SHORT COMMUNICATION

Somatic rearrangement of the tropomyosin-receptor-kinase (trk) oncogene is rare in gastrointestinal cancer

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Activation of cellular oncogenes plays a major role in the pathogenesis of malignant neoplasias. Three mechanisms have been described by which oncogenes can be activated; these include somatic point mutations in the oncogene itself, somatic DNA rearrangements and gene amplification (Bishop, 1987).

Recently, a novel oncogene, the tropomyosin-receptor-kinase (trk) oncogene or oncD was isolated from a human colonic carcinoma (Martin-Zanca et al., 1986; Pulciani et al., 1982). Molecular studies showed that the trk-oncogene is generated by a somatic DNA rearrangement adjoining a truncated non-muscle tropomyosin locus and a tyrosine-specific protein kinase locus. This gene rearrangement was detected as a novel band by DNA probes hybridising to the breakpoints of the joined loci. Since this novel band was present only in tumour DNA but not in DNA from normal colonic mucosa it seems likely that the trk-oncogene was generated during the development of this particular carcinoma rather than being an inherited genetic abnormality.

We were interested in whether the activation of the trk-oncogene by somatic DNA rearrangement represented a common feature of various human cancers, particularly gastrointestinal carcinomas.

The study included 46 cases of gastrointestinal carcinomas, a variety of other cancers (5 breast carcinomas, 1 carcinoma of the urinary bladder and 15 haematological malignancies), 4 colonic adenomas, 4 cases of Crohn's disease and 11 normal individuals (Table I). The diagnoses of the solid tumours were made by histological examination of biopsy material obtained at surgery. The leukaemias were diagnosed on the basis of peripheral blood films and bone marrow smears. Samples representing tumour DNA were obtained from tumour tissue and in case of the leukaemias from peripheral blood and bone marrow. Constitutional DNA of the patients was extracted from the following sources: adjacent normal tissue and peripheral blood leukocytes of normal subjects. No constitutional DNA was available in the 5 cases of leukaemia. All samples were collected before chemo- or radio-therapy was started.

DNA was extracted from peripheral blood and bone marrow as described (Maniatis et al., 1982). Solid tissue material was first ground to powder in liquid nitrogen and afterwards subjected to the same DNA extraction procedures used for blood samples. In each case 5μg DNA were digested with the restriction enzymes EcoRI and BamHI according to the manufacturer's guidelines. The DNA fragments were subjected to electrophoresis, constitutional and tumour DNA in adjacent tracks, in 0.8% (w/v) agarose gels at 50V for 16h and blotted onto nylon (Hybond-N)

| Table 1 | Cases included in the study |
|---------|-----------------------------|
| Diagnosis | Number of cases |
| Carcinoma of oesophagus | 1 |
| Carcinoma of stomach | 12 |
| Carcinoma of colon* | 24 |
| Adenoma of colon | 4 |
| Carcinoma of rectum* | 9 |
| Crohn's disease | 4 |
| Carcinoma of breast | 5 |
| Carcinoma of urinary bladder | 1 |
| Hodgkin's disease | 1 |
| Non-Hodgkin's lymphoma | 3 |
| Waldenstrom's disease | 2 |
| Multiple myeloma | 4 |
| Acute myeloblastic leukaemia (AML) | 4 |
| Chronic myelogenous leukaemia | 9 |
| Normal individuals | 11 |
| Total | 86 |

* - malignant neoplasias | 67 |

Carcinoma of the colon: 'Duke' category: A 3; B 12; C 7; D 2; 'Duke' category: A 0; B 5; C 4; D 0; FAB classification of AML: M2 2 cases; M4 1 case; M5 1 case.

Activation filters (Southern, 1975). The filters were hybridised to a probe derived from the kinase domain of ecdna, a 1.2kb Ball-EcoRI insert of pDM10-1 (obtained from the American Type Culture Collection, Rockville, Maryland, USA, deposited by Martin-Zanca, 1986) which was 32P-labelled as described by Feinberg and Vogelstein (1983). After hybridisation, the filters were washed under stringent conditions (Old & Higgs, 1983) and autoradiographed at −70°C with intensifying screens for 3–7 days.

Hybridisation of the pDM10-1 probe with DNA from all 67 cancer patients, the 4 colonic adenomas, the 4 cases of Crohn's disease and from the normal controls (Table I) revealed the normal 14kb and 2.3kb BamHI DNA fragments and the normal 23kb EcoRI DNA fragment as described in the original report. A novel rearranged 7kb BamHI DNA fragment was originally found in a single case of colonic cancer (Martin-Zanca et al., 1986). However, in none of our cases was this particular rearranged band or any other rearrangement seen on autoradiography, despite the use of an additional restriction enzyme (EcoRI). Overexposure of the autoradiographs with respect to the normal fragments failed to show any faint rearranged bands which might have been missed after 2–3 days exposure. Figure 1 shows a representative autoradiograph.

The trk oncogene was originally identified as a transforming gene present in a human colonic carcinoma and was shown by molecular analysis to be generated by a somatic gene rearrangement involving a tropomyosin locus and a tyrosine-specific kinase locus. Gene probes which recognise sequences within the breakpoints of the two loci permitted the detection of this rearrangement by DNA analysis. The fact that it was found only in the tumour DNA
enzymes might have to be based on a detailed restriction enzyme map of the breakpoint region of the tyrosine kinase locus which is not available in the original report by Martin-Zanca et al. (1986).

Activation of oncogenes by somatic DNA rearrangement appears to be rare in human carcinomas. For example, only 4% of human breast carcinomas showed a rearranged c-myc oncogene (Escot et al., 1986) and no evidence of rearrangements of c-myc, N-myc, K-ras, N-ras and fos oncogenes was found in a series of colonic cancers reported by Alexander et al. (1986). As reported here, activation of the trk oncogene by genetic rearrangement seems to be uncommon in gastrointestinal carcinomas. It would appear that other mechanisms of oncogene activation predominate in these cancers. Somatic point mutations of ras genes (particularly c-Ki-ras genes) have been detected in 40% of colorectal carcinomas (Bos et al., 1987; Forrester et al., 1987). Amplification of the c-erbB-2 oncogene, an oncogene resembling part of the gene encoding the receptor for epithelial growth factor, has been exclusively found in human adenocarcinomas including carcinomas of the stomach (Yokota et al., 1986a). Similarly, amplification of c-myc has been described in colonic cancer, especially in advanced tumour stages (Alexander et al., 1986; Yokota et al., 1986b).

In contrast, somatic rearrangements of oncogenes occur more commonly in lymphomas and leukaemias. For example, rearrangements of the c-myc oncogene involving a reciprocal chromosomal translocation with the immunglobulin gene loci are found in 100% of sporadic Burkitt lymphomas (Pelacci et al., 1986). It is not clear as to why lymphomas and leukaemias should show an apparently higher frequency of oncogene activation by somatic DNA rearrangements than carcinomas. Further studies are needed to establish whether this is a consistent difference between haematological neoplasias and carcinomas.

We wish to thank Professor Sir D.J. Weatherall and Dr J.S. Wainscoat, for support and C. Hesketh for excellent technical assistance. MFF is the recipient of a fellowship by the Swiss National Science Foundation and The Royal Society and is also supported by the Swiss Cancer League. SLT is a Wellcome Senior Research Fellow.

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