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Maintenance of Identical p53 Mutations Throughout Progression of Squamous Cell Carcinomas of the Tongue

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The occurence of mutations within the coding sequence of the p53 tumour suppressor gene is now well documented for squamous cell carcinomas of the head and neck region. However, evidence that these mutations are required for the maintenance and progression of squamous tumours is still formally lacking. To test this we have examined whether p53 mutations detected in primary squamous cell carcinomas of the tongue are also detected in the corresponding lymph node metastases. Three different p53 mutations were detected in each of three primary tongue squamous cell carcinomas (SCC), and in each case the same mutation was detected in a lymph node metastasis excised from the same patient. Although the sample number is small, the chance of obtaining the same p53 mutation independently in both the primary and metastatic tumour of each patient is at least 10^{-4}, therefore the results indicate that keratinocytes harbouring these p53 mutations possess a selective advantage throughout SCC progression.

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INTRODUCTION

GENE MUTATIONS or deletions in the p53 tumour suppressor gene have been described in almost every human cancer type ([1, 2] for reviews), including squamous cell carcinoma (SCC) of the epidermis [3–5], oral cavity [4, 6, 7] and larynx [4, 8]. Elevated levels of p53 protein suggestive of p53 mutation have also been reported in precancerous lesions at some of these sites [7, 9] and similar levels of p53 protein are seen in lymph node metastases and corresponding primary SCCs [4, 9].

However, elevated levels of p53 protein may reflect a response to genetic damage either as a prelude to cell cycle exit [10–12], apoptosis [13], or the underlying genetic instability of transformed cells [14].

Very recently, Chung *et al.* [15] reported that the p53 gene of one SCC of the subglottis and one SCC of the tongue shared the same single strand conformation polymorphism pattern as their corresponding regional lymph node metastases. This suggested, but did not formally prove, that the primary and metastatic tumours shared the same p53 mutation as has been reported in other human tumour types [16–19].

We report data here using the technique of direct sequencing which demonstrates for the first time that different mutations of the p53 gene found in three SCC patients are conserved throughout progression to lymph node metastases.

MATERIALS AND METHODS

The polymerase chain reaction (PCR) conditions and primers for the amplification and single strand direct sequencing have been described previously, together with the tumour collection, sectioning and staging [4]. DNA was isolated from a frozen section of each tumour and the extent of normal tissue present in each tumour section was assessed by a pathologist who examined a replicate section stained with haematoxylin and eosin.

Each sample was repeated twice using fresh PCR products each time.

RESULTS

Figure 1(a) shows the presence of a G→T mutation at the splice acceptor site on the boundary of exons 8 and 9 in tumour BICR-21 and a lymph node metastasis from the same patient (BICR-22). This results in a 19 bp deletion in the p53 transcript, a frame shift of codons 308 and beyond, and a stop mutation at codon 345 [4]. In addition to the mutant sequence, the normal sequence was also clearly detectable and was much stronger than the mutant sequence in tumour BICR-21 but not BICR-22 (Fig. 1a). This might suggest that most of the cells in BICR-21 were heterozygous for the p53 mutation since no normal tissue was present in the sample (Table 1).

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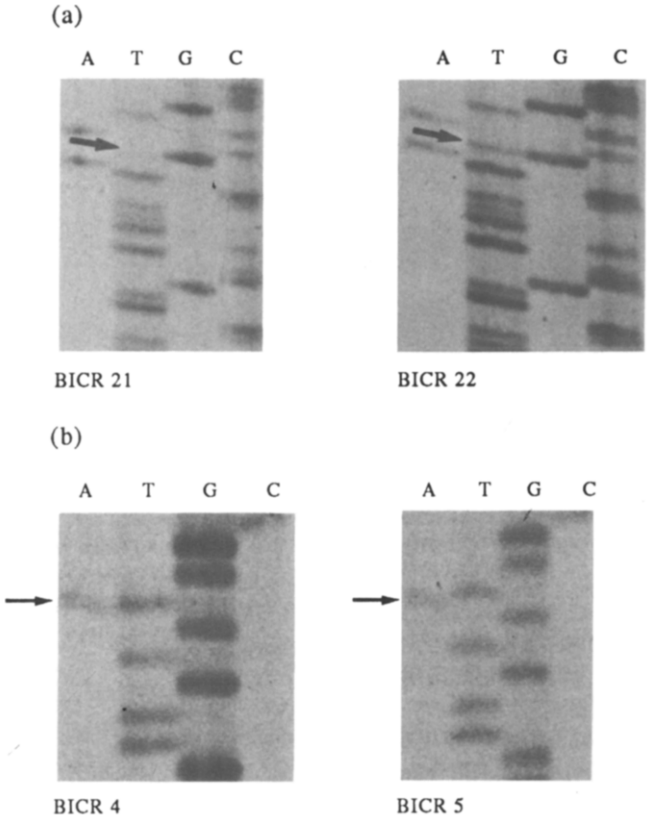

Fig. 1. (a) The p53 sequence of tumours BICR-21 and BICR-22 shows the presence of a G→T mutation at the splice acceptor site on the exon 8/exon 9 boundary. The mutation is indicated by an arrow. Note the faintness of the T signal in BICR-21 relative to BICR-22. (b) The p53 sequence of tumours BICR-4 and BICR-5 shows the presence of an A→T mutation at the first base of codon 174 which results in an Arg→Trp amino acid change. The normal sequence is visible in both BICR-4 and BICR-5 and the mutation is indicated by an arrow.

Figure 1(b) shows an A→T missense mutation at the first base of codon 174. This results in an arginine to tryptophan amino acid substitution in both the primary tumour BICR-4 and its lymph node metastasis, BICR-5. The mutation was probably responsible for the elevated levels of p53 protein previously noted in tumours BICR-4 and BICR-5 [4] since all other regions sequenced were normal (data not shown). Normal p53 sequence was clearly detectable in both BICR-4 and BICR-5 at codon 174 suggesting that no loss of heterozygosity had occurred in these samples, since neither BICR-4 nor BICR-5 were significantly contaminated with normal tissue (Table 1).

A TGA deletion spanning codons 173 and 174 results in elevated p53 protein levels in a cell line derived from the primary SCC, BICR-31. This deletion was also faintly detectable in both the primary tumour and a lymph node metastasis from the same patient, BICR-32. In both cases there was a large amount of normal sequence present, perhaps indicating heavy contamination of these tumours with normal tissue. Unfortunately, no sections were available from these tumour samples to enable us to assess the amount of tumour material present in them.

DISCUSSION

We have shown that three mutations of the p53 tumour suppressor gene which were detected in the lymph node metastases of tongue SCCs were also present in the primary tumours from the same patients (Fig. 1, Table 1). Although the number of primary/metastasis pairs we have studied is small, the number of possible mutations of the p53 gene which destroy its tumour suppressor function is likely to be close to 10^3 and there are over 100 commonly observed mutations [1, 2], therefore it is most unlikely (a probability of $<10^{-4}$) that even in a single patient the same p53 mutation arose independently in the primary and metastatic SCC.

In all cases the normal p53 sequence was clearly visible in both the primary and metastatic samples. This might indicate that there was no loss of heterozygosity in this particular tumour, since the BICR-4, BICR-5, and BICR-21 tumour samples were 95–100% tumour cells (Table 1) and at least 15% normal contamination is necessary to register a normal sequence signal using the direct sequencing technique [20].

It is nevertheless clear that the mutant p53 genes continue to be important for SCC progression even when presumably accompanied by accumulating additional gene mutations at other loci [21]. These results are consistent with the observed antiproliferative [22–25] or antitumorigenic [26–28] effects of normal p53 when re-expressed in a variety of human cancer cell lines obtained from advanced tumours.

Table 1.

Patient	Tumour number	Primary (P)/ metastatic (M)	Codon	Mutation	Amino acid change	Normal sequence detected	Percentage of tumour in biopsy specimen
4	BICR-4	P	174	AGG→TGG	Arg→Trp	Yes	95
	BICR-5	M	174	AGG→TGG	Arg→Trp	Yes	100
21	BICR-21	P	Exon 8/9	Splice site G→T	308+frame shift 345 stop	Yes	100
	BICR-22	M	Exon 8/9	Splice site G→T	308+frame shift 345 stop	Yes	30
31	BICR-31	P	173,174	TGA deletion	val,arg→gly	Yes	N.D.
	BICR-32	M	173,174	TGA deletion	val,arg→gly	Yes	N.D.

N.D. = not determined.

We have also previously shown that a cell line from a primary tumour SCC-25 [29] harbours the same p53 mutation [4] as the cells excised from a local occurence of this tumour [6].

All of the above results have significance in that they suggest that mutant p53 genes may be used to mark human SCC and this may be useful in distinguishing between clonal SCCs and genetically distinct SCCs which arise out of large field changes [30]. They also support the suggestion that the mutant p53 "signature" of a tumour could be used to distinguish between recurrent and second primary SCCs [15] and this might well have implications for the choice of therapy in some cases.

The p53 mutations in patients BICR-4/5 and BICR-31/32 both lead to the stabilisation of the p53 protein it is likely that these mutations would be of a dominant-negative type [31] however the mutation found in patient BICR-21/22 leads to a frame-shift and hence loss of function of the p53 protein. Our results therefore extend the studies of other tumour systems [16–19] to show that loss—as well as a gain—of function p53 mutations are required for tumour progression.

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