p53 Expression in ulcerative colitis: a longitudinal study

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Abstract

p53 Mutation is a late event in the development of sporadic colorectal carcinoma (CRC). The timing of p53 mutations in the development of ulcerative colitis associated colorectal carcinoma (UCACRC) is, however, less certain and some reports suggest that it may be a relatively early event. This study sought to establish the timing of p53 mutations in neoplasia arising in ulcerative colitis. Blocks of 10 resected colorectal specimens from patients who had had a biopsy positive for dysplasia at least one year prior to resection were retrieved from the archives of St Mark's Hospital. Immunohistochemistry using the monoclonal antibody DO-7, specific for both mutant and wild type p53, was performed on sections from both the resection specimens and dysplastic biopsy specimens. Seven of the 10 resection specimens were positive for p53. Two of these seven specimens showed p53 overexpression in specimens taken two years before the development of carcinoma or high grade dysplasia. Five of the seven specimens were negative for p53 overexpression between one and four years prior to resection. These results suggest that p53 overexpression is usually a late event in the development of UCACRCs.

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p53 Is a DNA binding protein that participates in both DNA repair and induction of apoptosis.^{1,2} Mutation of the p53 gene is probably the single most common mutation found in malignant tumours in humans.³ This mutation is generally deemed a late event in most carcinogenetic pathways. It occurs as one of the final steps in the adenoma-carcinoma sequence in sporadic colorectal carcinoma (CRC)^{4,5} and participates in high grade transformation in malignant lymphomas.⁶ In ulcerative colitis the picture is rather less clear. Most reports show that it is found far more frequently in high grade dysplasia than low grade dysplasia suggesting that p53 mutation is a late event.⁷⁻¹⁰ Some reports, however, suggest that it may be a relatively early event¹¹ and in one report mutations have been detected in up to 30% of biopsy specimens from non-dysplastic epithelium.¹² Another report from the same group has shown loss of heterozygosity, an event that usually occurs later than mutation,

in specimens negative and indefinite for dysplasia.⁸ We aimed to identify the timing of p53 mutations in a longitudinal retrospective study.

Selection criteria

Specimens were selected from a series previously described in a study by Connell *et al* in which all samples positive for dysplasia were reviewed.¹³ From those patients in which the specimens were still positive for dysplasia after review, inclusion into this study was accepted only if at least 12 months had elapsed between the dysplastic biopsy and the resection of the bowel. After application of these criteria only 10 cases were found to be suitable.

Immunohistochemistry

Standard immunohistochemistry was performed by the ABC method using a Vectastain ABC kit (Vector Laboratories). Fresh 4 µm thick sections were cut from the dysplastic specimens and sites anatomically corresponding with these samples in the resection specimen. In addition any other interesting sites from the specimens were also examined. Sections were dewaxed in xylene and rehydrated through graded alcohols. Endogenous peroxidase activity was blocked by incubating for 20 minutes in 0.5% hydrogen peroxide in methanol. Antigen retrieval was by boiling the sections for two minutes in an aluminium pressure cooker at 15 psi in sodium citrate buffer (0.01 M, pH 6.0). After preincubating for 30 minutes in normal horse serum, sections were incubated overnight at room temperature with the mouse monoclonal antibody DO-7 (Novocastra) at a dilution of 1:100. This antibody recognises a fixation resistant epitope of both mutant and wild type p53. Bound antibody was detected using the Vectastain ABC kit in accordance with the manufacturer's instructions with diaminobenzidine (Sigma) as the chromogen. Sections were then counterstained with haematoxylin and reviewed blind. Epithelial staining was scored in a semi-quantitative manner using the following scale: 0 = <10% positive cells; + = 10-25%; ++ = 25-50%; +++ = >50%.

Results

The Table shows the results of the positive cases. Only sections in which over 10% of epithelial cells showed clear staining were regarded as positive. In some areas there was pale background staining of normal epithelial nuclei. Nuclei were only counted as positive if

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Results of the p53 immunostaining of the dysplastic biopsy and resection specimens from the seven specimens that were positive for p53 in the resection. 0=<10% positive cells; +=10–25%; ++=25–50%; +++=>50%

Case	Biopsy p53 (histology)	Resection p53 (outcome)	Time: biopsy to resection (y)
1	0 (LGD)	+ (HGD)	2
2	++ (LGD)	+++ (Ca)	2
3	++ (HGD)	++ (HGD)	2
4	0 (LGD)	+ (LGD)	4
5	0 (LGD)	++ (Ca)	1
6	0 (HGD)	++ (HGD)	1
7	0 (HGD)	+++ (Ca)	2

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

both pathologists agreed that there was significantly greater staining than that seen in the background. From the 10 cases, seven were positive for p53 staining in the mucosa of the resection specimen. Of these seven, two were positive for p53 in the earlier dysplastic biopsy specimens (Figs 1 and 2). In both of these cases the specimens were taken two years prior to resection. One of these patients developed a mucinous carcinoma while the other showed high grade dysplasia in the resection. The

remaining five p53 positive specimens showed no evidence of p53 overexpression in the specimens and there was no patient in whom the biopsy result was positive but the resection specimen was negative. These results show that p53 overexpression can occur in low grade dysplasia at least two years prior to malignant transformation or development of high grade dysplasia. In most cases, however, the overexpression of p53 was a late event.

Discussion

The increased risk of development of CRC in ulcerative colitis is well reported. As ulcerative colitis associated colorectal carcinomas (UCACRCs) often arise from flat mucosa showing cellular atypia without the polypoid architectural changes seen in adenomas, these tumours provide an alternative model for the study of carcinogenesis – that is, a ‘dysplasia–carcinoma’ sequence in contradistinction to the ‘adenoma–carcinoma’ sequence. Similar genetic changes have been seen in UCACRCs as in sporadic CRCs and

Figure 1: p53 Staining and a haematoxylin and eosin section from case 3. Strong nuclear staining with the monoclonal antibody DO-7 in areas of high grade dysplasia in both the biopsy (A) and resection specimen (B). Haematoxylin and eosin section of high grade dysplasia from the resection specimen (C). (Magnification: (A)=×562; (B)=×288; (C)=×144.)

Figure 2: p53 Staining and a haematoxylin and eosin section from case 2. Strong nuclear staining with the monoclonal antibody DO-7 in areas of low grade dysplasia in the biopsy (A) and mucinous carcinoma in the resection specimen (B). Haematoxylin and eosin section of the biopsy specimen showing low grade dysplasia (C). (Magnification: (A)=×450; (B)=×225; (C)=×360.)

the putative 'dysplasia-carcinoma' pathway may share many features with the 'adenoma-carcinoma' pathway.

Most p53 studies in ulcerative colitis have shown that the frequency of immunohistochemical overexpression and point mutation increases with the severity of dysplasia and this suggests that it is probably a late event.⁷⁻¹⁰ Our aim was to investigate this further in a longitudinal study in which early biopsy specimens showing unequivocal dysplasia were investigated for p53 overexpression. Our selection criteria required that there be at least a 12 month interval between the biopsy and the resection. Only 10 cases fitted our criteria and, of these, seven were p53 positive. Two of these seven cases showed p53 overexpression two years prior to resection while the remaining five showed no such early overexpression. Our findings are significant in that they show that p53 overexpression is probably a late event in the development of UCACRCs and are in agreement with other studies. In this respect, the carcinogenetic pathway in UCACRCs is probably similar to that of sporadic CRCs.

One important function of p53 lies in its role in the induction of apoptosis.² The reduction in the ability to undergo apoptosis may lead to immortalisation of cells. This would tend to increase the accrual of mutations, which may eventually be sufficient for malignant transformation (as in the Epstein-Barr virus induced immortalisation of lymphocytes).^{14 15} In such a model the loss of ability to undergo apoptosis is an early event. Alternatively, if the 'transforming' mutations have already occurred then loss of ability to undergo apoptosis will allow these ordinarily lethal mutations to survive. In this model loss of apoptosis inducing processes occurs as a late event. Our findings suggest that p53 is a late mutation in the development of UCACRCs and suggest that, in terms of disease biology, loss of apoptosis may be a late event.

This study is, however, limited by too few cases to draw any important conclusions. None the less, only in a truly longitudinal study such as this can the natural history of a disease be followed. Another limitation is the use of immunohistochemical overexpression as an indication of probable mutation. We may thus have missed some non-sense mutations resulting in truncated protein. In addition, given the high degree of sensitivity of antigen retrieval methods, we may have detected overexpression of wild type protein – that is, appropriate

expression of non-mutant p53 – in an attempt to induce apoptosis in neoplastic cells. Unequivocal overexpression of p53 protein in over 5% of tumour cells has been shown to be very highly correlated with p53 point mutations in colorectal carcinomas.¹⁶ We set rigid criteria for the definition of positive staining to prevent false positive results – only when both pathologists were in agreement were cells accepted as positive. For completeness, however, direct genetic analysis of the p53 gene in these cases is required.

In summary, we conducted a longitudinal study in an attempt to identify the timing of p53 mutations in ulcerative colitis. Our results suggest that this is probably a late event in the 'dysplasia-carcinoma' pathway. In this we are in agreement with other workers and this suggests that a 'dysplasia-carcinoma' sequence has some features in common with the 'adenoma-carcinoma' sequence.

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