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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 leukosis 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 & Groudine, 1982; Dalla-Favera *et al.*, 1982; Crews *et al.*, 1982; Little *et al.*, 1983; Sümegi *et al.*, 1983; Mushinski *et al.*, 1983; Alitalo *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 (HNPCC) 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.*,

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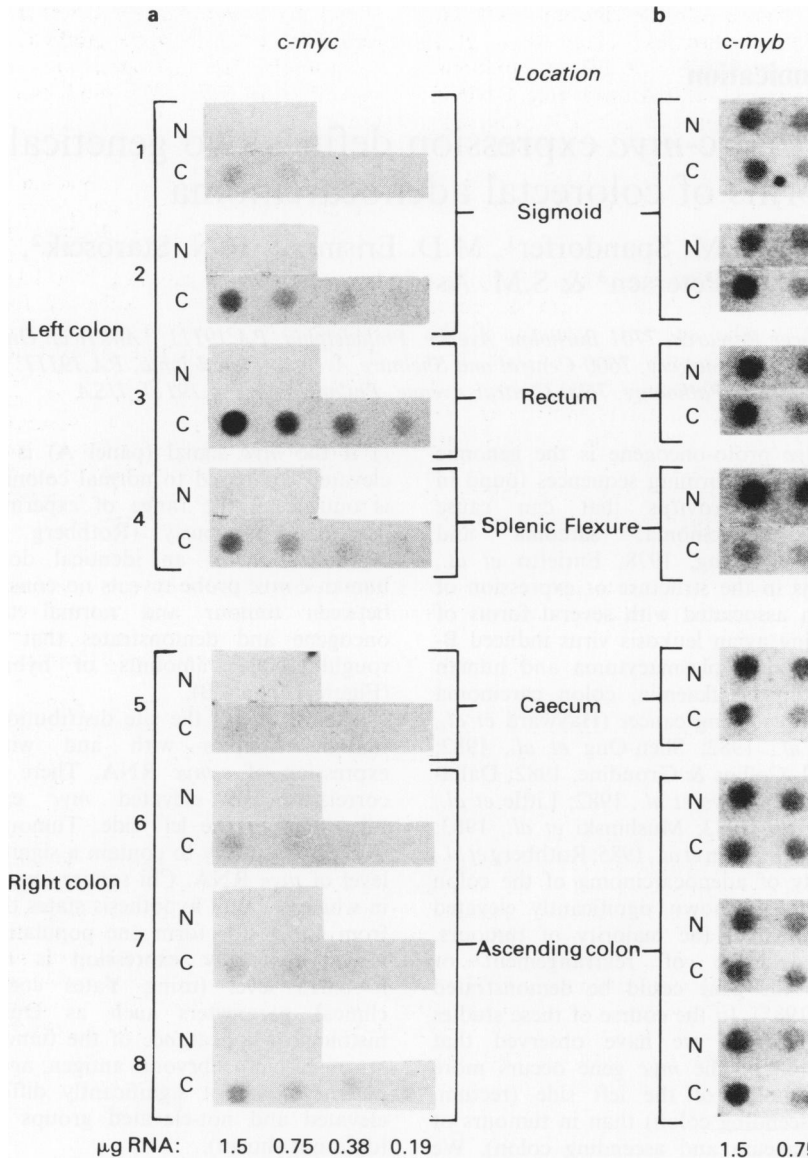

Figure 1 RNA dot blot hybridization analysis of oncogene expression in colorectal adenocarcinoma. The samples shown here were chosen to represent the asymmetry in *myc* expression seen between left and right sided carcinoma of the colon. The splenic flexure is the bend between the transverse and descending 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.

1977). Table I shows site distribution data for tumours from these diseases. There is a close similarity in the site distribution of the carcinoma samples with low *myc* RNA and the HNPCC patients (Table I, columns I and III). Likewise, there is a close correlation of the site distribution in FPC and the tumours we studied with a significantly elevated level of *myc* RNA (Table I, columns II and IV).

The data shown here support, but do not prove, the hypothesis that elevated expression of the *myc* gene is a marker of a distinct form of colon carcinoma that is the sporadic version of the inherited carcinoma of familial polyposis coli. It is also possible that elevated *myc* expression is an essential part of the neoplastic phenotype in this disease (Erisman *et al.*, 1985). The tumours with low *myc* expression would be the sporadic counterpart of hereditary non-polyposis colorectal cancer. This hypothesis predicts that FPC tumours would have significantly elevated *myc* expression regardless of site, while HNPCC tumours would consistently lack elevated *myc* expression, also regardless of site. This prediction remains to be tested.

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

	Myc *RNA level*[a]		*Inherited colon cancer*	
	I	*II*	*III*	*IV*
Location	*not elevated*[b]	*elevated*[b]	*HNPCC*[c]	*FPC*[d]
Left[e]	5 (42%)	22 (85%)	35%	84%
Right[f]	7 (58%)	4 (15%)	65%	16%
Total	12 (100%)	26 (100%)	100%	100%

[a]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; [b]Not elevated $<5X$ of normal controls $\leqq$ elevated; [c]Hereditary non-polyposis colorectal cancer. The data are from 220 patients summarized by Anderson (1980); [d]Familial polyposis coli. The data are from 263 patients studied by Bussey (1975); [e]Tumours of the left side are located in the rectum, splenic flexure, sigmoid colon and descending colon; [f]The tumours of the right side are in the caecum, hepatic flexure, ascending colon and transverse colon.

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