p53 in colorectal cancer: clinicopathological correlation and prognostic significance

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Summary p53 protein was detected by immunohistochemistry in 42% of 52 colorectal adenocarcinomas. Positive tumours were significantly more frequent in the distal colon, and demonstrated a higher rate of cell proliferation. No correlation was found with tumour grade, Dukes' stage, presence of DNA aneuploidy or patient survival. The role of p53 in colorectal carcinogenesis is discussed with particular reference to differences between proximal and distal large bowel cancers.

p53 is a 53KD nuclear protein, highly conserved in vertebrates, which is believed to regulate entry into and progression through the normal cell cycle (Mercer et al., 1984). Like c-myc it is induced during transition from G₀ to G₁ phase (Miner & Miner, 1981; Reich & Levine, 1984), and is present at low levels in most normal fetal and adult tissues (Rogel et al., 1985). Studies of p53 expression in cultured cells suggest that increased levels are associated either with an abnormal mutated protein (Finlay et al., 1988), or stabilisation of the protein in a complex with viral antigens, e.g. SV40 large T antigen (Lane & Crawford, 1979). Point mutations occurring in a highly conserved region of the gene are known to activate p53 in the primary rat embryo fibroblast transfection assay for dominant oncogenes (Hinds et al., 1989), whereas the wild type protein has a tumour suppressor action (Finlay et al., 1989). The presence of increased levels of protein may therefore provide a marker for mutated p53.

 Elevated p53 expression has been described in a number of human tumours including carcinoma of the breast (Cattoretti et al., 1984; Cattoretti et al., 1988; Thompson et al., 1990), colorectum (Crawford et al., 1984), and lung (Iggo et al., 1990). Colorectal cancer is characterised by frequent deletion of chromosome 1p close to the p53 locus (Vogelstein et al., 1988), and elevated protein levels have been found by radioimmunoassay in 44% of tumours (Crawford et al., 1984). These studies suggest that in some tumours hemizygous deletion of one p53 allele is accompanied by mutation and overexpression of the other. Recently van den Berg et al. (1989) described the immunohistochemical detection of p53 in 55% of 29 colorectal cancers. However no information was given regarding the relationship of p53 expression to clinicopathological variables several of which are believed to correlate with the biological aggressiveness and stage of progression of a tumour. The aim of the present study was to assess these relationships in a larger series and investigate the value of the immunocytochemical detection of p53 as a prognostic indicator.

Materials and methods

Fresh tumour tissue was obtained from 52 adenocarcinomas of the large bowel from 52 patients. A single 4μm frozen section was cut from each tumour, air dried overnight, and fixed for 15 min in acetone at 4°C.

In five cases tissue was available from up to five different areas of the same tumour. These were included in the main series in order to assess the effect of intra-tumour heterogeneity on the detection of p53.

Sections were incubated with Pab421, a monoclonal antibody to murine and human p53, at a dilution of 1:200 of ascites fluid for 30 min. Sections were washed in Tris buffered saline and incubated successively with biotinylated rabbit anti-mouse immunoglobulin for 10 min, streptavidin-peroxidase for 5 min, and amino-ethylcarbazole for 10 min (Zymed Laboratories Inc). Staining was controlled by omission of the primary antibody.

Follow-up was available for 41 out of 52 cases (mean follow-up = 35.4 months; range 1–84 months). Median age was 69 years and 52% of the series was male. Twenty-two tumours were located in the rectum; 11 in the sigmoid colon; three in the descending colon; two in the transverse colon; two in the ascending colon, and 12 in the caecum. For further analysis these were divided into left sided (rectum, sigmoid and descending colon) and right sided lesions. All cancers were staged at the time of resection, and reviewed by one of us (NS) for histological grade, type of margin (infiltrating vs expanding) and presence of a host lymphocyte response at the tumour edge.

Ploidy was determined by flow cytometry using an established technique previously described (Quirke et al., 1987). Proliferation was assessed in 24 diploid tumours using the Para 1 cell cycle analysis program (Bagwell, 1979) and expressed as the proliferative index (PI) which is the sum of %S and %G₂M phases. Median CV was 5.8%.

Statistics

Frequency of p53 positive tumours was compared for each variable using Chi square analysis with Yates correction. Proliferation was also compared in p53 positive and negative tumours using Students t test. Kaplan–Meier survival curves were constructed using the BMDP 1L statistics package and assessed using the Log rank test.

Results

p53 was detected immunohistochemically in 22 out of 52 (42%) adenocarcinomas. Staining was confined to malignant nuclei (Figure 1) and was never found in adjacent 'normal' mucosa. Although some variation was noted in the proportion of nuclei which contained p53, 70% of nuclei or more were stained in all positive cases. No variation was found between different areas of the same tumour in the five cases which were assessed for intra-tumour heterogeneity (Table I).

The relationship between p53 expression and several clinicopathological variables is summarised in Table II. No correlation was found with tumour grade, Dukes' stage, invasive margin, presence of a host lymphocyte response or tumour ploidy. A trend was seen towards a higher rate of cell proliferation in p53 positive diploid tumours (x = 27.3% vs 20.9%) which just reached statistical significance (P<0.05) using Students t test. Interestingly left sided cancers expressed p53 more often than right sided lesions (P<0.05).

Patient survival was predicted by Dukes' stage (P = 0.04); presence of a host lymphocyte response (P = 0.04), and
Tumour ploidy: margin: infiltrating grade: poorly differentiated site: left

Dukes' stage: A B C

Tumour margin: infiltrating expanding

Lymphocyte response: present absent

Tumour ploidy: DNA diploid DNA aneuploid

| Table 1 | p53 expression in different areas of the same tumour |
|---------|-----------------------------------------------------|
| Tumour  | Number of areas examined | p53 (%) |
| 1       | 2                      | 2/2     |
| 2       | 4                      | 4/4     |
| 3       | 2                      | 2/2     |
| 4       | 5                      | 0/5     |
| 5       | 2                      | 0/2     |

| Table 2 | p53 expression and clinico-pathological variables |
|---------|--------------------------------------------------|
| Clinico-pathological variable | Number of cases | p53 (%) positive |
| Sex: male | 27 | 54.5% | NS |
| female  | 25 | 33.3% | |
| Age: <69 | 26 | 35% | NS |
| >69      | 26 | 45% | |
| Tumour site: left colon | 36 | 52.8% | P<0.05 |
| right colon | 16 | 18.8% | |
| Tumour grade: poorly differentiated other | 13 | 46.1% | NS |
| Dukes' stage: A | 2 | 0% | |
| B        | 28 | 39.3% | NS |
| C        | 22 | 50% | |
| Tumour margin: infiltrating expanding | 12 | 50% | NS |
| Lympohocyte response: present absent | 40 | 40% | |
| Tumour ploidy: DNA diploid DNA aneuploid | 24 | 46.2% | NS |

Discussion

Oncogenes and tumour suppressor genes are believed to play a fundamental role in the initiation and progression of most neoplasms. Several of these genes are implicated in colorectal cancer. K-ras mutations and altered c-myc expression have been described in 47% (Vogelstein et al., 1988) and 72% (Erisman et al., 1985) of colonic cancers respectively, while the FPC locus on chromosome 5q, a putative tumour suppressor gene, is deleted in up to 35% of sporadic carcinomas (Vogelstein et al., 1988). More recently alterations in p53 expression have been described in between 44% (Crawford et al., 1984) and 55% (van den Berg et al., 1989) of large bowel tumours. Baker et al. (1989) has demonstrated mutation of the p53 gene in two tumours associated with increased mRNA production. Remvikos et al. (1990) found a significant association between elevated p53 and the presence of DNA aneuploidy but not Dukes' stage. They did not however investigate cell proliferation or prognosis. With the exception of the latter study, which investigated 41 tumours, no information is available on the relationship of p53 expression to other clinic-pathological variables in colorectal carcinoma, including patient survival.

We have confirmed the expression of elevated levels of p53 in 42% of tumours. No correlation was found with a number of pathological variables with the exception of cell proliferation in diploid tumours, and tumour site. The latter is particularly interesting as other studies support the biological distinction of left and right sided large bowel cancer. c-myc expression (Rothberg et al., 1985), 17p and 18q chromosome deletions (Kern et al., 1989; Delattre et al., 1989) are all commoner in left sided lesions. The demographic features of proximal tumours are known to differ from more distal ones (Moller-Jensen, 1984), and the incidence of caecal cancer is increasing whilst that of rectal cancer is in decline (Beart et al., 1983). It is reasonable to suggest therefore that aetiological factors and the molecular basis of neoplastic transformation differ around the colorectum.

The relationship of p53 expression to cell proliferation in diploid tumours is perhaps not surprising given the role of p53 in normal cells where it appears to regulate entry into the cell cycle. Constitutive expression of mutated p53 might conceivably prevent dividing cells from becoming quiescent.

The lack of correlation with established prognostic indicators such as tumour grade, Dukes' stage, type of margin, and tumour ploidy is consistent with the failure of p53 expression to predict survival. This contrasts with breast cancer where in two independent studies p53 expression has been related to estrogen receptor status, a known prognostic indicator (Cattoretti et al., 1988; Thompson et al., 1990).

Little information is available regarding the role of other oncogenes in determining prognosis in large bowel cancer. Kern et al. (1989) report that K-ras mutations and 5q deletions do not predict survival whereas deletions of 17p and

Figure 2 Survival in p53 positive and negative tumours.

Figure 1 Colorectal carcinoma: a, malignant nuclei are stained for p53 protein (× 400), b, control.
18q are significantly associated with distant metastasis and reduced survival. These studies suggest that loss of tumour suppressor function, as identified by chromosome deletion, may be more important in determining prognosis than proto-oncogene activation. Our observations would support this.

There is little doubt that alterations in p53 will be increasingly recognised in a variety of tumour types. The frequency of abnormal expression in colorectal adenocarcinoma, and its distribution around the bowel, would suggest that p53 plays an important role in colorectal carcinogenesis, and supports the belief that proximal and distal tumours are biologically distinct.

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