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Transforming genes of human bladder and lung carcinoma cell lines are homologous to the *ras* genes of Harvey and Kirsten sarcoma viruses

(human tumors/transfection/retroviruses)

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ABSTRACT **Blot hybridization analysis indicated that NIH 3T3 mouse cells transformed by high molecular weight DNAs of a human bladder and a human lung carcinoma cell line contained new sequences homologous, respectively, to the transforming genes of Harvey (*ras*^{H}) and Kirsten (*ras*^{K}) sarcoma viruses. The unique *ras* sequences were present in multiple independent NIH cell lines transformed in both primary and secondary transfection assays and corresponded to *ras* sequences normally present in human DNAs. The *ras* gene product was expressed in NIH cells transformed by bladder carcinoma DNAs and in the human bladder carcinoma cell lines at levels 2- to 4-fold greater than the level observed in nontransformed NIH 3T3 cells. These results indicate that the transforming genes of these human tumor cell lines are the cellular homologs of two retroviral transforming genes.**

Transfection of cellular DNAs has demonstrated that both normal and transformed cells contain genes capable of inducing oncogenic transformation (1, 2). DNAs of a variety of neoplasms from several different vertebrate species, including man, induce transformation of NIH 3T3 cells with high efficiencies, indicating that carcinogenesis can involve dominant genetic alterations resulting in the activation of cellular transforming genes (3–10). Analysis of the transforming genes activated in neoplasms has suggested that different transforming genes are activated in different types of neoplasms, but the same or closely related transforming genes are activated in independent neoplasms of the same type of differentiated cells (6–8, 10, 11).

One class of potential cellular transforming genes is composed of the cellular homologs of the transforming genes of highly oncogenic retroviruses. These viruses contain specific genes that are responsible for transformation and that are homologous to genes of normal cells (12). At least a dozen different viral transforming genes have been identified, each of which has a cellular homolog (13). These cellular sequences are highly conserved in vertebrate evolution, but their function in both normal and neoplastic cells has been unclear.

In the present study, we have investigated the possible relationship between the cellular homologs of retroviral transforming genes and the transforming genes detected by transfection of tumor DNAs. We report here that the transforming genes of human bladder and lung carcinoma cell lines are homologous to the transforming genes of Harvey and Kirsten sarcoma viruses.

MATERIALS AND METHODS

Cell Lines. NIH 3T3 mouse cells, EJ and J82 human bladder carcinoma cell lines, and NIH cells transformed by DNAs of EJ, J82, MCF-7 human mammary carcinoma cells, and human lymphoid neoplasms were previously described (4, 6, 10). The human lung carcinoma cell line LX-1 was derived from an undifferentiated lung carcinoma of a 48-year-old man (14) and was generously provided by S. Bernal and L. B. Chen (Sidney Farber Cancer Institute). DNA of LX-1 cells induced transformation of NIH 3T3 cells with efficiencies of approximately 0.05 transformant per μg of DNA.

Molecular Clones of Viral DNAs. Molecular clones containing the transforming sequences of Harvey sarcoma virus (*ras*^{H}) and Kirsten sarcoma virus (*ras*^{K}) in plasmid pBR322 were a generous gift of E. M. Scolnick (National Cancer Institute) and have been described in detail elsewhere (15, 16). BS9 contains a 0.5-kilobase (kb) fragment of *ras*^{H} and HiHi-3 contains a 1.0-kb fragment of *ras*^{K} sequences. Plasmid DNAs were ^{32}P-labeled by nick-translation (17) to specific activities of approximately 4×10^8 cpm/μg DNA for use as hybridization probes.

Blot Hybridization Analysis. Procedures for digestion of cellular DNAs with restriction endonucleases, electrophoresis in agarose gels, and transfer to nitrocellulose filters were as described (18). Prehybridization, hybridization, and washing procedures were as described by Hanahan and Meselson (19) except that 10% dextran sulfate was included in the hybridization buffer (20).

p21 Immunoprecipitation. The procedure used was a modification of that previously described (21). Cells were incubated for 18 hr at 37°C in the presence of [^{35}S]methionine (200 μCi/ml, 500 Ci/mmol, New England Nuclear; 1 Ci = 3.7×10^{10} becquerels), extracted, and clarified by centrifugation as described (21). Cell lysates were then incubated for 1 hr at 4°C with 50 μl of staphylococcal protein A-Sepharose (50% suspension) that had been precoated with rabbit anti-rat immunoglobulin G. The protein A-Sepharose was removed by centrifugation and lysates were incubated for 1.5 hr at 4°C with rat monoclonal antibody 259 against Harvey sarcoma virus p21 (generously provided by E. M. Scolnick). This monoclonal antibody recognizes broadly reactive p21 determinants which are present on cell-encoded, *ras*^{H}-encoded, and *ras*^{K}-encoded gene products (M. Furth and E. M. Scolnick, personal communication). Lysates were then incubated for an additional 1.5 hr at 4°C with 50 μl of a 50% suspension of protein A-Sepharose precoated with rabbit anti-rat immunoglobulin G. Immune precipitates were collected by centrifugation, washed, and analyzed by electrophoresis in sodium dodecyl sulfate/polyacrylamide gels as described (21).

RESULTS

Detection of *ras* Sequences in NIH Cells Transformed by Human Bladder and Lung Carcinoma DNAs. Molecular clones

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Abbreviation: kb, kilobase(s).

of viral transforming genes were used as probes for hybridization to Southern (22) blots of DNAs of NIH cells transformed by DNAs of a variety of neoplasms. Fig. 1 illustrates results of a representative analysis in which *Bam*HI-digested DNAs were hybridized with ^{32}P-labeled DNA of a plasmid containing the transforming gene (*ras*^{H}) of Harvey sarcoma virus. DNAs of NIH 3T3 cells and NIH cells transformed by DNAs of human lung and mammary carcinomas and several human lymphoid neoplasms contained only a 3.4-kb *Bam*HI fragment that represents the normal mouse *ras*^{H} gene (Fig. 1, lanes a and c–h) (23). In contrast, DNA of NIH cells transformed by DNA of the EJ human bladder carcinoma cell line contained an additional 6.6-kb *Bam*HI fragment that was not present in NIH 3T3 DNA (Fig. 1, lane i). This 6.6-kb *ras*^{H}-containing *Bam*HI fragment was also detected in DNAs of two additional independent NIH cell lines transformed by EJ bladder carcinoma DNA (lanes j and k) and in DNAs of two independent NIH cell lines transformed in secondary transfection assays by 10- to 15-kb fragments of DNA of NIH cells transformed by EJ bladder carcinoma DNA (lanes l and m). The presence of the 6.6-kb *ras*^{H}-containing *Bam*HI fragment in DNAs of three independent primary and two independent secondary lines of transformed NIH cells indicates that transformation by EJ bladder carcinoma DNA was mediated by transfer of these *ras*^{H} sequences.

The 6.6-kb *ras*^{H}-containing *Bam*HI fragment was also present in DNA of NIH cells transformed by DNA of a second human bladder carcinoma cell line, J82 (Fig. 1, lane n). However it is presently unclear whether or not this cell line is distinct from EJ or from a third bladder carcinoma cell line, T24, which also possesses transforming activity (8).

*Bam*HI digestion of human DNAs yielded two *ras*^{H}-containing fragments (Fig. 1, lane b), one of which was similar to the fragment present in NIH cells transformed by EJ DNA. Analysis with other restriction endonucleases (*Sac* I, *Pvu* II, and *Kpn* I) also indicated that similar *ras*^{H}-containing fragments were present in human DNA and in DNAs of NIH cells transformed by EJ DNA (data not shown). The *ras*^{H}-containing fragments of EJ and J82 DNAs were similar to those of other human DNAs. These results indicate that the unique *ras*^{H}-containing fragment present in NIH cells transformed by EJ DNA was derived from transfer of a human *ras*^{H} sequence. The intensity of hybridization to the fragment present in transformed NIH cells suggests amplification of this sequence (Fig. 1), as has previously been reported for other DNA sequences introduced by transfection (24).

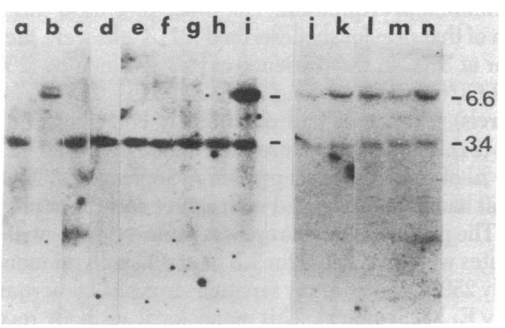

FIG. 1. Analysis of *ras*^{H} sequences in transformed NIH cells. Cellular DNAs (10 μg) were digested with *Bam*HI, electrophoresed in 0.8% agarose gels, transferred to nitrocellulose filters, and hybridized with ^{32}P-labeled BS9 DNA (*ras*^{H} probe). Lengths of *ras*^{H}-containing fragments are expressed in kb. Lane a, NIH 3T3 cells; lane b, human K562 cells; lane c, NIH cells transformed by human lung carcinoma LX-1 DNA; lane d, NIH cells transformed by human pre-B neoplasm 207 DNA; lane e, NIH cells transformed by human T lymphoma T10 DNA; lane f, NIH cells transformed by human mammary carcinoma MCF-7 DNA; lane g, NIH cells transformed by human Sezary T cell neoplasm DNA; lane h, NIH cells transformed by human myeloma 2132 DNA; lanes i–k, three independent lines of NIH cells transformed by human bladder carcinoma EJ DNA; lanes l and m, two independent lines of NIH cells transformed in secondary transfection assays by DNA of NIH cells transformed by EJ DNA; lane n, NIH cells transformed by human bladder carcinoma J82 DNA.

Fig. 2 presents the results of a similar analysis of *Eco*RI-digested DNAs with a probe containing the transforming gene (*ras*^{K}) of Kirsten sarcoma virus. The *ras*^{H} and *ras*^{K} genes encode similar proteins but do not cross-hybridize under stringent conditions (15). Only the normal mouse *ras*^{K}-containing *Eco*RI fragments of 12.0, 6.6, and 1.3 kb were detected in DNAs of NIH 3T3 cells (lane a), and no additional fragments were present in DNAs of NIH cells transformed by EJ DNA (lane d), J82 DNA (lane e), or human mammary carcinoma DNA (not shown). However, NIH cells transformed by DNA of the LX-1 lung carcinoma cell line (lanes b and f–l) contained additional *ras*^{K} sequences detected as an *Eco*RI fragment of 3.0 kb. The 3.0-kb *ras*^{K}-containing *Eco*RI fragment was present in DNAs of two independent NIH cell lines transformed by LX-1 DNA (lanes g and h) and in five independent NIH cell lines transformed in secondary transfection assays by DNA of one of these primary transformants (lanes b and i–l). Increased hybridization to the 6.6-kb *ras*^{K}-containing *Eco*RI fragment was also detected in some NIH cells transformed by LX-1 DNA (lanes f and i) and one line of transformed NIH cells contained an additional fragment of approximately 5.0 kb (lane g). *ras*^{K}-containing *Eco*RI fragments of 6.6 and 3.0 kb were present in both LX-1 DNA (compare lanes c and f) and in other human DNAs (data not shown) (15), indicating that transformation by DNA of the LX-1 lung carcinoma cell line was mediated by transfer of a human *ras*^{K} gene.

Expression of *ras* Gene Products in Bladder Carcinomas and Transformed NIH Cells. The *ras*^{H} and *ras*^{K} genes encode related 21,000-dalton proteins designated p21 (15). Because p21 is expressed at a low level in normal cells but at a high level in cells transformed by Harvey or Kirsten sarcoma viruses (25) it was of interest to investigate p21 expression in NIH cells transformed by human bladder carcinoma DNAs and in the human bladder carcinoma cell lines.

Cells were metabolically labeled with [^{35}S]methionine and

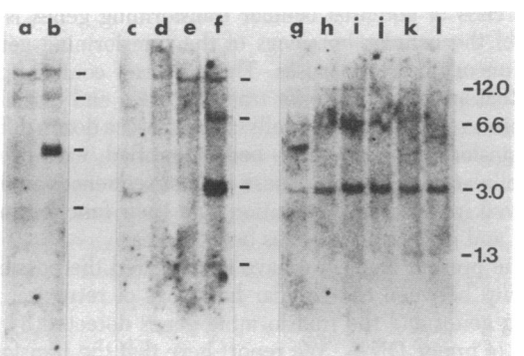

FIG. 2. Analysis of *ras*^{K} sequences in transformed NIH cells. DNAs were digested with *Eco*RI and analyzed as described for Fig. 1 except that hybridization was with ^{32}P-labeled HiHi-3 DNA (*ras*^{K} probe). Lane a, NIH 3T3 cells; lane b, NIH cells transformed in secondary transfection assays by DNA of NIH cells transformed by LX-1 human lung carcinoma DNA; lane c, human LX-1 cells; lane d, NIH cells transformed by EJ DNA; lane e, NIH cells transformed by J82 DNA; lanes f and h, one line of NIH cells transformed by LX-1 DNA; lane g, a second independent line of NIH cells transformed by LX-1 DNA; lanes i–l, four independent lines of NIH cells transformed in secondary transfection assays by DNA of NIH cells transformed by LX-1 DNA.

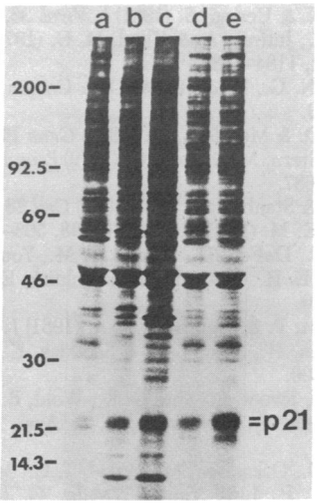

FIG. 3. Immunoprecipitation of p21. [^{35}S]Methionine-labeled cell extracts were immunoprecipitated with anti-p21 monoclonal antibody, analyzed by electrophoresis in sodium dodecyl sulfate/7.5–15% linear gradient polyacrylamide gels, and fluorographed. Molecular weights of marker proteins are indicated $\times 10^{-3}$: myosin, 200,000; phosphorylase *b*, 92,500; bovine serum albumin, 69,000; carbonic anhydrase, 30,000; soybean trypsin inhibitor, 21,500; lysozyme, 14,300. NIH 3T3 cells (lane a), EJ cells (lane b), J82 cells (lane c), NIH cells transformed by EJ DNA (lane d), and NIH cells transformed by J82 DNA (lane e). The p21 doublet was not detected in controls that lacked anti-p21 monoclonal antibody.

analyzed for p21 expression by immunoprecipitation with a broadly reactive rat monoclonal antibody against Harvey sarcoma virus-encoded p21. A representative analysis is shown in Fig. 3. The background observed in these gels is similar to that reported by others (25). The level of p21 expression in NIH cells transformed by EJ and J82 DNAs (lanes d and e) was increased in comparison to NIH 3T3 cells (lane a). In addition, the level of p21 expressed in the EJ and J82 cell lines (lanes b and c) was similar to that expressed in the transformed NIH cells. Quantitation of these results by densitometry (Table 1) indicated that the levels of p21 in NIH cells transformed by the bladder carcinoma DNAs and in the bladder carcinoma cell lines were 2- to 4-fold greater than in nontransformed NIH 3T3 cells.

DISCUSSION

NIH cells transformed by DNAs of the EJ and J82 human bladder carcinoma cell lines and the LX-1 human lung carcinoma cell line contained unique restriction fragments homologous, respectively, to the transforming genes of Harvey (*ras*^{H}) and Kirsten (*ras*^{K}) sarcoma viruses. In both cases, these unique *ras*-containing restriction fragments were found in multiple independent lines of NIH cells transformed in both primary and secondary transfection assays, indicating that they were not acquired by random cotransfer of donor DNA segments (26). Instead, these results indicate that the transforming genes detected by transfection of these human neoplasm DNAs represent the cellular homologs of these two retroviral transforming genes.

Table 1. Quantitation of p21 expression in human bladder carcinoma cell lines and in NIH cells transformed by human bladder carcinoma DNAs

Cell line	Relative level of p21
NIH 3T3	1.0
NIH(EJ DNA)	2.2
NIH(J82 DNA)	4.2
EJ	3.7
J82	4.2

The levels of p21 expression were quantitated from densitometer tracings of autoradiographs of gels after immunoprecipitation of cell extracts with anti-p21 monoclonal antibody. The level of p21 in NIH cells transformed by EJ and J82 DNAs [NIH(EJ DNA) and NIH(J82 DNA) cells] and in the EJ and J82 cell lines is expressed relative to the level of p21 in NIH 3T3 cells. Each number is an average of two to four independent experiments.

The unique *ras*^{H}- and *ras*^{K}-containing restriction fragments present in NIH cells transformed by human tumor DNAs comigrated with *ras*^{H}- and *ras*^{K}-containing restriction fragments of human DNAs, indicating that the *ras* sequences that mediated transformation of NIH 3T3 cells were derived from the human tumor DNAs. Furthermore, the *ras*-containing restriction fragments of EJ and LX-1 DNAs were indistinguishable from those of other human DNAs, indicating that activation of these transforming sequences in the human tumors did not involve DNA rearrangements detectable by this Southern blot analysis. Instead, activation of the transforming activity of these sequences may have involved point mutations or small rearrangements that were undetected in these experiments.

The gene product (p21) encoded by *ras*^{H} was expressed at an increased level (2- to 4-fold) in NIH cells transformed by human bladder carcinoma DNAs and in the EJ and J82 human bladder carcinoma cell lines. Transfection experiments with normal cell DNAs (2) and with molecular clones of the normal cell genes homologous to the transforming genes of Moloney sarcoma virus (*mos*) (27) and to *ras*^{H} (28) have indicated that altered expression of normal cell genes can result in transformation. In addition, abnormal expression of the cellular homolog of the transforming gene (*myc*) of avian myelocytomatosis virus has been implicated in the development of chicken B cell lymphomas (29, 30).

Although molecular clones of the normal rat *ras*^{H} genes lack transforming activity, recombination of these genes with viral transcriptional regulatory sequences results in activation of transforming activity that is correlated with an increased level of gene expression (28). It is thus possible that the transforming activity of *ras* genes in the human bladder and lung carcinomas is a consequence of mutations in regulatory sequences that result in abnormal gene expression. Alternatively, activation of the transforming activity of these genes in tumors may involve mutations affecting the activity of the gene products.

These results provide a link between the transforming genes of viruses and human neoplastic disease. The findings that the cellular homologs of the transforming genes of two sarcoma viruses induce efficient transformation upon transfection of DNAs of two human carcinoma cell lines and that the protein encoded by these genes is expressed in both transformed NIH 3T3 cells and the original human tumor cell lines strongly suggests a causal role for these genes in human bladder and lung carcinomas.

Because the *ras*^{H} and *ras*^{K} genes are members of a family of related human genes (15), the involvement of two different members of this gene family in human bladder and lung carcinomas further suggests the possibility that these genes may be involved in a variety of human neoplasms of epithelial origin. If this is the case, further studies of these genes and their gene products may contribute to understanding the etiology of a significant class of human tumors.

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1. Shih, C., Shilo, B. Z., Goldfarb, M. P., Dannenberg, A. & Weinberg, R. A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5714–5718.
2. Cooper, G. M., Okenquist, S. & Silverman, L. (1980) *Nature (London)* **284,** 418–421.
3. Cooper, G. M. & Neiman, P. E. (1980) *Nature (London)* **287,** 656–659.
4. Krontiris, T. G. & Cooper, G. M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 1181–1184.
5. Shih, C., Padhy, L. C., Murray, M. & Weinberg, R. A. (1981) *Nature (London)* **290,** 261–264.
6. Lane, M. A., Sainten, A. & Cooper, G. M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5185–5189.
7. Murray, M. J., Shilo, B. Z., Shih, C., Cowing, D., Hsu, H. W. & Weinberg, R. A. (1981) *Cell* **25,** 355–361.
8. Perucho, M., Goldfarb, M., Shimizu, K., Lama, C., Fogh, J. & Wigler, M. (1981) *Cell* **27,** 467–476.
9. Hopkins, N., Besmer, P., DeLeo, A. B. & Law, L. W. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7555–7559.
10. Lane, M. A., Sainten, A. & Cooper, G. M. (1982) *Cell* **28,** 873–880.
11. Shilo, B. Z. & Weinberg, R. A. (1981) *Nature (London)* **289,** 607–609.
12. Bishop, J. M. (1981) *Cell* **23,** 5–6.
13. Coffin, J. M., Varmus, H. E., Bishop, J. M., Essex, M., Hardy, W. D., Martin, G. S., Rosenberg, N. E., Scolnick, E. M., Weinberg, R. A. & Vogt, P. K. (1981) *J. Virol.* **40,** 953–957.
14. Giovanella, B. C., Stehlin, J. S., Williams, L. J., Shih-Shun, L. & Shepard, R. C. (1978) *Cancer* **42,** 2269–2281.
15. Ellis, R. W., DeFeo, D., Shih, T. Y., Gonda, M. A., Young, H. A., Tsuchida, N., Lowy, D. R. & Scolnick, E. M. (1981) *Nature (London)* **292,** 506–510.
16. Tsuchida, N. & Uesugi, S. (1981) *J. Virol.* **38,** 720–727.
17. Maniatis, T., Jeffrey, A. & Kleid, D. G. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1184–1188.
18. Copeland, N. G., Zelenetz, A. D. & Cooper, G. M. (1980) *Cell* **19,** 863–870.
19. Hanahan, D. & Meselson, M. (1980) *Gene* **10,** 63–67.
20. Wahl, G., Stern, M. & Stark, G. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3683–3687.
21. Der, C. J. & Stanbridge, E. J. (1981) *Cell* **26,** 429–438.
22. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
23. Ellis, R. W., DeFeo, D., Maryak, J. M., Young, H. A., Shih, T. Y., Chang, E. H., Lowy, D. R. & Scolnick, E. M. (1980) *J. Virol.* **36,** 408–420.
24. Goldfarb, M. P. & Weinberg, R. A. (1981) *J. Virol.* **38,** 125–135.
25. Langbeheim, H., Shih, T. Y. & Scolnick, E. M. (1980) *Virology* **106,** 292–300.
26. Wigler, M., Sweet, R., Sim, G. K., Wold, B., Pellicer, A., Lacy, E., Maniatis, T., Silverstein, S. & Axel, R. (1979) *Cell* **16,** 777–785.
27. Blair, D. G., Oskarsson, M., Wood, T. G., McClements, W. L., Fischinger, P. J. & Vande Woude, G. (1981) *Science* **212,** 941–943.
28. DeFeo, D., Gonda, M. A., Young, H. A., Chang, E. H., Lowy, D. R., Scolnick, E. M. & Ellis, R. W. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3328–3332.
29. Hayward, W. S., Neel, B. & Astrin, S. M. (1981) *Nature (London)* **290,** 475–480.
30. Cooper, G. M. & Neiman, P. E. (1981) *Nature (London)* **292,** 857–858.