Analysis of the c-Ha-ras-1 gene for deletion, mutation, amplification and expression in lymph node metastases of human head and neck carcinomas

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Summary The c-Ha-ras gene was analysed by Southern blot hybridisation in 67 specimens of lymph node metastases and in 25 specimens of primary tumours obtained from 85 untreated patients with head and neck squamous cell carcinoma. The loss of one c-Ha-ras allele was observed in 10/46 (22%) tumours from heterozygous patients for this locus. Different genes, located as the c-Ha-ras gene on the short arm of chromosome 11, were also found to be deleted suggesting that the deletion of other genes could play a role in aggressiveness of head and neck carcinomas. Using polymerase chain reaction, mutation at codon 12 was detected in only 2/54 (3.8%) tumours but no mutation involving codon 61 was found. Neither gene amplification nor gene rearrangement could be observed. Total RNA was prepared from 79 of these tumour specimens and analysed by Northern and slot blot hybridisation. A 1.2 kb c-Ha-ras transcript band was detected in all the RNA preparations. Relatively high c-Ha-ras transcript levels were found in 18% of lymph node metastases and in 21% of primary tumours, indicating no significant differences between these cancers. Moreover, the c-Ha-ras mRNA levels were not significantly greater in the primary tumours than in the normal mucosa in 10/12 cases for which both tissues were analysed. These data indicate that c-Ha-ras gene does not seem to be strongly involved in head and neck carcinomas at that advanced stage of the disease, as this was previously reported for earlier clinical stages.

It is now well established that genetic alterations are implicated in the biological deregulation of cancer cells and that the cellular oncogenes are involved in the cancer process (Bishop, 1987; Merkel & McGuire, 1988; Nordenskjold & Cavenee, 1988). Among those cellular oncogenes, the ras genes were thought to play an important role and many studies were initiated to detect alterations and aberrant expression of these genes. Somatic mutations, resulting in the substitution of a single base at particular positions in the gene locus were found to be responsible for oncogenic activity by transfection assay, in about 15% of cancers (Barbacid, 1988). Recently, using more sensitive methods, high rates of mutation affecting the c-Ki-ras gene were detected in DNA from pancreas and colon carcinomas (Bos et al., 1987; Forrester et al., 1987; Almoguera et al., 1988; Bos, 1989). In colon tissues the c-Ki-ras mutations were even found in the preneoplastic lesions which were assumed to progress in invasive carcinomas. Mutations of the ras gene were also found in thyroid adenomas and carcinomas (Lemoine et al., 1988; Suarez et al., 1988). C-Ha-ras mutation at codon 12 was shown to be associated with cervical cancers of poor prognosis (Riou et al., 1988). A c-Ha-ras restriction fragment length polymorphism (RFLP) (Capon et al., 1983) linked to susceptibility of individuals to cancers was reported (Krontiris et al., 1985) and deletions of the c-Ha-ras locus were observed in a variety of human cancers (Nordenskjold & Cavenee, 1988; Riou et al., 1988; Sheng et al., 1988). Other alterations of the ras gene consisting of amplification or rearrangement were more rarely found in human tumours (Barbacid, 1988). On the contrary, studies on the expression of ras genes indicated that high levels of ras specific messenger RNA and ras p21 protein were associated with tumour progression in human cancers of different origins (Viola et al., 1986; Clair et al., 1987). However studies have shown the presence of high levels of ras transcripts or proteins in normal tissues (Furth et al., 1987; Kerr et al., 1985) while others have reported that high levels of these gene transcripts or protein were not necessarily related to tumour progression (Spanidios & Kerr, 1984; Gallick et al., 1985; Chesa et al., 1987).

In this study we have analysed the c-Ha-ras-1 for allelic deletion, mutation, amplification and expression in the same specimens of a large series of lymph node metastases from patients with head and neck squamous cell carcinomas. Our aim was to determine whether the c-Ha-ras proto-oncogene by alteration of its structure and/or overexpression plays also a role in the progression of these cancers known to be of poor prognosis.

Materials and methods

Carcinomas and control specimens

Ninety-two specimens of head and neck squamous cell carcinoma of different locations were obtained by surgical excision from 85 untreated patients (Table I); 25 samples were primary tumours and 67 samples were lymph node metastases. We could obtain both primary tumours and lymph node metastases from 7 patients and primary tumours and normal mucosa from 14 other patients. In each case, normal mucosa was cut off distantly from the tumour. Histopathological examination was performed on all the tissue specimens (Figure 1a, b and c). Carcinomas were of squamous cell histological type. A large majority of these carcinomas were well differentiated. Lymphocytes were obtained from 65 healthy donors and 15 patients. The lymphocytes were fractionated in a Ficoll-Hypaque gradient. All the tissue and cell samples were immediately stored in liquid nitrogen.

Isolation of DNA and RNA

DNA and RNA were prepared from the same tissue samples corresponding to about 50–200 mg of fresh tissue. Frozen tissues were ground in liquid nitrogen and nucleic acids extracted by the guanidinium-thiocyanate method. DNA and RNA were fractionated by centrifugation in a CsCl gradient (Maniatis et al., 1982; Terrier et al., 1988). Nucleic acids were also prepared from EJ human cancer cell line. EJ cells originated from a bladder carcinoma with transitional cells exhibiting on the c-Ha-ras gene a point mutation at codon 12 involving a transversion G T.

Southern blots

DNA preparations (5 or 10 µg) were incubated with HpaII-MspI enzymes to analyse c-Ha-ras RFLP and allele loss and with SacI enzyme to analyse the structure of the locus.

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Southern blot hybridisations using the different probes were performed. Other genes located on the short arm of chromosome 11 were also studied for allelic loss. Different restriction enzymes and probes were used as noted in the legend of Figure 2.

Northern blots
Denatured total RNA samples (10 μg per well) fractionated on a 1.2% formaldehyde agarose gel and transferred to a Hybond C extra filter were analysed by Northern blot hybridisation as previously described (Maniatis et al., 1982). The quality and the quantity of total RNA were tested by a preliminary electrophoresis in a non-denaturing 1.2% agarose mini-gel. The filters were exposed at ~ 70°C to Kodak XAR 5 films for various periods of times.

Slot blots
Preparations of total RNA were applied to nitrocellulose filters (BA 85) using a slot blot apparatus (Schleicher & Schuell). Total RNA from EJ cell line was used to measure the levels of c-Ha-ras mRNA. Transcript level in this cell line was stable and arbitrarily considered as 1 unit. Transcript level was determined by densitometer scanning of the autoradiographic bands (Chromoscan 3, Joyce Loeb). To provide a control for the amount of RNA on the filters, the c-Ha-ras gene signal was removed and the same filters rehybridised with an actin probe.

Detection of mutation by polymerase chain reaction (PCR)
We have amplified sequences spanning 63–128 base pairs across codons 12, 13, and 61 of ras (Verlaan-de Vries et al., 1986). Amplification was carried out as described by Saiki et al. (1985) using Thermus aquaticus DNA polymerase (Perkin-Elmer Cetus). All the oligonucleotides used in our laboratory were synthesised by the solid phase triester method. The 5' ends of oligomers were labelled with T4 polynucleotide kinase (Maniatis et al., 1982). Aliquots (2 μl) of the PCR mixture were spotted to a nylon filter (Gelman) and hybridised to a panel of 20 mers synthetic oligonucleotide probes. Hybridisation and washing of filters were performed in a 3 M tetramethylammonium chloride salt solution (Verlaan-de Vries et al., 1986).

Probes
Two c-Ha-ras probes were used, the 2.5 kilobase pairs (kb) BamH1-SacI DNA fragment (probe 1) encompassing the Variable Tandem Repetitive (VTR) region (Capon et al., 1983) and the 2.9 kb SacI-SacI DNA fragment encompassing the c-Ha-ras coding exons (probe 2). These probes were obtained from the 6.6 kb c-Ha-ras-1 gene of EJ cells. Probes used as markers on chromosome 11 were: D11S12 from clone pHAD1762 (Barker et al., 1984); HBBC (γg and γA of the β globin cluster) from clone JW151 (Antonarakis et al., 1982); PTH (human parathyroid hormone) from clone pPTH 2.5 (Vasicek et al., 1983); CALC (calcitonin) from clone pHBT3 (Craig et al., 1982); CAT (catalase) from clone pCAT41 (Korneluk et al., 1984).

Results
Characteristics of head and neck carcinomas are presented in Table I. A large majority of the carcinomas which were analysed, were of poor prognosis since patients had lymph node involvement with extracapsular rupture, one of the most powerful indicator of prognosis in these types of cancer (Figure 1c). RFLP was analysed in DNA preparations from carcinomas and from lymphocytes of healthy blood donors. A RFLP for BamH1 orMspI (Capon et al., 1983; Krontiris et al., 1985) was found to occur in a region of variable tandem repetition (VTR) located at the 3' end of the c-Ha-ras locus (Figure 2). After Southern blot hybridisation using the c-Ha-ras-1 VTR probe (probe 1, Figure 2), several classes of allelic restriction fragments were observed, some of them were frequently detected while others rarely found (Krontiris et al., 1985). Single or 2 DNA fragments were revealed in cancer DNA preparations from homozygous and heterozygous patients respectively. Figure 3A shows representative patterns of hybridisation from tumour DNAs. Similar patterns were obtained from DNAs of unaffected population (data not shown). The size and the frequency of the different alleles are presented in Table II. The rare alleles occurred with similar combined frequencies in head and neck patients (7%) and in normal individuals (6.5%). As expected, identical c-Ha-ras-1 VTR alleles were detected in DNAs from tumours and lymphocytes or normal mucosa obtained from the same patients. Heterozygosity for this allele was found in 46 tumours and the loss of 1 allele was observed in the tumour samples from 10 (22%) of these heterozygous patients (Figure 3B).

Figure 1  Histological sections. Normal mucosa a, and squamous cell carcinoma b, were obtained from larynx by surgery. Carcinoma was composed of typically well differentiated squamous cells. Lymph node metastatic tumour exhibiting extracapsular rupture c, arrow. (Hematoxylin-eosin stain. a, b x 136; c, x 21.25).

Figure 2  Schematic representation of the 6.6 kb BamH1 DNA fragment carrying the human c-Ha-ras-1 gene obtained from EJ cells. The black boxes represent the 4 exons and the hatched box, the region of variable tandem repetition (VTR). The 2.5 kb SacI-BamH1 DNA fragment (probe 1) was used to detect RFLP and gene deletions, the 2.9 kb SacI-SacI DNA fragment (probe 2) encompassing the 4 exons was used to detect amplification and expression of the gene (B, BamH1 and S, SacI restriction sites).
Table I  Characteristics of squamous cell head and neck carcinomas and tissues

| Site of the primary tumour | Total no. of patients | No. of node positive patients | No. of patients with at least one node with extracapsular rupture | Primary tumour | Node metastasis | Normal mucosa
|---------------------------|-----------------------|-------------------------------|----------------------------------------------------------------|---------------|----------------|--------------|
| Hypopharynx*              | 22                    | 22                            | 20                                                              | 6             | 19             | 4            |
| Oral cavity               | 21                    | 21                            | 20                                                              | 5             | 18             | 1            |
| Larynx                   | 18                    | 14                            | 14                                                              | 5             | 14             | 5            |
| Oropharynx                | 14                    | 13                            | 12                                                              | 4             | 11             | 3            |
| Sinus and hard palate     | 5                     | 1                             | 1                                                               | 4             | 1              | 1            |
| Unknown                   | 5                     | 5                             | 5                                                               | 1*            | 4              | 1            |
| Total                     | 85                    | 76                            | 72                                                              | 25            | 67             | 14           |

*a,b,c,d Both primary tumour and metastasis from 3*, 2*b and 1*c patients were analysed. 
Lymphadenopathy with unknown site of the primary tumour. 
*DNA from the 92 tumour samples were analysed whereas RNA from 79 of these samples were analysed. 
Both normal mucosa and primary tumour from 14 patients were analysed.
*The sample quoted with primary tumour was a tumoral mass of unknown origin.

Figure 3  Southern blot analysis of c-Ha-ras-1 restriction fragment length polymorphism in head and neck squamous cell carcinomas and normal cells from patients (normal mucosa, N; lymphocytes, L). DNA samples (5 μg) were digested with HpaII-MspI, electrophoresed in 2% agarose gel and hybridised with the c-Ha-ras-1-VTR probe (probe 1 of Figure 2). DNA with similar-sized alleles were re-electrophoresed in the same gel in separated wells and in the same well, A, head and neck carcinomas. B, pairs L-Ca (carcinoma) and N-Ca from the same patients. Note the loss of 1 allele in Ca, Ca, and Ca. A faint hybridisation band to the deleted band probably due to the presence of normal cells in tumours was observed in Ca, and Ca.

Table II  C-Ha-ras allele frequencies in head and neck carcinomas and normal lymphocytes

| Allele size (kb) | Lymphocytes of healthy donors (65)* | Carcinomas (74)* |
|------------------|-----------------------------------|-----------------|
| Common alleles   |                                   |                 |
| 1.1              | 66.0                              | 54.9            |
| 1.6              | 14.5                              | 12.7            |
| 2.2              | 6.5                               | 9.9             |
| 2.6              | 6.5                               | 15.5            |
| Total            | 93.5                              | 93.0            |
| Rare alleles     |                                   |                 |
| 0.95 to 2.8      | 6.5                               | 7.0             |

In parentheses the number of samples analysed.

Several genes located in the vicinity of the c-Ha-ras locus on the short arm of chromosome 11 were also analysed to determine the extension of chromosome deletion. An allelic loss pattern after Southern blot hybridisation (Figure 4) and a schematic representation of the deleted genes (Figure 5) are presented. The allelic deletions for different loci on chromosome 11p were observed in 4 of the 11 head and neck carcinomas analysed. One HBBC allele belonging to the β globin cluster was lost in these 4 carcinomas together with 1 c-Ha-ras allele in 3 carcinomas and with 1 calctinon allele in the other carcinoma.

The presence of mutation at positions 12 and 61 on the c-Ha-ras locus was investigated in 54 carcinoma specimens using the PCR technique and hybridisation with synthesised specific probes. A mutation affecting the codon 12 as in the EJ cell lines was observed in only 2 carcinomas and not detected in the lymphocyte specimens. The same transversion G T was observed in the 2 cases of mutated c-Ha-ras genes (Figure 6). No mutation was detected at position 61. The presence of mutation at positions 12, 13 and 61 on the Ki-ras gene and of mutation at positions 12 and 61 on the N-ras gene was also investigated in 28 carcinoma samples. No mutation could be evidenced (data not shown). DNA preparations, after digestion with SacI, were analysed by Southern blot hybridisation with the probe 2 (Figure 2) encompassing the 4 c-Ha-ras exons. As expected a 2.9 kb DNA band was observed in all the DNA preparations. Neither gene amplification nor gross gene rearrangement were found in carcinomas (Figure 7).

The total RNA was prepared from 79 specimens of these carcinomas and from 12 specimens of normal mucosa. RNA preparations were analysed by Northern and slot blot hybridisation using the c-Ha-ras-1 gene probe 2 of Figure 2. As usually observed in human tissues, a 1.2 kb transcript band was detected in all RNA preparations. A representative Northern blot was shown in Figure 7. Blots were dehybridised then rehybridised with the actin probe which was assumed to be equally expressed in cancers. The levels of c-Ha-ras gene transcripts were determined by densitometric analysis of autoradiograms from slot blots and normalised against actin values (Figure 8). Relative levels were evaluated by comparison to c-Ha-ras transcript level obtained in EJ cells. Lymph node metastases and primary tumours were independently grouped according to their relative c-Ha-ras mRNA levels (Figure 9). The mean values (in arbitrary units ± standard deviation) were equal to 0.8 ± 0.9) in metastases and 0.9 ± 0.6) in primary tumours. The differences were not found to be significant (Student Fisher test p = 0.4). The c-Ha-ras mRNA levels found in individual primary tumours and normal mucosa node metastases are presented in Figure 10. In only 2 of 12 cases, the c-Ha-ras transcript level was found to be significantly higher in tumour than in normal mucosa. No significant difference was observed between primary tumours and lymph node metastases. As shown in Table III the level of c-Ha-ras RNA expression was not influenced by the state of c-Ha-ras gene in tumours (loss of allele, mutation, or presence of rare allele).
Allelic deletions on chromosome 11p in head and neck carcinomas. Southern blot hybridisation, DNA from normal mucosa (N) and primary tumour (T) of a head and neck patient with a larynx location (Patient d Figure 5). Restriction enzymes used were HpaII/MapI for c-Ha-ras and D11S12 loci, HindIII for HBBC, ParI for parathyroid hormone (PTH) locus and TaqI for calcitonin (CALC) and catalase (CAT) loci. Allelic deletions were observed for HBBC and CALC, the presence of a faint 8 kb hybridisation band was due to the presence of normal cells in these tumours. Only sizes in kb of DNA bands which could undergo an allele loss are given.

Map of the common region of 11p deletion. Positions of 6 genes or markers from chromosome 11p are shown, a black box indicates that 1 parental allele was lost in the carcinoma, a hatched box indicates that both parental alleles were retained in the carcinoma, an open box indicates that the marker was not informative (the patient’s normal mucosa was homozygous for the marker). An allele loss (apolipoprotein 1, Apo Al) on chromosome 11q is also shown.

Analysis of DNA and total RNA prepared from the same specimens of head and neck carcinoma for amplification and expression of the c-Ha-ras gene. EJ, cell line; Ca, carcinoma; N, normal mucosa. A, Southern blot analysis: DNA (5 μg per well) was incubated with SacI restriction endonuclease. B, Northern blot analysis of total RNA (10 μg per well). Filters were sequentially hybridised with the c-Ha-ras probe then actin probe; kb, kilobase pair, c-Ha-ras-1 probe 2 (Figure 2).

Analysis of c-Ha-ras transcripts by slot blot hybridisation (5 μg of total RNA in each slot). Filters were sequentially hybridised with the c-Ha-ras-1 probe (probe 2 of Figure 2) and the actin probe. Panels A, B, C lanes 1 to 10, RNA from head and neck carcinoma except in B lane 10, RNA from EJ cell line. Panel D, lanes 1–2, 3–4 and 5–6, carcinoma lymph-node metastasis and lanes 7–8 and 9–10, primary tumour-normal mucosa pairs from the same patients.
Table III  Expression of the c-Ha-ras gene according to its structural characteristics

| Characteristics of c-Ha-ras in individual carcinomas | Relative level of c-Ha-ras mRNA |
|---------------------------------------------------|--------------------------------|
| Allelic deletion                                   |                                |
| O1 *                                              | 0.39                           |
| O2                                                | 0.42                           |
| O3                                                | 0.62                           |
| O4                                                | 0.69                           |
| O5                                                | 0.85                           |
| O6                                                | 2                              |
| O7                                                | 3.4                            |
| Mutation at codon 12                              |                                |
| O2                                                | 0.38                           |
| O9                                                | 0.54                           |
| Rares alleles                                      |                                |
| O2                                                 | 0.4                            |
| O7                                                 | 0.69                           |
| O4                                                 | 0.92                           |
| O6                                                 | 1.2                            |
| O9                                                 | 1.3                            |

*Patient no.

Discussion

Head and neck cancers are among the commonest cancers world-wide (Parkin et al., 1984) and the large geographical variations in incidence have suggested the importance of environmental risk factors (Blot et al., 1988; Tuyns et al., 1988). Epidemiological studies have shown that these cancers, if we except carcinomas of the nasopharynx for which Epstein-Barr virus is the main aetiological factor, are strongly associated with a high intake of alcohol and tobacco smoking. The majority of carcinomas of this study developed in patients with poor prognosis since specimens were obtained from lymph node metastases exhibiting extracapsular rupture (Table I, Figure 1c).

Studies have reported that an aberrant expression of the c-Ha-ras gene was associated with tumoral progression (Viola et al., 1986; Clair et al., 1987; Spandidos et al., 1985; Azuma et al., 1987). If the c-Ha-ras gene was involved in head and neck tumour progression, we should expect to find c-Ha-ras gene alteration or overexpression with a high frequency in these very aggressive cancers. We have analysed the c-Ha-ras-1 RFLP for HpaII-Msp1 enzymes in these carcinomas comparatively to a control population of healthy blood donors and found no significant differences in the alleles frequencies. Consequently, the presence of variant alleles at the c-Ha-ras-1 locus does not seem to be an informative marker for the genetic predisposition to head and neck carcinomas, as has been suggested in cancers of other origins (Krontiris et al., 1985).

Recent studies have shown that allelic deletions could signal the presence of a tumour suppressor gene within the affected regions of the chromosome. When both allelic copies of such a gene are inactivated, suppression may be relieved resulting in an abnormal proliferation. This process was particularly well documented for retinoblastoma and Wilms’ tumour, 2 childhood tumours (Knaudson, 1987; Friend et al., 1988). The tumour suppressor gene concept was extended to other common adult malignancies but the numerous studies undertaken until now have still not clearly shown the involvement of such suppressor genes. Rather, these studies have reported chromosomal deletions affecting particular loci in certain carcinomas, suggesting a prevalent role of these alterations in the development of malignancies. Deletions on the short arm of chromosome 11 were considered as being a common and important event in the development of many cancers and sometimes in tumour progression. Of the 46 head and neck carcinomas whose patients were constitutionally heterozygous for the c-Ha-ras locus, the loss of 1 allele was detected in 10 (22%) specimens. The deletions were...
all found in the lymph node metastases exhibiting an extra-
capsular rupture (Figure 1c), and not detected in the 9 primary tumours from patients with negative nodes. Although this observation was in favour of a role of the c-Ha-ras allelic deletion in head and neck tumour progression, no definitive conclusion can be drawn because of the small number of tumours examined (Exact Fisher test, $P = 0.29$). Deletions of HBBC genes located on the short arm of chromosome 11 were also found in 4 of 11 carcinomas. These deletions were accompanied with the loss of 1 c-Ha-ras allele in 3 tumours and with the loss of 1 calecitinin allele in 1 tumour (Figures 4 and 5). This result confirms recent data obtained in breast carcinoma showing that, the most frequent loss of sequences occur between the $\beta$ globin and parathyroid hormone loci on the short arm of chromosome 11 (Ali et al., 1987).

Using the PCR technique and hybridisation with specific oligonucleotide probes our present data showed that mutations on the c-Ha-ras locus were not frequent in head and neck carcinomas since, of the 54 DNA preparations analysed, only 2 mutations were detected on codon 12 involving a G T transversion. These mutations were somatic since not observed in the constitutional DNA from patient lymphocytes. Mutations on the c-Ha-ras locus are not frequently found in human cancers, however, in a series of 76 invasive cell carcinomas of the uterine cervix, mutations involving codon 12 were found to be significantly associated with advanced stages of the disease ($P < 0.01$) (Ali et al., 1988). No mutations were found in any of the codons 12, 13 and 61 of the c-Ki-ras and of the codons 12 and 61 of the N-ras (data not shown).

It is now well established that amplification of proto-
oncogenes is a late event associated with advanced stages of the disease in some types of invasive cancers. This is the case for example of the myc gene family and of the c-erbB2 gene. The c-myc gene was found to be amplified in advanced stages of cervical carcinomas (Riou et al., 1984; Riou, 1988; Ocadiz et al., 1987) and the N-myc gene in advanced stages of neuroblastosomas (Seeger et al., 1985; Bourhis et al., 1989). The c-erbB2 gene was also detected in a relatively high proportion of breast carcinomas of poor prognosis as are those with multiple lymph node involvement (Salamon et al., 1987; Guérin et al., 1988a and Guérin et al., 1989b). To our knowledge, very few data have reported a c-Ha-ras gene amplification in fresh human cancers. Using the 6.6 kb BamH1 DNA restriction fragment as a probe, an amplification was observed in a limited series of cervical cancers of stages III and IV (Riou et al., 1985). In the present study, Southern blot analysis performed in the 81 DNA preparations from head and neck carcinomas did not reveal the presence of either amplification or gross rearrangement of the c-Ha-ras locus.

It is known that aberrant expression of ras oncogene, either at the qualitative or quantitative levels, can result in malignant transformation and/or differentiation (Barbacid, 1988). Immunohistochemistry studies performed in ultrathin sections in a variety of tumours have reported the presence of the p21 ras protein in higher concentration in the tumoral tissues than in the peripheral non-tumoral tissues (Barbacid, 1988; Viola et al., 1986; Clair et al., 1987). These data suggested that overexpression was associated with progression of the tumour and thus with a poor clinical prognosis. However some recent studies are controversial and support the findings that elevation of the p21 may be a common event in early stages of tumours, which is not related to the proliferative activity of a variety of human malignancies (Spondidos & Kerr, 1984; Gallic et al., 1985; Chesa et al., 1987). Furthermore, in neuroblastosomas the c-Ha-ras protein expression was correlated with a favourable prognosis and early stage of disease (Takana et al., 1988). These data suggest that the c-Ha-ras gene product may play a role in decreasing the aggressiveness of neuroblastosoma cells in vivo and may be related to cellular differentiation.

In the present study, we show that, in head and neck carcinomas, a transcript band displaying different degree of hybridisation intensity was observed in primary tumours as well as in lymph node metastases. The relative levels of transcripts were not found to be significantly different between primary tumours and lymph node metastases although the latter specimens were obtained from nodes with extracapsular rupture, one of the most powerful indicator of poor prognosis in these types of carcinomas. The transcript levels were not found to be significantly higher in primary tumours than in adjacent normal mucosa with the exception of 2 cases (Figure 10). Mucosa obtained at surgery were composed of normal cells as showed by histopathological examination. They were used as tissue controls allowing the detection of c-Ha-ras deletion (2 cases) and HBBC (1 case) deletions (Figures 3 and 4).

In conclusion, our data showed that the c-Ha-ras transcript does not seem to be a major event in the tumour progression of head and neck squamous cell carcinoma, as has been reported in other tumour types. It is quite possible that increase of c-Ha-ras transcripts and/or protein was important for the initiation step of head and neck carcinogenesis (Field et al., 1986) but that in later steps other genes may supplant the role of this gene.

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