SHORT COMMUNICATION

Frequent loss of heterozygosity on chromosome 17 at 17q11.2–q12 in Barrett’s adenocarcinoma

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Summary Allelic loss on chromosome 17 in 18 Barrett’s oesophageal tumours was analysed with 17 polymorphic microsatellite markers. Loss of heterozygosity (LOH) of one or more markers was seen in 72% (13 of 18) tumours on 17p and 56% (10 of 18) on 17q. The highest 17p losses were found at D17S799 (62%, five of eight) and D17S261 (55%, five of nine), while loss at the p53 locus was 31% (5 of 16). The highest loss on 17q was found at the TCF-2 (17q11.2–q12) locus with 66% (8 of 12) LOH. TCF-2 was the only marker lost in two of the tumour samples; furthermore, TCF-2 was lost in four other tumours which retained heterozygosity at the markers on either side of it, D17S261 and D17S740. Six markers were used to assess LOH at 17q11.2–q12, and five of eight of the tumour specimens which had LOH at TCF-2 had no other loss on 17q. No statistically significant correlations were found between loss on 17q or 17p and any clinicopathological parameters. We propose from these data that the 17q11.2–q12 region contains a novel predisposing gene in Barrett’s adenocarcinomas and may represent the site of a tumour-suppressor gene.

Keywords: Barrett’s adenocarcinoma; chromosome 17; loss of heterozygosity

Barrett’s columnar metaplasia of the squamous epithelium of the oesophagus is a consequence of chronic gastro-oesophageal reflux. It has been estimated that approximately 700 000 people in the United States have acquired Barrett’s oesophagus (Provenzale et al., 1994). The risk of developing adenocarcinoma of the oesophagus in these patients is 30- to 40-fold higher than in the general population (Fennerty et al., 1993; Stein and Stewart et al., 1993). Once diagnosed, many patients with Barrett’s oesophagus are entered into surveillance programmes in order to detect histopathological evidence of premalignant states, such as low-grade and high-grade dysplasia. Oesophagectomy for those observed to have early invasive carcinoma or high-grade dysplasia during such surveillance programmes results in improved survival.

A clear sequence from low-grade dysplasia to high-grade dysplasia to invasive carcinoma is observed to develop over a substantial period of perhaps 3–5 years (Cameron and Lombboy, 1992). During the last two decades the incidence of adenocarcinoma of the oesophagus has increased at a rate exceeding that of any other cancer, with an incidence of 500 cancers per 100 000 patients with Barrett’s metaplasia per year (Haggitt, 1992).

Conventional histopathology with the detection of dysplasia is currently the only means of early diagnosis of Barrett’s cancers. Oesophageal cancers share a number of molecular markers previously found in colorectal and gastric cancers, especially loss of heterozygosity (LOH) in chromosomes 5 and 17 (Vogelstein et al., 1988; Leister et al., 1990; Meltzer et al., 1991; Sano et al., 1991; Boynton et al., 1992; Huang et al., 1992; Blount et al., 1993; Meltzer et al., 1994). In addition, overexpression and mutations of the p53 tumour-suppressor gene are a frequent event in these tumours (Baker et al., 1990; Hollstein et al., 1990; Huang et al., 1993), and microsatellite instability has recently been demonstrated in Barrett’s cancers by Meltzer et al., (1994). To date, the majority of investigations into LOH on chromosome 17 in Barrett’s cancers have concentrated on the region containing the p53 gene. We have undertaken a detailed analysis of both chromosome 17 arms, using 17 microsatellite markers. The results of this investigation indicate the highest loss of heterozygosity on the q arm of chromosome 17 at 17q11.2–q12.

Materials and Methods

Specimens

Eighteen Barrett’s oesophageal tumour specimens were collected at the Royal Liverpool University Hospital, Department of Surgery, and at the Cardiothoracic Centre, Liverpool. Tumour samples obtained from surgical specimens were frozen in liquid nitrogen and stored at −70°C. The pathology of all these specimens was assessed by MM. The sections were dissected to yield more than 50% tumour cells for polymerase chain reaction (PCR) analysis.

DNA extraction

Genomic DNA was extracted from tumour specimens using the Nucleon II DNA extraction kit (Scilab) following the manufacturer’s instructions. Genomic DNA samples were stored at 4°C.

PCR and LOH analysis

Microsatellite repeat primers were obtained from Isogen (The Netherlands). PCR reactions were performed in a 25 μl reaction volume and contained 200 ng of genomic DNA, 200 μM dNTP, 5 pmol each of forward and reverse primers, 0.5 units of Taq polymerase (Advanced Biotechnologies) and 2.5 μl of 10× buffer (670 mM Tris-HCl pH 8.5, 166 mM ammonium sulphate; 67 mM magnesium chloride; 1.7 mg ml−1 bovine serum albumin (BSA); 100 μM β-mercaptoethanol; 1% (w/v) Triton X-100). The reactions were denatured for 5 min at 95°C and then the DNA was amplified for 30 cycles of 95°C for 30 s, 57°C for 30 s and 72°C for 30 s. A 10 μl volume of the PCR product was electrophoresed for 10 h on a 10% polyacrylamide gel at 250 V and viewed by silver staining.

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Results

We have undertaken a LOH study on 18 Barrett’s oesophageal tumours on chromosome 17, using 17 polymorphic microsatellite markers (Table I), in order to ascertain common regions of deletions on both arms of this chromosome and evaluate whether allelic loss was concentrated in any particular region. Loss of one or more markers was seen in 72% (13 of 18) of the specimens on 17p and in 56% (10 of 18) on 17q.

Significantly high frequencies of LOH (ie. > 30%) on 17p were found at D17S799 (63%, five of eight) and D17S261 (55%, five of nine), while loss of the p53 marker, TP53, usually occurred with one or all of the three markers located centromeric to it (D17S520, D17S799, CHRNBI), and was the sole 17p locus lost in only one case (patient 3). The highest loss on 17q was found to be at the TCF-2 locus, with 66% LOH (8 of 12). It is of note that the TCF-2 marker was the only marker lost in two of the Barrett’s oesophageal tumours (patients 8 and 18). Furthermore, TCF-2 was lost in four tumours which retained heterozygosity at informative markers on either side of it.

We have used six markers in the 17q11.2–q12 region as assigned by linkage mapping and show that five of eight of those Barrett’s oesophageal tumours which have a loss at TCF-2 have no other losses on 17q. Figure 1 demonstrates diagrammatically the region of minimal loss at the TCF-2 locus at 17q11.2–q12. No correlation was found between loss on 17p or 17q and any clinicopathological parameters or survival (Table II). Also, no clinical correlations were found between loss at the TCF-2 locus and any clinical parameters or survival.

Discussion

We have detected a high incidence of loss of heterozygosity at the TCF-2 locus (17q11.2–q12) on the q arm of chromosome 17. Loss at this site has not been previously reported in any oesophageal tumours including Barrett’s adenocarcinoma. Blount et al. (1993) reported 17p deletions in 12/13 (92%) Barrett’s oesophageal tumour specimens, while we have found 72% (13/18) LOH on the 17p arm. In comparison, on 17q we now report 56% (10/18) LOH, whereas there are no previous reports of LOH on 17q in Barrett’s adenocarcinoma. In none of these cases was the entire 17q arm lost, whereas 56% had partial or interstitial deletions on 17q. The nearest similar study is that of Mori et al. (1994), who studied losses on 17q in squamous cell carcinomas of the oesophagus. Their investigation centred around the BRCAl region located telomeric to that in which we are interested and the marker nearest to the TCF-2 locus they used was C117–316 (17q12–q21.1), which had a low LOH frequency.

There have been a number of investigations of other tumour types suggesting that there may be novel tumour-suppressor genes on both 17p and 17q. Apart from the p53 gene, several groups have reported the presence of a further gene at 17p13.3 in breast cancer (Coles et al., 1990; Sato et al., 1990; Thompson et al., 1990), a finding also seen in ovarian tumours (Eecles et al., 1990; Tsao et al., 1991; Foulkes et al., 1993). We have recently described the site of another putative tumour-suppressor gene in head and neck squamous cell carcinomas at CHRNBI (17p12–p11.1) (Adamson et al., 1994). Furthermore, a number of genes on 17q have previously been implicated in breast cancer, including BRCAl, NM23 and prohibitin (Hali et al., 1990; Leone et al., 1991; White et al., 1991; Futreal et al., 1994; Miki et al., 1994). To this can be added the oncogene c-erbB-2 (17q12), which most likely acts by increasing copy number (Van de Vijver et al., 1988). There are a number of possible candidate genes which have been assigned to the 17q11.2–q12 region, and these include NFI (neurofibromin 1), CSF3 (colony-stimulating factor 3), erbB-2 (epidermal growth factor) and ITB4 (integrin β).
Table II: Clinicopathological characteristics of the patients with Barrett's oesophagal tumours investigated in this study

| ID no. | Sex | Length (mm) | Status | Grade | Survival (months) | Fate | LOH at 17q |
|-------|-----|-------------|--------|-------|-------------------|------|-----------|
| F001  | M   | 025         | 2      | 0     | Moderate          | 20   | Alive NSR |
| F002  | M   | 035         | 1      | 0     | Good              | 19   | Alive NSR |
| F003  | M   | 060         | +VE    | 0     | Poor              | 8    | Dead rec  |
| F004  | M   | 025         | +VE    | 0     | Moderate          | 10   | Dead rec  |
| F005  | M   | 065         | +VE    | 0     | Poor              | 5    | Dead rec  |
| F006  | F   | ND          | ND     | ND    | Moderate          | 7    | Dead rec  |
| F007  | M   | 025         | 3      | +VE   | Poor              | 15   | Alive NSR |
| F008  | M   | 025         | 3      | +VE   | Poor              | 3    | Dead rec  |
| F009  | M   | 100         | 3      | ND    | Poor              | 1    | Dead rec  |
| F010  | M   | 025         | 1      | 0     | Moderate          | 1    | Op Death  |
| F011  | M   | 050         | 3      | +VE   | Moderate          | 2    | Dead rec  |
| F012  | M   | 025         | 3      | +VE   | Poor              | 1    | Dead rec  |
| F013  | M   | 075         | 1      | +VE   | Moderate          | 25   | Dead rec  |
| F014  | F   | 045         | 3      | +VE   | Poor              | 4    | Dead rec  |
| F016  | M   | 035         | 3      | +VE   | Moderate          | 1    | Op Death  |
| F017  | M   | 050         | 3      | +VE   | Moderate          | 18   | Alive NSR |
| F018  | M   | 025         | 3      | +VE   | Moderate          | 15   | Alive NSR |
| F019  | M   | 025         | 3      | +VE   | Poor              | 3    | Dead rec  |

ND: No data
Grade: histological differentiation of adenocarcinomas (moderate, good, poor)
Fate: Alive NSR, Dead rec
LOH: Loss of heterozygosity

The LOH data presented in this study suggest that the TCF-2 locus may represent an important predisposing gene in Barrett's adenocarcinomas and may indicate the site of a novel tumour-suppressor gene. The importance of this finding will have to await the analysis of a larger sample of Barrett's tumours, especially when specimens containing both Barrett's pre-malignant and malignant tissue are investigated with these markers. Such information will further our knowledge of the clonal ordering of allelic losses in Barrett's cancers, in which it has recently been proposed that 17p allelic losses occur before 5q allelic losses during neoplastic development of this disease (Blount et al., 1994).

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