

Prognostic Value of Cyclin D1 and P53 Protein in Colorectal Carcinoma

NANCY Y. ASAAD, M.D.; MONA A. KANDIL, M.D. and NADIA M. MOKHTAR, Ph.D.*

The Department of Pathology, Menoufyia, Faculty of Medicine and NCI, Cairo University*

ABSTRACT

The multistep carcinogenic process of colorectal cancer involves a series of events as oncogenes, inactivation of suppressor genes and abnormalities in cell cycle regulating proteins. This study concerns altered expression and prognostic role of cyclin D1 and p53 in colorectal cancer patients. We evaluate nuclear accumulation of cyclin D1 and p53 immunohistochemically in archival tissue specimens from 41 primary colorectal adenocarcinomas. They had undergone surgery with a median follow up of 23 months (range 1-85 months). Survival time was analyzed using Kaplan-Meier survival estimates and Cox proportional hazards model for nuclear accumulation of cyclin D1 and p53 with adjustments for other confounding demographic and clinical variables. The expression of cyclin D1 was identified in 41.5% while p53 was expressed in 58.5% of our cases. Cyclin D1 was statistically associated with p53, Dukes' stage, nodal state, histologic grade and vascular invasion, but not with age, gender, location, size, gross picture, tumor type, bilharziasis or stromal reaction. p53 was significantly related to male gender and to mucinous and signet ring tumor types but not to either age, location, size gross picture, Dukes' stage, nodal state, histologic grade, bilharziasis, stromal reaction or vascular invasion. Using Kaplan-Meier survival curve, cyclin D1, p53, size, Dukes' stage, nodal state and tumor type were significantly correlated with poor survival. By Cox multivariate regression analysis, p53 (relative risk 3.33, 95% confidence interval 1.39-7.95; $p = 0.006$), nodal state (relative risk 3.17, 95% confidence interval 1.31-7.68; $p = 0.01$) and Dukes' stage (relative risk 1.83, 95% confidence interval 1.06-3.15; $p = 0.027$) were independent prognostic indicators in our colorectal adenocarcinoma cases. Our data suggest that cyclin D1/p53 pathway represent a frequent target of the multistep evolution of colorectal carcinoma. Nuclear p53 accumulation combined with nodal state and Dukes' stage can predict the clinical behavior of a tumor and high risk colorectal cancer patients. Hence p53 might help to define, a subset of biologically unfavorable neoplasms and improve the prognostic accuracy for colorectal cancer.

Key Words: Colorectal carcinoma - Cyclin D1 - P53.

INTRODUCTION

Colorectal cancer is a frequent malignancy

as it is the seventh most common cancer in Egypt [30] risk factors for colorectal neoplasia include a positive family history, meat consumption, smoking and alcohol consumption. Important inverse associations exist with vegetables, non-steroidal anti-inflammatory drugs, hormone replacement therapy and physical activity [15,27].

There are several molecular pathways of colorectal cancer. At least four separate pathways of colorectal cancer exist: (A) adenomatous polyposis coli pathway in which B catenin-T cell factor-MYC factors are implicated (APC-B catenin-Tcf-MYC) in adenoma carcinoma sequence, (B) hereditary non-polyposis colorectal cancer pathway (HNPCC) characterized by loss of DNA mismatch repair by inherited or acquired mutation or methylation that results in microsatellite instability in tumor. Tissue specific hypermethylation of multiple genes, e.g. p16 lead to inactivation of cyclin D1-p16-retinoblastoma pathway; (C) the ulcerative colitis or dysplasia-carcinoma sequence that is usually not associated with APC mutation or polyp formation. p53 loss can occur early in this pathway; (D) hypermethylation silencing of the estrogen receptor gene, which may be part of a wider pattern of gene-specific hypermethylation-common in sporadic tumors. Expression of key genes in any of these pathways may be lost by inherited or acquired mutation or by hypermethylation [38].

The multistage process of carcinogenesis involves the progressive acquisition of mutations and epigenetic abnormalities in the expression of multiple genes that have highly diverse functions. An important group of these genes are in-

involved in cell cycle control including cyclin D1 and p53 [44]. Cyclin D1 is a 45 kD protein encoded by the CCND1 gene mapped to chromosome 11q13. Cyclin D1 is a part of the complex molecular system regulating the G1-S transition point of the cell cycle. This regulatory protein is frequently overexpressed in a variety of human cancers. Cyclin D1 plays a critical role in carcinogenesis because: (i) overexpression enhances cell transformation and tumorigenesis and enhances the amplification of other genes [44] and (ii) an antisense cyclin D1 cDNA reverts the malignant phenotype of carcinoma cells of the colon. Therefore, cyclin D1 may be a useful biomarker in molecular epidemiology studies and inhibitors of its function may be useful in both cancer chemoprevention and therapy [42,48].

By acting in conjunction with protein kinase cdk4, cyclin D1 may phosphorylate the retinoblastoma gene at the end of the G1 phase. Phosphorylation of the retinoblastoma gene leads to its inactivation and consequently to the unhindered progression of the cell to S phase [26]. Cyclin D1 overexpression may be caused by chromosomal rearrangements or translocations resulting in amplification of the CCND1 gene or its product, although the participation of transcriptional or post-transcriptional mechanisms cannot be excluded [21]. Cyclin D1 overexpression secondary to 11q13 amplification has been identified in a variety of tumors, including parathyroid adenoma, B-cell lymphoma, carcinoma of the colon, breast, liver, oesophagus, urinary bladder, head and neck, vulva and uterine cervix [4]. Moreover, cyclin D1 overexpression seems to confer a worse prognosis on oesophageal carcinomas [53], squamous cell carcinoma of the head and neck [28] and colonic carcinoma [32], however there was a controversy regarding the last association [36].

In addition to cyclin D1 overexpression, other genes regulating cell cycle progression as p53 suppressor gene have been implicated frequently in colorectal carcinoma [6]. The p53 gene, located on short arm of chromosome 17, encodes a 53 kD phosphoprotein capable of binding to DNA and acting as a transcription factor. The normal or wild type p53 protein is thought to have a role in inhibiting cell proliferation by arresting the cell cycle at the G1-S phase to allow DNA repair to take place. Loss

of this activity may lead to neoplastic transformation [18]. In the p53 gene, over 90 percent of mutations have been reported to fall within a 600 base-pair region encompassing exons 5-8, where most of the evolutionarily conserved amino acids are concentrated [14]. At the protein level, the wild-type p53 gene product usually does not accumulate in amounts detectable by immunohistochemistry because of a short half-life of 6-20 minutes. However, functionally inactive and stabilized p53 protein can be detected in the nucleus of the cells [22]. More recently, Mahdani et al. [24] found that not only p53 gene mutation but also p53 mRNA overproduction is a frequent event in colorectal tumors that is not predictive of the status of the gene, i.e. whether or not a mutation is present. Those authors also found that p53 protein overexpression does not only result from an increase in the half-life of mutated p53 but also suggested that inactivation of the p53 function in colorectal cancers involves at least two distinct mechanisms including p53 overexpression and/or mutation.

PATIENTS AND METHODS

Patients and tissue specimens:

We obtained formalin fixed, paraffin embedded, archival tissue blocks of colorectal adenocarcinomas, randomly selected, from the National Cancer Institute, Cairo University. All patients had been diagnosed with primary colorectal adenocarcinomas and had undergone surgery between 1993-1996. We obtained the surgical pathology reports for age, sex, location, size, gross picture and nodal state. Survival data were collected from computerized colorectal cancer data-base from the date of surgery to the date of death or last documented contact. We reviewed haematoxylin and eosin stained slides of all cases for tumor stage, differentiation, tumor type (adenocarcinoma or others), nodal state, prominent stromal reaction (lymphohistiocytic infiltrate around the tumor), vascular invasion and associated evidence of bilharzial infestation. The pathologic tumor staging was performed according to the Dukes' classification system, high lymph node involvement adjacent to the apical vascular tie indicating Dukes' stage C2 [17]. Histologic grade was coded as low, moderate and high [10]. Tumor typing was performed according to the recommendations of the World Health Organization

[45]. The anatomic location of the tumor was identified using the International Classification of Disease for Oncology Codes [46]. Based on the embryologic origin of the colorectum, the anatomic locations were grouped into proximal colon (the cecum to the right two-thirds of the transverse colon) and distal colorectum (the left one-third of the transverse colon to the rectum) [19]. Non-neoplastic area were available in all blocks.

Immunostaining analysis:

Heat induced epitope retrieval technique was used as described by Zymed to reverse the loss of antigenicity that occurs with some epitopes in formalin-fixed, paraffin-embedded tissues. We started the immunostaining protocol for all sections using Histostatin-Plus kits, second generation LAB-SA kits [Labeled-(strept)-avidin-biotin amplification] (Zymed Laboratories, Inc.) according to the manufacture's instructions. The primary antibodies used were: (1) mouse monoclonal antibody specific for cyclin D1, isotype: IgG2a-Kappa, Clone: AM29, second generation prediluted (Zymed Laboratories, Inc., Carlton Court, South San Francisco, Ca.); (2) mouse monoclonal antibody specific for p53 sensitive to both wild and mutant types, the epitope location is between amino acids 1 and 45 in the p53 protein product, Isotype: IgG2a-Kappa, Clone; BP53.12, second generation prediluted (Zymed Laboratories, Inc., Carlton Court, South San Francisco, Ca.). Sections were incubated overnight at room temperature. Positive staining was visualized with diaminobenzidine substrate solution and nuclei were counterstained with Mayer's haematoxyline. Sections were mounted in DPX and the percentage of tumor cells with brown staining of nuclei was assessed semi-quantitatively. A negative control section for each case was included. Sections know to stain strongly positive for cyclin D1 and p53 protein were included in each run as positive controls.

Assessment of nuclear accumulation of cyclin D1 and p53:

Cyclin D1: Only nuclear staining with or without simultaneous weak staining of the cytoplasm was accepted as positive staining [29]. This nuclear staining was considered positive if the chromogen was detected in at least 5% of the nuclei within a tissue section with cut-off value of 5% [1].

p53: We considered only tumor cells with distinct nuclear immunostaining for p53 as positive and considered the tumor positive only if nuclear accumulation was identified in at least 10% of all malignant cells in a tissue section, as it was found that the cut-off value of 10% positivity showed the highest concordance between immuno-histochemical detection of nuclear accumulation of p53 and point mutation of p53 gene [25].

Statistical analysis:

All analyses were performed using the statistical Package for the Social Sciences (SPSS, Chicago, IL USA). Non-parametric data were assessed using the Mann-Whitney U-test. Categorical variables were assessed by the X² test and Fisher Exact test. Kaplan-Meier survival curves were constructed and differences in survival between groups were compared using the log-rank test. Regression analysis was performed with the Cox proportional hazards model.

RESULTS

The clinicopathologic variables of our colorectal cancer patients are included in table (1). No cases with low histologic grade or Dukes' stage D were included in our study and only one case was Dukes' stage A was included.

Cyclin D1:

Cyclin D1 staining was predominantly nuclear and variable in terms of intensity from weak to strong. The normal mucosa showed an undetectable signal. The staining showed wide range of expression from 5 to 80%. Our colorectal carcinoma cases showed 41.5% positivity rate for cyclin D1 immunostaining (Fig. 1). Cyclin D1 expression occurred more often in more advanced Dukes stages ($p = 0.001$), lymph node metastasizing tumor ($p = 0.0001$), high histologic grade ($p = 0.002$), in tumors with vascular invasion ($p = 0.01$) and in p53 positive tumors ($p = 0.01$), but cyclin D1 expression was otherwise unrelated to patients' age, gender, tumor location, size, gross picture, tumor histologic type, bilharziasis or lymphocytic host reaction ($p > 0.05$) (Table 1).

P53:

Distinct nuclear staining was detected in malignant cells but not in normal mucosa (Fig. 2). The overall incidence of nuclear accumulation

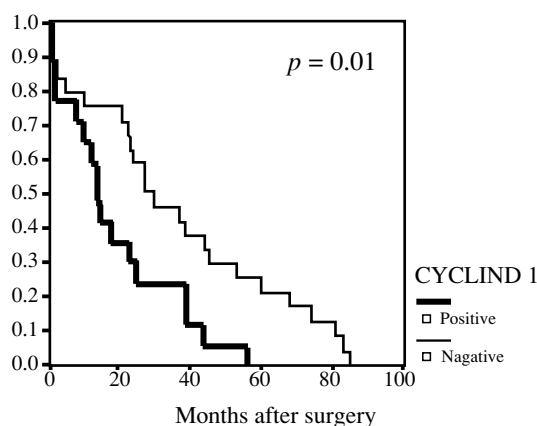
of p53 in colonic adenocarcinomas was 58.5%. In our cases, p53 immunopositivity was related significantly to male gender ($p = 0.02$) and to mucinous and signet ring tumor type ($p = 0.01$). There were no correlations between nuclear accumulation of p53 and patients' age, tumor location, size, gross picture, Dukes' stage, nodal state, histologic grade, bilharziasis, lymphocytic host reaction or vascular invasion ($p > 0.05$) (Table 2).

Survival analysis:

Follow up time in months for all patients was up to 85 months with median time of 23 months.

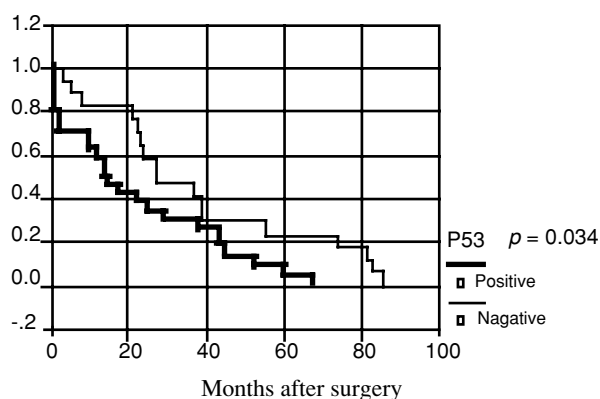
The correlation with survival was examined in two steps: first, univariate Kaplan-Meier survival plots were generated for our patients demonstrating that colonic adenocarcinoma cases positive for nuclear accumulation of cyclin D1 (log rank test = 5.89, $p = 0.01$) or p53 (log rank test = 4.45, $p = 0.034$) (Diagram 1,2) had poorer survival time compared to those with negative immunostaining. Also, our results demon-

strated a statistically significant difference in patient survival regarding, tumor size (log rank test = 9.73, $p = 0.0022$), Dukes' stage (log rank test = 16.23, $p = 0.0003$) (Diagram 3), nodal state (log rank test = 16.18, $p = 0.0001$) and tumor type (log rank test = 3.80, $p = 0.051$). Hence, patients with small tumor size, less advanced Dukes' stage, absence of nodal metastasis and conventional type adenocarcinoma had better overall survival. Second, multivariate analysis was performed to assess the relative influence of our clinicopathologic variable on survival. In a backward stepwise regression, the p53 abnormality (relative risk 3.33, 95% confidence interval 1.39-7.95; $p = 0.006$), nodal state (relative risk 3.17, 95% confidence interval 1.31-7.68; $p = 0.01$) and Dukes' stage (relative risk 1.83, 95% confidence interval 1.06-3.15; $p = 0.027$) were found to be independently associated with shorter survival (Table 3). In this model cyclin D1 abnormality did not provide additional significant information on survival. This may be explained by weaker correlation between p53 and nodal state, also Dukes stage compared with that of cyclin D1 abnormality.



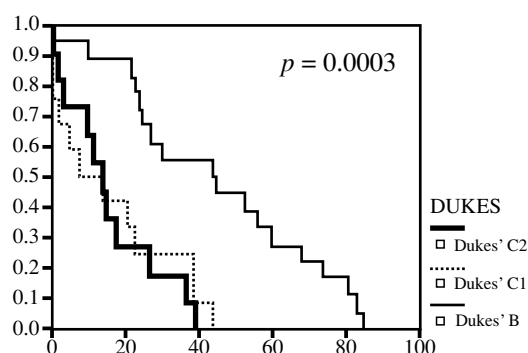
Kaplan Meier survival analysis of colorectal adenocarcinoma according to cyclin D1

Diagram (1): Median survival time cyclin D1 negative cases in months = 27 ± 7.96 standard error (SE) with 95% confidence interval (C.I) (11.40-42.6), while that of positive cases was 14 ± 2.06 SE, 95% CI (9.97-18.03). Log rank test = 5.89, $p = 0.01$. Survival rate of cyclin D1 negative patients = 87.5% while that of positive cases = 29%.



Kaplan Meier survival analysis of colorectal adenocarcinoma according to p53

Diagram (2): Median survival time of p53 negative cases in months = 27 ± 8.92 standard error (S.E) with 95% confidence interval (CI) of (9.52-44.48), while that of p53 positive cases was 14 ± 3.67 sE with 95% CI of (6.80-21.20). Log rank test = 4.45, $p = 0.034$. Survival rate of p53 negative cases = 82.35% while that of p53 positive cases = 50%.



Months after surgery
Kaplan Meier survival analysis of colorectal adenocarcinoma according to Dukes stage.

Diagram (3): Mean survival time in months of Dukes stage A + B = 44±15.91 SE with 95% CI = 12.82-75.18, that of Dukes stage C1 = 8±7.79 SE with 95% CI = 0-23.28, while that of Dukes state C2 = 14±2.75 SE, their 95% CI = 8.61-19.39. Log rank test = 16.23, $p = 0.0003$. Survival rate of Dukes stage A + B = 88.89%, stage C1 = 41.67 while that of C2 = 45.45.

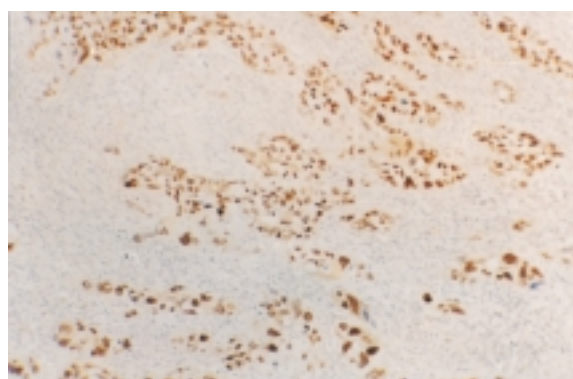


Fig. (1): Colorectal carcinoma strongly positive for nuclear staining of cyclin D1 (IP-Mayer's Hx. counterstain x 200).

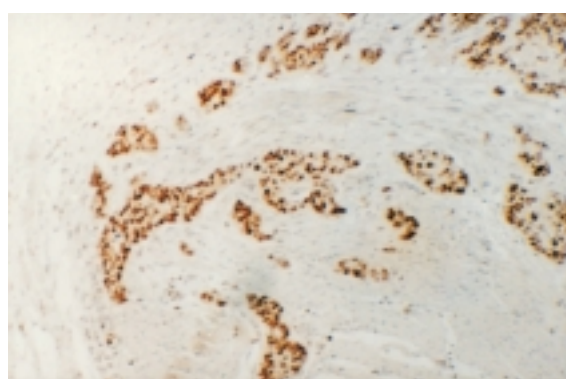


Fig. (2): Colorectal carcinoma with strong positive nuclear staining for p53 (IP-Mayer's Hx. counterstain x 200).

Table (1): Cyclin D1 immunostaining and clinicopathologic data.

Variables	Cyclin D1			p value	Signif.
	No.	+ve	-ve		
Age (yrs):		No. (%)	No. (%)		
≤ 40	11	7 (63.6)	4 (36.4)	> 0.05	NS
> 40	30	10 (33.3)	20 (66.7)		
Gender:					
Male	24	13 (54.2)	11 (45.8)	> 0.05	NS
Female	17	4 (23.5)	13 (76.5)		
Location:					
Proximal colon	10	4 (40)	6 (60)	> 0.05	NS
Distal colon	31	13 (41.9)	18 (58.1)		
Size:					
< 4 cm	10	2 (20)	8 (80)	> 0.05	NS
≥ 4 cm	31	15 (48.4)	16 (51.6)		
Gross picture:					
Ulcer	34	14 (41.2)	20 (58.8)	> 0.05	NS
Mass	7	3 (42.9)	4 (57.1)		
Dukes' stage:					
A+B	18	2 (11.1)	16 (88.9)	0.001	Sign.
C1	12	7 (58.3)	5 (41.7)		
C2	11	8 (72.7)	3 (27.3)		
Nodal state:					
-ve	18	2 (11.1)	16 (88.9)	0.0001	H. Sign.
+ve	23	15 (65.2)	8 (34.8)		
Tumo type:					
Adenocarcinoma	29	13 (44.8)	16 (55.2)	> 0.05	NS
Mucinous+signet ring	12	4 (33.3)	8 (66.4)		
Grade:					
II	26	6 (23.1)	20 (76.9)	0.002	Sign.
III	15	11 (73.3)	4 (26.7)		
Bilharziasis:					
-ve	34	16 (47.1)	18 (52.9)	> 0.005	NS
+ve	7	1 (14.3)	6 (85.7)		
Prominent stromal reaction:					
-ve	19	9 (47.4)	10 (52.6)	> 0.05	NS
+ve	22	8 (36.4)	14 (63.6)		
Vascular invasion:					
-ve	29	8 (27.6)	21 (72.4)	0.01	Sign.
+ve	12	9 (75)	3 (25)		
p53:					
-ve	17	3 (17.6)	14 (82.4)	0.01	Sign.
+ve	24	14 (58.3)	10 (41.7)		

Table (2): p53 immunostaining and clinicopathologic data.

Variables	p53			p value	Signif.
	No.	+ve	-ve		
<i>Age (yrs):</i>		No. (%)	No. (%)		
≤ 40	30	17 (56.7)	13 (43.3)	> 0.05	NS
> 40	11	7 (63.6)	4 (36.4)		
<i>Gender:</i>				0.02	Sign.
Male	24	18 (75.0)	6 (25)		
Female	17	6 (35.0)	11 (65)		
<i>Location:</i>				> 0.05	NS
Proximal colon	10	4 (40)	6 (60)		
Distal colon	31	20 (64.5)	11 (35.5)		
<i>Size:</i>				> 0.05	NS
< 4 cm	10	4 (40)	6 (60)		
≥ 4 cm	31	20 (64.5)	11 (35.5)		
<i>Gross picture:</i>				> 0.05	NS
Ulcer	34	21 (61.8)	13 (38.2)		
Mass	7	3 (42.9)	4 (57.1)		
<i>Dukes' stage:</i>				> 0.05	NS
A+B	18	9 (50)	9 (50)		
C1	12	8 (66.7)	4 (33.3)		
C2	11	7 (63.6)	4 (36.4)		
<i>Nodal state:</i>				> 0.05	NS
-ve	18	9 (50)	9 (50)		
+ve	23	15 (65.2)	8 (34.8)		
<i>Tumo type:</i>				0.01	Sign.
Adenocarcinoma	29	21 (72.4)	8 (27.1)		
Mucinous+signet ring	12	3 (25)	9 (75)		
<i>Grade:</i>				> 0.05	NS
II	26	15 (57.7)	11 (42.3)		
III	15	9 (60)	6 (40)		
<i>Bilharziasis:</i>				> 0.05	NS
-ve	34	20 (58.8)	14 (41.2)		
+ve	7	4 (57.1)	3 (42.9)		
<i>Promient stromal reaction:</i>				> 0.05	NS
-ve	19	10 (52.6)	9 (47.4)		
+ve	22	14 (63.6)	8 (36.4)		
<i>Vascular invasion:</i>				> 0.05	NS
-ve	29	16 (55.2)	13 (44.8)		
+ve	12	8 (66.7)	4 (33.3)		

Table (3): Multivariate Cox regression analysis to evaluate independent prognostic indicators in colorectal adenocarcinoma.

Variables	B	S.E.	p vlaue	r	Relative risk 95% confidence rate
P53	1.204	0.44	0.006	0.152	3.33 (1.39-7.95)
Nodal state	1.15	0.43	0.01	0.14	3.17 (1.31-7.68)
Dukes' stage	0.60	0.27	0.027	0.11	1.83 (1.06-3.15)

B: Beta coefficient of regression.

SE: Standard error of regression.

R: Regression.

DISCUSSION

In this study, we analyzed the status of two cell cycle regulators; cyclin D1 and p53 in a number of colorectal carcinomas and we observed alterations in both in a significant proportion of cases. It is now well established that cyclin D1 is upregulated in a subset of colorec-

tal carcinomas. Gillet et al. [11] found that cyclin D1 gene was overexpressed in the absence of any measurable increase in gene copy number, suggesting that mechanisms other than gene amplification can lead to upregulated expression of cyclin D1 and indicated that probably the most direct approach to search for cyclin D1 abnormalities is to examine the protein

abundance at the single-cell level. We detected cyclin D1 overexpression in 41.5% of our colorectal carcinoma cases. Previous reports evaluating cyclin D1 immunohistochemically, [1,3,4, 35,36] showed a range of positivity varying from 12 to 46 percent of colorectal carcinomas, which might be largely due to the variety of antibodies, antigen-retrieval techniques and scoring systems. The wild type p53 transactivates the WAF1/p21 gene, whose protein product prevents exit from the G1-phase by inhibiting cyclin/Cdk complex and in parallel, blocks replicative DNA synthesis by binding to proliferating cell nuclear antigen, hence DNA repair takes place [24]. The longer half life of the mutant p53 protein results in the accumulation of this phosphoprotein in the nuclei, facilitating its detection by immunohistochemical analysis with specific antibodies. The frequency of p53 nuclear immunostaining indicating abnormality in our patients was 56.5%, other several series recorded frequencies lower [12,13,20,23,25] and higher [2,24,37,43], but not statistically different from ours. The stability of p53 is not always associated with mutation; wild type p53 was reported to be associated with stable immunohistochemically detectable protein [22].

The complete negativity of cyclin D1 in apparently normal non-neoplastic colonic tissues is due to the fact that the expression of cyclin D1 may occur transiently and for only a brief period in normal tissues making its detection difficult by immuno-staining [49]. The heterogeneous staining pattern and the variation of staining intensity of cyclin D1 within one tumor sample are most likely due to variation in the amount of cyclin D1 protein in the tumor cells, which is related to a cell cycle-dependent oscillation with maximal levels of the protein found in the mid/late G1 phase [3,40]. Our findings in the form of the absence of detectable cyclin D1 expression in non-tumorous colonic mucosa adjacent to malignant tissues and its detection in 41.5% of malignant colonic tissues, significant statistical association of cyclin D1 overexpression with survival, Dukes stage, nodal state, grade and vascular invasion, strongly suggested an oncogenic role for cyclin D1 protein as it enhances not only cell transformation and tumorigenesis, but also tumor progression. The increased expression of this G1 regulatory protein may account for the increased cell proliferation and the autonomous growth of colonic carcinoma. Some preliminary studies have shown that

cyclin D1 could represent a feature of malignancy with prognostic significance. In hepatocellular carcinoma, Nishida et al. [34] reported an association between faster tumor growth and aggressive behaviour. In addition, Bartkova et al. [4] studied a large number of tumors (breast, colorectal, uterine, melanoma and soft tissue sarcoma) and their normal counterpart; the authors concluded that alterations of cyclin D1 expression represent a common feature of malignancy in different human cancers that ranged from weak to high according to the degree of malignancy. Also, Zang et al. [49] found that increased cyclin D1 positivity was associated with more severe dysplasia in intestinal adenomas from multiple intestinal neoplasia in mice.

Absence of p53 abnormal staining in the adjacent normal colonic mucosa in our study suggests that such p53 abnormalities may be due to somatic changes rather than hereditary; they may be acquired during tumor development. Mucinous carcinoma tends to present at a more advanced stage and their prognosis is somewhat worse than for the conventional type of colorectal adenocarcinoma. In addition, the prognosis of signet ring colorectal carcinoma is extremely poor [41]. Hence, mucinous and signet ring type carcinoma may constitute biologically different neoplasms than the conventional type adenocarcinoma, so it is not surprising to record a statistically significant higher frequency of p53 abnormality in mucinous and signet ring variants than in the conventional type colorectal adenocarcinoma. Our result goes in accordance with that of Mulder et al. [31], Poller et al. [37] and Manne et al. [25] who found statistically significant differences between histologic types of colorectal carcinoma regarding p53 abnormality. In our study, p53 abnormality was significantly more frequent in males. This could be due to more environmental exposure of males to exogenous carcinogens that may induce predominantly point p53 mutations. However, the association between p53 and gender is denied by others [25,31,37]. Our results suggest that p53 mutations are not associated with the size of the tumor, hence they may occur early in tumor development.

De Lattre et al. [7] and Kern et al. [16] demonstrated that the frequency of allelic loss of 17P in left sided colon cancer was significantly higher than that observed in right-sided tumors, so it has been proposed that the molecular

mechanism of carcinogenesis is different at these two sites. However, our results failed to detect any significant difference regarding the frequency of cyclin D1 and p53, survival or Dukes stage between left and right sided colonic carcinoma. Although, Watatni et al. [43] found that the overall incidence of p53 mutations in exons 5 to 18 in right-sided and in left sided colorectal cancer was similar, they detected the frequency of exon 8 mutations significantly lower in right-sided tumors. Hence, in our study depending only on immunostaining the absence of the difference between right and left sided colonic neoplasm does not exclude their difference in the process of carcinogenesis.

Cyclin D1 and p53 abnormalities were significantly associated with each other in our cases, 82.3% of cyclin D1 abnormal cases showed also p53 abnormality. Although, our finding is based on a limited number of cases, it suggests that during colorectal carcinogenesis there is selective advantage for cells to sustain both genetic alterations, overexpression of cyclin D1 and mutation of the p53 gene. It has been shown that p53 negatively regulates cell cycle progression through WAF-1/CIP-1, an inhibitor of cyclin-dependent kinases [8] WAF-1 associates with and inhibits multiple cyclin-dependent kinases and so prevents the cell from exiting G1. In tumors, loss of wild-type p53 function deactivates this growth-control-pathway and is thought to play a role in the uncontrolled growth of tumor cells [26]. In contrast, cyclin D1 acts as positive regulator of the transition from G1-phase to S-phase by association with and activation of cyclin D1-dependent kinase [47]. Therefore, loss of wild p53 function and overexpression of cyclin D1 may have a similar effect on the cell cycle, namely abrogation of G1 growth control.

We used Cox multivariate regression analysis to evaluate independent prognostic indicators in colorectal adenocarcinoma. In such an analysis, the p53 abnormalities are the first parameter to enter, followed by nodal state then Dukes stage. However, cyclin D1 lost its prognostic role in this model. This suppression of the prognostic significance of cyclin D1 is most likely caused by the significant association of cyclin D1 alteration with lymph node state and Dukes stages. Our results go in accordance with those of Palmquist et al. [35] and Bukholm and

Nesland [5] who failed to establish independent prognostic impact of cyclin D1 on colorectal cancer patients. However, Michalides et al. [28] who investigated the prognostic significance of cyclin D1 in squamous cell carcinomas of the head and neck, reported correlation with rapid cancer recurrence and poor survival. Cyclin D1 could represent an independent prognostic marker in their study, as it was not correlated with other clinical features (tumor size and stage). Consequently, the tumor-associated alterations of cyclin D1 expression appear to be a common feature of a considerably wider spectrum of human neoplasias than initially thought from DNA studies. Since there are already limited data suggesting that cyclin D1 overexpression correlates with an adverse prognosis, it would be of great interest to extend the immunohistochemical analysis of cyclin D1 to a larger number of cases and also other tumor types to see whether the elevated cyclin D1 protein correlates with the known prognostic indicators and has any significant bearing on the clinical outcome of the disease. Searches for prognostic information linked to the detection of p53 abnormalities demonstrated a significant association of p53 abnormality with shorter survival in non-small cell lung cancer and also in breast tumor without estrogen and progesterone receptors, a tumor phenotype associated with poor prognosis. Also, in colorectal carcinoma a strong correlation between the presence of p53 abnormality and short survival was observed [13,20,24,25,39]. Moreover, it is proved to be an independent adverse predictor of survival [13,20,25,39] and this goes in accordance with our results. However, these associations have not been observed in another study [9]. This could be due to restriction of our cases to a subset of particular patients (Dukes' stage C) and not on whole cohorts of patients. Hence, p53 may be an important event in a multistep process of colonic tumorigenesis and genomic wide instability as deregulated expression, it may perturb normal cell cycle control and thereby enhance tumor progression.

Conclusion:

Cyclin D1 and p53 abnormal staining are common events in colorectal carcinoma. The expression of these G1 regulatory proteins may account for the increased cell proliferation and autonomous growth of neoplastic colonic mucosa. It will be of interest to determine the

mechanisms involved in cyclin D1 and p53 co-expression in colonic neoplasia as such studies could lead to novel therapeutic strategies to prevent cancer formation or progression by designation of specific therapeutic agents.

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