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Genetics

A human oncogene of the *RAS* superfamily unmasked by expression cDNA cloning

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ABSTRACT **As an approach to identify human oncogenes, we generated an expression cDNA library from an ovarian carcinoma line. A potent transforming gene was detected by transfection analysis and identified as *TC21*, a recently cloned member of the *RAS* gene superfamily. A single point mutation substituting glutamine for leucine at position 72 was shown to be responsible for activation of transforming properties. While the cDNA clone possessed high transforming activity, the ovarian tumor genomic DNA, which contained the mutated *TC21* allele, failed to induce transformed foci. Thus, expression cDNA cloning made it possible to identify and isolate a human oncogene that has evaded detection by conventional approaches.**

Malignancies arise from a multistep process involving the activation of cellular protooncogenes and inactivation of tumor suppressor genes (1). By means of genomic DNA transfection–transformation assays with NIH 3T3 mouse fibroblasts, various oncogenes have been isolated from both human and rodent tumors. They represent diverse classes of growth-regulatory molecules, including growth factors, growth factor receptors, mitogenic signal transducers, and transcription factors (2). Despite the insights gained over the past few years, oncogenes have yet to be detected in a majority of human tumors. To detect an oncogene by conventional gene transfer, it must be small enough to be efficiently transfected, its promoter must allow a high level of expression in the recipient cell, and its signaling pathways must be intact. In an attempt to solve some of these problems, we have developed efficient cloning vectors allowing stable cDNA expression (3). This system combines the ability to generate high-complexity phagemid libraries containing a large proportion of full-length cDNAs with the ability to perform efficient rescue of integrated plasmids (4). In efforts to uncover additional human oncogenes by this strategy, we generated an expression library from an ovarian carcinoma line, a tumor type in which oncogenes remain for the most part uncharacterized.

MATERIALS AND METHODS

Library Construction. A cDNA expression library was constructed as described (4) from poly(A)$^+$ RNA generously provided by G. Kruh (Fox Chase Cancer Center). cDNAs were inserted directionally into the λpCEV29 eukaryotic expression vector, which is a derivative of the λpCEV27 vector (3) The cDNA library consisting of $\approx 10^7$ individual phage clones was amplified by a standard plate lysate method for DNA transfection experiments. Focus identification and plasmid rescue procedures were performed as described (3).

Cell Cultures. Cultures were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum (CS). NIH 3T3 cells carrying different plasmid constructs were derived by transfecting 1.5×10^5 cells with 1.0 μg of DNA by the calcium phosphate precipitation method (5). Transfectants were selected in Geneticin (750 μg/ml) and passaged twice prior to characterization of growth properties *in vitro* and *in vivo*.

Cell Proliferation Assay. For analysis of proliferation in semisolid medium, 1×10^4 and 1×10^3 cells were suspended in 0.4% agarose (SeaPlaque; FMC) in DMEM supplemented with 10% CS as described elsewhere (6). Colonies were stained with *p*-iodonitrotetrazolium violet (Sigma) and scored after 2 weeks. For analysis of tumor-forming capacity, $1–5 \times 10^5$ cells were injected subcutaneously into athymic nude mice as described (7). Tumor occurrence and size were monitored at least once weekly for 5 weeks.

Detection of Mutations. For reverse transcription PCR, 10 μg of total RNA was reverse-transcribed by murine leukemia virus reverse transcriptase (GIBCO/BRL) to synthesize first-strand cDNAs with random oligonucleotide primers in a final reaction vol of 50 μl. Four microliters of the first-strand reaction mixture was used in a 50-μl PCR mixture with primers a (5′-ATAGATGACAGAGCAGCCCGGCTA-3′) and b (5′-GATAGAGGCAGTTTTGAAGAAATC-3′) under the following cycling conditions: 94°C for 1 min, 57°C for 2 min, and 72°C for 3 min for 30 cycles. Reaction mixtures were separated on a 1.3% agarose gel and the 143-bp PCR-amplified products were extracted from the gel by a Qiaex kit (Qiagen, Chatsworth, CA). Purified fragments were then digested with *Bfa* I enzyme (New England Biolabs) and electrophoresis was performed on a 4% agarose gel. DNA fragments were then transferred onto a nitrocellulose membrane and hybridized to a ^{32}P-labeled oligonucleotide probe (*tc26*) under standard conditions (8). After stringency washing of the filter, the membrane was exposed to x-ray film (Kodak) at −70°C for 3 hr.

Northern Analysis. Total RNA was isolated from cell lines by RNazol solution (Cinna/Biotecx Laboratories, Friendswood, TX) as described by the manufacturer. After separating samples by electrophoresis on 1% denaturing formaldehyde agarose gel, RNAs were transferred to nitrocellulose filters (8). A tissue RNA blot was purchased from Clontech. Blots were hybridized at 42°C for 12 hr with ^{32}P-labeled DNA probes in 40% formamide/6× standard saline citrate (SSC)/5× Denhardt's solution/1% SDS/10% dextran sulfate/sonicated salmon sperm DNA (50 μg/ml). After the hybridization reactions, filters were washed twice in 1× SSC/0.1% SDS at room temperature and in 0.1× SSC/0.1% SDS at 55°C. Filters were dried and exposed to x-ray films at −70°C for various times.

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Abbreviation: ffu, focus-forming unit.

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RESULTS

Expression Cloning of a Transforming Gene from a Human Ovarian Tumor cDNA Library. A λpCEV29 cDNA expression library was generated from a late passage of the A2780 cell line established from a metastatic human ovarian carcinoma (9). For analysis of transforming cDNAs, library DNA was used to transfect NIH 3T3 cells. A distinct class of morphologically transformed foci consisting of rapidly growing, highly refractile cells (Fig. 1) was identified at a frequency of ≈1 focus-forming unit (ffu) per plate. Two independent foci (64-1 and 66-1) from separate plates were isolated and shown to exhibit G418 resistance, indicating that each had taken up and stably integrated the vector. Plasmid rescue was performed as described (3), and the transforming cDNAs were identified based on their high-titered transforming activities (>10^4 ffu/pmol) and their ability to confer a similar transformed morphology.

Restriction enzyme analysis revealed cDNA inserts of 1.9 and 2.4 kbp for plasmids rescued from foci 64-1 and 66-1, respectively. Moreover, the two cDNA clones displayed the same pattern with *Bam*HI, *Hind*III, and *Xba* I restriction enzymes, suggesting that they were different cDNAs generated from the same gene (Fig. 2*A*). The nucleotide sequence of clone 66-1 revealed an open reading frame of 612 bp flanked by 6 bp and ≈1.7 kbp of 5′ and 3′ untranslated regions, respectively. The open reading frame predicted a protein species of 204 amino acids with a calculated molecular mass of ≈23 kDa (Fig. 2*A*). A search in the GenBank data base uncovered extensive sequence identity to *TC21*, a member of the *RAS* superfamily (10). *TC21* was initially cloned from a human teratocarcinoma cDNA library by PCR methodology with degenerate oligonucleotides to the conserved region of the *RAS* genes (10).

***TC21* Oncogene Is Point-Mutated at Codon 72.** Detailed comparison between *TC21* and 66-1 identified nucleotide sequence disparities in two regions of the coding sequence. First, 3 additional nucleotides were present at positions 11, 20, and 33 in the N-terminal region of 66-1, leading to frameshifts, which resulted in replacement of amino acid residues from codons 5 to 10 (AGGRLR) in *TC21* with (GWRDGSG) in 66-1 (Fig. 2*B*). However, the addition of these 3 bp restored the reading frame at amino acid position 12 of TC21. Our sequence determined for 66-1 was identical to that of a cDNA clone we isolated from a normal human epithelial cell library, indicating that this region was identical in both the 66-1 oncogene and the wild-type allele (data not shown). Thus, we attribute differences from that reported for *TC21* in this region (10) to sequencing variations arising from the high G+C content in this region.

The second disparity involved a region in which the sequences of both the *TC21* and the normal human epithelial cell cDNA were identical. This alteration involved an A·T to T·A transversion in the second nucleotide of codon 72, resulting in the substitution of glutamine (CAA) by leucine (CTA) in the 66-1 oncogene (Fig. 2*C*). Gln^{72} corresponds exactly to Gln^{61} in the *HRAS* protooncogene product, a position frequently mutated and responsible for activation of the *HRAS* oncogene in a variety of human tumors (11, 12). The same mutational alteration was also present in clone 64-1, indicating that both transforming cDNAs were derived from transcripts expressed from a point-mutated allele of the wild-type *TC21* gene.

Codon 72 Mutation Activates *TC21* Oncogenicity. To assess the effects of the single A to T transversion on *TC21* biological properties, we compared transforming activities of the normal and mutant cDNAs by NIH 3T3 transfection analysis. As shown in Table 1, the mutant exhibited transforming activity of >10^4 ffu/pmol when either 64-1 or 66-1 plasmids were used. In striking contrast, the wild-type *TC21* allele expressed under the influence of the same promoter showed no detectable transforming activity. These results established the mutation as being responsible for *TC21* oncogene activation. Table 1 shows that the *TC21* oncogene was almost as active as a *HRAS* oncogene. However, the wild-type *HRAS* allele was significantly more active than the wild-type *TC21* (Table 1). We next analyzed mass populations of marker-selected cells for other properties of transformed cells including growth in semisolid agar-containing medium and tumorigenicity upon subcutaneous inoculation of athymic nude mice. Cells expressing the *TC21* mutant exhibited a highly transformed phenotype, inducing colony formation in agar and tumors in animals at efficiencies comparable to those of cells expressing an oncogenically activated *HRAS* mutant (Table 1). All these findings established that the mutation was responsible for activation of *TC21* oncogenic properties in transfected NIH 3T3 cells.

A2780 Ovarian Tumor Cells Contain the *TC21* Codon 72 Mutation. To establish that the codon 72 mutation was present in the ovarian tumor cell line, we took advantage of the creation of a polymorphic restriction enzyme site, *Bfa* I (C/TAG), when an A·T to T·A transversion occurs in the

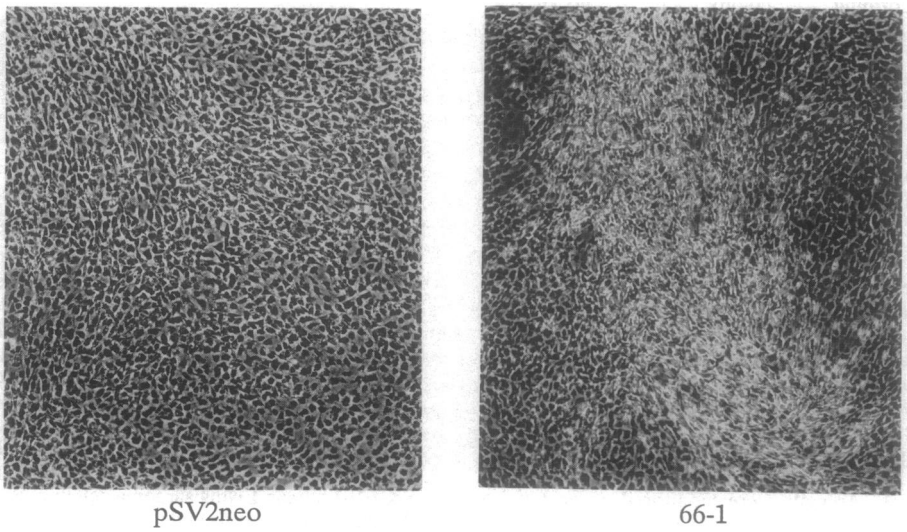

FIG. 1. Transformed focus induced by rescued plasmid 66-1. Around 1.5 × 10^5 NIH 3T3 cells were transfected with 0.01 μg of plasmid DNA. The morphology of a typical focus after 2 weeks in culture is shown.

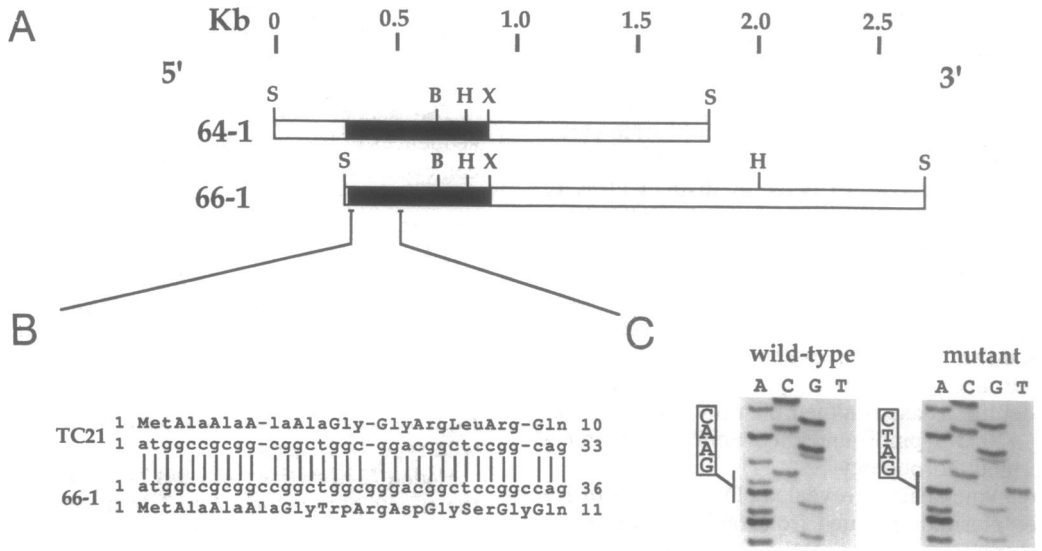

FIG. 2. Physical characterization of transforming cDNAs. (*A*) Schematic representation of the 1.7- and 2.4-kb cDNA inserts of 64-1 and 66-1 plasmids, respectively. Open reading frames encoding the *TC21* gene product are indicated by solid boxes and 5′ and 3′ untranslated regions are represented by open boxes. S, *Sal* I; B, *Bgl* II; H, *Hind*III; X, *Xba* I. (*B*) Sequence disparities in N-terminal coding regions of the *TC21* (10) and 66-1 cDNAs. Nucleotides at positions 11, 20, and 33 in 66-1 not present in *TC21* are shown, and the resulting changes in amino acid sequence are indicated. (*C*) Sequence analysis flanking nucleotide position 215 showing the wild-type and mutant *TC21* sequences. The A to T transversion in the second base pair of codon 72 of the *TC21* oncogene is boxed.

second base pair of the tetranucleotide wild-type sequence (CAAG) (Fig. 3*A*). PCR primers flanking the mutation site were generated to produce an amplified product of 143 bp. An additional *Bfa* I site 24 bp upstream from Gln^{72} was included in the PCR product to serve as an internal control for the restriction enzyme reaction. Total cellular RNAs were prepared from three different A2780 lines corresponding to early ($A2780^{E}$; ≈20), middle ($A2780^{M}$; ≈50), and late ($A2780^{L}$; >200) passage cells. In parallel, control cell RNAs were prepared from a normal mammary epithelial cell line, AB589, a Ewing sarcoma cell line, SK-ES-1, and NIH 3T3 cells transformed by the 66-1 plasmid.

As shown in Fig. 3*B*, two DNA fragments were observed in samples derived from normal human and Ewing sarcoma cells. The upper 143-bp band represented undigested DNA, and the lower 119-bp band represented the product generated by cleavage at the internal control *Bfa* I site. The restriction

Table 1. Transforming properties of the 66-1 oncogene

Transfectant	Transforming efficiency,* ffu/pmol	Soft agar colony formation,† %	Tumorigenicity,‡ no. of tumors/no. inoculated
pSV2neo	$<1.0 \times 10^{0}$	<1.0	0/6
$HRAS^{wt}$	2.5×10^{2}	ND	ND
$HRAS^{Val\text{-}12}$	5.0×10^{4}	23.6	7/7
$TC21^{wt}$	$<1.0 \times 10^{0}$	ND	ND
$TC21^{Leu\text{-}72}$§	6.0×10^{4}	21.6	7/7

ND, not determined.

*NIH 3T3 cells were transfected with different amounts of each plasmid DNA and the number of foci was scored after 3 weeks in culture. All three plasmid DNAs produced similar numbers of marker selectable colonies (≈10^4 colonies per μg).

†NIH 3T3 cells were transfected with 1 μg of each plasmid, and mass populations were marker selected. Each marker-selected culture was suspended in 0.4% semisolid agarose in medium supplemented with 10% CS. Colonies of >300 cells were scored after 14 days and results represent mean values of duplicate plates.

‡Marker-selected cells (≈4×10^4) were inoculated subcutaneously into athymic nude mice. Animals were monitored at least twice weekly for 5 weeks for the appearance of >0.5-cm^2 tumors at the inoculation site.

§The 64-1 and 66-1 plasmids exhibited similar transforming efficiencies.

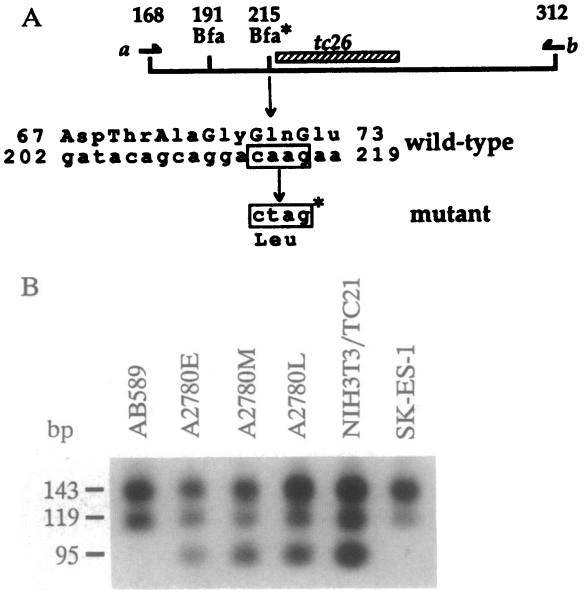

FIG. 3. Detection of *TC21* mutation in A2780 human ovarian carcinoma cells. (*A*) Schematic representation of the strategy for detection of mutations at position 215 by PCR showing primers a and b (arrows) used for amplification of the region between positions 168 and 312. The polymorphic *Bfa* I site generated as a result of the A to T transversion at position 215 is indicated by an asterisk. An additional *Bfa* I site at position 191, which serves as an internal control for restriction enzyme digestion, is also shown. Hatched bar represents the 45-bp oligonucleotide probe *tc26* used for detection of mutant-specific restriction fragments. (*B*) Southern analysis of *Bfa* I-digested PCR fragments generated from RNA samples derived from AB589 human mammary epithelial cells; A2780 ovarian carcinoma cells at early (E), medium (M), and late (L) passages; and the SK-ES-1 human Ewing sarcoma cell line. Samples were subjected to Southern blot analysis and hybridized with the *tc26* probe. The 95-bp mutant-specific fragment, the 143-bp band representing uncut DNA, and the 119-bp band representing the wild-type specific fragment obtained by digestion at position 191 alone are indicated.

fragments produced by *Bfa* I digestion of the PCR product from 66-1 transformed NIH 3T3 cells contained an additional 95-bp fragment, consistent with the size expected if cleavage occurred at both control and polymorphic *Bfa* I sites (Fig. 3*B*). This 95-bp mutant-specific restriction fragment was also observed in all three passages of the A2780 tumor cell line, indicating that the *TC21* gene was oncogenically activated in the initially established human ovarian cancer line.

Lack of Transforming Activity of A2780 Genomic DNA. To investigate the detectability of the mutant *TC21* oncogene within A2780 tumor cells by standard genomic transfer, we performed transfection experiments with high molecular weight DNAs isolated from both $A2780^E$ and $A2780^L$ cells. As controls, genomic DNAs were prepared from T24/EJ bladder carcinoma cells containing a mutated *HRAS* oncogene and NIH 3T3 cells transfected with the *TC21* oncogene cDNA. As shown in Table 2, genomic DNAs isolated from EJ as well as *TC21* transformed NIH 3T3 cells induced transformed foci at comparable efficiencies (20–50 ffu per plate). In striking contrast, A2780 genomic DNAs showed no detectable focus-forming activity in several experiments. These results establish that the *TC21* oncogene would have evaded detection by standard genomic DNA-mediated gene transfer approaches.

Expression of *TC21* Transcripts. We sought to characterize *TC21* transcripts present in normal cells and the A2780 ovarian tumor line. Fig. 4*A* demonstrates expression of a major 2.5-kb and a minor 1.7-kb transcript in A2780 cells at each of several different passage levels. *TC21* transcripts of the same respective mobilities were observed at similar relative levels in AB589 human epithelial cells. Thus, oncogene activation was not associated with any gross mRNA size alterations. It should be noted that the 2.4-kb cDNA isolated by expression cloning must represent essentially the full-length major transcript. NIH 3T3 cells also expressed two *TC21* transcripts of similar respective sizes at somewhat lower but detectable levels. Fig. 4*B* shows that the two *TC21* transcripts were ubiquitously present in all human tissues examined, with the highest levels in heart, placenta, and skeletal muscle. Moderate levels were detected in lung and liver; low levels were observed in brain, kidney, and pancreas.

DISCUSSION

Our present studies demonstrate the application of a stable expression cDNA cloning strategy in the isolation of a human oncogene. This oncogene represented a mutated allele of *TC21*, one of several *RAS* superfamily members recently cloned by means of degenerate PCR primers but otherwise uncharacterized (10). Members of this large superfamily of small guanine nucleotide binding proteins (G proteins) have been implicated in processes including cell proliferation and differentiation, motility, and intracellular trafficking (14).

Table 2. Transforming activities of different genomic DNAs

DNA source	Oncogene	Transforming efficiency,* ffu per plate		
		Exp. 1	Exp. 2	Exp. 3
EJ	$HRAS^{Val-12}$	≈30	30, 25, 30	20, 25
66-1 transfected NIH 3T3	$TC21^{Leu-72}$	≈50	ND	30, 30, 50, 50
$A2780^E$	$TC21^{Leu-72}$	0, 0, 0, 0	0, 0, 0, 0, 0	0, 0
$A2780^L$	$TC21^{Leu-72}$	0, 0, 0, 0	ND	0, 0, 0, 0

ND, not determined.

*NIH 3T3 cells were transfected with 50 μg of each genomic DNA per plate, and the number of foci was scored after 3 weeks in culture. Data shown here were generated from three independent experiments, and each number represents results from a single plate.

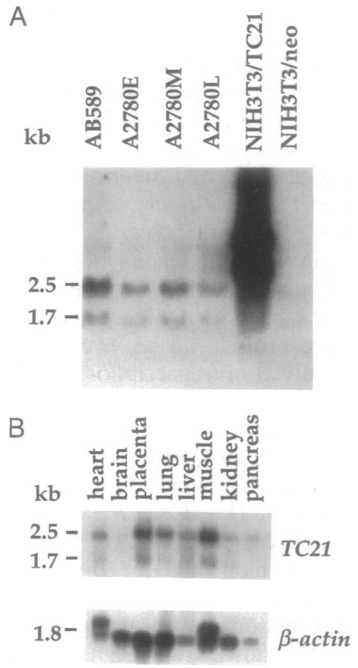

FIG. 4. *TC21* expression in cell lines and tissues. (*A*) Northern analysis of total cellular RNA obtained from AB589 human mammary epithelial cells; early, medium, and late passages of A2780 cells (A2780E, A2780M, A2780L); and NIH 3T3 cells transfected with the *TC21* transforming plasmid (NIH3T3/TC21) or with control plasmid pSV2neo (NIH3T3/neo). Around 20 μg of total cellular RNA was subjected to Northern blot analysis with a ^{32}P-labeled *TC21* cDNA probe. A major 2.5-kb and a minor 1.7-kb *TC21* transcript are indicated. Equal amounts of RNA were loaded in each lane as confirmed by ethidium bromide staining (data not shown). (*B*) Expression of *TC21* gene in tissues was analyzed with a commercially available filter (Clontech) with ≈2 μg of poly(A)$^+$ RNA loaded in each lane. After hybridization with a *TC21* cDNA probe, the filter was stripped and rehybridized with a control mouse β-actin probe for normalization of the amount of RNA loaded.

The oncogene contained a position 72 substitution, analogous to a 61st codon mutation commonly responsible for activating *RAS* oncogenes (11, 12) by reducing the intrinsic GTPase activity of the molecule. This lesion was shown to activate *TC21* oncogenic properties in tissue culture and *in vivo* and to be present in the initially established human ovarian carcinoma line. Thus, the mutated *TC21* allele was likely activated as part of the malignant process in this tumor.

Three previously identified *RAS* genes with transforming potential include *H-*, *K-*, and *NRAS*. Whereas *H-*, *K-*, and *NRAS* p21 proteins share 75% amino acid sequence identity, this similarity increases to >97% when their putative N-terminal catalytic domains (positions 5–120) are compared. In contrast, *TC21* shows 56% overall similarity and only 70% relatedness in the conserved catalytic domain. Structurally, *TC21* also contains an N-terminal 11-amino acid extension, which would result in a predicted 23-kDa product rather than the 21-kDa proteins observed for *H-*, *K-*, and *NRAS*. Of note, *TC21* is more closely related to the predicted human *RRAS* gene product with 64% identity throughout and 76% similarity in the most conserved domain. *RRAS* was initially identified by low-stringency hybridization using a viral *HRAS* probe (15). Yet efforts to mutationally activate *RRAS* as an oncogene *in vitro* were unsuccessful (16). Thus, it would not be possible to predict the oncogenic potential of *TC21* by comparison of its overall similarity or function with known *RAS*-related genes.

Among small G proteins examined so far, *TC21* is most related to the *Drosophila Ras2* gene product (17, 18) with an

overall similarity of 70%, which increases to 83% in the N-terminal catalytic domain. Three *Drosophila ras*-related genes, *Ras1*, *Ras2*, and *Ras3*, have been described (17). *Drosophila Ras1* is more similar to human *RAS* p21 proteins than to either *Ras2* or *Ras3*. Extensive genetic and biochemical studies have implicated the *Drosophila Ras1* gene product in mediating a signal that determines the fate of photoreceptor cells in the developing compound eye (19). This pathway, which is well conserved in the evolution of higher organisms (20), involves the channeling of signals generated from tyrosine kinase receptors through *RAS* p21 to downstream effectors such as the *RAF* serine kinase (21–25) and mitogen-activated protein kinase, eventually activating nuclear factors involved in transcription and DNA replication (13). No physiological function has as yet been assigned to *Drosophila Ras2*. However, expression of an activated *Ras2* gene in *Drosophila melanogaster* has been demonstrated to cause abnormal development in various tissues (26). The close relatedness of *TC21* and *Drosophila Ras2* gene products raises the possibility that these two molecules may be involved in analogous biochemical pathways.

A striking finding was the lack of *TC21* oncogene detection by NIH 3T3 transfection analysis using A2780 ovarian tumor cell line DNA. *TC21* downstream signaling pathways in NIH 3T3 cells must be intact, because this oncogene was almost as efficient in transformation as a *HRAS* oncogene under analogous long terminal repeat transcriptional control. The *TC21* oncogene including essential regulatory elements may be too large to allow efficient genomic transfer. Alternatively, species- or tissue-specific differences in transcriptional regulation of the genomic *TC21* sequence may prevent a sufficient level of its expression for induction of the transformed phenotype.

Previously identified *RAS* oncogenes have been implicated in a wide array of human malignancies. Greater than 90% of pancreatic carcinomas and >50% of colon carcinomas exhibit activating mutations of *H-* or *KRAS* alleles (12). Such oncogenes have also been identified in a variety of other carcinomas. In contrast, *NRAS* oncogenes seem to be preferentially observed in mesenchymal and hematopoietic malignancies (12). Substantial evidence from experimental models indicates that *RAS* oncogenes can be responsible for initiation of the malignant process as well as play important roles in later steps of tumor progression (27). Oncogenes have yet to be commonly detected in many human tumors. These include ovarian, breast, and prostate tumors as well as melanomas. Our discovery of an additional human oncogene of the *RAS* superfamily, which has evaded detection by previous approaches, raises the possibility that this oncogene may be widely implicated in human malignancies.

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1. Weinberg, R. A. (1991) *Science* **254**, 1138–1146.
2. Bishop, J. M. (1991) *Cell* **64**, 235–248.
3. Miki, T., Fleming, T. P., Crescenzi, M., Molly, C. J., Blam, S. B., Reynolds, S. H. & Aaronson, S. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5167–5171.
4. Miki, T., Matsui, T., Heidaran, M. & Aaronson, S. A. (1989) *Gene* **83**, 137–146.
5. Wigler, M., Silverstein, S., Lee, L. S., Pellicer, A., Cheng, Y. C. & Axel, R. (1977) *Cell* **11**, 223–232.
6. DiFiore, P. P., Pierce, J. H., Fleming, T. P., Hazan, R., Ullrich, A., King, C. R., Schlessinger, J. & Aaronson, S. A. (1987) *Cell* **51**, 1063–1070.
7. Heidaran, M. A., Fleming, T. P., Bottaro, D. P., Bell, G. I., DiFiore, P. P. & Aaronson, S. A. (1990) *Oncogene* **5**, 1265–1270.
8. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
9. Eva, A., Robbins, K. C., Andersen, P. R., Srinivasan, A., Tronick, S. R., Reddy, E. P., Ellmore, N. W., Galen, A. T., Lautenberger, J. A., Papas, T. S., Westin, E. H., Wong-Staal, F., Gallo, R. C. & Aaronson, S. A. (1982) *Nature (London)* **295**, 116–119.
10. Drivas, G. T., Shih, A., Coutavas, E., Rush, M. G. & D'eustachio, P. (1990) *Mol. Cell. Biol.* **10**, 1793–1798.
11. Yuasa, Y., Srivastava, S. K., Dunn, C. Y., Rhim, J. S., Reddy, E. P. & Aaronson, S. A. (1983) *Nature (London)* **303**, 775–779.
12. Bos, J. (1989) *Cancer Res.* **49**, 4682–4689.
13. Nishida, E. & Gotoh, Y. (1993) *Trends Biochem. Sci.* **18**, 128–131.
14. Chardin, P. (1991) *Cancer Cell* **3**, 117–126.
15. Lowe, D. G., Capon, D. J., Delwart, E., Sakaguchi, A. Y., Naylor, S. L. & Goeddel, D. V. (1987) *Cell* **48**, 137–146.
16. Lowe, D. G. & Goeddel, D. V. (1987) *Mol. Cell. Biol.* **7**, 2845–2856.
17. Neuman-Silberberg, F. S., Schejter, E., Hoffmann, F. M. & Shilo, B.-Z. (1984) *Cell* **37**, 1027–1033.
18. Mozer, B., Marlor, R., Parkhurst, S. & Corces, V. (1985) *Mol. Cell. Biol.* **5**, 885–889.
19. Simon, M. A., Bowtell, D. D. L., Dodson, G. S., Laverty, T. R. & Rubin, G. M. (1991) *Cell* **67**, 701–716.
20. McCormick, F. (1993) *Nature (London)* **363**, 15–16.
21. Vojtek, A. B., Hollenberg, S. M. & Cooper, J. A. (1993) *Cell* **74**, 205–214.
22. Zhang, X.-F., Settleman, J., Kyriakis, J. M., Takeuchi-Suzuki, E., Elledge, S. J., Marshall, M. S., Bruder, J. T., Rapp, U. R. & Avruch, J. (1993) *Nature (London)* **364**, 308–313.
23. Warne, P. H., Viciana, P. R. & Downward, J. (1993) *Nature (London)* **364**, 352–355.
24. Van Aelst, L., Barr, M., Marcus, S., Polverino, A. & Wigler, M. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 6213–6217.
25. Blenis, J. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 5889–5892.
26. Bishop, J. G., III, & Corces, V. G. (1988) *Genes Dev.* **2**, 567–577.
27. Barbacid, M. (1987) *Annu. Rev. Biochem.* **56**, 779–827.