Variability of Ha-*ras* (codon 12) proto-oncogene mutations in diverse thyroid cancers

Mourad Bouras^{1,2}, Jacques Bertholon², Nicole Dutrieux-Berger³, Parviz Parvaz^{1,2}, Christian Paulin⁴ and André Revol^{1,2}

¹Laboratoire de Biologie Moléculaire et Pédiatrique, Centre Hospitalier Lyon-Sud, Bât. 3B, 69475 Pierre-Bénite Cedex, France, ²Laboratoire de Biochimie Endocrinienne et Moléculaire, Institut des Sciences Pharmaceutiques et Biologiques, 8 avenue Rockefeller, 69373 Lyon Cedex 08, France, ³Laboratoire d'Anatomopathologie, Hôpital de l'Hôtel-Dieu, place de l'Hôpital, 69288 Lyon Cedex 02, France and ⁴Laboratoire de Cytologie, Centre Hospitalier Lyon-Sud, Bât 1G, 69475 Pierre-Bénite Cedex, France

(Correspondence should be addressed to A Revol, Laboratoire de Biochimie Endocrinienne et Moléculaire, Faculté de Pharmacie de Lyon, 8 Avenue Rockefeller, 69373 Lyon Cedex 08, France)

Abstract

Structural alterations to proto-oncogene sequences may be involved in the pathogenesis of human thyroid neoplasms. We studied 128 thyroid tumours (35 benign and 93 malignant) for *ras* gene point mutations in three different codons (12, 13 and 61) using a restriction fragment length polymorphism technique and direct sequencing of double-stranded DNA on polymerase chain-reaction-amplified tumour DNA. We found a high frequency of *ras* mutation for the Ha-*ras* codon 12 in follicular adenomas (7 of 35), particularly in atypical adenomas (5 of 17), in follicular carcinomas (6 of 19), with a high percentage for Hürthle cell carcinomas (6 of 11), and in papillary carcinomas (4 of 66). Point mutations for other *ras* genes in different codons studied were weak to absent. No mutation was found in undifferentiated carcinomas ($n = 8$). The predominant amino acid substitution both in the adenomas and in the differentiated tumours was glycine to valine (GGC to GTC) at position 12 of the Ha-*ras* gene.

Our results obtained on a large series confirm the frequent occurrence of Ha-*ras* codon 12 gene mutations both in adenomas and in carcinomas. The frequency of *ras* mutations is linked to the geographical origin of the population studied, and varies (0–85%) from one cancer type to another according to published data. Therefore, these mutations are merely an expression of cellular transformation.

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Introduction

The *ras* proto-oncogenes (Ha-, Ki-, and N-*ras*) are believed to have an important role (1) in the genesis of human cancer. Mutations at codons 12, 13 or 61 of *ras* proto-oncogenes are found in more than 30% of human tumours (2). A mutation in one allele is sufficient to convert the genes into active oncogenes (1).

At present, it is important to identify new biological markers, the presence of which in precancerous lesions could predict the risk of development of cancer. Studies of various types of benign and malignant thyroid tumours have revealed the presence of activated *ras* oncogenes in some of them (3–7). Although the number of tumours included in each study was relatively small, it appears that mutations in *ras* genes are frequent in thyroid tumours, and can affect all three *ras* genes. Lemoine *et al.* (4) have found an association between the frequency of *ras* activation observed in follicular and papillary carcinomas and the biological behaviour and progression of the tumour (8).

In this work, we have studied the frequency of *ras* gene mutations in 'hot' codons (frequently mutated) (12, 13 and 61), using a greater number of benign and malignant tumours and applying sensitive and specific techniques. We analysed 128 thyroid tumours: adenomas ($n = 35$), and follicular ($n = 19$), papillary ($n = 66$) and undifferentiated carcinomas ($n = 8$). When a mutation was observed, it was identified by a sequencing technique.

The results show a high frequency of Ha-*ras* codon 12 mutation in atypical adenomas and Hürthle cell carcinomas compared with a low frequency in papillary carcinomas and a complete absence of mutations in undifferentiated carcinomas. For the other *ras* genes, the frequency of *ras* mutations was low to nil.

Codon 12 Ha-*ras* gene mutation may participate in the processes of transformation and progression of thyroid tumours. However, cellular malignant transformation requires the association of several gene anomalies, including the activation of some (trk and ret) and the mutation of tumour suppressor genes (p53 and Rb).

Table 1 Histological classification of different thyroid tissues studied.

Adenomas	
Atypical	17
Macrofollicular	2
Microfollicular	5
Normofollicular	7
Toxic	4
Carcinomas	
Papillary	66
Follicular	19
Undifferentiated	8
Total	128

Materials and methods

Tissues

Human normal and tumorous thyroid tissues, identified according to the World Health Organization (WHO) histological classification of thyroid tumours (9), were provided by the Department of Pathology, Services of the Lyon-Sud Hospital Centre and Antiquaille Hospital. Samples were taken from 128 patients after total or partial thyroidectomy. The tumours included 35 adenomas and 93 carcinomas (Table 1). Pathological samples and samples of normal tissue (ten patients) from sites remote from the lesion were immediately frozen in liquid nitrogen and kept at -70°C .

Primers and probes

Oligonucleotides were synthesized by Genset (Genset Oligos, Paris, France). We used oligonucleotides encoding PCR primers, and wild-type or mutated *ras* sequences around codons 12, 13 and 61, described by Jiang *et al.* (10).

The sequence corresponding to nucleotides 1872–1892 (5'-CTGCATGGTGTGGCCCT-3') was used as a probe for Southern blot analysis of Ha-*ras* codon 12 gene. The oligonucleotide primers for nested-PCR of Ha-*ras* codon 12 gene were: 5'-GACGGAATA-TAAGCTGGTGG-3' (exon 1: 1671–1691), 5'-CTGCTT-ATGCTGTGGTGATATCT-3' (exon 1: 1779–1757). For the sequencing of Ha-*ras* codon 12 gene, one primer of the nested-PCR set primer was used.

Nucleic acid extraction

Several samples from the same pathological tissue were used for DNA extraction. About 40 mg tissue was ground twice in 0.5 ml lysis buffer (0.2 mol/l Tris-HCl, pH 7.5; 20 mmol/l EDTA; 0.3 mol/l NaCl). The ground tissue was incubated overnight in a 50°C water bath with 2% SDS and 9 U proteinase K (Amresco, Solon, OH, USA).

DNA isolation was performed with two phenol–chloroform–isoamylalcohol (25:24:1) extractions (Amresco), followed by two chloroform–isoamylalcohol (24:1) extractions. Ammonium acetate (final concentration, 2 mol/l) was added to the aqueous layer, which was then ethanol precipitated. DNA was pelleted, air dried, and resuspended in water. After quantification by absorption at 260 nm, extracts were stored at -20°C until required for assay.

PCR amplification of thyroid tissue DNA

The amplification was performed according to a modification of the technique of Jiang *et al.* (10). Genomic DNA (1 μg) was amplified for 30 cycles with 2.5 U Taq DNA polymerase (Promega Corp., Madison, WI, USA) and 1 $\mu\text{mol/l}$ of each oligonucleotide primer, in a total volume of 50 μl .

For DNA amplification, the sample was first denatured at 94°C for 10 min and then rapidly chilled in an ice bath before amplification in a Hybaid Thermalcycler (Hybaid, Ceralabo, Aubervilliers, France) (94°C for 1 min, 56°C for 2 min and 72°C for 3 min). Each PCR experiment included a negative control with distilled water in place of DNA and a positive control DNA, extracted from peripheral blood leucocytes of normal individuals. After completion of PCR, 15 μl of the PCR product was analysed on 2% agarose (Tébu FMC, Rockland, ME, USA) gel electrophoresis in $1\times\text{TBE}$ buffer (45 mmol/l Tris–borate; 1 mmol/l EDTA, pH 8.0) and visualized, after ethidium bromide staining, under u.v. light. The HaeIII digest of ΦX174 (Sigma) was used as molecular size marker.

Detection of mutations after DNA enzyme digestion

The experiments were performed according to modifications of the techniques of Scott *et al.* (10). One microlitre digestion buffer (10 $\times$) (10 mmol/l Tris-HCl, pH 7.5; 10 mmol/l MgCl_2 ; 1 mmol/l dithiothreitol) was added to 10 μl amplified products and incubated with 2 μl restriction enzyme (MspI) overnight at 37°C . After this digestion, gel electrophoresis in a 4% NuSieve–agarose 3:1 (Tébu, FMC, Rockland, ME, USA), was performed to separate the restricted fragments (restriction fragment length polymorphism, RFLP). For Ha-*ras* codon 12 gene mutation, when the sample was positive for the mutation (heterozygous), mutated fragments were cut out, purified and reamplified using nested-PCR primers (see above).

DNA preparation and Southern blot analysis

After electrophoresis of digested PCR products, the fragments were transferred onto nylon membranes (Hybond N, Boehringer Mannheim, Grenoble, France) according to the standard method of Southern (11):

10 µg DNA digested with MspI, separated on 2% agarose gel (Tébu, USA) were blotted onto nylon membranes for 3 h using a Vacu-Aid (Ceralabo). The filters were then hybridized with Ha-ras DNA probe that was end-labelled by the Dig Oligonucleotide 3'-End Labeling Kit (Boehringer Mannheim). The hybridization was performed at 43 °C overnight in 5×SSC, 2% blocking solution, 0.1% N-laurylsarcosine, and 0.02% SDS. The membranes were then washed twice in 2×SSC, 0.1% SDS for 5 min and twice in 0.1×SSC, 0.1% SDS for 15 min at 43 °C.

Detection was performed with the Dig Luminescent Detection Kit for Nucleic Acids (Boehringer Mannheim). Afterwards, these membranes were exposed to Kodak X-OMAT AR-5 film (Eastman Kodak, Rochester, NY, USA) for 30 min.

Direct sequencing of the mutated DNA fragment

A gene-mutated DNA fragment comprising 108 base pairs of Ha-ras codon 12 was obtained after nested-PCR using the primers (see above) and performed in the same conditions as were used for the first PCR protocol. The PCR products were sequenced directly by the dideoxy chain termination method of Sanger *et al.* (12), as follows. Template DNA was denatured at 100 °C for 2 min, annealed with 1 pmol labelled sequencing primer, and sequenced with Sequenase PCR Product Sequencing Kit (United States Biochemical, Cleveland, OH, USA). High-specific-activity dATP-5'-α [³⁵S] (sp. act. 1000 Ci/mmol) was obtained from Amersham Corp., Arlington Heights, IL, USA. The samples were then run for 2 h in a 6% polyacrylamide denaturing gel (Promega Corp., Madison, WI, USA) in 1×taurine buffer (Tris base–Na₂EDTA, 2H₂O–taurine), pH 8.0. After gel transfer onto 3 MM membranes (Whatman, Maidstone, UK) and air drying by heating at 80 °C for 45 min, the membrane-bound samples were exposed to Kodak Biomax MR film (Eastman Kodak,) for 48 h.

Results

Our series comprised 35 adenomas (17 atypical, 2 macrofollicular, 5 microfollicular, 7 follicular, and 4 toxic adenomas); the malignant tumours examined comprised 93 carcinomas (19 follicular, 66 papillary, and 8 undifferentiated carcinomas) (Table 1). For certain specimens, a sample was taken from a normal region of the same thyroid tissue.

Selective amplification of the region around the studied codons of the *ras* oncogene was performed by PCR after extraction of genomic DNA. RFLP and Southern blot analysis were then performed to detect the presence of Ha-ras codon 12 gene mutation and the DNA sequencing method was used to confirm the mutation. For purposes of interpretation of our results,

we considered that, after DNA digestion, the presence of a single band of 291 bp (Fig. 1A, lane 4) was evidence of a homozygous mutation, and the presence of two bands of 236 bp and 291 bp was the mark of a heterozygous mutation (Fig. 1A, lanes 5 and 6). In all positive cases, we observed two bands corresponding to wild-type and mutated alleles (236 bp and 291 bp respectively).

Thyroid adenomas

DNA of 35 benign thyroid tumours was examined. A point mutation in codon 12 of the Ha-ras gene was found in 7 (20%) of them (Table 2, Fig. 1A).

Codon 12 Ha-ras gene mutation was identified in 5 atypical adenomas of the 17 tested. There were only two macrofollicular adenomas in our series and one of the two (50%) was found to be positive for this mutation. Among the microfollicular type, one out of five tested (20%) was found to be positive.

In our study, 30 adenomas were negative for Ha-ras codon 12 mutation after PCR-RFLP analysis, but activated Ha-ras codon 12 gene was detected in 2 more cases by the Southern blot analysis (Fig. 1B, lanes 4 and 5). Sequence analysis show that the seven observed mutations in these thyroid adenomas were G to T transversion (glycine to valine substitution; Fig. 1C). Only one codon 61 Ha-ras gene mutation was found among seven normofollicular adenomas analysed (14.3%).

Thyroid carcinomas

We examined 93 malignant thyroid tumours. Mutations were identified in the Ha-ras codon 12 gene in 4 of 66 (6%) papillary thyroid carcinomas (three in papillary carcinomas and one in papillary carcinomas with follicular differentiation), and 6 of 19 (32%) follicular carcinomas (only in Hürthle cell carcinomas) (Table 2, Fig. 1A). We found no point mutation of the Ha-ras codon 12 in any of the undifferentiated thyroid tumours (*n* = 8).

Activated Ha-ras codon 12 was detected by the Southern blot analysis in one more case (Fig. 1B, lane 2). The analysis of mutated Ha-ras codon 12 sequences also revealed a G to T transversion (Fig. 1C). A low frequency of mutations was found in the other *ras* genes (Table 2): one mutation of the codon 61 Ha-ras gene among 60 papillary carcinomas (1.7%), and two codon 12 N-ras gene mutations (one in a follicular carcinoma and the other in a papillary carcinoma).

In contrast, all normal thyroid samples showed negative results for mutation.

Discussion

Mutations in all three families of *ras* oncogenes have been detected in both benign and malignant thyroid

Figure 1 (A) Ha-ras codon 12 gene mutation in thyroid tumours. Lane 1: amplified non-digested fragment (311 bp); lane 2: Φ X174 HaeIII digest DNA marker; lane 3: internal control (99 bp of the Ki-ras gene); lane 4: reamplified band of 291 bp as an amplification control; lanes 5, 6: mutated DNA fragment (236-bp and 291-bp fragments), respectively from an atypical adenoma and a Hürthle cell carcinoma; lane 7: digested non-mutated fragment. (B) Southern blot hybridization technique. Mutations of the Ha-ras locus were observed in two adenomas (lanes 4, 5) and in one carcinoma (lane 2). Lane 1: normal band of 236 bp (wild type); lanes 3, 6–10: amplified non-digested fragment (311 bp). (C) Direct sequencing performed only for the mutated DNA fragment, cut from gel electrophoresis after MspI digestion (from Hürthle cell carcinomas). We detected a G to T transversion in the second position of codon 12 (arrowhead), responsible for a glycine-to-valine amino acid substitution.

tumours (3–7, 13–23). Mutations of the *ras* (Ha-, Ki-, N-*ras*) proto-oncogene have been reported in 20–60% of thyroid tumours, particularly in follicular types (3, 5, 6, 15, 17), and more frequently in iodine-deficient areas (16). For this reason, *ras* mutations have been suggested as an important prognostic marker for thyroid cancer (17).

In our study, point mutations of the Ha-ras codon 12 gene were detected by RFLP and hybridization, and positive cases were controlled by a sequencing technique. Validation of our results, and of the reliability and reproducibility of our techniques, was performed using DNA (positive for the mutation in codon 12 of the gene Ha-ras) extracted from carcinomas of the cervix (data

Table 2 Activated *ras* oncogenes in human thyroid adenomas and carcinomas. Values for gene and sex frequency are given as number (%).

Histological type	Mean age (years)		Gene frequency					Sex frequency	Amino acid substitution
			Ha- <i>ras</i>		Ki- <i>ras</i>	N- <i>ras</i>			
	F	M	Codon 12	Codon 61	Codon 12	Codon 12	Codon 13		
Adenomas									
Atypical	46	41	5/17 (29.4)	—	—	—	—	F (5/10, 50)	GGC to GTC
Macrofollicular		40	1/2 (50)	—	—	—	—	M (1/2, 50)	GGC to GTC
Microfollicular	46		1/5 (20)	—	—	—	—	F (1/5, 20)	GGC to GTC
Normofollicular	42	35	—	1/7 (14.3)†	—	—	—	F (1/6, 16.7)	—
Toxic	47	59	—	—	—	—	—	—	—
Carcinomas									
Follicular	43	35	—	—	—	1/6 (16.7)	—	F (1/4, 25)	—
Follicular with oxyphilic cells	45	56	6/11 (54.6)	—	—	—	—	F (4/11, 36)	GGC to GTC
								M (2/11, 18)	
Follicular insular variant	49	76	—	—	—	—	—	—	—
Papillary	48	42	3/60 (5)	1/60 (1.7)	—	1/60 (1/7)	—	M (3/60)—M (1/60)	GGC to GTC
Papillary with follicular differentiation	60	53	1/4 (25)	—	—	—	—	M (1/1, 100)	GGC to GTC
Papillary and atypical adenomas*	49	76	—	—	—	—	—	—	—

† These mutations were screened using only PCR-RFLP techniques.* Nodule comprising an atypical adenoma and a papillary carcinoma.

not shown). For the other *ras* genes, point mutations were only detected by PCR-RFLP techniques.

In our experimental conditions, when mutations affected only one allele of Ha-*ras* codon 12 (heterozygous) or when a homozygous cell was present among heterozygous or normal cells, both the 291- and the 236-bp DNA fragments were observed after electrophoretic migration. If only a single 291-bp fragment was observed in the tumour DNA, the mutated allele was assumed to be in a homozygous state.

In this study, we examined 128 thyroid tumours and normal tissues. None of the ten normal thyroid tissue samples had *ras* point mutations. However, we did not perform control experiments with NIH 3T3 cells in culture conditions to establish if these DNA extracts from normal thyroid tissue are unable to induce transformation in such cells in culture, as was reported by Lemoine *et al.* (4, 5). In our series of 128 thyroid tumours, we detected a Ha-*ras* codon 12 point mutation in 17 (16.4%) (Fig. 1A, lanes 5 and 6). *ras* point mutations were present in 23% of 35 thyroid adenomas, with a high frequency for atypical adenomas (5 of 17, 29%; Table 2), in 36.8% of 19 follicular carcinomas, and in 9% of 66 papillary carcinomas (Table 2). With our detection method, we were unable to confirm the presence of homozygous mutations.

After DNA digestion and in all positive cases, heterozygous mutated bands of 291 bp were cut from NuSieve-agarose 3:1 gel, purified and reamplified using a nested-PCR primer set. The fragment obtained after DNA reamplification was sequenced using a nested-PCR primer. The only point mutation observed was G to T transversion in the second nucleotide of codon 12 and was a valine for glycine substitution. We do not know the pathological significance of this substitution in thyroid tumours.

Our results suggest an association between the presence of the Ha-*ras* codon 12 mutation and the histological nature of adenomas. Seven positive mutations were distributed as follows: in five of seventeen atypical adenomas, in one of two macrofollicular adenomas, and one of five microfollicular adenomas. No point mutation was found in four toxic and seven normofollicular adenomas (Table 2). Our findings concerning Ha-*ras* codon 12 gene mutations in microfollicular adenomas (in one of five, 20%) were intermediate compared with other published data (4, 5, 7, 15) (Table 3). It appears that atypical adenomas are mutated more frequently than other adenomas (toxic adenomas excluded). However, the histological identification of atypical adenomas is a difficult problem (9).

In our study of thyroid follicular carcinomas, we observed that 6 of 19 follicular carcinomas (32%) carried the Ha-*ras* codon 12 gene mutation and, in all cases, the mutation was found only in follicular carcinomas with oxyphilic cells (6 of 11 such carcinomas). This mutation was identical with others found in

adenomas. No point mutation was observed in six follicular and two insular carcinomas (Table 2). The number of mutations described elsewhere in the literature is more restricted and the findings vary, depending on the histological nature of the tumour studied. Lemoine *et al.* (4, 5) and Du Villard *et al.* (7), respectively, observed the presence of Ha-*ras* codon 12 gene mutation in 7% and 14% of follicular carcinomas; however, other authors (15, 17, 20) have failed to find any Ha-*ras* codon 12 gene mutations in follicular carcinomas. In papillary carcinomas, Ha-*ras* codon 12 mutations were found in 4 of 66 (6%) cases. Suarez *et al.* (20) and Du Villard *et al.* (7) found a high frequency of mutations: 50% and 44% of cases respectively, (Table 3), but Wright *et al.* (6) and Manenti *et al.* (13) observed no mutations in these cancers. In eight cases of undifferentiated carcinomas, we detected no point mutation of Ha-*ras*, in agreement with the findings of Suarez *et al.* (20) and Du Villard *et al.* (7). Lemoine *et al.* (5), however, observed two mutations among ten undifferentiated carcinomas.

The variation of the frequency of *ras* point mutations in thyroid tumours observed by different authors (5, 14, 16) could be explained by the selection of tumours for which the histological degree of differentiation, their genetic susceptibility, or environmental factors such as dietary iodide supply (16) are different.

Ha-*ras* codon 12 gene mutation has been implicated in many spontaneous and experimental cancers. These mutations have been found in cancer of the bladder (24), laryngeal carcinoma (25), soft-tissue malignant fibrous histiocytomas (26), chemically induced rat mammary carcinomas (27), and bronchogenic carcinoma (28). It is of interest that thyroid tumours induced by chemical carcinogens in rats involve activation of Ha-*ras* (21). The role of Ha-*ras* codon 12 gene mutation in cell transformation has been reported by *in vitro* gene transfer experiments (29); this process of cell proliferation, with preservation of differentiated thyroid functions, is manifested in the phenotype of an adenoma *in vivo* (30).

We have also studied other *ras* point mutations (N-*ras* 12 and 13, Ha-*ras* 61, and Ki-*ras* 12). However, we have found only a low frequency of N-*ras* codon 12 and Ha-*ras* codon 61 point mutations, in 2 papillary carcinomas from a total of 66 (3%) and in 2 of 19 follicular carcinomas (11%). No Ki-*ras* codon 12 gene point mutation was observed in any of the tissues we studied (Table 3). Some mutations have been detected in the Ha-*ras* gene on codon 61 of thyroid tumours (3–5, 16, 20, 23). However, Ha-*ras* is not the only member of the *ras* proto-oncogene family that carries these mutations in benign and in malignant thyroid tumours: N-*ras* and Ki-*ras* gene point mutations have also been reported (3–6, 14–19, 21, 23, 31, 32). In thyroid carcinomas with oxyphilic cells, Schark *et al.* (19) observed a variable frequency (4–17%) of mutation of the codon 61 N-*ras* gene, depending on the stage of malignancy.

Table 3 Frequency of *ras* mutations at various positions in different histological types of thyroid tumours. Values are given as number (%).

Reference	Adenomas				Follicular carcinomas			Papillary carcinomas	Undifferentiated carcinomas
	Macro	Micro	Normo	Atypical	Spontaneous	FC	HCC		
4, 5	–	H12: 1/16 (6) H61: 2/16 (13) K12: 1/16 (6) N12: 1/16 (6) N61: 3/16 (19)	–	–	–	H12: 1/15 (7) H61: 4/15 (27) K12: 1/15 (7) N61: 2/15 (13)	–	–	H12: 2/10 (20) H61: 1/10 (10) K12: 2/10 (20) N12: 1/10 (10)
15	–	H12: 4/24 (17) H61: 2/24 (8)	–	–	–	–	–	H12: 3/14 (21)	–
20	H61: 1/5 (20) K12: 1/5 (20) N12: 1/5 (20) N13: 1/5 (20) N61: 1/5 (20)	–	–	–	–	–	–	H12: 4/13 (31) K12: 2/13 (15) N13: 1/13 (8) N16: 1/13 (8)	K12: 1/10 (10)
19	–	–	–	–	–	–	N61: 8/70 (11)	–	–
17	–	–	–	–	–	N61: 2/14 (14)	–	H12: 1/15 (7)	–
13	–	–	–	–	–	H61: 3/21 (24) N61: 2/21 (27)	–	–	K12: 1/5 (20)
31	–	–	–	–	H12: 3/30 (10) K12: 3/30 (10) N12: 1/30 (33) N13: 2/30 (7)	H12: 1/20 (5) K12: 3/20 (15) K61: 1/20 (5) N13: 2/20 (10)	–	H12: 4/35 (11) H61: 1/35 (3) K12: 6/35 (17) K61: 1/35 (3) N13: 1/35 (3) N61: 2/35 (6)	–
7	–	–	–	–	H12: 2/14 (14) H61: 2/14 (14)	H12: 1/7 (14)	–	H12: 6/16 (38) H61: 1/16 (6)	H12, H61: 0/1
32	–	–	–	–	–	–	–	K13: 1/15 (6)	K13: 1/15 (7)
Our results	H12: 1/2 (50)	H12: 1/5 (20)	H61: 1/7 (14)	H12: 5/17 (29)	–	N12: 1/6 (17)	H12: 6/11 (55)	H12: 4/66 (6) H61: 1/66 (2) N12: 1/66 (2)	H12, H61, N12, N13, K12: 0/8

Macro, macrofollicular; Micro, microfollicular; Normo, normofollicular; FC, follicular carcinomas; HCC, Hürthle cell carcinomas. H12 and H61, Ha-ras gene codons 12 and 61; K12, K13 and K61, Ki-ras gene codons 12, 13 and 61; N12, N13 and N61, N-ras gene codons 12, 13 and 61.

The Ha-ras codon 12 gene mutation seems to be predominant in those thyroid tumours presenting as Hürthle cells carcinomas, and to a lesser degree in atypical adenomas, and this gene mutation may be more characteristic for these two types of tumour. Other mutations involving the three *ras* genes are very rare, the greatest frequencies being reported by Du Villard *et al.* (7) and Suarez *et al.* (20): 38% and 31% respectively for the Ha-ras codon 12 gene mutations in papillary carcinomas.

Experimental data, together with the early mutation that is found in the Ha-ras codon 12 proto-oncogene in thyroid tumours, strongly suggest that this activation may constitute an initiatory event in the process of tumorigenic transformation of thyroid cells (30). Perhaps, we have found here the origin of the initiation, by Ha-ras, of Hürthle cell carcinomas, which appears first in adenomas and is conserved in carcinomas of the same origin. This would seem to justify the association, adenoma then follicular carcinoma (30). Further research on the Ha-ras codon 12 gene mutation may be valuable to the prognosis of the two forms of tumours, benign (atypical adenoma) and malignant (Hürthle cell carcinoma). Nevertheless, the fact remains that the frequency of Ha-ras codon 12 gene mutation in thyroid adenomas and carcinomas varies according to the type of cancer and seems to change from one population to another.

We conclude that alterations at codon 12 of the Ha-ras locus occur frequently in thyroid cancers and that they contribute, in association with several other oncogenes, to the transformation and progression of the cancer.

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