Combined Expression of P27 and P53 in Human Gastric Carcinoma

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Abstract: Purpose: Disregulation of the cell cycle is required for the formation of various malignant tumors including gastric carcinoma. This study aimed to evaluate cell-cycle-regulators, p27 and p53, expression in gastric carcinoma by immunohistochemistry and to correlate their expression with other clinicopathological findings. **Material and Methods:** Eighty-four cases of gastric carcinoma were included in this study. They were classified into intestinal and diffuse types according to Lauren's classification. P27 and P53 expression were correlated with patient's age, histologic type, pathologic grade, lymph node metastasis and tumor tissue invasion. **Results:** There was non significant correlation between p27 and p53 expression with patient's age or histologic type of the tumor. Loss of expression of p27 was significantly correlated with pathologic grade, lymph node metastasis and tumor tissue invasion. P53 over-expression was more frequently detected in high grade tumors, tumors with lymph node metastasis and high t-stage, but the correlations were statistically non-significance. A significant inverse correlation was detected between p27 and p53 expression. **Conclusion:** Reduced expression of p27 may influence the progression and metastases to lymph node in gastric tumors. In addition, combined expression of cell-cycle regulators, p27 and p53, may play an important role in the biological behavior of human gastric carcinoma.
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Introduction

Gastric cancer is the second most common cancer worldwide and remains a significant problem in global health terms, with a wide range in geographic distribution [1,2]. The highest incidence rates are reported in Korea, Japan and Eastern Asia. A high incidence is also observed n Eastern Europe and parts of Latin America, while in Western Europe and USA, the disease is in constant decline[3]. Gastric Carcinoma formed 2.12% of all cases of the digestive tract malignancies referred to National Cancer Institute (N.C.I) in Egypt during 2003-2004 [4].

The interaction of both environmental and gastric factors contributes to the etiology and pathogenesis of these aggressive cancers, mainly smoking, alcohol consumption, besides dietary habits and bacterial infection by Helicobacter pylor [5].

Despite the advance in therapeutic option, less than 20% of patients survive 5 years after diagnosis [3]. In Western countries, gastric cancer has a poor prognosis compared with Asian patients. The better results obtained in Japan are attributed to aggressive approaches, early detection and extensive surgery [6].

Gastric tumors arise from multiple genetic and epigenetic alterations that involve oncogenes, tumor-suppressor genes, cell cycle regulators and cell adhesion molecules, however its pathogenesis is still unknown [5].

The P27 and P53 proteins are important regulators of the cell cycle. P27 is a member of cyclin dependent kinase (CDK) that affects the activity of Kinase complexes controlling the G-S transition. P27 protein is expressed broadly in human tissue with similar mRNA levels in quiescent and proliferating cells. Its levels are reduced in many carcinomas, including those of the breast, colon, bladder, prostate, esophagus and lung carcinoma [7-12]. Loss of p27 function therefore, accelerates cell cycle progression to malignant transformation. Decreased expression of p27 has been also associated with high aggressiveness and poor prognosis in a large variety of malignant tumors [13].

The p53 protein acting as a tumor suppressor gene is present in very low levels in normal cells and involved in regulation of cell proliferation and apoptosis [14]. In the absence of functional p53 protein, cells fail to repair DNA damage or to undergo apoptosis and are more susceptible to neoplastic transformation. Both of p27 and p53 act by modulating cell proliferation via control of G1 arrest check point of cell cycle [15]. Although many studies suggest a prognostic significance of p53 expression

in gastric carcinoma [16-18], other few reports do not support this finding [19].

This study aimed to evaluate cell-cycle-regulators, p27 and p53, expression in gastric carcinoma by immunohistochemistry and to correlate their expression with other clinicopathological findings.

Material and Methods

Formalin-fixed paraffin-embedded tissue samples from patients, who had undergone partial or total gastrectomy were analyzed in this study. They are retrieved from Specialized-Ain-shams Hospital and a total of 84 cases were identified that contained suitable material. The pathology reports and hematoxylin and eosin stain slides were revised and the histological type according to Lauren's classification [20] was determined as diffuse and intestinal types. Tumor grading was performed according to WHO classification [21]. The patient age, presence of lymph node metastasis and T-stage were reviewed.

Immunohistochemistry:

P27 and p53 expression was examined in all tissues using streptavidin-biotin technique. Two slides from each case were deparaffinized, hydrated and incubated in 3% hydrogen peroxide for 30 minutes to block the internal peroxidase activity. Antigen retrieval was done by microwave pretreatment for 10 minutes in 0.01M citrate buffer. For each case, one slide was incubated at 4ºC overnight with monoclonal antibodies against p27 (Medicopharma trade) with a dilution 1:200.The second slide was incubated with monoclonal antibodies to p53 at a dilution 1:500 (Dako Corporation). These steps were followed by 30 minutes incubation with biotinylated horse anti-mouse antibody at room temperature, avidin-biotin peroxidase complex for 50 minutes at room temperature and finally diamiobenzidine (DAB) for 3-5 minutes. The slides were counterstained with hematoxylin, dehydrated and mounted.

In the negative control group, 1% bovine serum albumin was used in place of the primary antibody. Breast carcinoma and tonsillar tissue were used as positive controls for p27 and p53 respectively. In addition, lymphocytes were used as internal positive control for p27.

Staining interpretation:

The p27 and p53 stained sections were assessed semiquantitatively by two pathologists. The immunostaining for each patient was determined as positive when > 20% of the tumor cells showed distinct nuclear staining according to Al-Moundhri et al., [16]. For evaluation, ten fields within the tumor were selected and a total of 1000 tumor cells (100 for each field) were counted microscopically under high power (X200).

Statistical Analysis:

Chi-square (X^2) test was applied to examine the correlation between p27 and p53 expression and age of the patients, histologic grade, lymph node metastases, and tumor tissue invasion (T-staging). P-value less than 0.01was considered significant.

Results

Routine histopathological examination was performed on 84 gastric carcinoma cases and evaluated by p27 and p53 immunostaining. Sixteen cases were ≥ 65 years while 68 were < 65 years. Seventy-two cases were of intestinal type and 12 cases were of diffuse type. Fourty cases were found to be grade I-II and the remaining 44 cases were GIII. Lymph nodes were positive for metastasis in 44 cases and 40 cases were lymph node negative. Twenty-eight cases were T2 and 56 cases were T3.

Expression of p27 in cases of gastric carcinoma

Immunoreactivity for p27 was found in both normal and neoplastic gastric tissues (figures 1-3). P27 immunoreactivity was predominantly localized in the nuclei of the tumor cells. Occasionally, a weak or moderate cytoplasmic staining could be seen in some cases in addition to nuclear activity (figure 2), but we did not classify as positive. Small lymphocytes scattered in the entire gastric wall were positive and served as internal positive control (figure 2-3). The cells with p27 positive nuclei were variably distributed through the tumor section. The intensity of the staining was generally moderate, with only few positive tumor cells showing strong immunoreactivity.

This study showed that 40 cases out of the 84 cases of gastric carcinoma (47.6%) were p27 positive. It was found that p27 expression was more frequently expressed in patients younger than 65 years and tumors classified histologically as intestinal type, but the correlations were statistically non-significant. Howevever, its expression was inversely correlated with pathologic grade of the tumors, lymph node metastasis and tumor tissue invasion where p27 was frequently positive in low grade tumors, tumors without lymph node metastasis and tumors with T2 stage (table-1).

Expression of p53 in cases of gastric carcinoma

In the present study, tumors were considered to be positive for p53 only if definite nuclear staining was present in more than 20% of tumor cells. Uniform negative staining was observed in normal gastric epithelium. Most cases showed moderate nuclear

staining for p53 with few cases showed strong immunoreactivity (figure 4).

Sixty cases out of 84 cases (83.3%) expressed p53. Its expression was higher in patients younger than 65years, tumors classified histologically as intestinal type, high grade tumors, tumors with lymph node metastasis and tumors with T3 stage, but all these correlations were statistically non-significant (table-1).

Correlation between p27 and p53 expression

In this study, 63.7% (38 out of 60) of p53 positive cases were p27 negative and 18 out of 24 p53 negative cases (75%) were p27 positive revealing a significant inverse correlation between p27 and p53 expression (table-2).

Tbable (1): Relationship between p27 and p53 expression and clinico-pathological parameters in gastric carcinoma

Parameters	P27 expression				P53 expression			
	+ve	-ve	Total	P-value	+ve	-ve	Total	P-value
Age (years)								
<65	32 (80%)	36 (82%)	68		50 (83.3%)	18 (75%)	68	
≥65	8(20%)	8 (18%)	16	P>0.01	10(16.7%)	6 (25%)	16	P>0.01
Total	40	44	84		60	24	84	
Lauren's type								
Intestinal	34 (85%)	38 (86.4%)	72		52 (86.7%)	20 (83.3%)	72	
Diffuse	6 (15%)	6 (13.6%)	12	P>0.01	8 (13.3%)	4 (16.7%)	12	P>0.01
Total	40	44	84		60	24	84	
Tumor grade								
I-II	26 (65%)	14 (31.8%)	40		24 (40%)	16 (66.7%	40	
III	14 (35%)	30 (68.2%)	44	P<0.01	36 (60%)	8 (33.3%)	44	P>0.01
Total	40	44	84		60	24	84	
Lymph node status								
+ve	10 (25%)	30 (68%)	44		26 (43.3%)	14 (58.3%)	40	
-ve	30 (75%)	14 (32%)	40	P<0.01	34 (56.7%)	10 (41.&%)	44	P>0.01
Total	40	44	84		60	24	84	
T-staging								
T2	24 (60%)	4 (9%)	28		22 (36.7%)	6 (25%)	28	
T3	16 (40%)	40 (91%)	56	P<0.01	38 (63.7%)	18 (75%)	56	P>0.01
Total	40	44	84		60	24	84	

Table (2): Correlation between P27 and p53 expression in gastric carcinoma cases

	P53 expression			
	+ve	-ve	Total	P-value
P27 expression				
+ve	22 (36.7%)	18 (75%)	40	
-ve	38 (63.3%)	6 (25%)	44	P<0.01
Total	60	24	84	

Discussion

Gastric carcinoma is a major cause of morbidity and mortality world wide. The reliable prognostic factors are tumor stage and complete excision, however tumor grade and histological type may be also useful prognostic factors [(22)].

The development of human cancers is a multistep process, and phenotypic changes during cancer progression reflect the sequential accumulation of genetic alterations in cells. Cellular proliferation follows an organized and timely regulated progression through the cell cycle, which is regulated by cell-cycle-regulators including p27 and p53 [(15,23)].

This study aimed to evaluate cell-cycle-regulators, p27 and p53, expression in gastric carcinoma by immunohistochemistry and to correlate their expression with other clinicopathological findings.

Our study demonstrated that epithelial cells of normal glands and lymphocytes in the entire gastric wall showed expression of p27, while 47.6% of

gastric carcinoma were positive for p27. In previous studies of Liu et al. [24] and 17-Al-Moundhri et al. [16], the p27 expression in gastric carcinoma was detected in 38.6% and 39.7% of cases respectively. This finding is compatible with preservation of p27 in quiescent state of normal gastric tissue and loss of p27 protein in malignant tumors.

Prognostic significance of p27 expression has been reported in various human tumors [24-26]. Results of the present study showed that loss of expression of p27 protein was significantly correlated with tumor grade, lymph node metastases and T-stage. A number of studies were in accordance with our results [16,27,28]. These studies demonstrated that low expression of p27 protein tended to be associated with deeply invasive tumors, lymph node metastases and/ or poor clinical out come.

Previous study of Jordan et al. [29] showed decreased expression of p27 with increasing grade of gastric dysplasia. This finding implies that graded alterations in p27 expression might occur from premalignant lesions to early cancer and to advanced cancers.

In the present study, statistical significant correlation was found between reduced p27 expression and gastric carcinoma exhibiting high histologic grade. These results are consistent with the findings of Ma et al. [30] studying gastric carcinoma. In other previous studies, there was evidence of positive correlation between low p27 expression and high histologic grade in carcinoma of breast, prostate, colon and thyroid [12,31-33]. These results suggest that loss of p27 expression may participates not only in aggressive behavior, but also in differentiation of malignant tumors.

As regard p27 expression in relation to patient age and histologic type, p27 showed non-significant correlations with both parameters. Similar results were found by Kim et al. [34], while Al-Moundhri et al. [16] showed a significant association between p27 expression and patient age but non-significant correlation between p27 expression and histological type. Liu et al. [24] showed that p27 was negative in tumor classified histologically as diffuse type.

In the current study reduced p27 expression in cases of gastric carcinoma was found to be higher in patients with positive lymph node metastases. Previous studies of Mori et al. [35] , Yasui et al. [36] and Kim et al. [34] had shown also a significant correlation between reduced p27 expression and lymph node metastases. P27 has also been shown to have a function in adhesion-dependent cell growth [37]. This finding suggests that loss of p27 protein may aid in tumor cell growth in the presence of altered extracellular matrix properties and altered adhesion, and this could allow cells to escape from the primary tumor and facilitate metastasis.

In the current study, reduced p27 expression was significantly correlated with high T-stage. This was consistent with results of Kim et al. [34]; Liu et al. [24] and Kim (2007) [13] suggesting that loss of p27 protein may result not only in cellular proliferation, but also in tumor development and progression.

In the present study, the overexpression of p53 was shown in tumor classified as intestinal type, high grade tumor, tumors with lymph node metastases and tumors with T3 stage, but these correlations were statistically non-significant. These results, although non-significant, agree with those previously reported studies by Aoyagi et al. [38] and Bando et al. [39] who indicated that p53 overexpression is an independent prognostic marker for gastric carcinoma.

Liu et al. [24] and 17-Al-Moundhri et al. [16] reported that the aggressive characteristics of a tumor are often closely associated with abnormal expression of cell-cycle-related proteins. Reduced expression of p27 acting as a negative cell-cycle regulator would be predicted to increase cyclin-dependent kinase (CDC) activity, resulting in active proliferation of tumor cells. On the other hand, mutated p53 protein loses its inhibitory effect on cell cycle progression [40]. This is in agreement with the current study, where a significant inverse correlation between p27 and p53 expression was observed, suggesting that the relative co-expression of cell-cycle regulating proteins may have a role in the biologic behavior of gastric tumors.

So, in conclusion, reduced expression of p27 may influence the progression and metastases to lymph node in gastric tumors. In addition, combined expression of cell-cycle regulators, p27 and p53, may play an important role in the biological behavior of human gastric carcinoma. Further studies on gene expression in human cancer may (are recommended to) provide essential information for better understanding the mechanisms regulating gastric carcinogenesis.

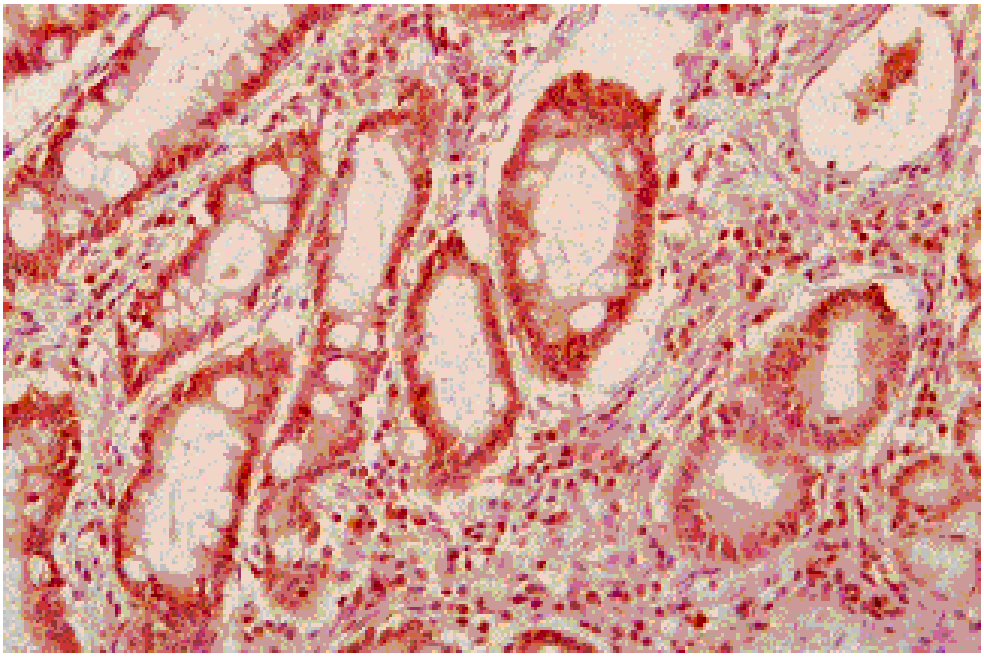

Figure (1): Normal gastric mucosa showing nuclear staining for p27. (Immunoperoxidase X 300)

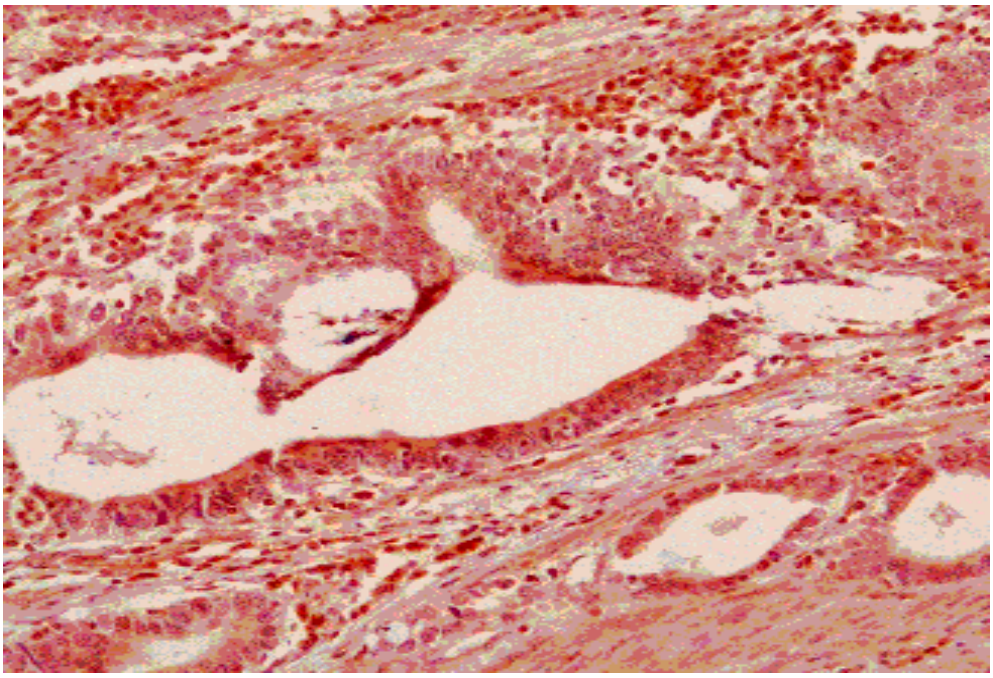

Figure (2): Infiltrating gastric carcinoma showing cancer cells with positive nuclear staining for p27. Lymphocytes used as positive control are stained for p27. (Immunoperoxidase X 300).

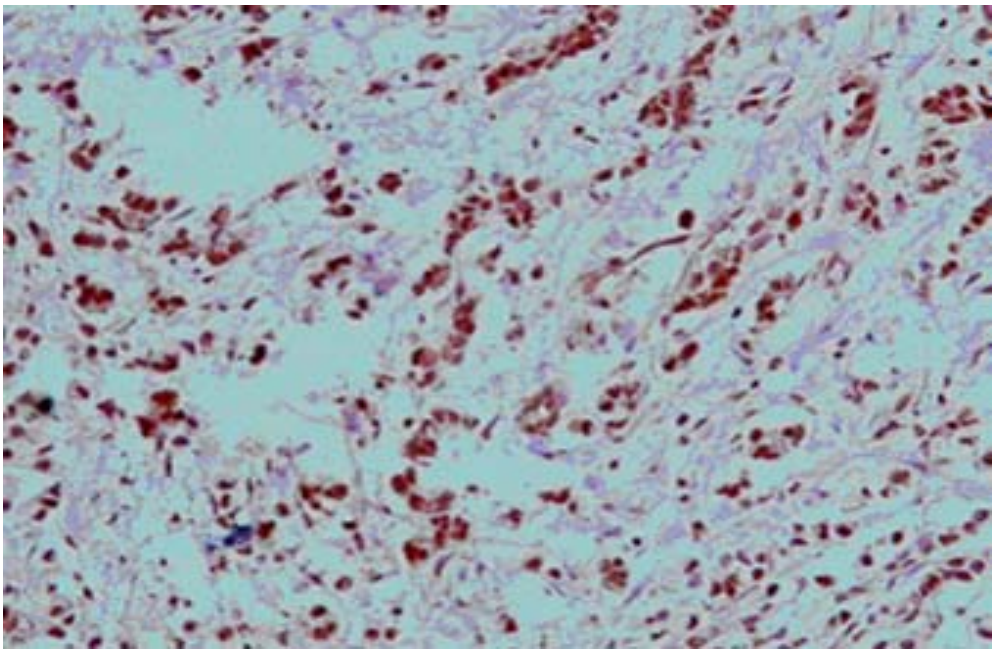

Figure (3): Infiltrating gastric carcinoma with p27 positive staining in cancer cells and lymphocytes.
(Immunoperoxidase X 200)

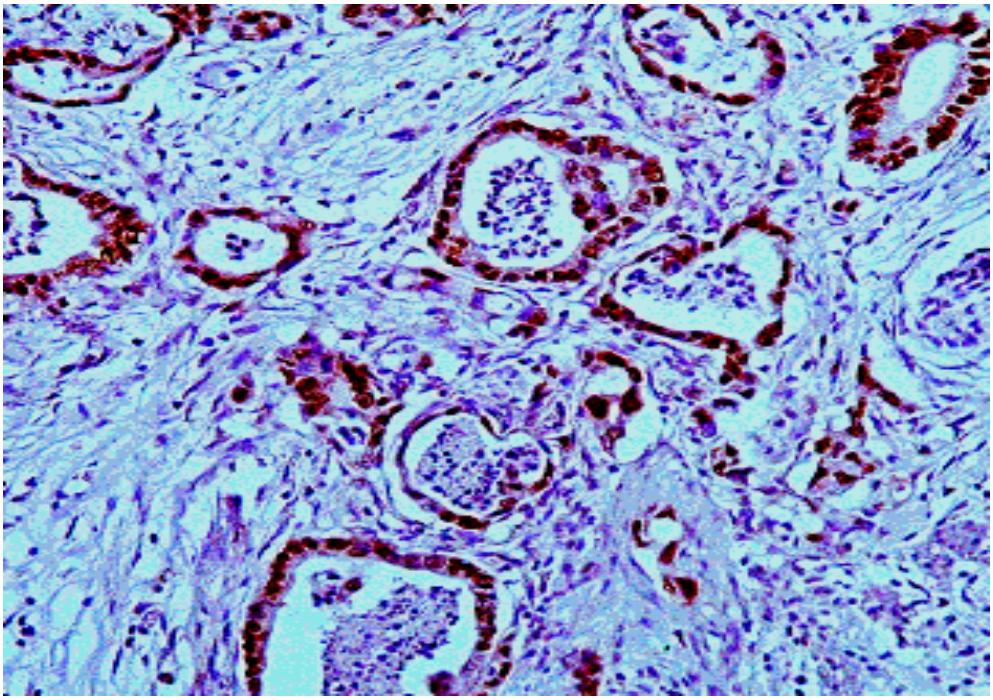

Figure (4): P53 nuclear staining in a case of gastric adenocarcinoma.
(Immunoperoxidase x 300)

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