# Restriction Fragment Length Polymorphism of the c-Ha-*ras*-1 Proto-Oncogene as a Marker of Genome Alterations and Susceptibility to the Development of Some Human Carcinomas

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## A. Introduction

The c-Ha-*ras*-1 proto-oncogene is highly polymorphic in a human population. A restriction fragment length polymorphism (RFLP) of Ha-*ras*, identified by a set of restriction endonucleases (*Bam*HI, *Msp*I, *Taq*I) was ascribed to change in the number of variable tandem-repeated units (VTR) closely linked to the Ha-*ras* coding sequences from the 3′ end [1, 2]. Restriction analysis established four common and several rare alleles of c-Ha-*ras*-1 [3]. A RFLP of c-Ha-*ras*-1 may be useful in detecting deletions and/or rearrangements of alleles in human DNA. Frequent (20%–60%) nonrandom loss of one of the Ha-*ras* alleles has been shown in Wilms' tumors [4], bladder carcinomas [5], breast carcinomas [6], and rhabdomyosarcomas [7].

The loss of normal cellular sequences is thought to unmask recessive mutations [8]. On the other hand, a deleted locus may represent an "antioncogene" that acts normally to constrain cellular proliferation. The suggestion should not be ruled out that some modifications of the proto-oncogene might turn it into an oncogene, whereas an intact one plays the role of an antioncogene, being involved in the same sequence of molecular events.

This study covered the distribution of c-Ha-*ras*-1 alleles in lung, ovarian, and thyroid cancer patients. Structural alterations of the c-Ha-*ras*-1 proto-oncogene

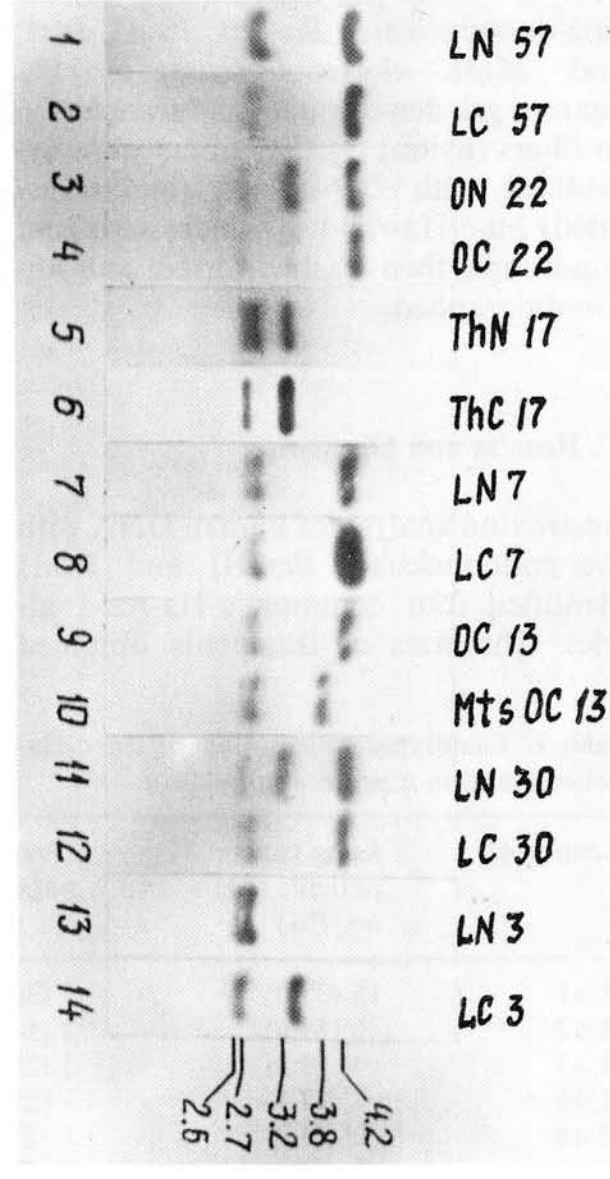


**Fig. 1.** Alterations of the c-Ha-*ras*-1 locus in tumor DNA of lung, ovarian, and thyroid cancer patients: *1, 3, 5, 7, 9, 11, 13*, constitutional genotypes of cancer patients; *2, 4, 6*, deletion of Ha-*ras* allele with the shorter fragment length; *8*, amplification of a4 allele; *10, 12, 14*, changes of allele fragment length. The size of *Pvu*II restriction fragment (in kb) of each allele is given at the right side of the figure. *OC, LC, ThC*, ovarian, lung and thyroid cancers; *ON, LN, ThN*, normal ovarian, lung and thyroid tissues; *MtsOC*, metastasis of ovarian cancer

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and their role in carcinogenesis in different allele combinations were analyzed.

## B. Materials and Methods

Genomic DNAs from tumors of the lung, ovary and thyroid and homologous normal tissues were prepared as previously described [9], digested with restriction endonucleases *Bam*HI, *Pvu*II, *Pst*I, and *Msp*I, electrophoresed in 1% agarose gel, denaturated, and transferred to filters (nylon) [9]. The filters were hybridized with $^{32}$P-labeled (nick-translated) hu-c-Ha-*ras*-1 [1] under stringent conditions, then washed, dried, and autoradiographed.

## C. Results and Discussion

Restriction analysis of human DNA with the endonucleases *Bam*HI and *Pvu*II identified four common c-Ha-*ras*-1 alleles. The sizes of fragments obtained were as follows: 6.6, 7.1, 7.7, 8.1 and 2.7, 3.2, 3.8, 4.2 kb, respectively. The major a1 allele possessing the shortest VTR region was found in more than 80% of cancer patients, which is consistent with the literature data concerning the distribution of the a1 allele in healthy donors (Table 1). Based on a comparison of the frequency of a2, a3, and a4 alleles in genotypes of lung, ovarian, and thyroid cancer patients and in a normal population, the following conclusions might be drawn: (a) The frequency of the a2 allele was approximately two times higher in thyroid cancer patients coupled with a lower incidence of a3 and a4 alleles; and (b) the a4 allele was more frequently observed in lung and ovarian cancer patients (Table 1).

Deletions of the a1 allele were found in three of seven thyroid carcinomas with an a1/a2 allele combination (Fig. 1, lanes 5, 6). The only two cases of Ha-*ras* alteration (3- to 4-fold and 50- to 80-fold amplifications) were identified in human DNA from 22 thyroid cancer patients lacking the a2 allele.

**Table 1.** Genotypic distribution of the c-Ha-*ras*-1 gene in lung, ovarian, and thyroid cancer patients and in a normal population

| Genotype | Lung cancer patients (41) no. (%) | Ovarian cancer patients (14) no. (%) | Thyroid cancer patients (29) no. (%) | Normal controls (419)[a] (%) |
|---|---|---|---|---|
| a1/a1 | 16 (39.0) | 5 (36) | 12 (41.4) | 41.3 |
| a1/a2 | 6 (14.6) | 2 (14) | 8 (27.6) | 14.9 |
| a1/a3 | 4 (9.8) | 3 (21) | 4 (13.8) | 14.0 |
| a1/a4 | 7 (17.1) | 3 (21) | 3 (10.3) | 12.2 |
| a2/a4 | 2 (4.9) | 1 (7) | 1 (3.4) | 2.2 |
| a2/a2 | 0 (0) | 0 (0) | 0 (0) | 1.3 |
| a3/a3 | 0 (0) | 0 (0) | 0 (0) | 1.2 |
| a4/a4 | 1 (2.4) | 0 (0) | 0 (0) | 0.9 |
| a2/a3 | 1 (2.4) | 0 (0) | 0 (0) | 2.5 |
| a3/a4 | 1 (2.4) | 0 (0) | 0 (0) | 2.1 |
| a3/8.5[b] | 1 (2.4) | 0 (0) | 0 (0) | 7.1[c] |
| 6.8/a2 | 1 (2.4) | 0 (0) | 0 (0) | 0.3[d] |
| 6.3/a2 | 1 (2.4) | 0 (0) | 0 (0) | |
| a1/7.5 | 0 (0) | 0 (0) | 1 (3.4) | |

[a] Summarized data [3, 10–13] calculated according to Hardy-Weinberg test.
[b] *Bam*HI restriction fragment length (kb) of rare alleles.
[c] Common/rare genotypes.
[d] Rare/rare genotypes.

Specific rearrangements (amplification of the a4 allele, deletion of another allele, and change of size of one allele) were established in five of 11 lung tumors (Fig. 1, lanes 3, 4, 7, 8, 11, 12) and in three of four ovarian tumors (Fig. 1, lanes 1, 2, 9, 10) possessing the a4 allele. On the other hand, rearrangements of c-Ha-*ras*-1 were a rare event in tumor DNA obtained from lung and ovarian cancer patients lacking the a4 allele and were detected in two of 40 tumors tested (Fig. 1, lanes 13, 14).

Since the frequency of a2 and a4 alleles were found to be increased in thyroid cancer patients and lung and ovarian cancer patients respectively, and the above changes in Ha-*ras* were observed in a2- and a4-bearing patients, these alleles may perhaps be viewed as genetic markers of predisposition to thyroid, lung, and ovarian cancers in combination with other clinical parameters.

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