A TAQI RFLP OF THE HUMAN TGFα GENE IS SIGNIFICANTLY ASSOCIATED WITH CUTANEOUS MALIGNANT MELANOMA

Nicholas Hayward¹, Derek Nancarrow, Kay Ellem, Peter Parsons and Chev Kidson
Queensland Institute of Medical Research, Bramston Terrace, Herston, Brisbane, 4006, Australia.

A TaqI restriction fragment length polymorphism (RFLP) of the human transforming growth factor alpha (hTGFα) locus was analyzed in DNA from 45 normal individuals, 34 malignant melanoma (MM) cell lines, and 18 melanoma biopsy specimens. The frequency of a 2.7-kb allele (0.18) in MM cell lines was significantly higher (p < 0.01) than in lymphoblastoid cell lines (LCLs) derived from unaffected controls (0.05). The frequency (0.16) in MM biopsies was similar to that in MM cell lines although, owing to the small numbers investigated, it was not significantly higher than in controls. In the case of 5 MM patients who were constitutionally heterozygous for alleles at the TGFα locus, no apparent losses of heterozygosity were observed in the corresponding tumour DNA. Thus, the constitutional presence of the 2.7-kb allele may be a risk factor for melanoma.

Transforming growth factor alpha (TGFα) is a polypeptide of 50 aminocoids which is structurally related to epidermal growth factor (EGF) (Marquardt et al., 1984; Derynck et al., 1984) and competes for the same receptor (Todaro et al., 1980; Massagué, 1983). It can induce the reversible phenotypic transformation of normal mammalian cells in culture (DeLarco and Todaro, 1978; Roberts et al., 1981, 1983) and transfection with a recombinant TGFα gene can lead to malignant transformation of immortalized rat fibroblasts (Rosenthal et al., 1986). A total of 115 DNA samples derived or obtained from Caucasian individuals were analyzed for TaqI RFLPs at the hTGFα locus. These comprised 63 DNA samples from lymphoblastoid cell lines (LCLs) established from unaffected controls, 34 DNA samples from cultured cutaneous malignant melanoma (MM) cell lines (28 derived from metastatic tumours, 2 from level-V primary tumours, 3 from level-IV and 1 from level-III) and 18 DNA samples extracted from fresh MM biopsies (1 level-II, 2 level-III, 1 level-IV, 1 level-V and 13 of metastatic origin). In addition, DNA was also available from 1 LCL corresponding to 1 of the MM cell lines, and from 12 samples of normal skin corresponding to samples of fresh MM biopsy material.

DNA extraction and electrophoresis
DNA was extracted from cell lines or fresh tissue (Hayward et al., 1988). Five micrograms of DNA were digested overnight at 65°C with 20 units of TaqI (New England Biolabs, Beverly, MA). Digested DNA samples were electrophoresed in 1.1% agarose (FMC, Rockland, ME) gels using a buffer at pH 8 containing 0.04 M Tris, 0.02 M sodium acetate, 1 mM EDTA and 5 μg/ml ethidium bromide. Size standards employed were lambda DNA double-digested with EcoRI and HindIII. Gels were Southern blotted onto nylon membranes (Amersham, Little Chalfont, UK) according to the protocol of Maniatis et al. (1982). DNA was fixed to the filters by UV-crosslinking for 2 min.

DNA probes and hybridization
Plasmid pHTGF1-10-925 (Murray et al., 1986) containing the 5' portion of the hTGFα cDNA was nick-translated to a specific activity of >1 × 10^6 cpm/µg using [32P]-dCTP (Amersham). Pre-hybridization, hybridization and washes were carried out as previously described (Hayward et al., 1988). Briefly, pre-hybridization was carried out for 18 hr in 6 × SSC (1 × SSC is 0.15 M NaCl, 15 mM trisodium citrate), 5 × Denhardt’s solution (1 × Denhardt’s is 0.02% Ficoll, 0.02% BSA, 0.02% polyvinylpyrrolidone), 50 mM sodium phosphate buffer pH 6.5, 0.1% SDS, 5 mM EDTA, 250 μg/ml sheared, denatured salmon testis DNA, at 68°C. Hybridization was done at 68°C in 6 × SSC, 5 × Denhardt’s solution, 20 mM sodium phosphate, 0.1% SDS, 5 mM EDTA containing 100 μg/ml salmon DNA with approximately 1 × 10^6 cpm/ml of labelled probe. Filters were washed at 65°C for 1 hr in 2 × SSC/0.1% SDS followed by 0.1 × SSC/0.1% SDS for a

Received: March 1, 1988.
biopsy material was compared to that found in lymphoblastoid cell lines derived from peripheral blood lymphocytes of unaffected controls. Constant bands appear at 2.1, 1.8 and 1.2 kb. Further 90 min at the same temperature. Autoradiography was carried out at -70°C for 1 to 5 days.

**Statistical analysis**

Statistical analyses were carried out using the contingency Chi-squared test with Yates’ correction.

**RESULTS**

TaqI-digested DNA from Caucasian subjects most commonly hybridize TGFα cDNA to a 3.0-kb fragment, but occasionally a 2.7-kb allele is detected. Probes containing either the 5' (pHTGF1-10-925) or the 3' (pHTGF1-10-3350) region of the TGFα cDNA both identify this same infrequent RFLP (Hayward et al., 1987). Individuals are either homozygous for fragments of 3.0 kb (“a” allele) or 2.7 kb (“b” allele), or are heterozygous for these alleles.

Figure 1 depicts the hybridization pattern obtained with DNA from several MM cell lines probed with pHTGF1-10-925. The 3 possible genotypes a/a, a/b and b/b are clearly apparent (e.g., lanes 4, 1 and 5 respectively). Constant bands occur at 2.1, 1.8 and 1.2 kb. For one MM cell line and 12 MM biopsies, matching somatic DNAs were also available for analysis. For all paired samples the somatic DNA hybridization patterns (8 × a/a, 5 × a/b) were identical to those of the corresponding tumour-derived DNAs (results not shown). The frequency of the rarer “b” allele in MM cell lines or fresh biopsy material was compared to that found in lymphoblastoid cell lines derived from peripheral blood lymphocytes of unaffected controls (Table 1). The frequency (0.18) detected in MM cell lines was significantly higher (p<0.01) than in controls (0.05). Furthermore, 3/34 MM cell line DNAs were homozygous for the “b” allele compared to 1/63 control DNAs. The frequency of “b” alleles detected in fresh MM biopsy material (0.14) was comparable to that for MM cell lines, although this value was not statistically different from that for controls due to the small number of samples in the MM biopsy group.

Digests of some MM cell line DNAs heterozygous for the TaqI polymorphism consistently showed that the “b” allele was more strongly hybridizing than the “a” allele (e.g., Fig. 1, lanes 1 and 2). It is therefore possible that we are here analyzing a mixed population of cells with a/b and b/b genotypes, the latter possibly arising through loss of somatic heterozygosity at this locus which may lead to selection of certain subclones in vitro. However, for 5 matching pairs of MM biopsy and somatic DNAs where the patients were constitutionally heterozygous at the TGFα locus, no loss of somatic heterozygosity was observed in the tumour DNAs (results not shown).

**DISCUSSION**

Transforming growth factor alpha (TGFα) is produced by a variety of human tumours, including melanoma (Marquardt et al., 1983; Derynck et al., 1987; Ellem et al., 1988), and is believed to stimulate the growth or maintenance of these tumours in an autocrine fashion (Sporn and Todaro, 1980). TGFα is a potent angiogenic agent (Schreiber et al., 1986) and may play a crucial role in the neo-vascularization associated with solid tumours. In this regard, TGFα is not produced by tumours of haemopoietic origin where angiogenesis is unnecessary (Derynck et al., 1987). Abnormal amounts of TGFα have been found in the urine of melanoma patients (Ellis et al., 1987) as well as in the urine of patients with breast (Stromberg et al., 1987) or hepatocellular carcinoma (Yeh et al., 1987). Thus, this factor may serve as a useful marker for monitoring tumour burden in certain cancer patients.

We have found a significant association between an RFLP at the TGFα locus and melanoma. It is not yet clear how this finding is related to the aetiology of the disease. Further studies need to be carried out to localize the site of this polymorphism within the gene and to determine whether it relates to altered expression or stability of TGFα mRNA. On the other hand, the TaqI RFLP presented here may be in genetic linkage with a melanoma susceptibility gene. TGFα has been mapped to chromosome band 2p13 (Brissenden et al., 1985; Tricoli et al., 1986) and it is of interest that a locus on the short arm of chromosome 2 appears to be involved in the genesis of uveal melanomas as judged by specific loss of somatic heterozygosity in this region of the genome (Mukai and Dryja, 1986). However, in the present study, there was no apparent reduction in heterozygosity of the TGFα locus in any of the 5 melanoma DNAs where the somatic genotypes were informative.

The frequency of 0.18 found for the rare TGFα allele in melanoma cell lines is similar to the frequencies of unusual alleles of c-Ha-ras-1 reported to be significantly associated with several malignancies (Krontiris et al., 1985, 1986; Lid-
ereau et al., 1986; Hayward et al., 1988). The frequency of activation, by point mutation, of members of the ras proto-oncogene family in a number of human tumour types falls within the range 0.1–0.4 (Barbacid, 1986; Bos et al., 1987; Forrester et al., 1987). Changes observed in the 30 loci detected with a single minisatellite probe in fingerprints of tumour DNA, compared with paired somatic DNA, occurred with a frequency of 0.29 (Thein et al., 1987). In melanoma-derived material the generation of homozygosity at different loci scattered over several chromosomes occurred with a similar frequency, and an apparent lack of specificity (Dracopoli et al., 1985).

Clearly, the multi-step nature of tumour development and progression (Foulds, 1958) may not only result from random acquisition of the many necessary changes in gene activity, but may well represent several alternative combinations of genetic changes capable of resulting in the final common phenotype of malignancy. Thus, any one tumour may not acquire all the possible changes which could contribute to the deregulated behaviour of other tumours in the same class. Consequently, surveys of any one tumour type, bearing the same histopathological label, may be expected to have less than total but nonetheless significant frequencies of association with any of a variety of single gene alterations. The accumulating experience of cancer-associated RFLPs (including those generated by activation of the ras genes) may ultimately be useful in assessing the variety of possible changes which can result in a single type of cancer. These changes could be either constitutional or the result of somatic mutation/rearrangement. If they are constitutional, then a distinct familial association may occur in certain pedigrees. Whatever the case, the data presented in the current study further indicate that RFLPs at specific growth factor or proto-oncogene loci may be useful in risk assessment for certain human cancers.

ACKNOWLEDGEMENTS

This project was supported by the Queensland Cancer Fund and the National Health and Medical Research Council of Australia. We are indebted to the many pathologists and surgeons who contributed material to this project. We are grateful to M. McMannett, M. Ash, B. Kerr and M. Walters for excellent technical assistance and to H. Matthews for photography. We thank D. G. Bell for supplying the TGFα cDNA probe and D. R. MacLennan for his help in establishing the specimen collection program.

REFERENCES


KONTRIS, T.G., DIMARTINO, N.A., COLB, M., and PARKINSON, D.R., Unique allelic restriction fragments of the human Ha-ras locus in leuco-


