Short Communication

Evidence that c-myc expression defines two genetically distinct forms of colorectal adenocarcinoma

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The human c-myc proto-oncogene is the genomic homologue of the transforming sequences found in MC29, an avian retrovirus that can cause myelocytomatosis, carcinoma, sarcoma and lymphoma (Graf & Beug, 1978; Enrietto et al., 1983). Alterations in the structure or expression of c-myc have been associated with several forms of neoplasia including avian leukaemia virus induced B-cell lymphoma, rodent plasmacytoma and human Burkitt's lymphoma, leukaemia, colon carcinoma and variant small cell lung cancer (Hayward et al., 1981; Payne et al., 1982; Shen-Ong et al., 1982; Taub et al., 1982; Collins & Grouiners, 1982; Dalla-Favera et al., 1982; Crews et al., 1982; Little et al., 1983; Sümegi et al., 1983; Mushinski et al., 1983; Altalal et al., 1983; Erisman et al., 1985; Rothberg et al., 1984). In a study of adenocarcinoma of the colon and rectum we have shown significantly elevated expression of c-myc in the majority of tumours, although no evidence of rearrangement or amplification of the gene could be demonstrated (Erisman et al., 1985). In the course of these studies on unselected tumours we have observed that elevated expression of the myc gene occurs more frequently in tumours of the left side (rectum, sigmoid, and descending colon) than in tumours of the right side (caecum and ascending colon). We discuss this finding in view of published reports of similar asymmetries in site distribution in inherited forms of colon cancer.

Figure 1 shows a dot blot analysis of the myc RNA level in several colorectal adenocarcinomas and in samples of nearby uninvolved tissue. Expression of myc in a sample is designated as significantly elevated (patients 2,3,4 and 8 in Figure 1) if the myc signal (panel A) is at least 5-fold elevated compared to normal colonic mucosa. This is outside of the range of experimental error as described previously (Rothberg et al., 1984). Hybridization of an identical dot blot with a human c-myc probe reveals no consistent difference between tumour and normal tissue for this oncogene and demonstrates that all lanes have roughly equal amounts of hybridizable RNA (Figure 1, panel B).

Table I shows the site distribution of unselected tumour samples with and without elevated expression of c-myc RNA. There is a significant correlation of elevated myc expression with carcinoma of the left side. Tumours of the right side are less likely to contain a significantly elevated level of myc RNA. Chi square analysis of the data in which the null hypothesis states that the tumours from either side form one population with respect to elevated myc expression is rejected at the p<0.025 level (using Yates correction). Other clinical parameters such as Dukes stage and histological appearance of the tumour, the level of serum carcinoembryonic antigen, age and sex of the patient were not significantly different when the elevated and not-elevated groups were compared (data not shown).

Published reports on site distribution in the inherited forms of colon cancer may provide an explanation for the asymmetry in myc expression we have found in unselected tumours. Familial polyposis coli (FPC) is an autosomal dominant trait characterized by numerous adenomatous polyps and eventual colon carcinoma usually occurring in the distal colon (Anderson & Williams, 1985; Bussey, 1975). Hereditary non-polyposis colorectal cancer (HNPPC) is also an autosomal dominant trait, but the tumours appear predominantly on the right side of the large bowel and are not preceded by the development of multiple polyps (Anderson & Williams, 1985; Anderson, 1980; Lynch et al.,
Figure 1. RNA dot blot hybridization analysis of oncogene expression in colorectal adenocarcinoma. The samples shown here were chosen to represent the asymmetry in myc expression seen between left and right sided carcinoma of the colon. The splenic flexure is the bend between the transverse and descending colon near the spleen. 'N' indicates normal mucosa which was located a few centimeters away from the tumour indicated by 'C'. Panel A: Preparation of the dot blot and hybridization with exon 3 of the human c-myc gene was done as described in Erisman et al. (1985). Panel B: Hybridization with human c-myb was done with a probe (0.7 kb, BamHI-XbaI) obtained from A. Begue (Leprince et al., 1983) using the same conditions as for myc. The myb blot was washed several times in 2 × SSC (1 × SSC is 0.15 M sodium chloride, 0.015 M sodium citrate), 0.1% sodium dodecylsulfate (SDS) at room temperature, then at 33° in 80% formamide, 3 × SSC, 0.1% SDS before exposure at −70° with an intensifying screen. The myc blot was exposed 19 h, and the myb blot was exposed 160 h.
CLINICAL CORRELATES OF C-MYC EXPRESSION IN COLON CANCER

Table I Site distribution in colorectal adenocarcinoma: Elevated myc expression compared with inherited colon cancer syndromes. Percentages at each site are given.

| Location | Myc RNA level | Inherited colon cancer |
|----------|---------------|------------------------|
|          | I not elevated | II elevated | III HNPCC | IV FPC |
| Left*    | 5 (42%)       | 22 (85%)  | 35%        | 84%   |
| Right†   | 7 (58%)       | 4 (15%)   | 65%        | 16%   |
| Total    | 12 (100%)     | 26 (100%) | 100%       | 100%  |

*c-myc RNA levels were determined by dot blot hybridization as shown in Figure 1 and confirmed by Northern blot analysis in several cases (Erisman, et al., 1985). The actual number of patients in this unselected study is shown with the percentages in parenthesis; †Not elevated <5X of normal controls ≤ elevated; *Hereditary non-polyposis colorectal cancer. The data are from 220 patients summarized by Anderson (1980); †Familial polyposis coli. The data are from 263 patients studied by Bussey (1975); *Familial polyposis coli. The data are from 263 patients studied by Bussey (1975); †The tumours of the right side are in the caecum, hepatic flexure, ascending colon and transverse colon.

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