Adenocarcinoma arising in Barrett's oesophagus: evidence for the participation of p53 dysfunction in the dysplasia/carcinoma sequence

R H Hardwick, N A Shepherd, M Moorghen, P V Newcomb, D Alderson

Abstract

Adenocarcinoma arising in Barrett's oesophagus is often preceded by mucosal dysplasia, but little is currently known about the aetiology or natural history of this dysplasia/carcinoma sequence. To investigate the participation of the tumour suppressor gene p53 in this sequence, an immunohistochemical analysis of p53 protein overexpression, which is known to closely correlate with point mutation of the p53 gene, was conducted in 30 patients with Barrett's adenocarcinoma. Adjacent Barrett's mucosa was dysplastic in 21 (70%) patients. Sixteen (53%) tumours overexpressed p53, 10 of which had adjacent dysplastic Barrett's mucosa. In all 10 patients, this dysplastic mucosa also overexpressed p53, predominantly in areas of high grade compared with low grade dysplasia. In contrast, none of the dysplastic mucosa adjacent to 11 tumours lacking p53 overexpression showed detectable values of p53. These results suggest that p53 dysfunction may participate in the progression from dysplasia to carcinoma in some patients with Barrett's oesophagus.

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In 10-15% of patients with chronic gastro-oesophageal reflux disease areas of the distal squamous oesophageal epithelium are replaced by metaplastic columnar mucosa,¹⁻⁴ a condition known eponymously as Barrett's oesophagus.^{5,6} This unique mucosa consists of a mosaic of distinct cell types, which represent the variable differentiation of an as yet unidentified multipotent stem cell.⁷⁻¹⁰

Patients with Barrett's oesophagus have an increased risk of developing oesophageal adenocarcinoma.¹⁰⁻¹³ Carcinoma is predominantly associated with specialised intestinal type mucosa and preceded by dysplasia.^{14,15} High grade dysplasia is often associated with oesophageal adenocarcinoma and its discovery should prompt an extensive search for microinvasive malignancy.¹⁶⁻²⁰ Low or indefinite grade dysplasia is of less certain prognostic significance. It is difficult to characterise reliably²¹ and has an uncertain natural history, although flow cytometry can detect clonal genomic instability in dysplastic Barrett's mucosa, which may be useful in predicting the risk of progression to high grade dysplasia or cancer.²²

Mutations and deletions of the tumour suppressor gene p53 have stimulated considerable interest because of their high frequency in human cancer.²³⁻²⁵ Current evidence suggests

that p53 mediates a G1 cell cycle arrest after genomic damage,²⁶ and that loss of p53 function may permit immortalisation of this damage by its incorporation into the next cycle of synthesis and mitosis.²⁷ Many mutant forms of p53 protein are 'overexpressed' secondary to a poorly understood increase in post-translational stability, which makes them detectable immunohistochemically.^{28,29}

17p allelic deletions (the site of the p53 gene) are common in colorectal adenocarcinomas, especially in conjunction with p53 mutation in the remaining allele.³⁰ 17p deletions are also a common finding in Barrett's adenocarcinomas³¹ and Barrett's mucosa showing high grade dysplasia.³² In addition, p53 overexpression has been detected in Barrett's adenocarcinoma and in some patients with dysplastic Barrett's mucosa.³³ In contrast with these immunohistochemical findings, DNA sequence analysis has found p53 missense mutations in the columnar mucosa adjacent to some Barrett's adenocarcinomas, but not in the tumour themselves.³⁴

To investigate this apparently conflicting evidence, an immunohistochemical investigation was performed, comparing p53 expression in Barrett's adenocarcinomas and the surrounding dysplastic columnar mucosa from which these tumours are presumed to have arisen.

Materials and Methods

TISSUES

Thirty patients with oesophageal adenocarcinoma arising in Barrett's oesophagus (mean age 68 years, M:F 22:8) were identified from histopathology computer records of the Bristol Royal Infirmary, Gloucestershire Royal Hospital, and Frenchay Hospital. These were defined as adenocarcinomas arising in the tubular oesophagus associated with specialised intestinal type Barrett's mucosa.³⁵ All tissues had been routinely fixed in buffered formal saline. Twenty patients with non-dysplastic Barrett's oesophagus (mean age 62 years, M:F 14:6) receiving endoscopic surveillance acted as a control group. Haematoxylin and eosin stained sections were graded for dysplasia^{36,37} by two consultant pathologists independently (NAS and MM), with consensus scoring for those sections where there was initial disagreement.

Methods

Tissue sections of 5 µm were cut from each block and floated onto poly-L-lysine (Sigma) coated slides and left to dry overnight before

University Departments
of Surgery and Pathology,
Bristol Royal Infirmary,
Bristol
R H Hardwick
M Moorghen
P V Newcomb
D Alderson

Department of
Histopathology,
Gloucestershire Royal
Hospital, Gloucester
N A Shepherd

Correspondence to:
Mr R H Hardwick, University
Department of Surgery,
Bristol Royal Infirmary,
Bristol BS2 8HW.

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being dewaxed in histoclear (Cell Path plc) and rehydrated in graded alcohols. The polyclonal antibody CM-1 (Novacastra) was used to stain for p53 protein as previously described.³⁸ The StrAvisGen (Biogenex) detection system was used according to the manufacturer's instructions and 3,3 diaminobenzidine with 0.3% nickel sulphate were used as chromogens. A light haematoxylin counterstain was applied to permit identification of morphology.

Two sections of breast tumour with a characterised p53 gene mutation causing p53 protein overexpression were stained with each batch of slides to act as positive and negative controls, the latter being incubated without primary antibody.

Staining was assessed independently by two of the authors (RHH and PVN) in the following manner. At low power, the area of interest was identified and 10 random fields of 50 cells were examined under high power. The numbers of unequivocally positive staining nuclei were counted and expressed as a percentage of the total. Four patterns of staining were seen: I=no staining, II=<1%, III=1–9%, and IV=10–100%. Patterns III and IV were considered positive.

Results

p53 EXPRESSION IN BARRETT'S ADENOCARCINOMA
Sixteen (53%) of the 30 Barrett's adenocarcinomas showed overexpression of p53 (Table I). Staining was exclusively nuclear, specific for neoplasia, and commonly heterogeneous (Fig 1). In two tumours, only occasional isolated staining was seen, amounting to less than 1% and these were considered negative.

p53 EXPRESSION IN COLUMNAR MUCOSA ADJACENT TO TUMOURS

The columnar mucosa adjacent to 18 of the tumours showed high grade dysplasia (HGD) with or without low grade dysplasia (LGD). LGD alone was found in three cases, bringing the total number of tumours with adjacent dysplasia to 21 (70%). Of the 16 carcinomas overexpressing p53, 10 had high grade dysplastic Barrett's mucosa adjacent to them. In all 10 cases this dysplastic mucosa overexpressed p53 (Table II). The pattern of overexpression in dysplasia was predominantly type III (Fig 2), although two cases showed more extensive pattern IV staining (Fig 3). Of the three cases with LGD alone, one was p53 positive. Of the 14 carcinomas not overexpressing p53 11 had areas of adjacent dysplasia, none of which showed overexpression (Table II). None of the non-dysplastic columnar

mucosa associated with any of the tumours overexpressed p53.

p53 EXPRESSION IN CONTROL GROUP
No p53 overexpression was detected in any of the columnar biopsy specimens from 20 patients with non-dysplastic Barrett's oesophagus.

p53 EXPRESSION IN DYSPLASTIC COLUMNAR MUCOSA BEFORE DISCOVERY OF CARCINOMA
Three patients (numbers 1, 2, and 30) had endoscopic oesophageal biopsy specimens taken before the detection of invasive carcinoma, 6, 11, and 12 months respectively. All three patients had p53 positive HGD and in addition, patient 1 and 30 had small areas of LGD that also showed p53 positivity. The oesophageal adenocarcinomas that subsequently developed in each patient also overexpressed p53.

Patient number 30 was a 72 year old man with a 6 cm segment of columnar lined oesophagus with a small Barrett's ulcer. Multiple biopsies before treatment showed LGD and HGD in specialised intestinal type mucosa. He was treated with omeprazole 20 mg daily with surveillance endoscopy at three monthly intervals. The dysplasia at the time of initial diagnosis did not overexpress p53 and despite good symptom relief and significant regression of the columnar lined segment, biopsy specimens continued to show LGD and HGD. Between 12 to 18 months after diagnosis, weak pattern III p53 expression was noted in LGD and more intense pattern III staining in HGD. By 24 months p53 positive HGD and areas of early focal stromal invasion were seen in biopsy specimens and he had surgery. The resection specimen confirmed the presence of p53 positive multifocal microinvasive T1N0 adenocarcinoma.

Discussion
This study has shown a high prevalence of p53 overexpression in Barrett's adenocarcinoma and in the adjacent dysplastic columnar mucosa from which these tumours are thought to arise. There is a striking positive correlation between p53 overexpression in a carcinoma and the adjacent dysplastic Barrett's mucosa. When present, p53 overexpression in dysplastic mucosa is found mainly in high grade dysplasia with a type III staining pattern. p53 overexpression in carcinomas tended to be more extensive and this is similar to the p53 expression seen in dysplastic colorectal polyps and adenocarcinomas.^{39–41}

There is substantial evidence that p53 overexpression results in most cases, from missense mutation of the p53 gene.^{42–47} Wild type p53 protein is rapidly degraded and not normally detectable immunohistochemically,⁴⁸ whereas mutant forms of p53 are detectable because of their extended half lives.^{43,49} Doubts, however, concerning the specificity of p53 overexpression have been expressed⁵⁰ as wild type p53 can be detected in response to DNA damaging agents^{26,51} and in some cells during periods of rapid proliferation.⁵² Ultra violet irradiation of normal human skin induces a temporary over-

TABLE I p53 expression in Barrett's adenocarcinoma (n=30)

Pattern	n	
I	12	'p53 negative'
II	2	
III	2	'p53 positive'
IV	14	

TABLE II Patterns of p53 overexpression in dysplastic columnar mucosa adjacent to Barrett's adenocarcinoma (n=21)

Tumour status	Adjacent dysplasia	Pattern I	Pattern II	Pattern III	Pattern IV
p53 positive	HGD	0	0	7	2
p53 positive	LGD	0	0	1	0
p53 negative	HGD	8	1	0	0
p53 negative	LGD	1	1	0	0

HGD=high grade dysplasia, LGD=low grade dysplasia.

Figure 1: Pattern IV p53 overexpression in a Barrett's adenocarcinoma. Most neoplastic cell nuclei stain with the polyclonal antibody CM-1, but with varying intensity (original magnification $\times 200$).

Figure 2: Pattern III p53 overexpression in Barrett's mucosa showing high grade dysplasia. Three glands with variable staining are seen adjacent to a gland with no staining. Overall, 8% of dysplastic nuclei in this biopsy specimen stained positive (original magnification $\times 200$).

Figure 3: p53 Overexpression (pattern IV) in high grade dysplasia in Barrett's mucosa (original magnification $\times 200$).

expression of wild type p53 and proliferating cell nuclear antigen (PCNA).⁵³ This probably results from the physiological stabilisation of p53 as it participates in a G1 cell cycle arrest in response to DNA damage,⁵⁴ and PCNA is required for DNA excision repair.⁵⁵

It is not clear if there is a cut off point for p53 overexpression below which the association between immunohistochemical detection and gene mutation is lost (D Lane personal communication). Widespread overexpression (pattern IV staining) does correlate well with gene mutation,^{43, 45} but without analysing the coding regions of the p53 gene, interpretation of pattern III staining must be more guarded. We can postulate, however, that the strong association between pattern III p53 positivity in dysplastic Barrett's mucosa and pattern IV positivity in Barrett's adenocarcinoma makes it highly probable that the lesser values of p53 expression do indeed represent evidence of p53 mutation. If the staining seen in dysplasia resulted from some 'stress' phenomenon we would expect to see staining in dysplasia associated with p53 positive and negative tumours, but this is not the case. p53 positive dysplasia is only associated with p53 positive carcinoma.

Casson *et al*³⁴ investigated exons 5–8 of the p53 gene in neoplastic Barrett's oesophagus using the polymerase chain reaction and single strand conformational polymorphism. They found clonal exon 5 missense mutations in four areas of 'minimally' dysplastic columnar mucosa adjacent to seven Barrett's adenocarcinomas, but unexpectedly, no mutations in the carcinomas. They concluded that any part p53 mutations might have in the formation of these tumours was indirect. Ramel *et al*³³ examined 15 Barrett's adenocarcinomas and 11 patients with HGD but without carcinoma using flow cytometric analysis of p53 protein, and found overexpression in 53% and 45% respectively. This study and our own, suggests that p53 mutation may participate directly in the development of Barrett's adenocarcinoma in about 50% of cases. Furthermore, Flejou *et al*⁵⁶ have shown similar results in a series of 11 patients with Barrett's adenocarcinomas. These findings are important to our understanding of malignant transformation in Barrett's oesophagus as loss of p53 function, through allelic deletion and mutation of the remaining allele, may permit cells with an accumulated number of activated proto-oncogenes to break away from the normal constraints on cell cycle progression and proliferate unchecked.^{57, 58} In the dysplasia/carcinoma sequence of Barrett's oesophagus, p53 dysfunction seems to occur at a genotypic stage that coincides with the phenotypic events we currently recognise as high grade dysplasia. Whether p53 mutation occurs late in the progression from low grade to high grade, or early in the transition from high grade dysplasia to invasive carcinoma is currently unclear. These results do not suggest that p53 mutation is essential for malignant transformation in columnar lined oesophagus, rather that it is common. Its prevalence is possibly even higher than we have shown, as the influence of technical factors such

as fixation delay on the detection of p53 are uncertain. In addition, loss of both p53 alleles or a nonsense mutation resulting in a truncated p53 protein will not result in overexpression.

p53 overexpression may prove useful clinically in diagnosing high grade dysplasia in Barrett's oesophagus and possibly help identify a subgroup of patients who are at risk of progressing from dysplasia to invasive malignancy. Barrett's oesophagus offers an unrivalled opportunity to observe the premalignant stages of tumour development in vivo in the same patient. Follow up studies of patients with dysplastic Barrett's oesophagus should show if there is a clinical application for p53 overexpression and provide valuable insight into the role of this important tumour suppressor gene in the multistep development of human malignancy.

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