Molecular Mechanism of Alteration of H-Ras I Oncogene in Human Breast Carcinomas: G to T Transversion in 12th Codon of the Undeleted Allele in the Case of the Loss of the Other Gene Allele

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Introduction

It is widely accepted that tumor development and progression are due to illegitimate activation of cellular oncogenes by point mutation, retroviral insertion, chromosomal translocation and amplification or deletion of the gene [1–3]. Nonrandom deletions of chromosomal regions 13q14 and 11p13 have been detected in retinoblastoma [1] and Wilm's tumor [2, 3]. It has been proposed that these rare childhood cancers result from the deletion of dominant-acting genes, permitting the expression of tumorigenic recessive alleles [1]. Moreover, restriction fragment length polymorphism (RFLP) analysis has demonstrated loss of H-*rasI* oncogene allele (chromosome 11p15) in primary bladder, breast, ovarian, and lung carcinomas [4–7].

On the other hand, another important mechanism of activation of *ras* oncogene (including H-*rasI*) have been shown in 10–15% of certain types of human tumors, which involved a point mutation, causing an alteration at amino acid positions 12, 13 or 61 of the *ras* gene product $p21^{ras}$ [8].

The study discusses the possible suppressive action of the wild-type H-*rasI* allele on the mutant one in breast cancer.

Material and Methods

The RFLP of H-*rasI* oncogene was analyzed in 76 primary breast carcinomas as described [7]. H-*rasI* sequence spanning 145 base pairs across codon 12 was amplified in vitro by *Thermus thermophylus* DNA polymerase [9]. Subsequent *MspI* digestion allowed us to detect the mutation in "hot spot" due to the loss of the restriction site for *MspI* in the case of substitution in the 12th codon of H-*rasI* [10].

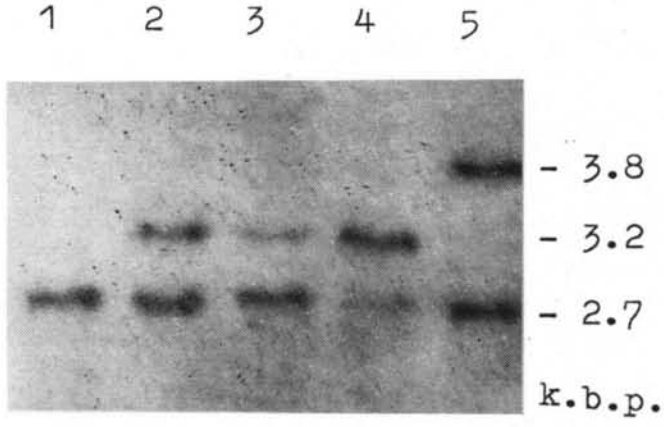

Fig. 1. Deletions of one of H-*rasI* allele in breast carcinomas (BC), identified by means of PvuII restriction of DNA samples, Southern blotting and hybridization with 6,6-fragment of pEJ [11]. Samples of DNA were derived from (*1*) BC12 – genotype A1/A1; (*2*) leukocytes of BC9 – constitutive genotype A1/A2; (*3*) BC9 – A1/A2, deletion of A2 allele; (*4*) BC5 – A1/A2, deletion of A1 allele; and (*5*) BC31 – genotype A1/A3. Slight hybridization signals at the place of lost A2 (*3*) and A1 (*4*) alleles are due to the contamination of the tumors by normal cells. In BC107 and BC109 the same deletions were detected as in BC9 (*3*)

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Haematology and Blood Transfusion Vol. 35
Modern Trends in Human Leukemia IX
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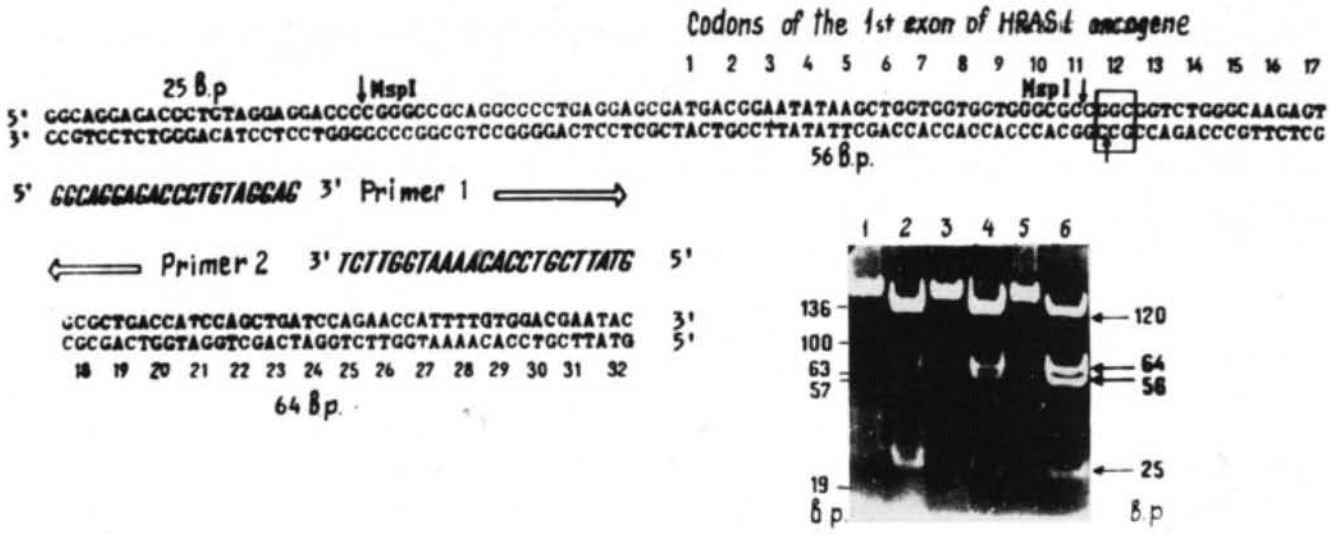

Fig. 2. Detection of H-*rasI* mutation in codon 12. The 145 base pair (bp) DNA sequence across 12th codon (in the *frame*) was amplified in vitro using the oligonucleotide primers (*large open arrows* indicate the 5′ 3′ orientation of the primers [9]. *MspI* digestion of the amplified sequences result in three fragments (25, 56, and 64 bp) in the case of wild-type allele, and two fragments (25 and 120 bp) in the case of substitution in codon 12 that altered *msp* site of restriction (*vertical arrows*). The *photograph* demonstrates the products of amplification of BC5 (*1*), BC9 (*3*), and BC107, BC109 (not shown) DNA samples and *MspI* restriction (*3*, *4*, and *6*, respectively). *Arrows on the right* of the *photograph* indicate bands corresponding to mutant and wild-type alleles. *Alu* fragments of pBR322 DNA (standard) are pointed out on the *left of the photo*

Results and Discussion

Restriction analyses of 76 DNA samples from primary breast carcinomas revealed deletions of one of the H-*rasI* allele in ten out of 41 (25%) heterozygous patients (Fig. 1). Enzymatic amplification and Msp restriction showed the presence of point mutation in the undeleted H-*rasI* allele in four out of ten carcinomas with the allelic loss (Fig. 2). All four mutations identified were the G-to-T transversion in the second position of codon 12 (Fig. 3).

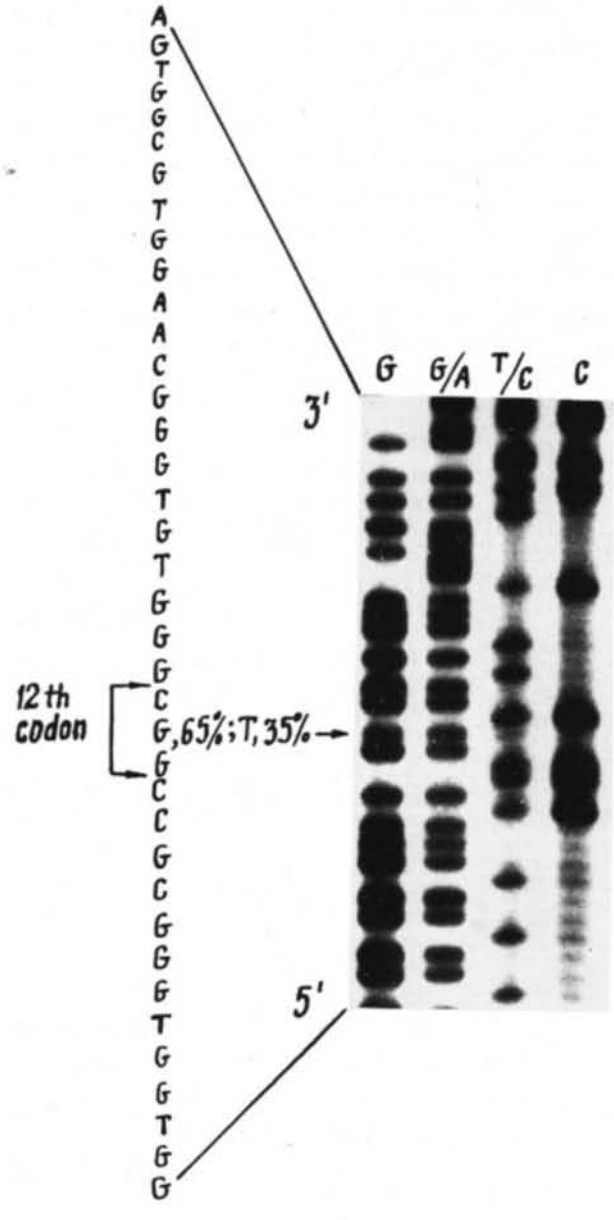

Fig. 3. Nucleotide sequence of the H-*rasI* oncogene fragment, containing the 12th codon with G-to-T transversion. The sequence was determined by means of a 8% polyacriamide sequencing gel and the Maxam and Gilbert procedure [12]. *Arrows* indicate the codon 12 and the mutated band

The loss of intact H-*rasI* allele and consequently its product – $p21^{ras}$ – normally involved in transport of mitogenic signal in the cell, might potentiate transforming activity of the oncoprotein, coded by the mutant allele. The deletion of wild-type allele of H-*rasI* oncogene is likely to unmask the mutant one.

Nevertheless it is possible that another cellular constraint of growth is present on chromosome 11p13-p15, and that the loss of this suppressor locus leads to activation of normally repressed class of genes.

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