Overexpression of p53 protein in Barrett's syndrome with malignant transformation

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Abstract

Aims—To study the overexpression of p53 protein in Barrett's oesophagus with adenocarcinoma, and to correlate this expression with the pathological features of Barrett's syndrome.

Methods—Immunohistochemical staining was performed on frozen sections with a monoclonal antibody directed against wild type and mutated p53 protein (Pab 1801). Eleven cases of Barrett's adenocarcinoma were studied, seven of which had extensive sampling of benign Barrett's mucosa.

Results—Eight of 11 adenocarcinomas overexpressed the p53 protein. Both early and advanced tumours were positive. In Barrett's mucosa around the p53 positive tumours, high grade dysplasia was positive; low grade dysplasia and non-dysplastic mucosa were negative.

Conclusions—P53 gene mutation with ensuing p53 protein overexpression is a common feature of Barrett's adenocarcinoma, both at early and advanced stages. This mutation appears as a relatively late event during the neoplastic transformation of Barrett's oesophagus.

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TP53 is a tumour suppressor gene located on the short arm of chromosome 17.¹ It codes for a 53 kilodalton nuclear protein with probable cell cycle regulatory function. The loss of p53 protein wild type function may have a role in malignant transformation. This loss usually occurs in two stages that includes mutation of one copy of the p53 gene and deletion of the remaining wild type allele.²⁻⁴ Gene mutation results in the synthesis of a mutant protein with a much longer half-life than that of the wild type protein. Therefore, many groups have used immunohistochemistry to demonstrate the presence of the mutant form because the wild type product cannot be detected by this method. Overexpression of p53 protein has been shown immunohistochemically in several human tumours, including cancers of the lung, colon, breast and liver.⁵⁻¹⁰ Correlation of p53 protein expression with clinicopathological factors has been diversely appreciated in different tumours. p53 protein overexpression is usually confined to malignant tissue, except in rare cases of breast fibroadenoma,¹¹ and in some adenomas of the colon, as a premalignant lesion.⁶

Barrett's oesophagus predisposes to the

development of oesophageal adenocarcinoma.¹² p53 protein overexpression has been shown in some Barrett's adenocarcinomas, but no correlation has been established with the clinicopathological features of the tumour.¹³ p53 protein overexpression has also been demonstrated by flow cytometry in cases of Barrett's oesophagus without cancer.¹⁴ This has been proposed as a putative indicator of an increased risk for progression to carcinoma. However, the stage of neoplastic progression at which p53 protein overexpression develops in Barrett's oesophagus has not been clearly established.

Methods

Eleven patients with resected Barrett's syndrome were included in the study. All patients were caucasian males aged 53 to 80 years.

All patients underwent oesophagogastrctomy. Surgical specimens were received fresh, opened, and samples were snap frozen in liquid nitrogen within one hour of surgery and stored at -80°C . In four cases only malignant tissue was frozen. In seven, the whole specimen was systematically sampled: two mucosal pieces were taken every centimeter on both sides of the oesophagus, together with gastric tissue, comprising a total of eight, nine, 10, 10, 11, 12, and 13 frozen blocks for cryostat sections.

The whole affected oesophagus was then processed in paraffin wax and serially sectioned, and analysed for the type of mucosa according to the method of Paull *et al*¹⁵ (specialised, cardiac, and fundic types). The presence and grade of dysplasia was analysed according to Riddell *et al*¹⁶ (non-dysplastic mucosa, low grade dysplasia, high grade dysplasia). Tumour differentiation, degree of infiltration, and lymph node status were assessed.

Sections (5 μm) from frozen tissue blocks were serially cut and stained with the primary monoclonal antibody Pab 1801 using a three step immunoperoxidase technique as described by Mason *et al*.¹⁷ Monoclonal antibody Pab 1801 (Novocastra, Newcastle upon Tyne, England) recognises a denaturation resistant epitope in human p53 protein located between amino acids 32 and 79.¹⁸ Sections were dried overnight at room temperature and fixed for 10 minutes in acetone immediately before use. After rehydration in TRIS-buffered saline (TBS) tissue sections were either exposed to primary antibody at a

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Figure 1 Diagram of a specimen of oesophagogastricomy for adenocarcinoma developed in Barrett's oesophagus, with histological features and p53 protein immunohistochemical reactivity.

dilution of 1 in 50 or to the dilution buffer for 30 minutes (negative control). They were then sequentially incubated for 40 minutes with peroxidase labelled species specific immunoglobulin antibodies. Peroxidase-labelled rabbit anti-mouse immunoglobulin (Dakopatts N° p161) and swine anti-rabbit immunoglobulin (Dakopatts N° p217) were diluted 1 in 20 in TBS. Visualisation was with diaminobenzidine (0.6 mg/ml) in the presence of 0.03% hydrogen peroxide. Endogenous peroxidase was not inhibited.

Haematoxylin was used to counterstain lightly nuclei. The inclusion in each run of HT29 cells (a human colonic carcinoma cell line known to express a high level of p53)¹⁹ was used as a positive control.

Results of immunohistochemistry were reported as either positive or negative, and compared with the histological features observed in the following frozen sections stained with haematoxylin and eosin and in the corresponding paraffin wax embedded blocks.

Results

Eight of the 11 adenocarcinomas stained positively with the human p53 specific mono-

Table 1 Relation between histological features of Barrett's adenocarcinoma and p53 tumour status

	p53 positive	p53 negative
Degree of invasion:		
Superficial*	3	0
Advanced	5	3
Differentiation:		
Well differentiated	2	0
Moderately differentiated	4	2
Poorly differentiated	2	1
Lymph node metastasis:		
Absent	6	1
Present	2	2

*Invasion limited to mucosa and submucosa

clonal antibody Pab 1801. The staining was exclusively nuclear, with all malignant cells stained and with stroma cells unstained. Three carcinomas were superficial (limited to the submucosa), and lymph node metastasis was absent in seven cases. Table 1 shows that most of the tumours expressed p53, irrespective of the stage of infiltration and grade of differentiation. Six of seven tumours without lymph node metastases and two of four with lymph node metastases were p53 protein positive.

In six of seven cases with multiple frozen specimens, the tumour was p53 protein positive (table 2). In those cases, squamous epithelium, normal fundic mucosa, non-dysplastic Barrett's mucosa, and Barrett's mucosa with low grade dysplasia were p53 protein negative; Barrett's mucosa with high grade dysplasia was p53 protein positive as was the carcinoma. A representative case is presented in figs 1 and 2. In some cases, areas of non-dysplastic mucosa and high grade dysplasia were present in the same section. In these cases, staining was limited to dysplastic glands.

Discussion

In this study, we have shown a high prevalence of p53 protein overexpression (73%) in Barrett's adenocarcinoma. In a similar immunohistochemical study, Jankowski *et al* found seven positive cases among 15 tumours (47%).¹³ Using a flow cytometric assay, Ramel *et al* found eight positive adenocarcinomas out of 15 cases (53%).¹⁴ This prevalence of p53 protein overexpression in Barrett's adenocarcinoma is comparable with that observed in other human solid neoplasms including colon, breast, and lung carcinomas. This result confirms that p53

Table 2 Relation between histological aspect of Barrett's mucosa and p53 protein expression in seven specimens of resected Barrett's oesophagus with adenocarcinoma

Case No	Squamous epithelium	Fundic mucosa	Non-dysplastic Barrett's mucosa			Dysplasia		Carcinoma
			Fundic	Cardiac	Specialised	Low grade	High grade	
Case 1	NA	-	NA	-	-	-	+	+
Case 2	NA	NA	-	-	-	-	+	+
Case 3	-	-	NA	-	-	NA	+	+
Case 4	-	-	NA	-	-	-	NA	+
Case 5	-	-	NA	-	-	NA	+	+
Case 6	-	-	NA	-	-	NA	NA	-
Case 7	-	-	NA	-	-	NA	+	+
Total	5-/0 +	6-/0 +	1-/0 +	7-/0 +	7-/0 +	3-/0 +	0-/5 +	1-/6 +

NA, not available on frozen sections.

Figure 2 Frozen sections of a specimen of Barrett's oesophagus with adenocarcinoma (same case as in fig 1). (A) Non-dysplastic Barrett's mucosa; (B) with no detectable p53 protein expression; (C) Barrett's adenocarcinoma; (D) with strong nuclear overexpression of p53 protein (A, C: haematoxylin and eosin; B, D: indirect immunoperoxidase with Pab 1801).

mutation and overexpression may be the most common genetic alteration in the development of human malignancies.^{4 20}

Correlation of p53 protein expression with clinicopathological factors has been diversely appreciated in various tumours. In colon cancer no correlation was found with the degree of tumour differentiation, tumour staging, or patient survival.^{6 7 21} In breast cancer, p53 protein expression was related to oestrogen receptor control,²² a known prognostic indicator, and to shortened survival.²³ Our study gives the first indications about the correlation between pathological features of Barrett's adenocarcinoma and p53 protein overexpression. As p53 protein was largely expressed both in superficial (early) and advanced adenocarcinomas, a prognostic value of this finding seems unlikely. This assumption is reinforced by the p53 protein positivity observed in six of seven lymph node negative tumours. However, larger prospective studies are clearly needed to reinforce these preliminary results.

The results that we observed in non-dysplastic and dysplastic mucosa are different from those obtained by Casson *et al.*²⁴ Using the polymerase chain reaction to detect p53 gene mutation on paraffin wax embedded material, these authors only found one mutated tumour among 14 oesophageal adenocarcinomas, including seven with Barrett's mucosa adjacent to tumour. Mutation was localised to exon 8, at codon 273 (CGT- > CAT). Interestingly, point mutations of the p53 gene were detected in four of seven specimens with Barrett's oesophagus with minimal or no dysplasia, with no mutation detected in the corresponding adenocarcinomas. Although difficult to compare, as different techniques were used, these results are different from those we obtained. In the six p53 positive cases from which multiple frozen specimens were available, p53 protein overexpression was limited to carcinoma and high grade dysplasia, and no reactivity was found in non-dysplastic mucosa and low grade dysplasia. Therefore, in our series, p53 protein

overexpression appears as a relatively late event in the neoplastic transformation of Barrett's oesophagus. Our results cannot exclude that in some cases mutation and overexpression occur at an earlier stage, as suggested by Ramel *et al* in their flow cytometric study.¹⁴ In this study, p53 overexpression was found in 5% of Barrett's metaplasia negative for dysplasia, 15% of mucosa with low grade dysplasia, and 45% of mucosa with high grade dysplasia. However, in this later study, the possibility of more severe histological features present in the biopsy specimens studied by flow cytometry cannot be entirely excluded, as different specimens were used for the morphological grading and the flow cytometric study.

The management of high grade dysplasia in Barrett's oesophagus remains controversial. When oesophageal biopsy specimens show high grade dysplasia, there is a high probability of finding an invasive carcinoma.²⁵⁻²⁶ Moreover, the surveillance of patients with Barrett's oesophagus and high grade dysplasia has enabled different groups to find early carcinomas with a good prognosis.²⁵⁻²⁷ Therefore, several authors have supported the recommendation that prophylactic oesophagectomy should be carried out in patients with persistent high grade dysplasia.²⁵⁻²⁸ However, Reid *et al* reported two patients who underwent radical surgery for high grade dysplasia, but in whom no cancer was found in the corresponding surgical specimen.²⁹ In an other study Hameeteman *et al* observed four patients with persistent dysplasia, one high grade, who did not develop any carcinoma, with a follow up ranging from 3.5 to eight years.³⁰ When high grade dysplasia is diagnosed, therefore, complementary data that could indicate a high risk of associated or rapidly developing carcinoma are required. In our series all surgical specimens with high grade dysplasia were p53 positive both in carcinoma and high grade dysplasia. This result suggests that p53 protein overexpression in high grade dysplasia could be an indicator of associated or incipient carcinoma. This hypothesis needs to be verified in a prospective follow up study of patients with Barrett's oesophagus which includes p53 protein evaluation together with histological surveillance. We are currently undertaking such a study.

This study adds further data confirming that mutation of the p53 gene, as indicated by the abnormal expression of p53 protein defined immunohistochemically, is frequently involved in Barrett's adenocarcinoma. A prognostic value for this mutation seems unlikely, and in our study this mutation appeared as a relatively late event in the multistep process in which the characteristic epithelium in Barrett's syndrome progresses to dysplasia and eventually to adenocarcinoma.

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