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Short Communication

ras Mutations are Uncommon in Nasopharyngeal Carcinoma

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NASOPHARYNGEAL CARCINOMA (NPC) is one of the most common cancers in Southern China. The *ras* gene was detected in these tumours by a previous transfection study [1]. However, there has been no report of direct examination of the status of the *ras* genes in these tumours.

In this study, we examined 18 NPC tumours for mutations of *Ha-ras*, *Ki-ras* and *N-ras* at codons 12, 13 and 61, which are the mutational hotspots of this gene family, using allele-specific oligonucleotide hybridisation as described by Verlaan de-Vries *et al.* [2]. The tumour samples included three xenografts (666, 1915, 2117) which were WHO III (undifferentiated), three WHO II (poorly differentiated) and 12 WHO III biopsies. Four of the WHO III biopsies were obtained from secondary tumours located at the regional lymph nodes and the others were primary tumours. Four control cell lines: HT1080, HL60, SW480 and MOLT4, were used as positive controls for hybridisation, as described by Verlaan de-Vries *et al.* [2].

DNA was extracted from the tumours as described in [3]. The primers and oligonucleotide probes for PCR were the same as those used by Verlaan de-Vries *et al.* [2]. The PCR cycles used were: 94°C, 30 s; 55°C, 1 min and 72°C, 1 min (for *Ha-ras* and *Ki-ras*), while the annealing temperature for *N-ras* was 49°C. Thirty cycles were performed, followed by an extension at 72°C for 7 min, using TC-1 tempcycler (Perkin–Elmer). The PCR products were run on a 2–2.5% TBE gel and Southern-blotted. Hybridisation was performed according to the procedures in [2].

Under the hybridisation conditions used, only PCR products from cell lines carrying *ras* mutations exclusively hybridised with probes which were specific for the corresponding mutant alleles. PCR products from the tumour samples studied hybridised only with wild-type probes while the water blank control consistently showed no hybridisation. An example of the results, in this case *N-ras*, is shown in Fig. 1.

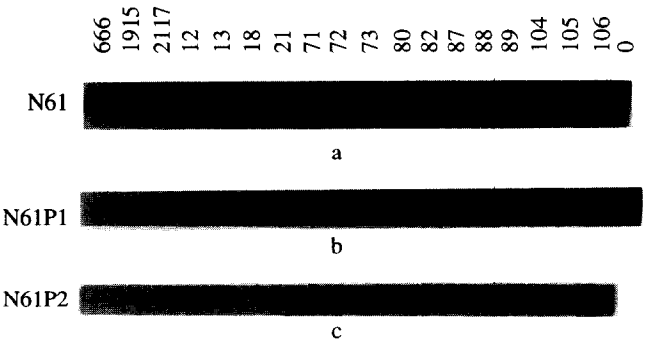

Fig. 1. Analysis of *N-ras* codon 61 mutations. Hybridisation using wild-type oligoprobes identified PCR products (103 base pairs) of all NPC samples. Lane 0 is the DNA blank control (a). Hybridisation with mutant probe pools N61P1 (b) and N61P2 (c) encompass all possible mutations in the first and second base positions of codon 61, respectively. Only the positive cell lines, HT1080 (b) and HL60 (c) gave positive signals.

The patterns of hybridisation were confirmed by reproducing the experiments at least twice.

None of the NPC specimens examined in this study contained *ras* mutations at the hotspots. However, the possibility that novel mutations occur in other locations of the *ras* genes cannot be excluded, even though these are rarely found in natural tumours. Recently, the ras products have been found to be overexpressed in NPC [4], suggesting the possible involvement of ras proteins in the pathogenesis of NPC. It seems possible that some epigenetic events may occur that lead to upregulation of the proteins. The ras proteins have been shown to be transforming when overexpressed, even though the proteins are wild type [5].

Alternatively, *ras* activation may be replaced by other events that produce similar effects. It has been shown that nearly all NPCs harbour deletions on chromosome 3p, near the *raf-1* locus [6]. Since this gene encodes a serine/threonine kinase that interacts directly with the ras products, aberration of this gene may substitute *ras* mutations. In addition, a human herpes virus, EBV, is regularly associated with NPC. One EBV transforming protein, LMP-1, shares homology to the rhodopsin family [7]. This family comprises receptors coupled with G proteins. Since the ras proteins are members of the G protein family, expression of LMP-1 may obviate the need for *ras* mutations in those tumours that express this protein.

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In conclusion, activating *ras* mutations at codons 12, 13 and 61 are relatively uncommon in NPCs. However, in view of the reported overexpression of ras products in NPCs [5], the involvement of the *ras* genes in NPC cannot be entirely excluded. Alternatively, other events that mimic the effects of *ras* mutation may occur in NPCs. The exact nature of such events warrants further investigation.

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