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Transforming activity of human tumor DNAs

(transfection/cellular transforming genes/bladder carcinomas)

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ABSTRACT **High molecular weight DNAs of 26 human tumors and tumor cell lines were assayed for the presence of transmissible activated transforming genes by transfection of NIH 3T3 mouse cells. DNAs of two bladder carcinoma cell lines induced transformation with high efficiencies (≈0.2 transformant per μg of DNA), whereas DNAs of the other tumors studied lacked detectable transforming activity. These findings suggest that dominant mutations or gene rearrangements can result in the activation of cellular transforming genes in some human tumors.**

Transfection of cellular DNAs has recently been used to investigate the biological activity, in both normal and neoplastic cells, of genes that are capable of inducing oncogenic transformation (1–3). The potential transforming activity of normal cell genes was indicated by the observation that DNA fragments of normal avian and murine cells induced transformation of NIH 3T3 mouse cells with low efficiencies (≈0.003 transformant per μg of DNA) (1). High molecular weight DNAs of NIH cells transformed by normal cell DNA fragments induced transformation with high efficiencies (0.1–1 transformants per μg of DNA) in secondary transfection assays, indicating that these transformed cells contained activated transforming genes that could be efficiently transmitted by transfection (1). The activation of normal cell transforming genes observed in these experiments may result from DNA rearrangements leading to abnormal gene expression, such as integration of a potential transforming gene derived from normal cell DNA in the vicinity of a transcriptionally active promoter in the genome of transformed recipient cells.

The presence of activated cellular transforming genes in neoplastic cells was initially demonstrated by the finding that high molecular weight DNAs of 5 out of 15 chemically transformed mouse cell lines efficiently transformed NIH 3T3 cells (2). Activated cellular transforming genes detectable by efficient transformation of NIH 3T3 cells with high molecular weight DNAs also have been found in neoplasms induced by avian lymphoid leukosis viruses, a class of weakly oncogenic retroviruses apparently lacking virus-encoded transforming genes (3). Some transforming lesions induced by chemicals or viruses thus appear to involve dominant mutations or gene rearrangements leading to the activation of cellular transforming genes to a state in which they can be efficiently transmitted by transfection.

In the present experiments, we have investigated the transforming activity of DNAs of 26 human tumors and tumor cell lines. High molecular weight DNAs of two bladder carcinoma cell lines induced high-efficiency transformation of NIH 3T3 cells, indicating that activated transforming genes in some human tumors can be detected by transfection.

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MATERIALS AND METHODS

Human Tumors and Cell Lines. The human tumor cell lines RAMOS (Epstein–Barr virus-negative Burkitt lymphoma), LAZ221 (null-cell acute lymphocytic leukemia), H-AJG-1 (melanoma), 734B (breast carcinoma), SK-N-MC (neuroblastoma), and CEM (acute lymphocytic leukemia) were provided by H. Lazarus (Sidney Farber Cancer Institute); JCJC (squamous cell carcinoma) was from J. Rheinwald (Sidney Farber Cancer Institute); EJ and J82 (bladder carcinomas) were from I. Summerhayes and L. B. Chen (Sidney Farber Cancer Institute). Frozen tumor tissues were obtained from S. Schlossman (Sidney Farber Cancer Institute) (one acute myelogenous leukemia and five acute lymphocytic leukemias), from H. Lazarus (two ovarian carcinomas), and from the Biological Carcinogenesis Branch of the National Cancer Institute (five lymphomas, one sarcoma, one lung carcinoma, one colon carcinoma, and one esophageal carcinoma). Normal human ovary cells (W434 cells) were obtained from H. Lazarus, and normal human embryo lung fibroblasts (IMR90 cells) were from J. Rheinwald.

Transfection Assays. Tumor tissues were homogenized in a Sorvall Omnimixer. DNAs were extracted from tissue homogenates and cell suspensions as described (4) except that the initial Pronase digestion was omitted. The molecular weights of DNAs prepared from both tumor tissues and cell suspensions were greater than 30×10^6 as estimated by electrophoresis in agarose gels.

The transforming activities of high molecular weight DNAs were assayed by transfection of NIH 3T3 mouse cells as described (5, 6). Recipient cells were exposed to 20 μg of DNA per culture. In most experiments, foci of transformed cells were counted 13–15 days after exposure to DNA. In some experiments, DNA-treated cultures were transferred into soft agarose medium 7 days after exposure to DNA and colonies of transformed cells were counted after 2–3 weeks of further incubation (1).

Virus Assays. Avian leukosis virus DNA sequences were assayed by hybridization of Southern blots of *Eco*RI-digested DNAs to [^{32}P]cDNA probe representative of the entire avian leukosis virus genome (4). Positive control DNA was from transformed NIH cells containing a single Rous sarcoma virus genome (6). Virus production was assayed by sedimentable DNA polymerase activity (5) and by focus formation on NIH 3T3 mouse and F2408 rat cells. Virus rescue experiments were performed by infection of human cells with NZB xenotropic murine leukemia virus and by infection of transformed NIH cells with Moloney murine leukemia virus as described (5). Infected cells were cultivated until production of high levels of sedimentable DNA polymerase activity were detected (8–12 days); super-DNAs of NIH(EJ DNA) and NIH(J82 DNA) cells in secondary

Table 1. Transforming activity of tumor DNAs

Donor DNA	Foci, no./μg DNA
Primary tumors:	
Lymphomas (5)	<0.005–0.02
Leukemias (2)	<0.005–0.01
Sarcomas (1)	<0.005
Carcinomas:	
Ovary (2)	<0.005
Lung (1)	<0.005
Colon (1)	<0.02
Esophagus (1)	<0.005
Tumor cell lines:	
SK-N-MC (neuroblastoma)	0.01
JCJC (squamous cell carcinoma)	<0.005
734B (breast carcinoma)	<0.005
RAMOS (lymphoma)	0.005
H-AJG-1 (melanoma)	<0.005
LAZ221 (leukemia)	<0.005
EJ (bladder carcinoma)	0.23
J82 (bladder carcinoma)	0.17
Controls:	
Normal ovary	0.005
Normal HEF	0.004
Salmon sperm	0.003
Bacteriophage λ	0.01

DNAs purified from tumors and tumor cell lines were assayed by transfection of NIH 3T3 cells. The number of different samples of each tumor type is listed in parenthesis. Colon carcinoma DNA (140 μg) was assayed on seven recipient cultures. At least 200 μg of DNA of each of the other tumors was assayed on 10 recipient cultures. The data presented are compiled from several experiments, each of which included control transfections with DNAs from normal cells. DNA from Rous sarcoma virus-transformed cells was also included as a positive control in all assays and induced transformation with efficiencies of 0.5–1.5 foci per μg of DNA. Foci were counted 13–15 days after transfection. HEF, human embryo fibroblasts.

natant media were then assayed for focus formation. Positive controls for virus rescue experiments were superinfection of Kirsten sarcoma virus-transformed normal rat kidney (NRK) cells with NZB virus. Immunoprecipitation assays for simian virus 40 large tumor antigen were performed as described (7), using simian virus 40-transformed human cells (SV80 cells) and NIH cells transformed by SV80 DNA (5) as positive controls. Assays for Epstein–Barr virus nuclear antigen were performed by immunofluorescence (8) with Epstein–Barr virus-transformed human lymphocytes (JY cells) used as a positive control.

RESULTS

Transforming Activity of Tumor DNAs. High molecular weight DNAs of the human tumors and cell lines were assayed for transforming activity by transfection of NIH 3T3 mouse cells. To facilitate the screening of multiple samples, 21 of these 26 DNAs were assayed for induction of foci in monolayer cultures. The results of these experiments (Tables 1 and 2) indicated that DNAs of only two of the tumors, bladder carcinoma cell lines EJ and J82, induced transformation at a level significantly above the background of spontaneous transformation in control cultures exposed to high molecular DNAs of normal human cells, salmon sperm DNA, or bacteriophage λ DNA. DNAs of five additional acute lymphocytic leukemias and of six of the tumors used in the experiments presented in Table 1 (RAMOS, LAZ221, H-AJG-1, and 734B cell lines, a sarcoma, and one ovarian carcinoma) were also negative for transformation when assayed by colony formation in soft agarose medium (data not shown). Thus, DNAs of 24 tumors and cell lines lacked significant transforming activity. DNAs of the EJ and J82 bladder carcinoma cell lines induced transformation with efficiencies of approximately 0.2 focus-forming unit per μg of DNA, values 30- to 50-fold higher than the level of spontaneous transformation in control cultures. Foci of NIH cells transformed by EJ and J82 DNAs were similar in appearance (Fig. 1).

Table 2. Transforming activity of bladder carcinoma DNAs

Donor DNA	Total foci, no./ total recipient cultures	Foci, no./μg DNA
EJ (bladder carcinoma)	126/27	0.23
J82 (bladder carcinoma)	38/11	0.17
Normal HEF	3/32	0.005
Normal ovary	1/10	0.005
Salmon sperm	0/9	<0.006

Data from three transfection assays of EJ DNA and two assays of J82 DNA are presented with the accompanying controls. HEF, human embryo fibroblasts.

Properties of NIH Cells Transformed by EJ and J82 DNAs. NIH cells transformed by EJ and J82 DNAs [NIH(EJ DNA) and NIH(J82 DNA) cells] were grown to mass cultures (10^7–10^8 cells) for further study. The cells retained transformed appearance, grew to high saturation densities, and formed colonies in soft agarose medium with efficiencies of approximately 10% (data not shown).

To determine whether NIH(EJ DNA) and NIH(J82 DNA) cells contained transmissible transforming genes, these cells were used as donors of DNA in secondary transfection assays. As reported (1, 3, 6), high molecular weight DNAs of spontaneously transformed NIH cells isolated from control cultures exposed to bacteriophage λ, salmon sperm, or high molecular weight normal human embryo fibroblast DNAs lacked significant transforming activity in secondary transfection assays (Table 3). DNAs of transformed cells grown from foci that occurred at control levels in recipient cultures exposed to lymphoma and neuroblastoma DNAs in the experiments presented in Table 1 were also used as donors of DNA in secondary transfection assays. DNAs of these cells lacked significant transforming activity, indicating that the occasional foci induced by lymphoma and neuroblastoma DNAs were spontaneous transformants. In contrast, high molecular weight DNAs of NIH(EJ DNA) and NIH(J82 DNA) cells induced transformation with high efficiencies, indicating that these cells contained transmissible activated transforming genes. The appearance of foci induced by

Table 3. Secondary transfection assays of DNAs of transformed NIH cells

Donor DNA	Total foci, no./ total recipient cultures	Foci, no./μg DNA
NIH(EJ DNA)	134/11	0.61
NIH(J82 DNA)	72/12	0.30
NIH(LYM DNA)	2/18	0.006
NIH(NB DNA)	0/12	<0.004
NIH(HEF DNA)	1/6	0.008
NIH(SS DNA)	0/6	<0.008
NIH(λ DNA)	0/6	<0.008

Transformed cells grown from foci isolated in the experiments described in Table 1 were used as donors of DNA in secondary transfection assays. LYM, lymphoma; NB, neuroblastoma; HEF, normal human embryo fibroblasts; SS, salmon sperm.

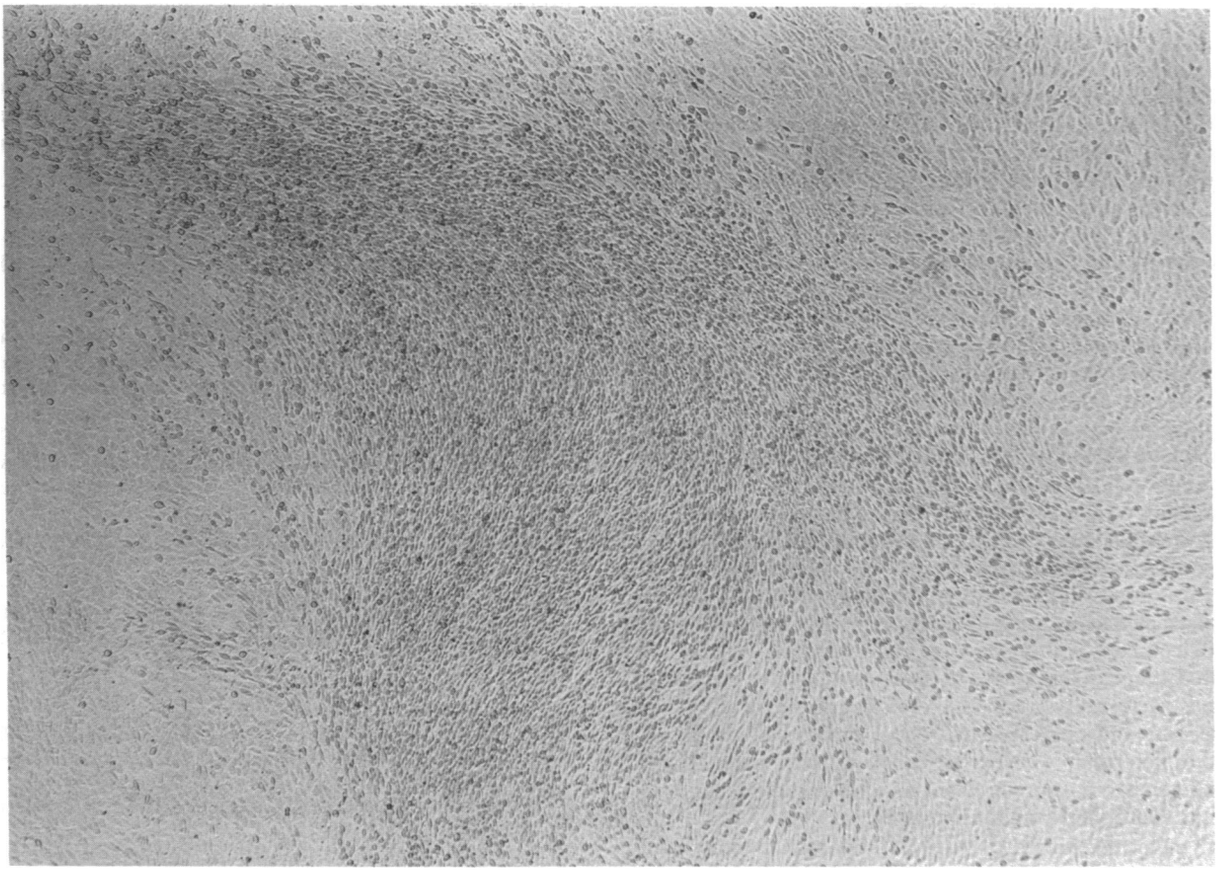

FIG. 1. Representative focus of NIH cells transformed by EJ bladder carcinoma DNA. (×25.)

transfection assays was similar to that of the cells used as DNA donors.

Restriction Endonuclease Digestion of EJ and J82 DNAs. The transforming activities of DNAs of the EJ and J82 bladder carcinoma cell lines were further characterized by investigating their sensitivity to digestion with the restriction endonucleases *Eco*RI, *Hin*dIII, and *Kpn* I. The transforming activities of both EJ and J82 DNAs were inactivated by digestion with all three restriction enzymes (Table 4). Similar results were obtained with DNA of NIH(EJ DNA) cells in secondary transfection assays (data not shown). Because the transforming activities of DNAs of cells transformed by fragments of normal cell DNA were not inactivated by digestion with these restriction enzymes under identical conditions (1), these results indicate that transformation by EJ and J82 DNAs was mediated by specific DNA segments. However, no conclusion can be drawn concerning the relationship between the transforming genes of EJ and J82 cells.

Characterization of EJ and J82 Bladder Carcinoma Cell Lines. The EJ and J82 cell lines were derived in different laboratories from transitional cell bladder carcinomas (9–11). Both cell lines were epithelial, although they were morphologically distinct. The human origin of the cell lines used in the present studies was verified by analysis of Giemsa-stained metaphase chromosomes. Both cell lines were aneuploid but could be distinguished by the presence of several marker chromosomes in J82 cells, as has been reported (10).

The possibility that the transforming genes of these cells were derived from infection by oncogenic viruses was also investigated. No DNA sequences homologous to avian leukosis viruses could be detected by hybridization of Southern blots of *Eco*RI-digested DNAs of EJ, J82, or NIH(EJ DNA) cells to [^{32}P]cDNA probe representative of the entire avian leukosis virus genome. Mammalian type C retrovirus particles containing DNA polymerase activity were not produced by EJ or NIH(EJ DNA) cells, nor could transforming viruses be detected in supernatant media of these cells. In addition, defective transforming mammalian type C retroviruses could not be rescued from EJ cells by superinfection with NZB xenotropic murine leukemia virus (which replicated efficiently in these cells) or from NIH(EJ DNA) cells by superinfection with Moloney murine leukemia virus. Extracts of NIH(EJ DNA) cells were negative in immunoprecipitation assays for simian virus 40 tumor antigen, which would also detect tumor antigens of the related human papovaviruses BK and JC (12). Both EJ and J82 cell lines were also negative in immunofluorescence assays for Epstein–Barr virus nuclear antigen.

Table 4. Restriction endonuclease digestion of bladder carcinoma DNAs

DNA	Foci, no./μg DNA			
	Undigested	*Eco*RI	*Hin*dIII	*Kpn* I
EJ	0.17	0.01	0.02	0.01
J82	0.15	0.01	0.02	<0.01

EJ and J82 DNAs (100 μg) were digested to completion with the designated restriction endonuclease. Bacteriophage λ DNA (2 μg) was included in a separate aliquot of the reaction mixture which was incubated in parallel to monitor the extent of digestion. Transforming activities of undigested and digested DNAs were assayed by focus formation.

DISCUSSION

The finding that high molecular weight DNAs of EJ and J82 human bladder carcinoma cell lines induced transformation of NIH 3T3 cells with high efficiencies indicated that these two tumor cell lines contained transmissible activated transforming genes. These transforming genes did not appear to correspond to avian leukosis-sarcoma viruses, mammalian type C retroviruses, simian virus 40-related papovaviruses, or Epstein–Barr virus. Although the possibility that the transforming genes present in the EJ and J82 cell lines were of viral origin cannot be excluded, we think the more likely interpretation of these results is that the transforming genes of these two tumor cell lines represent activated cellular transforming genes, as reported in some chemically transformed mouse cell lines (2) and in lymphoid leukosis virus-induced chicken neoplasms (3).

The significance of the observation that the only two tumor DNAs which efficiently induced transformation were from the two bladder carcinoma cell lines studied is unclear. The EJ and J82 cell lines were established in two independent laboratories (9–11), were distinguishable by appearance and by karyotype, and have been reported to differ in their colony-forming efficiency in soft agar and their tumorigenicity in *nude* mice (13). Additional characterization of the transforming genes of the EJ and J82 cell lines will be necessary to determine whether the activated transforming genes of these two bladder carcinoma cell lines are related to each other. Further studies of additional bladder carcinoma cell lines and tumor tissues will also be required to determine whether activation of transmissible transforming genes is a common event in bladder carcinomas or in cell lines established from these tumors.

High molecular weight DNAs of 24 other human tumors and cell lines did not induce transformation of NIH 3T3 cells, suggesting that the majority of human tumors do not contain activated transforming genes detectable in this assay. It is possible that the NIH 3T3 mouse cells used as recipients in the transfection assay were not transformable by the transforming genes activated in these tumors. Alternatively, the lesions resulting in neoplastic transformation in these tumors may have involved epigenetic changes, recessive mutations, or mutations in multiple unlinked genes required for transformation. In contrast, activation of transforming genes to a state in which they can be efficiently transmitted by transfection, as in the EJ and J82 cell lines, may be a consequence of dominant structural gene mutations, mutations in *cis*-acting regulatory sequences affecting expression of potential transforming genes, or gene rearrangements leading to abnormal gene expression, possibly analogous to the low-frequency activation of transforming genes upon transfection with fragments of normal cell DNA (1). Molecular cloning of the activated transforming genes of these tumor cell lines should permit elucidation of their origin, organization, and expression in normal and malignant cells.

Note Added in Proof. NIH cells transformed by EJ and J82 DNAs are tumorigenic in BALB/c mice and contain human DNA sequences detectable by hybridization with the human repetitive DNA probe described by Jelinek *et al.* (14).

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