomatous polyps that is weakly aggressive, size 0.5-1.1 cm.

Expression of p53, p21 and Ki-67 proteins in CRC and adenoma patients: The histopathological expression of p53 protein (Fig. 1) in the tumorous tissue sections of CRC and adenoma patients revealed that 39 CRC patients (78%) and 2 adenoma patients (14.3%) showed p53 protein overexpression, the range of scores was 4 to 5, while none (0%) of the corresponding non-tumorous tissue sections of both CRC and adenoma patients showed similar overexpression of p53 protein, the scores range was from 0-3, ([Table 2](#)).

The histopathological expression of p21 protein (Fig. 1) revealed that 17 CRC patients (34%) and 9

Table 2: Frequency distribution of p53 and p21 expression scores in colorectal cancer and adenoma patients

p53 staining	Tumorous tissue sections		Non- Tumorous tissue sections	
	CRC patients N (%)	Adenoma patients N (%)	CRC patients N (%)	Adenoma patients N (%)
P53 negative:				
Score 0 (0%)	0	0	12 (24)	6 (42.86)
Score 1 (1-5%)	0	1 (7.1)	10 (20)	4 (28.58)
Score 2 (5-15%)	0	5 (35.7)	17 (34)	3 (21.42)
Score 3 (16-25%)	11 (22)	6 (43)	11 (22)	1 (7.14)
P53 positive:				
Score 4 (26-75%)	35 (70)	2 (14.3)	0	0
Score 5 (>75%)	4 (8)	0	0	0
Total	50(100)	14 (100)	50(100)	

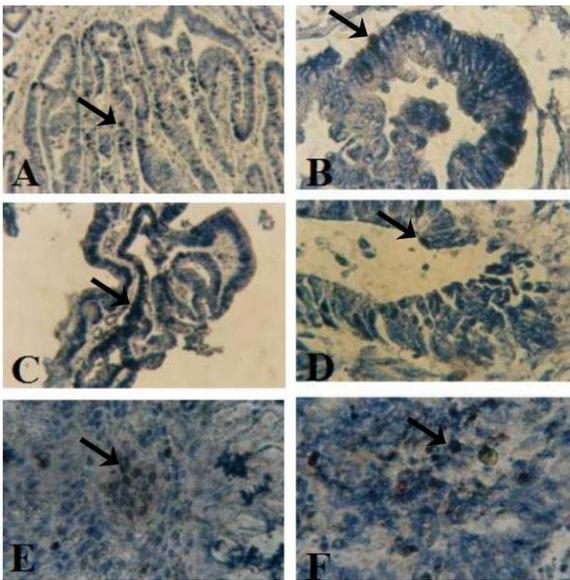


Fig. 1: Immunohistochemical staining of colorectal carcinoma tissue sections showing positive signals by DAB stain counterstained with Meyers' Hematoxylin. (a) Positive Ki-67 staining of CRC tissue section at X100 (b) The same field at X400. (c) Positive p53 staining (score 4) of CRC tissue section at X100 (d) The same field at X400. (e) Positive p21 staining (score 3) of CRC tissue section at X100. (f) The same field t at X400

adenoma patients (64.2%) showed positive p21 expression, while it was shown that 48 out of 50 (96%) non-tumorous tissue sections of CRC patients and 14 out of 14 (100%) non-tumorous tissue sections of adenoma patients were positive for p21 expression (the positively stained cells was >5%), (Table 3).

Chi-square and Fisher's exact test were used for evaluating the difference in the level of expression of p53 and p21 proteins according to the scoring system adopted in this study. P53 protein overexpression was

0% in the non-tumorous tissue sections of both CRC and adenoma patients in that overexpression of p53 was confined solely to the tumorous tissue sections of CRC and minimally to adenoma, indicating that the histologically normal tissues and most of the adenomatous cells still express p53 protein normally without exaggeration. On the contrary, the positive expression of p21 protein was extremely higher in the non-tumorous tissue sections, 48/50 patients (96%) in CRC and 14/14 (100%) in adenoma patients than the tumorous sections of the corresponding CRC, 34% and adenoma, 64% tissue sections ($p < 0.05$).

Most importantly, it was found that p53 overexpression (scores 4 and 5) in tumorous tissue sections of CRC patients was much higher than that of adenoma patients ($p < 0.0001$). On the other hand, p21 positive expression in tumorous tissue sections of CRC patients was much lower than that in adenoma patients ($p = 0.04$), (Fig. 2). Taken that, the positive control section of p53 was score 4 and the negative control section was score 0, while the positive control section of p21 was score 3 and the negative control section was score 0.

In order to validate the adapted scoring systems in this study and to keep a solid scientific results, quantitative statistical measures were applied using the percentage of the positively stained cells rather than the qualitative scoring systems. Student t- test results showed that the percentage of cells with p53 overexpression in the tumorous tissue sections of CRC patients 46.04% was significantly higher than that of adenoma patients 17.92% ($p < 0.0001$), (Fig. 2). For p21 protein, Mann-Whitney test was used which showed the percentage of positively stained cells for p21 protein in the tumorous tissue sections of adenoma patients 14.42% was significantly higher than that of CRC patients 6.06% ($p = 0.014$) (Fig. 2).

Pearsons' correlation coefficient for p53 with p21 in CRC patients showed that the correlation was inversely significant ($r = - 0.37$, $p < 0.01$), which therefore clearly indicates that the higher the expression

Table 3: Frequency distribution of p21 expression scores in colorectal cancer and adenoma patients

p21 staining	Tumorous tissue sections		Non- Tumorous tissue sections	
	CRC patients N (%)	Adenoma patients N (%)	CRC patients N (%)	Adenoma patients N (%)
p21 negative:				
Score 0 (0%)	18 (36)	1 (7.1)	0	0
Score 1 (1-5%)	15 (30)	4 (28.6)	2 (4)	0
p21 positive:				
Score 2 (5-15%)	11 (22)	3 (21.4)	17 (34)	5 (35.72)
Score 3 (16-25%)	4 (8)	3 (21.4)	18 (36)	6 (42.85)
Score 4 (26-75%)	2 (4)	3 (21.4)	12 (24)	3 (21.43)
Score 5 (>75%)	0	0	1 (2)	0
Total	50 (100)	14 (100)	50(100)	14(100)

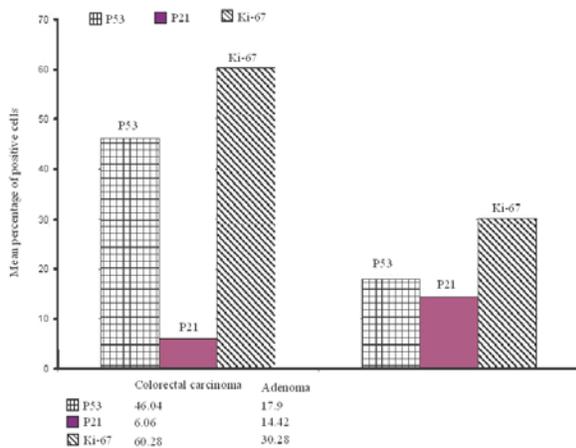


Fig. 2: Mean percentage of positively stained cells by immunohistochemistry assay for p53, p21 and Ki-67 in both CRC and adenoma patients

of p53 in CRC patients the lower the expression of p21 proteins and vice versa (Fig. 3).

Regarding Ki-67 and by using student t-test, it was found that Ki-67 expression index in CRC patients (KI 60.28) was significantly higher than that in adenoma patients (KI 30.29) ($p < 0.0001$) indicating that the proliferative index (KI) in CRC patients is far higher than that in adenoma patients, (Fig. 2). On the other hand, the non-tumorous tissue sections of CRC (KI 8.34) and of adenoma (KI 7.64) were significantly lower than KI of the corresponding tumorous tissue sections ($p < 0.05$).

Expression of p21, p53 and Ki-67 with staging and grading of CRC: There was a significant difference of p21 protein expression among the studied groups of CRC staging ($p < 0.05$) favoring stage B in which 6/8 (75%), 4/12 (33.3%) at stage C and 8/30 (26.6%) at stage D were p21-positive. Furthermore, there was a significant difference of p21 expression between poorly 7/30 (23.3%) and mild-moderate differentiated CRC tissue sections 10/20 (50%) ($\chi^2 p = 0.05$, Fisher

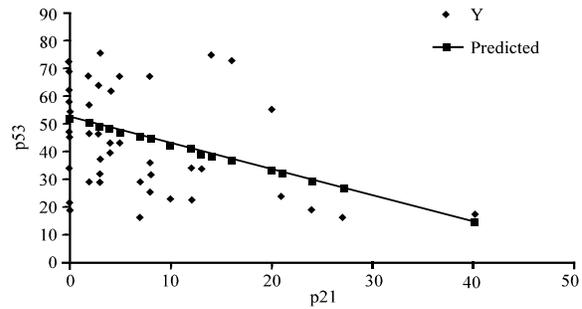


Fig. 3: Correlation coefficient between p21 and p53 expression, using Pearson/Spearman's test

$p = 0.048$). Regarding p53 and Ki-67, they were not related to the staging or the grading of CRC.

DISCUSSION

There are two major pathways in colorectal carcinogenesis, the chromosomal instability pathway (adenoma-carcinoma sequence), which is characterized by allelic losses on chromosome 5q (APC), 17p (p53) and 18q (DCC/ SMAD4) and the other is a pathway that involves microsatellite instability^[40]. In colon cancer, p53 gene on chromosome 17p is usually found to be mutated by partial deletions or mis-sense base changes, mainly located in four highly conserved regions of exons 5-8, such mutations have been observed in 30% of adenomas and 50-75% of carcinomas^[31,41,42]. Wild-type p53 has a short half-life of about 15 min 22 and is turned over rapidly by an ATP-abiquitin degradation pathway and mutant p53 protein has a greater stability with half-life prolonged up to 20 h^[43]. In this study, p53 protein overexpression was found solely in the tumorous tissues rather than the adjacent histologically-normal tissues of both CRC and adenoma patients. It is noteworthy to mention that no previous study was conducted to explore the expression of p21 and p53 proteins in the histologically normal adjacent tissues to adenomas and CRC^[30-36].

Furthermore, p53 overexpression was found far higher in CRC tumor cells than adenoma cells indicating an exclusive role to play in the transformation process from normal colorectal mucosa, 0% overexpression, to premalignant adenoma, 14.3% overexpression and finally from adenoma to malignant carcinoma, 78% overexpression. These results are supported by another study^[32] who stated that p53 positivity in CRC was 70% but they didn't include the non-tumorous sections of CRC in the study nor adenoma tissue sections. On the contrary, another study^[33] revealed that there is a complete absence of p53 expression in CRC which represents an adverse prognostic effect.

In order to investigate the controversial p53 overexpression in CRC and since p21 protein was identified as the product of the gene activated by wild-type p53^[20], the immunohistochemical analysis of p21 protein expression was studied and compared with that of p53 protein. According to the transcriptional activation of p53 towards p21, the level of p21 protein expression of both CRC and adenoma patients would clarify whether p53 protein in CRC or adenoma tumors is dysfunctionally mutated or not. Our study showed that the expression of p21 protein was highest in the non-tumorous adjacent tissues to CRC and adenoma and was significantly higher than that of the tumorous sections ($p < 0.05$). Moreover, it was higher in adenoma tumor cells than CRC tumor cells ($p < 0.05$). These findings showed that p21 protein expression decreases with the progression of the transformational changes from normal mucosa to adenoma and from adenoma to carcinoma which is exactly the opposite to p53 protein expression. This indicates that p53 gene in CRC tumors was mutated and its protein became cryptic enough to not act as a competent transcriptional factor for p21 and its overexpression is most probably a compensatory mechanism. Moreover, the statistically significant inverse pearsons' correlation observed in our study between p53 and p21 has confirmed this suggestion. Our results contradicted the results of previous studies^[32,33] which revealed that no inverse correlation was found between p21 and p53, while^[31,35] revealed such correlation.

Since mutated p53 is not found overtly overexpressed in adenoma such as the case in CRC, most of p53 proteins might still in the wild type as long as adenoma not yet converted to cancer and p53 dysfunctional mutation seems to act most probably as the key for ending the curbing control of wild p53 against the carcinogenetic tendencies initiated by the accumulation of a number of oncogens. In addition to the loss of p53 tumor suppressor function, p21 downregulation as a consequence adds an additional

breach to the tumor suppressor capability of the cells as Roninson et al. stated that p21 protein accounts for the induction of differentiation markers and mitosis inhibition, whereas subsequent downregulation could be related to mitotic catastrophe and cell proliferation would be unleashed^[44].

Interestingly, our study revealed no association between p53 protein expression and CRC staging or grading, while p21 protein associated significantly with both staging and grading of CRC unlike the study^[36] that revealed p21 has no relation to the staging of CRC and another study revealed that mutation of either p53 or Ki-ras gene did not correlate with Dukes's staging and tumor differentiation^[45]. While other studies showed that the expression of p21 could be used as a marker to determine the degree of malignancy of colorectal and gastric cancers^[46,31-33]. This might be explained by Yasui who stated that in colon cancer, abnormality of p21 genes is not found, but attenuation of expression of p21 genes is reported to be correlated with the degree of malignancy^[47]. Since p21 protein is not under the strict impact of the two-hit theory of inactivation such as p53^[48] and p21 protein showed a declining level of expression with the oncogenesis progression, so p21 level of expression has shown to be related to the degree of differentiation and staging of malignancy quantitatively.

The proportion of Ki-67 labeled cells in a given cell population (Ki-67 index) provides a measure of the growth fraction^[49]. Our results showed the proliferative index (KI) in the tumorous sections of CRC patients was far higher than that in adenoma patients. This was another player might help understanding the nature of interplay between p53 and p21 in CRC and adenomas. At adenoma stage, the growth is still under control represented by values of KI when the wild p53 expression is still not overtly overexpressed and the expression level of p21 is still relatively high. On the contrary in CRC, the dysfunctional p53 overexpression is seen widely along with a sharp decline in p21 expression and sharp upsurge of the growth of tumor cells. This plot refers to the vital role of tumor suppressor proteins might play in the carcinogenesis of colorectal mucosa.

Accordingly, it was concluded from our study that p53 overexpresses in CRC more than in adenoma and in adenoma more than in normal tissues significantly. This overexpression is shown to act as a compensatory mechanism for the functional impairment of the wild p53. And the conversion of wild p53 to the dysfunctionally mutant and overexpressed p53 most probably act as a threshold for the conversion from benign premalignant tumor, adenoma, to overtly

malignant tumor, CRC. And this role of p53 was understood and confirmed by the associated crippling behavior of p21 which in turn has been quantitatively lowered to a very shallow level in CRC that cripples even more the overall lowered tumor suppressor potential of the affected cells. This study showed that the histologically-normal adjacent tissues express the normal high levels of p21 protein and the normal levels of p53 protein although many studies confirmed that the adjacent tissues of adenoma or CRC are full of oncogens^[5,9,11,12,14,42]. This sheds light to the exclusive need by the colorectal mucosal cells to the functional impairment/overexpression of p53 and the grave decrease in p21 to unleash these oncogens to transform normal cells to tumor cells and later to malignant cells. This study also confirmed the significance of the transcriptional activatory role of the wild type p53 towards p21 expression. Furthermore, p21 level of expression seems to be a reliable index for the evaluation of the disease prognosis and the staging unlike p53. We recommend involving other carcinogenetic factors in the future studies like p27, COX-2, PGE2, TGF-beta, beta-catenin and APC using larger samples of adenoma/CRC patients and correlating their role in the adenoma-carcinoma transformation to that of the currently shown interplay of p53 and p21 in the progression of colorectal tumors. Moreover, we recommend focusing more on p53 and p21 proteins as potential targets for any futuristic CRC therapy whatever the type of accumulated oncogenes were because this study confirmed that the transformation process of adenoma to carcinoma needs by all means the currently shown p35/p21 interplay in order to proceed further in the transformation pathway reaching to the level of malignancy.

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