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Gülen DOĞUSOY
Süha GÖKSEL
Işıl PAKİŞ
Haydar DURAK
Gökhan ERSOY
Selda GÖÇENER

An Immunohistochemical Study of Gastric Neuroendocrine Tumors: Expression of Proliferating Cell Nuclear Antigen and P53 Oncoprotein

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Department of Pathology, Cerrahpaşa Faculty of Medicine, University of Istanbul, Istanbul-Turkey

Abstract: The aim of this study is to examine expression of Proliferating cell nuclear antigen (PCNA) and p53 oncoprotein in gastric neuroendocrine tumors. 35 gastric neuroendocrine tumors were analyzed for first histopathologic features and then, immunohistochemical study was done to detect labeling index of PCNA and accumulation of p53 protein by using monoclonal antibodies and the Labeled strepavidin-biotin method. PCNA labeling index and p53 protein expression were compared with histopatholoic features in statistical methods of Fisher's exact, chi square and logistic regression tests. No significant correlation was found between them except PCNA score and depth of invasion. In conclusion, our findings show that not p53 but PCNA appears to be useful in detecting malignant potential of these tumors.

Key Words: Gastric neuroendocrine tumors, PCNA, p53 oncoprotein.

Introduction

Gastric neuroendocrine tumors (GNET) are recently classified according to their degree of differentiation. Klöppel et al (1, 2) and Rindi (3) proposed new classification schemes. Klöppel et al's classification deals with all aspects of the pathology of GNET (1, 2). Rindi classifies these tumors on the basis of their differentiation status as: 1) well differentiated: argyrophil cell tumors, mainly composed by enterochromaffin-like (ECL) cells or gastrin-producing (G) cells or 2) poorly differentiated: neuroendocrine carcinomas (NECs) (3, 4).

Incidence of GNET raised to 11-41% in recent series, from 0.3% of all gastric tumors in the earlier literature(1, 3-6). The remarkable increase in their incidence is most likely related to our increased awareness of these lesions and the widespread use of endoscopy in gastroenterology practice (1, 3, 4).

Proliferating cell nuclear antigen (PNA) is an auxillary protein for DNA polymerase delta that plays an important role in DNA synthesis and is thought to be synthesized in nuclei, particularly during the proliferative period of late G1 and S-phases. PC10, a monoclonal antibody that can be used on tissues that are rountinely fixed and embedded in paraffin wax, has been found to recognize PCNA. Recently, this antigen was used to estimate the correlation between PCNA and the malignant potential in some neoplasms (5).

The p53 is a tumor supressor gene located on the short arm of chromosome 17 and its functional inactivation through mutation or allelic deletion plays an important role in the development of many human tumors like carcinomas of the lung, colon and breast. Mutations in the gene often increase the stability of the p53 protein, causing it to be detectable by immunostaining (6, 7, 8). In gastric carcinomas, the frequency of p53 mutations varies from 4 to 70% (8-12). These great variatioons are due to two different factors: a) the detection of p53 gene alterations has been done using different techniques; b) the series that have been studied are often small, involving only one particular histologic type. It is still controversial whether p53 mutations are associated with clinicopathologic features and prognosis (8).

We therefore studied the immunohistochemical staining of Proliferating cell nuclear antigen (PCNA) and expression of p53 protein in gastric neuroendocrine tumors and compared the results with histologic features to find any association with the malignant potential.

Material and Methods

Thirty-five gastric tumors which showed evidence of neuroendocrine differentiation histologically were randomly collected from the archieves of Department of Pathology, Cerrahpaşa Medical School, University of Istanbul, since 1985 to 1996. Tissues were fixed in formalin and routinely processed in paraffin. Serial sections were stained with hematoxylin and eosin for histological evaluation and conventional histopathology identified 26% well differentiated GNET and 74% poorly differentiated GNET or neuroendocrine carcinomas. Tumor analysis comprised determining the size, site, level of infiltration.

Immunohistochemical studies were performed according to the manufacturers instructions for identifying NE differentiation in these tumors, by Labeled strepavidin-biotin method with markers; Chromogranin A, Synaptophysin, neuron-specific enolase, gastrin, glucagon, serotonin, somatostatin DAKO.

Immunohistochemical tests used to detect p53 protein (DO-7, DAKO) and PCNA (PC 10, DAKO) were performed using the Labeled strepavidin-biotin method described before (13).

Only the nuclei were stained, whereas the cytoplasm and cell membrane remained unstained by PCNA and p53 staining. Therefore, the labeling indexes of the samples stained with anti PCNA and accumulation of p53 were determined by counting the number of positive nuclei per 1000 tumor cells. PCNA labeling index was calculated as the percentage of positive nuclei. The cases were classified into three subgroups; low score group for which the PCNA labeling indexes were less than 30%, moderate score group for which the PCNA labeling indexes were between 31-60% and high score group for which the PCNA labeling indexes were more than 61%. P53 expression was scored in all tumors as positive or negative regadless of some variation in the intensity of the immunostain in different positive cells.

For statistical analysis, Fisher's exact, chi square and logistic regression tests were used. Statistical significance was defined as $p<0.05$.

Results

In 35 gastric tumors which showed histopathologic features of GNET, the method for establishing NE differentiation was relied on immunohistochemical reactions. General neuroendocrine antibodies; Synaptophysin and Chromogranin A were found more frequent positive (87.5% and 70% respectively) than Neuron Specific Enolase (53%).

Glucagon (87.5%) and Gastrin (47%) were the most common antigens among specific hormones. Positivity with serotonin was 22% and with somatostatin was 12.5%.

PCNA labeling index stayed within the range of 5-90%. Of the 35 cases, 10 had low, 19 had moderate and 6 had high PCNA score. Table 1 shows the relationship between PCNA labeling index and histopathologic features. There was only statistically significant association found between PCNA and depth of invasion. The mean PCNA labeling index in patients with tumors extending to serosa and beyond serosa was significantly higher. ($p=0.0105$ in Fisher's exact test and $p=0.0190$ in chi square test when low and intermediate score compared, $p=0.0040$ in Fisher's exact and $p=0.0057$ in chi square tests when low and intermediate plus high score compared). No statistically significant association was found between PCNA and other histopathologic characteristics.

Of the 35 cases, 12 had p53 accumulation (34%). The number of positive cells and staining intensity varied from one case to another. The range of positive nuclei was 5-75%. The results of relationship of p53 expression and histopathologic features were summarized in Table 1.

No significant difference was found between p53 accumulation and histopathologic features.

Normal gastric mucosa and areas of intestinal metaplasia adjacent to carcinomas were completely negative for p53 but moderate or low PCNA labeling index was observed in glands with intestinal metaplasia and dysplasia.

Also presence of associated lesions in the neighbouring mucosa were compared with PCNA and p53 expression but no statistical significance was found (Table 2).

Discussion

In the earlier literature, GNET accounted for about 0.3% of all gastric tumors. However, recently, GNET comprise 11 to 41% of all gastrointestinal neuroendocrine tumors (NET). The remarkable increase in their incidence is most likely related to our increased awareness of the lesions and the widespread use of endoscopy in gastroenterology practice. Experimental studies on hypergastrinemia induced by lifelong antisecretory treatment or proximal gastric resection has

Table 1. Correlation between histopathologic features and PCNA index and p53 expression.

Features	PCNA Index			p53 expression		p
	Low	Intermediate	High	Negative	Positive	value
Location						
Body-Fundus	4	8	2	11	3	NS
Antrum	6	10	4	12	8	NS
Transitional mucosa	0	1	0	0	1	NS
Differentiation						
Well	5	3	2	6	4	NS
Poorly	5	16	4	17	8	NS
Pattern						
Mixed	5	14	4	15	8	NS
Trabecular	2	1	1	3	1	NS
Diffuse	0	2	0	1	1	NS
Insular	3	2	1	4	2	NS
Depth of invasion						
Muscularis	5	1	0	2		
Serosa	4	16	4	16	8	0.01*
Beyond serosa	1	2	2	3	2	
Lymphatic invasion						
Negative	8	15	6	18	11	NS
Positive	2	4	0	5	1	NS
Venous invasion						
Negative	1	6	1	6	2	NS
Positive	9	13	5	17	10	NS
Lymphnode metastasis						
Negative	6	9	5	11	9	NS
Positive	4	10	1	12	3	NS

NS: not significant

*(PCNA labeling index increased significantly when the depth of invasion increased, p=0.01 between low and intermediate score, p=0.005 between low and intermediate+high score)

been shown to result in GNET, raised concerns about a possible tumorigenic effect of prolonged antisecretory therapy in human subjects (3).

In 1980, WHO classification of endocrine tumors applied the term carcinoid to all tumors of the diffuse NE system, and subdivided carcinoids on the basis of various silver and other granule staining techniques in i) EC cell carcinoids; ii) gastrin cell carcinoids, and iii) other carcinoids. The broad use of the WHO terminology, however has proved difficult and often created confusion among pathologists and clinicians. This is primarily due to the fact that the wide application of progressively refined tecniques in pathology, has revealed a great diversity among NET. Secondly, the clinically characterized carcinoid syndrome only relates to a certain type of carcinoid, the EC-cell carcinoid. Thirdly, WHO classification vaguely considers the biological behavior of NET. For this reasons, a number of investigators, proposed to replace the term "carcinoid" by the more uncomitting name "NET". This name includes the entire NET spectrum, with the classic carcinoid at one hand and the undifferentiated carcinoma at the other. They also proposed a new classification of scheme that deals with all aspects of the pathology of NET. The first principal is that

Table 2. Correlation between associated lesions in the nontumorous mucosa and PCNA index and p53 expression.

Features	PCNA Index Low	Intermediate	High	p53 expression Negative	Positive	p value
Chronic atrophic gastritis						
Positive	7	11	4	15	7	NS
Negative	3	8	2	8	5	NS
Intestinal metaplasia						
Positive	7	6	3	12	4	NS
Negative	3	13	3	11	8	NS
Endocrine cell hyperplasia						
Positive	5	4	1	8	2	NS
Negative	5	15	5	15	10	NS

NS: not significant

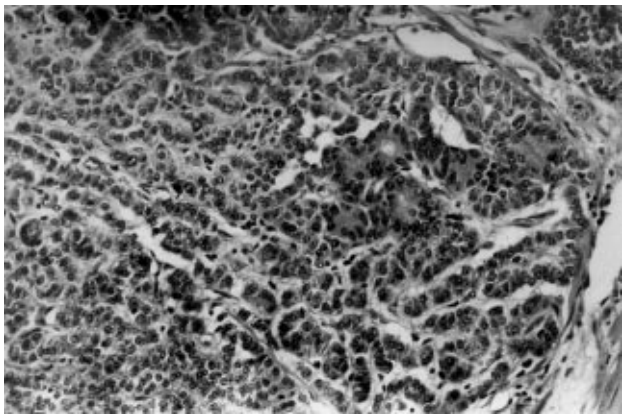

Figure 1. A gastric neuroendocrine tumor which shows mixed microscopic pattern. (HEX 200)

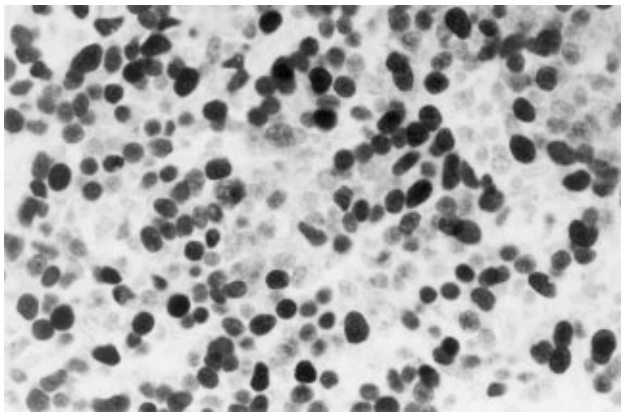

Figure 2. High PCNA labeling index in a poorly differentiated gastric neuroendocrine tumor (PCNAX400)

the tumors are distinguished according to the site of origin. The second principal is to subdivide the neoplasms into 1) tumors with benign behavior 2) tumors with uncertain behavior, 3) tumors with low grade malignant behavior 4) high grade malignant tumors. The main criteria for this biologic categorization are histologic differentiation, angioinvasion, direct invasion of neighboring organs, and the presence of metastases and size. The third principal is to incorporate the hormonal function and various clinical associations of NET. (1, 2). Rindi classifies these tumors on the basis of their differentiation status as: 1) well differentiated: argyrophil cell tumors, mainly composed by enterochromaffin-like (ECL) cells or gastrin-producing (G) cells or 2) poorly differentiated: neuroendocrine carcinomas (NECs) (3, 4).

Histologically, well differentiated NET show mostly mixed architectural pattern of insular and trabecular patterns. Microaciner pattern is seen in a minority. Tumor cells are monomorphic and medium-sized with regular shape and round nuclei. They shown no atypia and occasional mitoses. In poorly differentiated NET, the dominant pattern is trabeculae or diffuse sheets, frequently with central necrosis. Cells are intermediate in size, round to oval-shaped, with large nuclei, prominent nucleoli, and frequent mitoses (3, 4). In our study, histological evaluation and conventional histopathology identified 26% well differentiated GNET and 74% poorly differentiated GNET or neuroendocrine carcinomas.

PCNA is an auxiliary protein of DNA polymerase delta, which plays a major role in synthesizing DNA and is

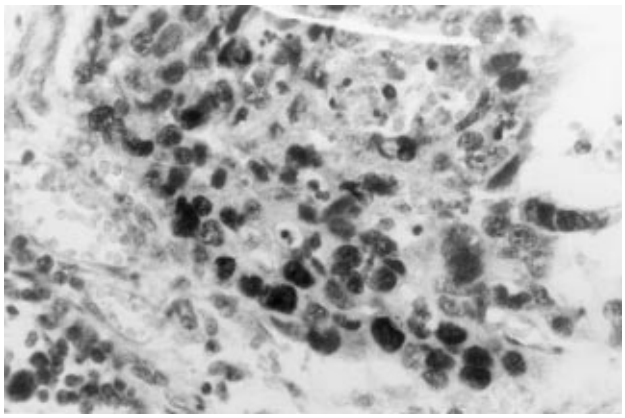

Figure 3. P53 oncoprotein positivity in a poorly differentiated gastric neuroendocrine tumor (p53X400)

thought to be expressed in the nuclei, particulary in late G1 and S phases (8, 14). PCNA has drawn attention as one of the parameters for cell proliferation kinetics in recent years and cell proliferative activity was suggested to be effective in judging the malignant potential of various carcinomas. The relationship between PCNA and the malignant potential of various cancer also has been reported. Some authors suggested that PCNA labeling index can be used as an indicator of malignant potential (8). In this study, we used PCNA as an indicator of cell proliferation activity in gastric neuroendocrine tumors. Ooi observed that endocrine-differentiated cancer cells are in a quiescent state, most likely arrested in the G0 and G1 phase of the cell cycle and they represent terminally differentiated cells as they do not stain for PCNA. This, however, does not necessarily imply that cancers with endocrine differentiation are less malignant (14). In another study although there was a significant difference in the incidence of PCNA immunoreactivity between NET of the jejunum and ileum and those of the appendix, no significat difference was observed in gastric neuroendocrine tumors (15). PCNA labeling index was found to be increased in gastric tumors in relation to some histopathologic features such as lymph node metastasis (16), depth of invasion, grade and vessel invasion (17). Our study showed that PCNA labeling index is not so how in fact and show high score in 23% of GNET. However, a significant association was found only between depth of invasion and PCNA but not with differentiation degree or other clinicopathologic features and PCNA.

Abnormalities in p53 have been demonstrated in a wide variety of common human malignancies (9). Variations in p53 positivity according to tumor histology have been observed in gastric carcinomas. P53 positivity is found higher in intestinal type compared to diffuse type in many studies (8-10, 12, 18-20). The variations in p53 in the different types of gastric cancers indicate that their carcinogenesis is different. This may reflect the fact that many differentiated adenocarcinomas of the stomach are thought to arise from areas of metaplasia that resemble intestinal epithelium (10, 18). P53 is important in gastric tumors of the intestinal type. This histological type has various oncogenes in common with colon tumors. The presence of p53 protein has been preferentially described in advanced stages of gastric cancer (18-21) and a significantly higher frequency of p53 positive staining was observed in carcinomas which show cardia location (20), necrosis (17), vessel invasion (12, 17, 22) and lymph node metastasis (22-24) but, in many studies, no relationship between p53 protein accumulation and histopathologic features and prognosis are established (8-10, 25). In our study, we examined the expression of p53 in only gastric neuroendocrine tumors and like these studies, we couldn't found any significant association between p53 protein expression and histopathologic features. Some authors, reported that the 5 year survival rate for patients with stomach cancers positive for p53 was significantly lower than for those with stomach cancers negative for p53 (9, 22).

In conclusion, PCNA labeling index may be a good prognostic indicator in patients with gastric neuroendocrine carcinoma. P53 protein accumulation is not rare in gastric neuroendocrine tumors and as p53 seems to play an important role in the carcinogenesis and as its expression significantly correlates with some prognostic indicators like depth of invasion and vascular invasion, this oncogen appears to be useful in detecting malinant potential of these tumors.

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