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Amino Acid Substitution at Position 13 of the N-*ras* Gene in a Non-Hodgkin's Lymphoma Patient

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A. Introduction

In many human tumors, cellular proto-oncogenes are structurally modified by gene translocation, gene amplification, or point mutations. Modification by the latter is observed in members of the *ras* gene family. Single-base substitutions occurring at defined positions and resulting in a change in the corresponding amino acid significantly alter the biological properties of *ras*-encoded p21 protein [1]. The relevance of "activated" p21 in the neoplastic development of the cell is not yet understood, but it is thought that such mutations contribute to the development of malignancy. *Ras* genes activated by point mutations have been demonstrated in 10%–20% of certain human tumors, e.g., acute leukemias [2, 3]. Here one observes mainly mutations in the N-*ras* gene at positions 12 and 61 [1, 4]. More recently, an altered codon 13 of N-*ras* leading to Gly → Val or Asp substitution was reported in acute myeloid leukemia (AML) cases [5]. A novel point mutation at codon 13 of N-*ras*, leading to Gly → Cys change, which was detected in a T-cell malignancy is described in this paper.

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B. Results and Discussion

The NIH/3T3 transfection assay combined with a direct in vivo selection of transfected

Table 1. Tumorigenicity of DNA-transfected NIH/3T3 cells in nude mice

DNA used for transfection	Experiment	Number of tumors/inoculation sites	Latency period (days)
Calf thymus/pSV$_2$neo	A	0/6	–
	B	1/8	42
Patient DNA/pSV$_2$neo	A	7/12	39–53
	B	12/18	21–35
Calf thymus/pEJ$^{(H\text{-}ras)}$/pSV$_2$neo	A	4/4	14
	B	2/2	10

Results from two independent experiments (A and B) are presented. 37.5 μg of cellular DNA and 0.375 μg of plasmids were precipitated with calcium phosphate into 100-mm plates seeded 1 day previously with 5×10^5 NIH/3T3 cells. After 6 h, the precipitate was washed off and cells incubated in Dulbecco's modified Eagle's medium + 10% calf serum for 20 h. Cells were then trypsinized and each plate seeded into 3, containing selective medium with 1 mg of G418. After 12 days, colonies were pooled and 2.5×10^6 cells were injected subcutaneously into each of 2 sites in nude mice.

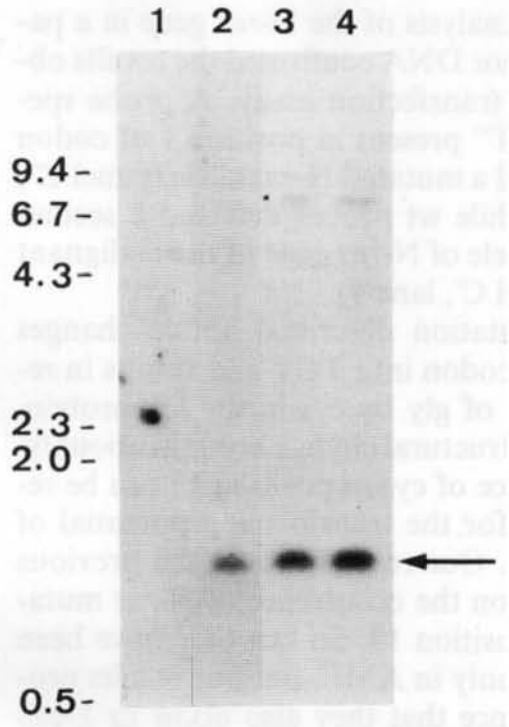

Fig. 1. Detection of N-*ras* sequences in DNA from primary NIH/3T3 tumors induced by patient's DNA. Southern blot of *Eco*RI-*Hind*III-digested DNA was probed with an isolated portion of the intron of the human N-*ras* gene (*Eco*RI-*Sac*I) fragment. *Lane: 1,* mouse fibroblast DNA; *lane 2,* patient's DNA; *lanes 3* and *4,* DNA from two 1° tumors induced by NIH/3T3 cells transfected with patient's DNA. An *arrow* marks the position of a 1-kb *Eco*RI-*Hin*dIII fragment of the human N-*ras* gene

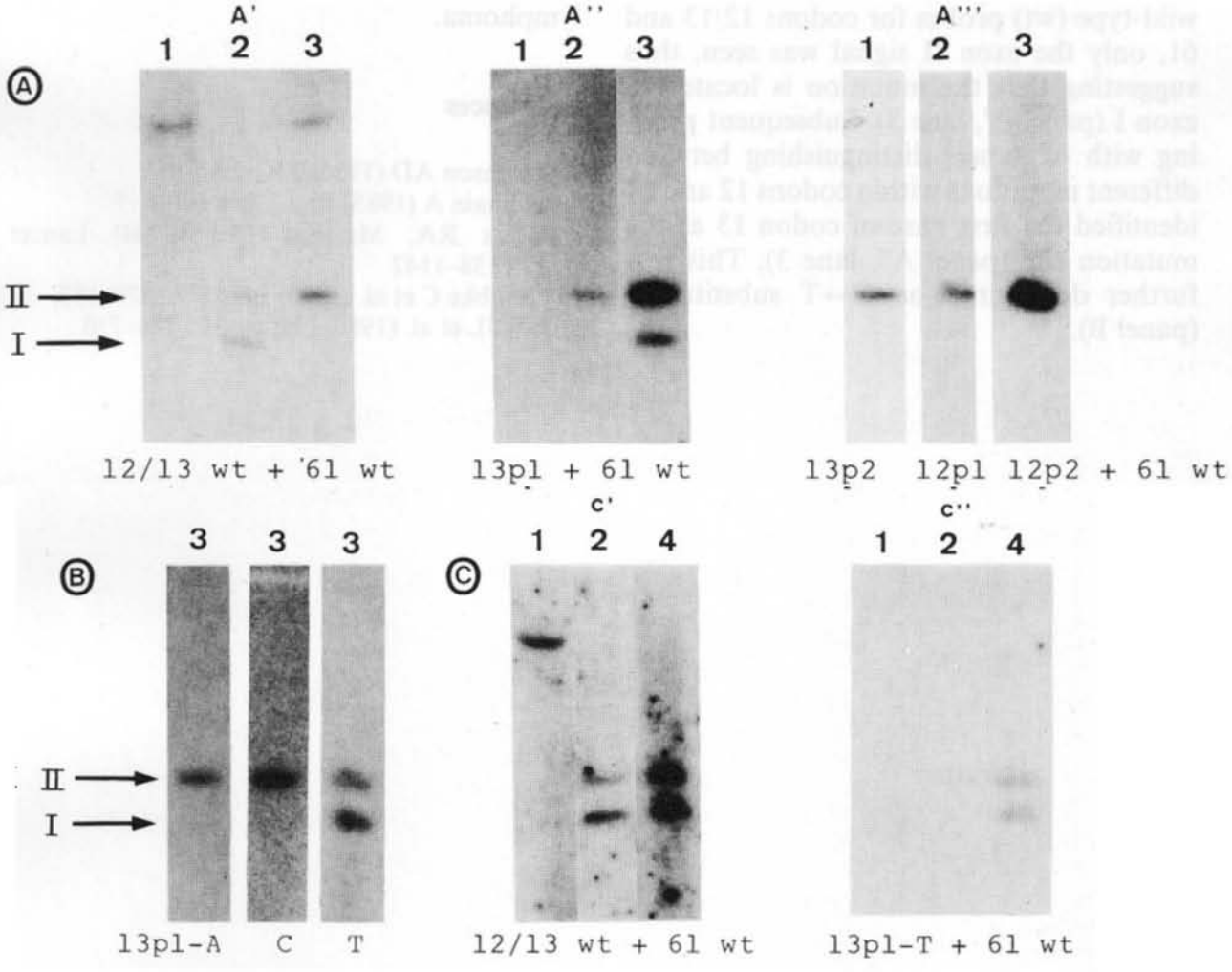

Fig. 2. Hybridization of synthetic oligonucleotide probes to genomic DNA from NIH/3T3 primary tumor induced by patient's DNA (*panels A* and *B*) and to DNA isolated from patient's malignant lymphoblasts (*panel C*). *Pst*I-digested DNA was hybridized directly in agarose gel to the synthetic oligomer probes. *Lane 1,* mouse fibroblast DNA, *lane 2,* human fibroblast DNA; *lane 3,* DNA from the primary NIH/3T3 tumor; *lane 4,* DNA from patient's malignant lymphoblasts. Probes: wt 12/13 and 61 – nonmutated exons I and II; 12 and 13, p1 and p2 – mutated exon I at codons 12 or 13, base 1 or 2. *Arrows* mark positions of human *Pst*I N-*ras* gene fragments (exons I and II)

cells in nude mice has been used to identify a transforming gene in DNA from malignant lymphoblasts of a non-Hodgkin's lymphoma patient. Table 1 presents data on the tumorigenicity of that DNA. Mice developed tumors at the site of inoculation after 3–5 weeks, and DNA from those tumors contained human *alu* sequences. Southern blot analysis with a human-specific N-*ras* probe revealed the presence of human N-*ras* sequences in 1° transfectants (Fig. 1). To analyze the transfected gene for mutations at the critical codons (12, 13, and 61), we used synthetic oligonucleotide probes specific for the normal and various mutated sequences (Fig. 2). Digestion of the N-*ras* gene with Pst I separates exons I and II, containing codons 12/13 and 61, respectively. When 1° transfectant DNA was analyzed with the wild-type (wt) probes for codons 12/13 and 61, only the exon II signal was seen, thus suggesting that the mutation is located in exon I (panel A′, lane 3). Subsequent probing with oligomers distinguishing between different mutations within codons 12 and 13 identified the first base of codon 13 as the mutation site (panel A″, lane 3). This was further determined as G→T substitution (panel B).

Direct analysis of the N-*ras* gene in a patient's tumor DNA confirmed the results obtained by transfection assay. A probe specific for "T" present in position 1 of codon 13 detected a mutated N-*ras* allele (panel C″, lane 4), while wt probes detected a second normal allele of N-*ras* gene of the malignant cells (panel C′, lane 4).

The mutation described above changes the GGT codon into TGT and results in replacement of gly by cys in the *ras* protein. Hence, a structural change brought about by the presence of cys in position 13 can be responsible for the transforming potential of N-*ras* p21. Our results extend the previous report [5] on the occurrence of N-*ras* mutations in position 13. So far, they have been observed only in AML, but our results provide evidence that they also occur in T-cell lymphoma.

References

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