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Evidence against a tumour-specific *Eco*RI RFLP of the c-*mos* locus

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*Eco*RI restriction fragment length polymorphisms (RFLP) at the human c-*mos* locus were analysed in DNAs of normal individuals and tumour patients. Two alleles with fragment lengths of 2.6 kb (A1) and 5.6 kb (A2) respectively were detected. The allele distribution among the tumour group was similar to that of the control group. No difference was found between the allele frequencies in leucocytes and tumour tissue DNA of the same patients.

c-*mos* gene; DNA rearrangement; Tumor tissue; Allele distribution

1. INTRODUCTION

A variety of genes are able to transform normal fibroblasts into tumour cells [1,2]. In the human genome such genes – named proto-oncogenes – exist, which appear to be involved in tumourigenesis [3,4] after activation by different mechanisms such as point mutation, translocation, amplification or coming under the control of specific promotor/enhancer elements [5–7].

If we assume that somatic mutations are the origin of tumour development [8], the question arises as to whether some alleles at oncogene loci may be linked to a susceptibility to cancer. Examination of RFLP frequencies at the c-*sis*, c-*myc*, c-*Ha-ras* and c-*mos* loci supports this proposal [9]. At the moment the relationship between the occurrence of unique *Ha-ras* alleles and cancer risk assessment is highly controversial. While one group [10,11] found an association between rare alleles and tumour development, subsequent studies did not confirm these results [12–14].

With respect to c-*mos*, Lidereau et al. [15] reported the occurrence of an *Eco*RI polymorphism exclusively in the DNA of some breast cancer patients. Breast cancer exhibits a hereditary component by virtue of its familial aggregation [16]. Analyses of the frequency and tumour specificity of oncogene alleles may therefore be a useful approach to search for genetic markers indicating a predisposition to cancer.

The human DNA sequence c-*mos*, homologous to the transforming gene of the Moloney murine sarcoma virus (Mo-MSV), is located on chromosome 8 [17,18]. So far studies of the transforming potential of c-*mos* showed that the gene can only be activated by linkage to the long terminal repeat (LTR) of the Mo-MSV [19,20].

We have studied DNAs from a control group and patients with gynaecological tumours and lymphomas. The *Eco*RI polymorphism was detected in non-affected individuals and in DNAs derived from various tumour tissues. Their frequency of occurrence among tumour patients and controls is comparable. This allele does not therefore seem to be tumour-specific but it is rare in the general population.

2. MATERIALS AND METHODS

DNA samples from 140 (80 women, 60 men) normal healthy individuals, 75 patients with breast cancer, 46 patients with ovarian cancer, 11 patients with cancer of the cervix, 9 patients with cancer of the uterus and 15 patients with lymphomas were examined. There were no discernible differences in the ethnic composition of the control and tumour group. Different types of cancer were diagnosed by pathological investigation in the clinics.

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DNA of the control group was extracted from white blood cells. If available, DNA from cancer patients was isolated from tumour tissue and leucocytes. Tumour biopsies were immediately frozen at −20°C followed by homogenization, lysis, deproteinization and precipitation of DNA [14]. For DNA isolation from leucocytes, 20 ml of peripheral blood were taken in the presence of EDTA. Extraction was carried out using techniques described previously [21].

DNA (5 μg) was digested with *Eco*RI and partly with *Bam*HI, separated on 0.8% agarose gels and transferred to nitrocellulose filters by the Southern procedure [22]. Blots were hybridized to a ^{32}P-labelled 2.6 kb *Eco*RI fragment [23] recognizing the 3'-end of the c-*mos* locus (fig.1). The conditions were those of Maniatis et al. [24].

3. RESULTS AND DISCUSSION

Among the 140 *Eco*RI-digested DNA samples derived from unaffected individuals, five exhibited the presence of two fragments of 2.6 kb (A1) and 5.6 kb (A2), while the other probes yielded only a single band of 2.6 kb (fig.1). RFLP analyses of the tumour patients resulted in the detection of allele A2 in six out of 156 investigated cases. This fragment was distributed among the different types of cancer (fig.2). The allele frequencies are listed in table 1.

While the majority of DNA probes examined showed homozygosity for the 2.6 kb fragment, allele A2 appeared only in a heterozygote linkage with allele A1. The intensity of the fragments was in the ratio 1:1 and no amplification of the 5.6 kb fragment, as has been observed in a pro-monocytic CM-S line [25], was detected.

Altogether the allele distribution between control and tumour groups is comparable. Significant differences between specific types of tumours were not ascertainable (table 1). In all investigated cases, both normal and tumour tissue contained polymorphism when present.

To determine the scope of the rearrangement responsible for the 5.6 kb fragment, probes generating two fragments were digested with *Bam*HI. Hybridization with the c-*mos* probe resulted in a single band of about 8.8 kb (fig.1) excluding a gross structural alteration of the c-*mos* locus. In view of previous studies [15,25], the 5.6 kb fragment seems to be due to a base change.

In contrast to the results of Lidereau et al. [15] we detected the *Eco*RI polymorphism even within the normal population. Nevertheless, the distribution of allele A2 between the sexes is striking. While four out of 60 normal male individuals (6.7%) carry an *Eco*RI polymorphism, only one

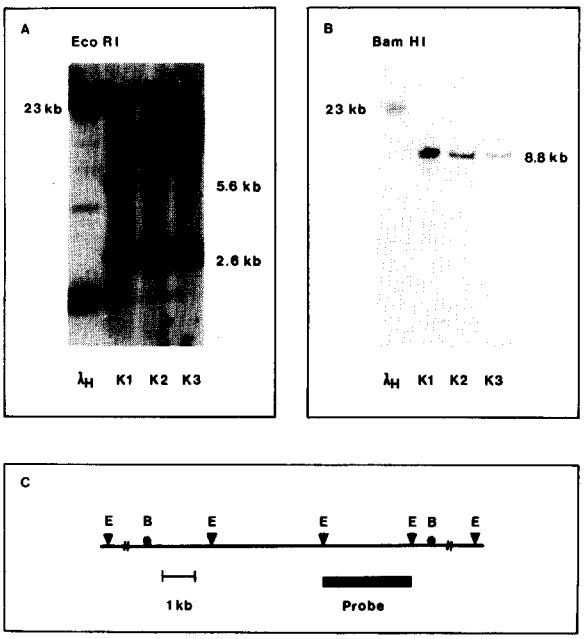

Fig.1. (A) Southern blot analysis of c-*mos* in *Eco*RI-digested DNA from three controls (lanes K1–K3) after hybridization with the *Eco*RI fragment of the c-*mos* specific probe. (B) Hybridization of the same DNA samples after *Bam*HI digestion. (C) Restriction map of c-*mos*. Only those restriction sites which are useful for understanding of the present results are indicated. Restriction sites: E, *Eco*RI; B, *Bam*HI; λ_H, λ DNA digested with *Hin*dIII.

Table 1

Frequency (%) of the c-*mos* genotypes in tumour patients

	A1/A1	A1/A2
Controls (n = 140)	96.4	3.6
Tumour patients (n = 156)	95.5	4.5
Breast cancer (n = 75)	97.3	2.7
Ovarian cancer (n = 46)	95.7	4.3
Cervix cancer (n = 11)	90.9	9.1
Uterus cancer (n = 9)	100.0	0.0
Lymphomas (n = 15)	93.3	6.7

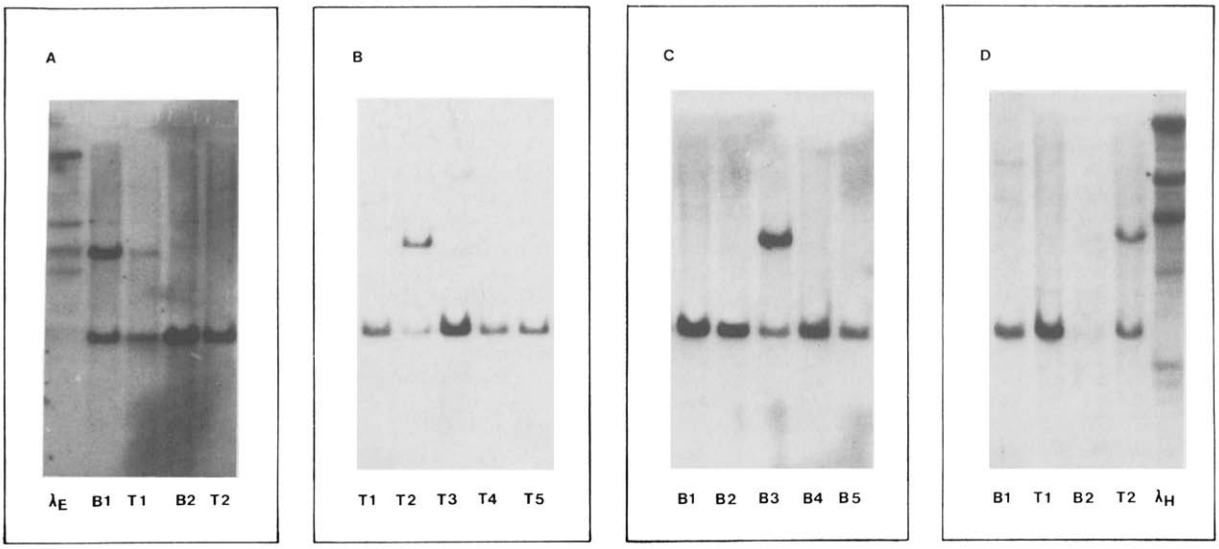

Fig.2. RFLPs at the c-*mos* locus of tumour patients after digestion with *Eco*RI. DNA from: (A) two patients with breast cancer; (B) five patients with ovarian cancer; (C) five patients with lymphomas; (D) two patients with cervical cancer. λ_E, λ DNA digested with *Eco*RI; λ_H, λ DNA digested with *Hin*dIII; B, DNA from leucocytes of patients; T, DNA from tumour tissue of patients.

DNA (1.25%) of the female population contains the 5.6 kb fragment. In this context, studies of the expression of *mos* in mice are worth mentioning. Depending on the course of development, distinct patterns of expression in female and male mouse germ cells have been observed and the transcripts differ from each other [26,27]. To what extent this suggested possible function of the murine *mos* gene in germ cell differentiation is connected with the occurrence of different allele frequencies between the two sexes in man remains under consideration.

As might be expected from previous studies [25,28] the *Eco*RI polymorphism is distributed among various kinds of tumours. Since the frequency of allele A2 is not significantly increased in tumour patients a general predisposition to malignancy seems to be unlikely. Reports of absent expression of c-*mos* in tumour tissue [4,29] support this view.

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