Characterization of Human Transforming Genes from Chemically Transformed, Teratocarcinoma, and Pancreatic Carcinoma Cell Lines

Colin S. Cooper, Donald G. Blair, Marianne K. Oskarsson, Michael A. Tainsky, Lou A. Eader, and George F. Vande Woude

Laboratory of Molecular Oncology, National Cancer Institute-Frederick Cancer Research Facility, Frederick, Maryland 21701

ABSTRACT

Dominant transforming genes that were transferred to mouse NIH3T3 cells by cellular DNAs prepared from a chemically transformed human cell line (MNNG-HOS), a human teratocarcinoma cell line (PA1), and a human pancreatic carcinoma cell line (A1165) were characterized (a) analyzing the repetitive human DNA sequences that were associated with the transforming gene and (b) determining their relationship to the oncogenes of the Harvey (ras<sup>H</sup>) and Kirsten (ras<sup>K</sup>) sarcoma viruses and to the human neuroblastoma transforming gene (ras<sup>N</sup>). The results show that the transforming gene activated in the teratocarcinoma cell line is identical to the neuroblastoma transforming gene and that the transforming gene of the pancreatic carcinoma cell line is a human homologue of ras<sup>H</sup>. In contrast, the transforming gene activated in the chemically transformed human cell line showed no detectable homology to ras<sup>H</sup>, ras<sup>K</sup>, and ras<sup>N</sup>.

INTRODUCTION

Recently, investigators in several laboratories (14, 15, 33, 34, 36) have shown that DNA samples prepared from some chemically transformed cells are able to induce efficient transformation of nontransformed mouse NIH3T3 cells. In contrast, DNAs from nonneoplastic cells do not transform these cells with reasonable efficiency, suggesting that chemical carcinogens may induce genetic alterations that give rise to dominant transforming genes. Chemically transformed cells that contain cellular genes capable of transforming NIH3T3 cells include a DMBA<sup>1</sup>-induced transplantable mammary tumor (14), mineral oil-induced B- and T-lymphocyte neoplasms (15), 4 independent lines of 3-methylcholanthrene-transformed mouse fibroblasts (34, 36), a cell line derived from (a) a benzo(a)pyrene-induced rabbit bladder carcinoma, (b) a nitrosoethylurea-induced mouse glioblastoma, and (c) 3 nitrosoethylurea-induced neuroblastomas (33). Transforming genes have also been detected in many established human tumor cell lines (1, 13-15, 20, 22, 24, 26, 33) and in some primary human tumors (15, 25).

Active cellular transforming genes have been characterized by determining their sensitivity to digestion by restriction endonucleases and, for transforming genes from human cells, by analyzing repetitive DNA sequences that are associated with the transforming gene (20, 22, 24, 25). The relationship between cellular transforming genes and viral oncogenes has also been studied. Cloned retrovirus onc genes were used in Southern blotting experiments to analyze the DNAs of NIH3T3 cells that had been transformed in gene transfer experiments by cellular transforming genes. The initial studies showed that NIH3T3 cells transformed by DNA from EJ and J82 human bladder carcinoma lines contained a new DNA sequence that was homologous to the oncogene (ras<sup>E</sup>) of Harvey sarcoma virus and that NIH3T3 cells transformed by DNA from the LX-1 human lung carcinoma line contain a new DNA sequence homologous to the oncogene (ras<sup>L</sup>) of Kirsten sarcoma virus (7, 23, 32). More recently, Pulciani et al. (25) and Der and Cooper (6) demonstrated that several other transforming genes, including those transferred by DNA from a primary pancreatic carcinoma, are also closely related to ras<sup>E</sup>. Shimizu et al. (37) have provided evidence that a transforming gene that is transferred to NIH3T3 cells by DNA from the SK-N-SH human neuroblastoma cell line is also a member of the ras gene family. This gene, which has been designated ras<sup>N</sup>, was also activated in 2 human sarcoma cell lines, HT1080 and RD (12).

Several of the transforming genes detected in transfection-transformation assays have been isolated as biologically active molecular clones. A transforming gene that is present in human bladder carcinoma cells has been isolated independently by 3 groups (9, 23, 26, 35), and comparisons of this biologically active gene with homologous DNA sequences isolated from nontransformed cells showed that activation of the gene resulted from a single base change in the portion of the gene encoding the protein (28, 41, 42). The isolation of transforming genes that are transferred by DNA from a chicken B-cell lymphoma (10) and a human neuroblastoma (37) has also been reported.

In the present study, the transforming genes transferred by DNA from a chemically transformed human cell line and cell lines derived from a human teratocarcinoma and a human pancreatic carcinoma were characterized by analyzing the repeated human DNA sequences that are associated with the transforming genes. The relationship between the transforming genes and ras<sup>H</sup> and ras<sup>K</sup> were also examined, and in some cases the sensitivity of the transforming activity to digestion with restriction endonucleases was determined.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. Human DMBA-HOS and HOS cells were obtained from J. M. Rhim. Human teratocarcinoma cells (PA1) and renal leiomyosarcoma cells (G-402) were provided by the American Type Culture Collection. Early-passage human fibrosarcoma cells (FS-1) were provided by P. Scuderi. Human fibroblasts (MC7) were obtained from the American Type Culture Collection. Mouse NIH3T3 cells were obtained from J. DeLarco.

Cell lines were cultured either under the optional conditions stated in the references that are cited in Table 1 or under conditions recommended by the American Type Culture Collection. Mouse NIH3T3 cells were maintained as monolayer cultures in Dulbecco's modified Eagle's medium supplemented with calf serum (10%, v/v), penicillin (50 µg/ml), streptomycin (50 µg/ml), and neomycin (50 µg/ml) in an atmosphere containing 5% CO₂.
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CO2 (12%, v/v). Stocks of NIH3T3 cells were stored frozen, and cells to be transfected were either used directly after revival from frozen stocks or passaged no more than twice at subconfluent densities. Foci of morphologically transformed NIH3T3 cells were picked using cloning cylinders, and mass populations of the transformed cells were maintained in Dulbecco's modified Eagle's medium supplemented with calf serum (5 to 10%, v/v) and antibiotics.

**Transfection Assays.** DNAs were extracted from cultured cells (39) and tumor tissues (43) as described. The sizes of all DNAs were approximately 50 kilobases as estimated by electrophoresis in agarose gels. DNA transfections of NIH3T3 cells were performed by modifications of established procedures (11, 16, 40), 20 to 25 μg of cellular DNA in CaCl2 (0.5 mM, 0.325 ml) were added slowly to 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (50 mM, 0.3 ml, pH 7.2) containing sodium phosphate (1.4 mM), NaCl (274 mM), KCl (10 mM), and dextrose (12 mM). The mixture was incubated for 30 min at 27° to allow the precipitate to form and then added to a 60-mm culture dish that contained 4 x 10^6 NIH3T3 cells and 3.5 ml of Dulbecco's modified Eagle's medium supplemented with calf serum (10%, v/v). Cells were incubated at 37° for 4 hr in the presence of the precipitate and then treated with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (25 mM, 1.0 mM, pH 7.2), 0.7% sodium phosphate (0.7 mM), NaCl (137 mM), KCl (5 mM), dextrose (6 mM), and glyceral (15%, v/v) for 4 min. After incubation for a further 18 hr in fresh culture medium, the cells were trypsinized and reseeded in two 100-mm culture dishes that contained Dulbecco's modified Eagle's medium supplemented with calf serum (10%, v/v). Cultures were refed with the same culture medium at intervals of 2 to 4 days.

In some experiments, the transformation of NIH3T3 cells was assessed by examining the cultures of cells for foci of morphologically transformed cells after 10 to 20 days. Alternatively, transformed cells were selected by injecting mass populations of transfected cells into athymic nude mice (1). One to 5 days after transfection, cells from 4 or 5 independent recipient cultures were pooled, and 1 to 5 x 10^6 of these cells were injected into athymic nude mice. Mice were examined at 7- to 10-day intervals for the appearance of tumors.

**Nude Mouse Tumors.** Tumors that were induced in nude mice by transfected NIH3T3 cells were allowed to grow until their diameters were 5 to 10 mm. The tumors were then either (a) maintained by transplanting portions of the tumors to other nude mice, (b) used for the preparation of DNA as described (43), or (c) used to prepare lines of transformed cells that could be maintained in tissue culture. To prepare cell lines, tumors were minced using a razor, and the minced tissue was incubated in culture flasks that contained Dulbecco's modified Eagle's medium supplemented with 10% calf serum and antibiotics. After 2 to 5 days, transformed cells grew out from the minced tissue and covered the surface of the flasks. The transformed cell lines prepared in this way could be readily subcultured and stored frozen.

**Digestion of DNA by Restriction Endonucleases.** Cellular DNAs were digested with restriction endonucleases (Boehringer Mannheim, Indianapolis, Ind.) using the conditions recommended by the supplier except that a 3- to 5-fold excess of enzyme was used. The extent of digestion of some of the DNAs was monitored by adding bacteriophage λ to portions of the digestion mixture, which were subsequently analyzed for the digestion of the λ DNA by electrophoresis in agarose gels.

**Detection of Repeated Human DNA Sequences.** Ten μg of DNA extracted from tumors or cell lines were digested with a restriction endonuclease and subjected to electrophoresis in 0.7% agarose gels. Extracted from tumors or cell lines were digested with a restriction endonuclease and subjected to electrophoresis in 0.7% agarose gels.

**Southern Blotting.** Southern blots of restriction endonuclease-digested DNAs were prehybridized, hybridized, and washed as described above, except that in experiments with the probes prepared from ras' the filters were washed 3 times with 2 x SSC and SDS (0.1%, w/v) at room temperature and 3 times with 0.3 x SSC and SDS (0.1%, w/v) at 47°. ^32P-Labeled probe that remained hybridized to the filter was detected by autoradiography for 1 to 7 days at ~70° using Kodak XR-5 film and an intensifying screen.

**Detection of DNA Sequences Homologous to the Oncogenes of Kirsten and Harvey Sarcoma Viruses and to the Human Neuroblastoma Transforming Gene.** Plasmids containing cloned transforming sequences from Kirsten sarcoma virus (ras"), Harvey sarcoma virus (ras"), and the human neuroblastoma gene (ras") were used as probes to detect related sequences in human DNA and in DNA from NIH3T3 cells that were transformed with DNA from transformed human cells. Plasmids BS9, HII3, and pnp1 which contained, respectively, ras", ras", and ras" DNA sequences (8, 37) were labeled with ^32P by nick translation. Southern blots of restriction endonuclease-digested DNAs were prehybridized, hybridized, and washed as described above, except that in experiments with the probes prepared from ras' the filters were washed 3 times with 2 x SSC and SDS (0.1%, w/v) at room temperature and 3 times with 0.2 x SSC and SDS (0.1%, w/v) at 63°.

**RESULTS**

**Detection of Dominant Transforming Genes.** DNAs prepared from a chemically transformed human cell line and from cell lines that were derived from human tumors were examined for their abilities to transform NIH3T3 cells. High-molecular-weight DNA samples were applied to NIH3T3 cells using the calcium phosphate coprecipitation technique. The NIH3T3 cells that were transformed by cellular DNAs were selected either by reseeding the transfected cells and scoring for foci of morphologically transformed cells after 10 to 20 days or by injecting the transfected cells into athymic nude mice (1), which were then examined periodically for the appearance of tumors. In control experiments, DNA from the EJ bladder carcinoma cell line efficiently induced foci and tumors, while no tumors and only very low levels of foci were observed when NIH3T3 cells were transfected with DNA from NIH3T3 cells, MC7 human fibroblasts, and calf thymus (Table 1). We reported previously (1) that DNA from MNNG-transformed human cells (MNNG-HOS) could morphologically transform NIH3T3 cells (15 foci in 12 recipient cultures) and that cells transfected with this DNA could induce tumors in nude mice (3 of 5 mice with tumors in 5 weeks). The results obtained in the present study showed that DNA samples prepared from a DMBA-transformed human cell line (DMBA-HOS) and the HOS human cell line, from which the MNNG-HOS and DMBA-HOS chemically transformed cell lines were derived, were unable to induce foci of morphologically transformed cells and that cells transfected with DNA from these cells did not efficiently induce tumors in nude mice (Table 1); cells transfected with DNA from HOS cells gave rise to one tumor, but the late appearance of this tumor and the absence of human DNA sequences in the DNA prepared from this tumor (see below and Table 1) indicated that it arose through a background transformation event.

The results also show that DNA from a human teratocarcinoma cell line (PA1) morphologically transformed NIH3T3 cells with a low efficiency but that no transforming activity was detected using the nude mouse assay. To determine whether human DNA sequences were present in these foci, DNA derived from the teratocarcinoma primary transfectants were examined for the presence of the alu family of highly repeated DNA sequences.

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Several investigators have used this method to detect the presence of the transfected DNA sequences in the transformed NIH3T3 cells (22, 24, 26). Samples of EcoRI-digested DNA were subjected to electrophoresis in agarose gels, transferred to nitrocellulose filters according to the method of Southern (38), and probed for the presence of human alu sequences. The data presented in Fig. 1 show that all 3 primary foci examined contained high levels of human DNA sequences.

The transforming gene transferred by DNA from the teratocarcinoma cell line (PA1) and the transforming genes previously detected by us (1) in MNNG-HOS cells and a cell line derived from a human pancreatic carcinoma (A1165) were screened for human repeated DNA sequences that are associated with the transforming gene and examined for their relationship to the ras family of oncogenes.

**Transforming Gene of a Chemically Transformed Human Cell (MNNG-HOS).** In a previous study, we showed that MNNG-HOS primary tumors and foci contained high levels of human DNA sequences (1). To remove the majority of the human DNA sequences that are not closely associated with the transforming gene, DNA from the primary transfectants were used in a second round of transfection to produce secondary foci and tumors. DNA from 3 MNNG-HOS primary foci and 2 primary tumors were used to induce secondary tumors and foci (Table 2). DNAs prepared from these secondary foci and tumors were digested with restriction endonucleases and subjected to Southern analyses using the human alu probe. Most of the secondary transfectants contained 11-, 5.7-, and 3.7-kilobase human EcoRI DNA fragments. Ten secondary transfectants were examined (Fig. 2A); of these, 9 contained the 11-kilobase DNA fragment, 6 contained the 5.7-kilobase DNA fragment, and 8 contained the 3.7-kilobase DNA fragment, indicating that these 3 DNA fragments are closely, but not invariably, associated with the transforming gene. Some of the bands observed in these experiments were very faint (e.g., the 5.7-kb EcoRI fragments in Fig. 2A, Lanes d and e), and variations in the relative intensities of the 3 conserved bands were observed when the different secondary transfectants were compared. Since the tumors used in these experiments were induced by nude mice injections of mass populations of transfected cells, each tumor should contain several different populations of transformed cells that arise through independent transformation events. The relative intensities of the conserved bands observed in each tumor would then be expected to reflect the relative abundance of the different transformed cells. Notably, the same conserved DNA fragments were observed, irrespective of whether the transformed cells were detected using the tumor or focus assay. This suggested that the 2 methods detected the same transforming genes. Further evidence that the focus and tumor assays detect the same transforming gene was obtained in experiments in which DNA from secondary foci and tumors were digested with other restriction endonucleases. With BglII, both MNNG-HOS secondary foci and foci contained 5.9-, 2.9-, 2.1-, and 1.8-kilobase alu-positive human DNA fragments; while with BamHI, both contained a large (>23-kilobase) human DNA fragment.

Probes prepared from the human neuroblastoma transforming gene and the transforming genes of the Harvey and Kirsten sarcoma viruses were hybridized to Southern blots of transfec-

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**Unpublished data.**
tant DNAs to determine whether the transfectants contained human DNA sequences that are homologous to these transforming genes. The results obtained when EcoRI-digested DNA was probed with the transforming gene of the Kirsten sarcoma virus (ras") are shown in Fig. 4A. For NIH3T3 cells and for transfectants prepared using DNA from MNNG-transformed human cells, only the 12.5-, 10.0-, 1.6-, and 0.9-kilobase EcoRI fragments that represent the endogenous mouse ras" sequences hybridized to the probe. Results obtained when BamHI-digested DNAs were probed with the oncogene of the Harvey sarcoma (ras") are presented in Fig. 4B. The probe hybridized to a 7.0-kilobase DNA fragment in BamHI-digested human DNA but hybridized to the endogenous mouse ras" gene only in transfectants of the chemically transformed human cell line. DNA prepared from MNNG-HOS secondary transfectants also failed to hybridize to a probe prepared from the human neuroblastoma transforming gene (ras") (Fig. 5). When taken together, these results indicate that the MNNG-HOS transforming gene is not related to ras", ras", and ras". The transforming gene transferred by DNA from MNNG-HOS cells was further characterized by determining its sensitivity to digestion by a series of restriction endonucleases. The results (Table 3) show that the transforming gene was inactivated by digestion with EcoRI, HindIII, BamHI, and SacI.

Transforming Gene of a Human Teratocarcinoma (PA1). Samples of DNA prepared from 6 secondary foci that were induced using DNA from 3 independent teratocarcinoma primary foci were also examined for the presence of human DNA sequences. The results of these analyses with EcoRI-digested DNA showed that all of the DNA samples contained some fragments of human DNA but that none of the DNA fragments were common to all secondary transfectants (Fig. 3A). Southern analysis of transfectant DNAs with probes prepared from ras", ras", and ras" showed that all of the secondary transfectants contained human DNA sequences that hybridized to ras" (Figs. 4 and 5), and indicated that the transforming gene activated in the PA1 teratocarcinoma cell line is the same as that activated in the SK-N-SH human neuroblastoma cell line. Although the ras" probe hybridized to a 9-kilobase DNA fragment in EcoRI-digested human DNA, the size of ras"-hybridizing DNA fragments present in EcoRI-digested DNA from PA1 secondary transfectants varied from 7 to 20 kilobases (Fig. 5). Similar heterogeneity in the size of this ras"-hybridizing EcoRI DNA fragment was observed in a previous study with SK-N-SH neuroblastoma transfectants and most probably results from the loss, during transfection, of an EcoRI site that is adjacent to the ras" transforming gene (37). Additional evidence that the same transforming genes are activated in the PA1 teratocarcinoma cell line and the SK-N-SH neuroblastoma cell line was provided by comparing their sensitivities to digestion with restriction endonucleases. Thus, the pattern of sensitivity of the teratocarcinoma transforming gene to digestion by restriction endonucleases (Table 3) is consistent with the restriction map reported previously for the neuroblastoma transforming gene (37).

Transforming Gene of a Human Pancreatic Carcinoma (A1165). Southern analysis of transfectant DNA with probes prepared from ras", ras", and ras" showed that the pancreatic carcinoma transfectants contained 7.0- and 3.2-kilobase EcoRI human DNA fragments that hybridized to ras" (Fig. 4A). The observation that these ras"-hybridizing fragments are present in both primary and secondary pancreatic carcinoma transfectants and correspond to 7.0- and 3.2-kilobase EcoRI fragments that were detected in analogous experiments with human DNA strongly suggest that the transforming gene transferred by DNA from the pancreatic carcinoma cell line was a human homologue of the oncogene of the Kirsten sarcoma virus.

When a sample of EcoRI-digested DNA from one secondary transfectant was examined for the presence of human DNA sequences, 7 alu-positive human DNA fragments were detected (Fig. 3B). Interestingly, at least 4 of these DNA fragments have sizes (6.8, 4.5, 3.4, and 2.7 kilobases) that are very similar to those of human DNA fragments detected by Perucho et al. (24) and Pulciani et al. (25) in similar analyses of other human transforming genes that are homologous to ras".

DISCUSSION

The results of previous studies have shown that the HOS cell line, which was derived from a HOS (21), is an immortal cell line that exhibits a flat morphology when grown in culture and is a poor inducer of tumors in nude mice (29, 30). Although human cells are in general very resistant to transformation by chemical carcinogens in vitro, HOS cells, which can be considered to be partially transformed human cells, can be converted into more transformed cells after treatment with chemical carcinogens. Thus, when HOS cells were treated either with DMBA or MNNG (29, 30), they were converted into cells called, respectively, DMBA-HOS and MNNG-HOS, that exhibited a characteristic transformed morphology and that efficiently induce tumors in nude mice (29, 30). In the present study and in one previous study, we have examined the abilities of DNA from MNNG-HOS, DMBA-HOS, and HOS cells to transform NIH3T3 cells. When taken together, our results show that DNA from MNNG-HOS cells can morphologically transform NIH3T3 cells and that NIH3T3 cells transfected with this DNA can induce tumors in nude mice. In contrast, DNA from DMBA-HOS cells and HOS cells were inactive in both the focus and tumor assays, suggesting that MNNG may cause genetic alterations in HOS cells that give rise to a dominant transforming gene. Since DNA and RNA can be prepared easily from these cells lines and the chemistry interactions of simple methylating carcinogenes, such as MNNG, with cells are particularly well defined (18), a comparison of MNNG-HOS cells with HOS cells may provide an interesting model system for examining the mechanism of activation of
Transforming genes were also activated when mouse cells were treated with chemical carcinogens in vitro. Thus, Shilo and Weinberg (36) showed that transforming genes were presenting 3 independent cell lines that were derived by treating mouse C3H10T½ cells with 3-methylcholanthrene. Most transforming genes have been detected by examining the abilities of DNA prepared from transformed cells to produce foci of morphologically transformed NIH3T3 cells in culture (1, 13-15, 20, 22, 24-26, 33, 34, 36), although in one study the transforming genes were also detected by examining the ability of NIH3T3 cells transfected with cellular DNAs to grow in soft agar (4). Recently, Blair et al. (1) have demonstrated that nude mice may be used successfully as a vehicle for selecting transformed cells from mass cultures of transfected cells, and this method was used to select NIH3T3 cells that were transformed by DNA from MNNG-HOS cells, a human pancreatic carcinoma cell line (A1165), and a human fibrosarcoma cell line (HT1080). In the present study, we have compared the MNNG-HOS transforming gene that was detected using the nude mouse assay with that detected using the conventional focus assay by analyzing the pattern of repeated human DNA sequences that are associated with the transforming genes. The results showed that DNA from secondary MNNG-HOS tumors and secondary MNNG-HOS foci exhibited a common pattern of human DNA fragments after digestion with EcoRI, BamHI and BglII and indicated that both the tumor assay and focus assay detected the same transforming gene.

Southern analyses of transfectant DNAs indicated that the transforming gene transferred by DNA from the human teratocarcina cell line (PA1) to NIH3T3 cells is the same as that transferred by DNA from the human neuroblastoma cell line. Thus, a probe prepared from the human neuroblastoma transforming gene, ras(5), hybridized to human DNA sequences present in all teratocarcinoma transfectants. Evidence presented here also indicates that the dominant transforming gene transferred by DNA from the human pancreatic carcinoma cell line (A1165) to NIH3T3 cells is the human homologue of ras(4). Thus, after digestion with EcoRI, DNA from both primary and secondary transfectants contained unique 7.0- and 3.2-kilobase ras(5)-hybridizing DNA fragments that had the same sizes as did ras(5)-hybridizing fragments present in EcoRI-digested human placental DNA. Evidence obtained by other investigators have indicated that several other transforming genes are human homologues of ras(5). Der et al. (7) and Der and Cooper (6) demonstrated that transfectants prepared using DNA from human lung (LX-1 and CaLu-1) and colon (SW480 and SK-CO-1) carcinoma cell lines contained unique 3.0- and 6.6-kilobase EcoRI DNA fragments that hybridized to ras(4); while Pulciani et al. (25) showed that the transfectants prepared using DNA from primary human rhabdomyosarcoma, from lung and pancreatic carcinomas, and from cell lines derived from human colon (A2233), lung (A427 and A2182), gall bladder (A1004), and urinary bladder (A1698) carcinomas contain the same 2 ras(5)-hybridizing EcoRI DNA fragments. When taken together, these results strongly suggest that the transforming gene present in A1165 pancreatic carcinoma cells and these 11 other human tumors arise through the activation of the same human gene. Comparisons between the pattern of the human repeated DNA sequences that are associated with the transforming genes provided further confirmation that the A1165 pancreatic carcinoma transforming gene was related to the ras(4)-hybridizing transformed genes detected by other investigators. Thus, the pattern of EcoRI-cleaved human DNA fragments observed for a secondary transfectants was very similar to those observed in analogous experiments with primary rhabdomyosarcoma, pancreatic and lung carcinomas (25), and cell lines derived from colon (SK-CO-1 and SW480) and lung (CaLu-1) carcinomas (22, 24).

Evidence obtained in several studies originally indicated that the same transforming gene would be repeatedly activated in independent tumors derived from the same differentiated cell type. For example, Lane et al. (14) found that 7 mouse and human mammary tumors contained the same or closely related transforming genes. Similarly, the same transforming gene was detected in 3 transformed cell lines that were derived by treating mouse C3H10T½ cells with 3-methylcholanthrene while a transforming gene related to ras(4) was detected in 5 independent lung carcinomas (6, 7, 25). In contrast, at least 2 different transforming genes may be activated in pancreatic carcinomas. The transforming gene present in the A1165 cell line and that detected by Pulciani et al. (25) in a primary pancreatic carcinoma were both human homologues of ras(4), but the transforming gene present in the PaCa-2 human pancreatic carcinoma was not closely related to ras(4) (6). Similarly, at least 2 different transforming genes may be activated in both colon carcinomas (6, 22, 24, 25) and in rhabdomyosarcomas (20, 25).

Since the sizes of ras(4)-hybridizing EcoRI DNA fragments present in primary and secondary A1165 transfectants were similar to those present in other human tissues, it is possible that activation of the transforming gene does not result from gross rearrangements of the ras(4)-related DNA sequences. Activation may have involved small insertions or deletions, point mutations, or small rearrangements that were not detected in these Southern blotting experiments. Several investigators have demonstrated that the activation of the human ras(4) gene present in the EJ bladder carcinoma cell line involves a single G to T point mutation in the DNA sequence encoding amino acid 12 of the ras(4) gene product (28, 41, 42). It is conceivable that an analogous point mutation may be involved in the activation of the ras(4) gene present in the A1165 pancreatic carcinoma cell line. Alternatively, activation could, in principle, result from mutations in regulatory sequences that affect the level of expression of the transforming gene. Results of experiments with molecular clones of normal rat and human ras(4) genes and of the normal mouse homologue of the transforming genes of the Moloney murine sarcoma virus (mos) have shown that alterations in the level of expression of these genes can result in activation of their transforming potential (2, 3, 5). Thus, although the normal genes are unable to efficiently transform NIH3T3 cells, recombinant clones containing these genes ligated to viral long terminal repeat sequences, which contain DNA sequences that regulate transcriptional activity, can cause alteration in the expression of these genes that result in activation of their transforming activity. Changes of the types discussed here may also have been involved in the activation of the transforming genes detected in the MNNG-HOS and PA1 teratocarcina cell lines.

The transforming genes transferred to NIH3T3 cells by DNA from the MNNG-HOS cells and from PA1 teratocarcina cells were also characterized by examining the sensitivity of the transforming activity to digestion by several restriction endonu-
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cles. Comparisons of the results obtained in our experiments with those obtained in analogous experiments with other transforming genes show that our data are consistent with the hypothesis that the transforming gene activated in the teratocarcinoma cell line is the same as that activated in the human neuroblastoma cell line (ras) and indicate that the MNNG-HOS transforming gene is not related to the transforming genes detected in mouse and human mammary carcinomas (14), chemically transformed C3H10T½ cells (36), and T- and B-lymphocyte neoplasms (15).

In conclusion, the data presented here show that (a) the transforming gene detected in a human pancreatic carcinoma cell line (A1165) is a human homologue of ras (1), (b) the transforming gene activated in the teratocarcinoma cell line (PA1) is the same as that activated in a human neuroblastoma cell line (37) and in some other human tumors (12), and (c) the transforming gene activated in the chemically transformed human cell line (MNNG-HOS) shows no detectable homology to ras (1), ras (6), and ras (7). Molecular cloning of these transforming genes is in progress, and comparisons of the cloned genes with analogous sequences isolated from nontransformed human cells should allow the molecular mechanisms of activation of the transforming genes to be defined more precisely.

ACKNOWLEDGMENTS

We thank M. Wigler for the generous gift of the ras⁺ clone and S. Showell for his excellent technical assistance.

REFERENCES

4. Cooper, G. M., Okerquist, S., and Silverman, L. Transforming activity of DNA sequences isolated from nontransformed human cells should indicate that the MNNG-HOS human neuroblastoma cell line (rasN) and indicate that the MNNG-HOS transforming gene activated in the teratocarcinoma cell line (PA1) is the ras gene family located on chromosome 1. Nature (Lond.), 303: 396-400, 1983.

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941-943, 1981.


Fig. 1. Detection of human DNA sequences in NIH3T3 primary transfectants derived from a human teratocarcinoma cell line (PA1). DNA (10 µg) from each transfectant was digested with EcoRI, subjected to electrophoresis in agarose gels, and transferred to nitrocellulose filters as described in “Materials and Methods.” The filters were probed with the Blur 8 plasmid. Lane a contains NIH3T3 DNA, and Lanes b to d contain DNA from primary teratocarcinoma foci that are called, respectively, NIH3T3(PA1)-F1, -F2, and -F3. The positions of migration and sizes of the HindIII-digested λ DNA markers are indicated. kb, kilobase.
Fig. 2. Detection of human DNA sequences in NIH3T3 secondary transfectants derived from the MNNG-HOS chemically transformed human cell line. DNAs from secondary foci and tumors were probed using the Blur 8 plasmid as described in Fig. 1. DNAs were digested with EcoRI (A), BamHI (B), and BglII (C). DNAs were from NIH3T3 cells (A, Lane a), secondary tumors induced using DNA from primary MNNG-HOS tumors (A, Lanes b and f; B, Lane a; C, Lane a), secondary foci induced using DNA from primary MNNG-HOS foci (A, Lane e; B, Lanes b and c; C, Lane b), a secondary tumor induced using DNA from a primary MNNG-HOS focus (A, Lane d), and a secondary focus induced using DNA from a primary MNNG-HOS tumor (A, Lane c). The positions and sizes of the HindIII digested λ DNA markers are indicated. Arrowheads, positions of the human DNA fragments that were common to all or most transfectants. kb, kilobase.

Fig. 3. Detection of human DNA sequences in NIH3T3 secondary transfectants derived from the PA1 human teratocarcinoma and A1165 pancreatic carcinoma cell lines. DNAs from secondary foci were digested with EcoRI and probed using the Blur 8 plasmid as described in Fig. 1. Lane a, DNA from NIH3T3 cells; Lanes b to g, DNA from secondary foci induced using DNA from the teratocarcinoma primary focus NIH3T3(PA1)-F1 (Lanes b and c), NIH3T3(PA1)-F1 (Lanes e and f), and NIH3T3(PA1)-F3 (Lanes f and g); Lane h, DNA from a secondary A1165 pancreatic carcinoma focus induced using DNA from a primary transfectant. The positions and sizes of markers produced by digesting λ DNA with restriction endonucleases are indicated. kb, kilobase.
Fig. 4. Detection of sequences related to the oncogenes of the Kirsten and Harvey sarcoma viruses in NIH3T3 primary and secondary transfectants. A, Southern blots of EcoRI-digested DNAs were probed using a plasmid (HiHi-3) that contained a portion of ras*. DNAs were from NIH3T3 cells (Lane a), a primary and a secondary A1165 pancreatic carcinoma transfectant (Lanes b and c), a secondary MNNG-HOS transfectant (Lane d), a secondary PA1 teratocarcinoma transfectant (Lane e), and a secondary HT1080 human fibrosarcoma transfectant (Lane f). Arrowheads, positions of the bands observed in analogous experiments with human DNA. The positions of migration and sizes of SamHI- and EcoRI-digested λ DNA markers are indicated. B, Southern blots of BamHI-digested DNAs were probed using a plasmid (BS9) that contained a portion of ras*. DNAs were from human placenta (Lane a), a spontaneous nude mouse tumor (Lane b), a MNNG-HOS secondary transfectant (Lane c), a PA1 teratocarcinoma secondary transfectant (Lane d), NIH3T3 cells (Lane e), and a HT1080 fibrosarcoma secondary transfectant (Lane f). The positions of migration and sizes of HindIII-digested λ DNA markers are indicated. Ab, kilobase.
Fig. 5. Detection of sequences related to the human neuroblastoma transforming gene (ras\textsuperscript{\textit{\textasciitilde}}) in secondary transfectants. Southern blots of EcoRI-digested DNAs were probed using plasmid pNP1 that contained a portion of ras\textsuperscript{\textit{\textasciitilde}}. DNAs were from NIH3T3 cells (Lane a), human placenta (Lane b), a secondary HT1080 human fibrosarcoma transfectant (Lane c), secondary PA1 human teratocarcinoma transfectants (Lanes d and e), and secondary MNNG-HOS transfectants (Lanes f and g). The positions of migration and sizes of HindIII-digested \lambda DNA markers are indicated, kb, kilobase.
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Colin S. Cooper, Donald G. Blair, Marianne K. Oskarsson, et al.


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