



OVER - EXPRESSION OF p53 PROTEIN IN GASTRIC ADENOMAS

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Summary: *The p53 gene is a nuclear phosphoprotein that regulates DNA replication, cell proliferation and cell death. Although the precise mechanisms by which p53 acts as a tumor suppressor gene are not known, accumulating evidence suggests that normal wild-type p53 acts as a "molecular policeman", preventing propagation of genetically damaged cells. The accumulated wild-type p53 binds to DNA and causes cells to arrest in the G1 phase of the cell cycle. With loss of normal p53 gene cells which are exposed to mutagenic agents replicate the damaged DNA and the mutations become fixed in the genome.*

To prove a stepwise fashion of tumor progression at the molecular level, we compared the results of immunohistochemical expression of p53 protein in gastric adenomas with results of immunohistochemical expression of p53 protein in gastric adenocarcinomas.

p53 was not expressed in the normal gastric mucosa but it was expressed in 7 of 10 adenomas with low grade of atypia and in all 6 adenomas with high grade of atypia. Among gastric adenocarcinomas, p53 protein was expressed in 15 of 16 adenocarcinomas. The incidence of p53 expression in gastric adenocarcinomas increased with depth of invasion (without any correlation with mentioned clinical factors) and with the grade of undifferentiation.

Our findings suggest that the p53 gene plays an important role in the origin of human gastric tumors.

Key words: *p53 expression, stomach adenoma, immunohistochemistry*

Introduction

Mutations of the p53 tumor - suppressor gene are the most common genetic alterations in human cancer, found in approximately 50% of all tumors (1, 2, 3, 4, 5). The importance of p53 in human cancer attracts attention in molecular studies dealing with the pathogenesis, diagnosis and prognosis in tumor pathology (3, 5, 6).

The truly neoplastic adenoma, including the adenomatous polyp (adenoma with glandular pattern) and papillary or villous adenoma, has malignant potential and because of that should be removed, by total or piecemeal endoscopic polypectomy (7, 8). The examination of multiple sections is essential, particularly when more marked degrees of dysplasia are present, to detect any areas of invasion of neoplastic cells through the basal membrane of the glands and pits.

Having in mind that the distinction between highly differentiated intramucosal adenocarcinoma and severe dysplasia in adenoma on histological grounds is often very difficult and that p53 alteration may play a part in the neoplastic progression of adenomas, we have undertaken the following study: a comparative investigation of histological characteristics and the expression

of p53 protein in stomach adenomas with low and high grade of dysplasia.

Materials and Methods

Endoscopically resected 16 adenomas and 16 surgically resected stomachs harboring differentiated type carcinomas (positive control) and non - neoplastic mucosa surrounding the carcinoma (negative control). All materials were fixed in 10% formalin and embedded in paraffin. The adenomas were divided according to the presence of low - grade and high - grade dysplasia. Low - grade dysplasia is characterized by a cigar - shaped hyperchromatic nuclei in a palisading arrangement with only slight stratification. High - grade dysplasia is recognized by the presence of swollen nuclei with prominent nucleoli and stratification extending to the apical surface. HE, PAS, HID - AB, pH = 2, 5 and Avidin - biotin complex methods were used. The specimens were treated by micro wave (in citrate buffer pH = 6.0 in 75°W), three times for 5 minutes, before the reaction with MAb DO - 7 (9). The percentage of p53 - immunoreactive tumorous cells was estimated in relation to all tumor cells present in the stained

specimens.

The adenomas were divided into four groups according to the results of p53 staining:

- (1) p53 - negative adenomas;
- (2) tumours with under 5% adenoma positive cells;
- (3) tumours with 5 - 10% adenoma positive cells and
- (4) tumours with more than 10% adenoma positive cells.

Results

Clinicopathological data

There were 10 males and 6 females, aged from 34 years to 78 years for males (mean 63 years) and from 43 to 80 years for females (mean 65 years) (Table 1). Ten tumors were involved in the antrum and six were located in the corpus.

The same clinical data for positive controls (adenocarcinomas) were pointed out in Table 1.

Table 1. Patients with p53 protein expression in stomach lesions

Lesions	Patients	m : f	Mean age	p53 expression
Adenomas	16	10 : 6	63 (m), 65 (f)	13 (81.3%)
Adenocarcinomas	16	11 : 5	66 (m), 72 (f)	15 (93.7%)

Histopathologic analysis

Adenomas were sessile or broad based lesions, with irregular surface of villous (2 cases), tubulo - villous (8 cases) and tubular architecture (6 cases). Histologically, adenomas were composed of closely packed tubular and/or papillary projections, lined by dark - staining colonic - type epithelial cells with reduced secretory activity and various degrees of cellular pleomorphism. Normal gastric glands, often cystic, were found in the base (Fig. 1)

Adenocarcinomas showed various grade of differentiation.

Immunohistochemical analysis

Immunohistochemical results were summarized in Table 2. Of 16 cases, nuclear positivity for p53 protein in adenomas with low grade of atypia, was detected in less than 5% of tumorous cells in 5 cases (50%) and in 5 - 10% in 2 adenomas (20%). Negative immunoreactivity was observed in 30% of adenomas with low grade of atypia.

In the adenomas with high grade of atypia less than 5% of positive tumour cells was observed in 2 cases (33.3%) and in 3 adenomas p53 was expressed in 5 - 10% of tumorous cells (50%). Only one adenoma with high - grade of atypia showed very high p53 protein expression (more than 10%) (Fig. 2.).

In the positive control group (differentiated type of carcinoma) 16 cases were studied. The expression of p53 was found in 93, 7% of carcinomas (Table 2.). In positive cancer cases p53 protein expression was scattered (less than 5% of tumorous cells), or densely

distributed (from 5 - 10% of tumorous cells). In 8 adenocarcinomas p53 protein expression was evident in more than 10% of cancer cells (Fig. 3).

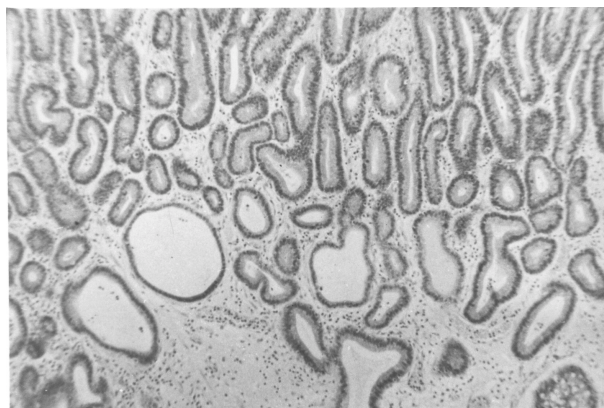


Fig. 1. Histomorphology of gastric tubular adenoma. HE×250

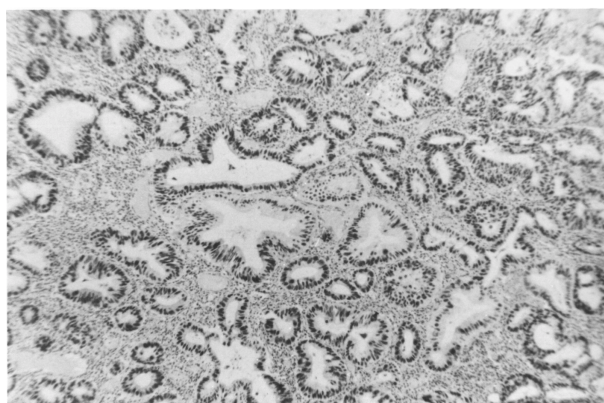


Fig. 2. p53 protein expression in tubular gastric adenoma with high grade dysplasia. ABC×250

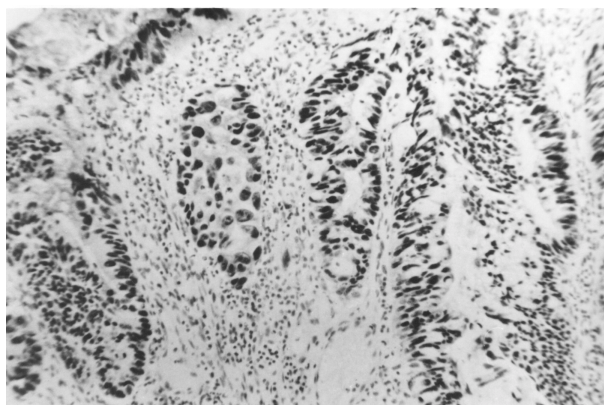


Fig. 3. p53 protein expression in gastric carcinoma. ABC×300

Significant correlation was found between p53 protein expression and grade of anaplasia (densely distributed in the region with more prominent nuclear atypia). Correlation was observed also between the p53 expression and the depth of invasion.

Normal gastric mucosa (negative controls) did not show p53 protein expression (Table 2).

Table 2. p53 immunoreactivity in adenomas, carcinomas and normal mucosa

Lesions		p53 immunoreactivity			
		negative	positive		
		No (%)	<5% No (%)	5-10% No (%)	>10% No (%)
Adenomas	(n=16)				
low grade	(n=10)	3 (30%)	5 (50%)	2 (20%)	0 (0%)
high grade	(n=6)	0 (0%)	2 (33.3%)	3 (50%)	1 (16.6%)
Adenocarcinomas	(n=16)	1 (6.25%)	3 (18.75%)	4 (25%)	8 (50.0%)
Normal mucosa	(n=16)	16 (100%)	-	-	-

Discussion

The p53 gene is known to be a tumor suppressor gene localized to chromosome arm 17p13 and to code for a 53 - kD nuclear protein regulating cell cycle in a still unclarified fashion (10). Its functional inactivation through mutation or allelic loss plays an important role

in the development and progression of a variety of human tumors. Immunohistochemical detection of the p53 protein product suggests the presence of genetic alterations, since the steady - state levels of the normal protein which are very low due to rapid turnover, are usually undetectable by this method. It has recently been reported that expression of p53 protein is common in gastric cancers (11, 12, 13, 14).

In this study we used a recently developed DO - 7 antibody, which readily detects p53 protein in routine formalin - fixed and paraffin - embedded tissues (9, 10). We demonstrated a high incidence of nuclear expression of p53 protein in more than 10% of tumorous cells in the adenomas with high grade dysplasia. Because of that, we suggest that p53 alteration plays a part in the dysplastic progression of adenomas. Other authors have observed that p53 protein expression corresponded to the presence of aneuploid cells (2, 9, 11). Finally, some of them have not demonstrated any significant association between p53 expression and clinical features, such as tumor stage, depth of invasion and metastasis (6, 12). However, in our results, positivity for p53 was significantly higher inside the fields with severe dysplasia without any correlation with mentioned clinical factors.

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EKSPRESIJA p53 PROTEINA U ŽELUDAČNIM ADENOMIMA

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Kratak sadržaj: Nuklearni fosfoprotein, p53 gen, reguliše DNA replikaciju, ćelijsku proliferaciju i ćelijsku smrt. Mada nisu poznati mehanizmi pomoću kojih p53 deluje kao supresorni gen, dokazano je da normalni "wilde - type" p53 deluje kao "molekularni policajac", sprečavajući propagaciju genetski oštećenih ćelija. Akumulisani "wilde - type" p53 se vezuje za DNA i izaziva mirovanje ćelija u G1 fazi ćelijskog ciklusa. Sa gubitkom normalnog p53 gena, ćelije koje su izložene mutagenim agensima replikuju oštećenu DNA, pa samim tim mutacije postaju fiksirane u genomu.

Da bi se dokazao stepeničasti način tumorske progresije na molekularnom nivou, uporedili smo rezultate imunohistohemijske ekspresije p53 proteina u adenomima želuca sa rezultatima imunohistohemijske ekspresije p53 proteina u adenokarcinomima želuca. Nema ekspresije p53 proteina u normalnoj mukozi želuca ali je ima u 7 od 10 adenoma sa nisko - stepenom atipijom i u svih 6 adenoma sa visoko - stepenom atipijom. U adenokarcinomima, p53 proteinska ekspresija je nađena u 15 od 16 ispitivanih adenokarcinoma. Učestalost p53 proteinske ekspresije je povećana sa dubinom invazije i sa stepenom dediferencijacije, dok nikakve korelacije nije bilo sa istaknutim kliničkim faktorima.

Naši nalazi sugerišu da supresorni p53 gen igra važnu ulogu u nastajanju želudačnih tumora u ljudi.

Ključne reči: p53 ekspresija, želudačni adenom, imunohistohemija

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