Identification of an antigen associated with transforming genes of human and mouse mammary carcinomas

(human tumors/mouse mammary tumor virus/chemical carcinogenesis/transfection/tumor antigen)

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Communicated by Edward A. Boyse, February 22, 1982

ABSTRACT Sera from tumor-bearing mice immunoprecipitated a 96,000-dalton glycoprotein from extracts of NIH cells transformed by human mammary carcinoma DNA. This antigen was not immunoprecipitated from extracts of NIH 3T3 cells, spontaneously transformed NIH cells, NIH cells transformed by normal human DNA, NIH cells transformed by human bladder carcinoma DNA, or NIH cells transformed by Rous sarcoma virus DNA. In addition, sera from mice bearing tumors induced by NIH cells transformed by either normal human DNA or human bladder carcinoma DNA did not immunoprecipitate this antigen from extracts of NIH cells transformed by human mammary carcinoma DNA. However, this antigen was immunoprecipitated by sera from mice bearing tumors induced by NIH cells transformed by mouse mammary carcinoma DNA and from mice bearing primary mammary carcinomas. These results indicate that this glycoprotein represents an antigen that is specifically associated with expression of the transmissible transforming genes of human and mouse mammary carcinomas.

Recent studies have demonstrated that transforming genes of cellular origin can be detected by the biological activity of cellular DNAs in transfection assays. DNA fragments of normal cells have a low transforming activity which is thought to represent activation of potential transforming genes as a consequence of DNA rearrangements during donor DNA integration (1). DNAs of various transformed cells induce transformation with high efficiencies indicating that, in some transformed cells, carcinogenesis has involved DNA alterations leading to activation of dominant cellular transforming genes which can then be efficiently transmitted by transfection. Neoplastic cells in which activation of transmissible transforming genes has been detected include chemically transformed mouse fibroblasts (2), chicken bursal lymphomas and a nephroblastoma (3), human, rabbit, and mouse carcinomas (4, 5), a mouse lung carcinoma (5), mouse and rat neuroblastomas and gliomas (5), a human colon carcinoma (6), a human promyelocytic leukemia (6), human and mouse mammary carcinomas (7), and human and mouse B- and T-lymphocyte neoplasms (8).

Restriction endonuclease analysis of the transforming genes of mammary carcinomas indicated that the same or closely related transforming genes were activated in a human mammary carcinoma cell line and in six independent mouse mammary carcinomas induced by either viral or chemical carcinogens (7). Similar analysis of the transforming genes of human and mouse lymphoid neoplasms indicates that specific transforming genes are activated in neoplasms representative of specific stages of B- and T-lymphocyte differentiation (8). However, the transforming genes activated in mammary carcinomas and lymphoid neoplasms are distinct from each other and from transforming genes activated in human bladder carcinomas (4), chemically transformed mouse fibroblasts (9), and NIH cells transformed by normal chicken DNA (1). In addition, analysis of repetitive DNA sequences associated with the transforming genes of a human bladder carcinoma, a human colon carcinoma, and a human promyelocytic leukemia indicates that different transforming genes are activated in these three different neoplasms (6). These results thus indicate that specific transforming genes are activated in neoplasms of specific types of differentiated cells.

Here we report the identification of an antigen associated with the transforming genes of human and mouse mammary carcinomas. These findings further support the involvement of a common transformation pathway in a human mammary carcinoma cell line, independent virus-induced mouse mammary carcinomas, and a chemically induced mouse mammary carcinoma. The identification of this antigen may provide an approach to the study of the biochemical events that mediate transformation in this system.

MATERIALS AND METHODS

Tumors and Cell Lines. The human mammary carcinoma cell line MCF-7 (10) was provided by L. B. Chen (Sidney Farber Cancer Institute). Primary mouse mammary tumor virus (MMTV)-induced mammary carcinomas were obtained from C3H/He SFC Nu+ retired breeder females. NIH cells transformed by human and mouse mammary carcinoma DNAs (7), human bladder carcinoma DNAs (4), and Rous sarcoma virus DNA (11) were as described. NIH cells transformed by normal human DNA were isolated by transfection with DNA fragments of normal human embryo lung fibroblasts (IMR 90 cells) (12) as described (1).

Preparation of Antisera. BALB/c mice (8–10 weeks old) were injected intraperitoneally at 3- to 4-day intervals with 5–10^3 NIH cells transformed by mammary carcinoma DNAs. As reported (7), these mice developed multiple focal tumors throughout the peritoneal cavity within 10–14 days. Sera were obtained from tumor-bearing mice 14–21 days after initial inoculation. Control sera were obtained from BALB/c mice injected in parallel with NIH 3T3 cells at 16 days after the initial injection; none of these mice developed tumors. Additional control sera were obtained from BALB/c mice bearing tumors induced by NIH cells transformed by human bladder carcinoma DNA or by normal human DNA. Sera were also obtained from normal primaparous C3H mice and from C3H mice bearing MMTV-induced mammary carcinomas.

Immunoprecipitation Assays. Cells were plated at a density of 3–5 × 10^5 cells per 60-mm culture dish in Temin's modified Eagle's medium supplemented with 10% (vol/vol) calf serum.

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Abbreviation: MMTV, mouse mammary tumor virus.

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After overnight incubation at 37°C, the cells were washed three times with methionine-free Dulbecco's modified Eagle's medium and incubated for 3 hr at 37°C in this medium containing [35S]methionine (250 μCi/ml; >500 Ci/mmol; New England Nuclear; 1 Ci = 3.7 × 1012 becquerels) and supplemented with 5% dialyzed fetal calf serum. Cells were also labeled with [3H]leucine (330 μCi/ml; >110 Ci/mmol; New England Nuclear) for 3 hr in Hank's balanced salts solution containing CaCl₂ and MgCl₂ and 5% dialyzed fetal calf serum or with [3H]glucosamine (330 μCi/ml; 30–60 Ci/mmol; New England Nuclear) for 3 hr in glucose-free Dulbecco's modified Eagle's medium. Cells were rinsed with ice-cold phosphate-buffered saline containing 1% Trasylol and lysed in 1 ml of RIPA buffer (0.1% NaDodSO₄/1% deoxycholate/1% Nonidet F-40/1% Trasylol/0.15 M NaCl/0.01 M sodium phosphate, pH 7.2) for 30 min at 4°C (13).

After lysis, the culture dishes were scraped with a rubber policeman and the contents were centrifuged in an Eppendorf microcentrifuge for 15 min at 12,000 × g at 4°C. Lysates were then incubated with 50 μl of either a 50% (wt/vol) suspension of protein A-Sepharose beads (Pharmacia) or a 10% (wt/vol) suspension of formaldehyde-fixed Staphylococcus aureus (Pansorbin, Calbiochem–Behring). After incubation for 10 min at 4°C with agitation, the lysates were centrifuged and this step was repeated. Lysates were then agitated for 30 min at 4°C with 5 μl of control serum from BALB/c mice that had been inoculated with NIH 3T3 cells and for an additional 30 min after addition of 5 μl of rabbit anti-mouse immunoglobulin antisera (Calbiochem–Behring) and 50 μl of either a 50% suspension of protein A-Sepharose beads or a 10% suspension of formaldehyde-fixed S. aureus.

These lysates were centrifuged, divided in half, and incubated with 10 μl of serum from either control or tumor-bearing mice for 5–6 hr at 4°C. They were then incubated for an additional hour with 10 μl of rabbit anti-mouse immunoglobulin antisera and 100 μl of a 50% suspension of protein A-Sepharose beads. Bead immunocomplexes were recovered by centrifugation, washed four times with 1 ml of RIPA buffer, re-suspended in 30 μl of sample buffer [62.5 mM Tris-HCl, pH 6.8/2% NaDodSO₄/2 mM EDTA/10% (vol/vol) glycerol/5% (wt/vol) 2-mercaptoethanol/0.01% bromphenol blue], and heated for 5 min at 100°C. Samples were analyzed by electrophoresis in NaDodSO₄/polyacrylamide gels as described by Laemmli (14) using 4% polyacrylamide stacking gels and 10% polyacrylamide separation gels. Gels of [35S]methionine-labeled extracts were stained with Coomassie blue, destained, dried, and autoradiographed on Kodak XR-5 film for 3 days. Gels of [3H]leucine- and [3H]glucosamine-labeled extracts were analyzed by fluorography (15).

RESULTS

Identification of Antigens Expressed in NIH Cells Transformed by Human Mammary Carcinoma DNA. [35S]Methionine-labeled extracts of NIH cells transformed by DNA of the MCF-7 human mammary carcinoma cell line were immunoprecipitated with sera from mice bearing tumors induced by these cells. Sera from mice bearing tumors induced by one such transformed cell line [NIH(MCF-7) cl 2 cells] precipitated an 86,000-dalton protein from extracts of these transformed cells which was not precipitated from NIH 3T3 cells and was not precipitated from NIH(MCF-7) cells by control sera from mice immunized with NIH 3T3 cells (Fig. 1). Sera from NIH(MCF-7) cl 2 tumor-bearing sera also precipitated the 86,000-dalton protein from extracts of two independent NIH cell lines transformed by MCF-7 DNA [NIH(MCF-7) cl 1 and NIH(MCF-7) cl 4 cells]. Similar results were obtained with individual sera of three different NIH(MCF-7) cl 2 tumor-bearing mice and with pooled sera of two NIH(MCF-7) cl 1 tumor-bearing mice.

The 86,000-dalton protein detected in immunoprecipitates of [35S]methionine-labeled NIH(MCF-7) cell extracts was also detected in immunoprecipitates of [3H]leucine- and [3H]glucosamine-labeled cell extracts (Fig. 2) but not in [3P]orthophosphate-labeled cell extracts (data not shown). This protein thus appears to be a nonphosphorylated glycoprotein with an apparent molecular weight of approximately 86,000 which is expressed in NIH cells transformed by MCF-7 DNA. We will refer to this protein hereafter as "gp86." In addition to gp86, the experiment presented in Fig. 2 reveals other antigens that occasionally were detected in extracts of NIH(MCF-7) cells. These include a protein of approximately 70,000 daltons, which was marginally detected in [35S]methionine-labeled cell extracts but was clearly visualized in [3H]leucine-labeled extracts, and a low molecular weight (19,000) protein which was detected in [35S]methionine- and [3H]leucine-labeled NIH(MCF-7) cell extracts. The molecular weight of this smaller was determined by electrophoresis in 15% polyacrylamide gels. Neither of these two proteins appeared to be labeled with [3H]glucosamine. However, immunoprecipitation of [3H]glucosamine-labeled NIH(MCF-7) cell extracts revealed a diffuse band of approximately 72,000–76,000 daltons.
DNA of NIH cells transformed by MCF-7 DNA induce efficient transformation when used as donor DNA in secondary transfection assays, indicating that these cells contain transmissible activated transforming genes derived from the mammary carcinoma DNA (7). To determine whether expression of gp86 was linked to secondary transmission of this transforming gene, [35S]methionine-labeled extracts of four independent lines of NIH cells transformed by DNA of NIH(MCF-7) cl 2 cells in secondary transfection assays were immunoprecipitated with sera from NIH(MCF-7) cl 2 tumor-bearing mice (Fig. 3). All four lines of NIH secondary transformants expressed gp86 and the 19,000-dalton protein, indicating that expression of these antigens was common to NIH cells transformed in either primary or secondary transfection assays by MCF-7 DNA.

To determine the specificity of gp86 expression in transformed NIH cells, sera from NIH(MCF-7) cl 2 tumor-bearing mice were used to immunoprecipitate extracts of other transformed NIH cells. This serum did not precipitate gp86 from [35S]methionine-labeled extracts of spontaneously transformed NIH cells, NIH cells transformed by normal human DNA, NIH cells transformed by human bladder carcinoma DNA, or NIH cells transformed by Rous sarcoma virus DNA (Fig. 4). Conversely, sera from mice bearing tumors induced by NIH cells transformed by either normal human DNA or human bladder carcinoma DNA did not precipitate gp86 from [35S]methionine-labeled extracts of NIH cells transformed by MCF-7 DNA (Fig. 5). These results indicate that gp86 is not generally expressed in transformed NIH cells but is instead specific for NIH cells transformed by MCF-7 DNA.

Relationship Between gp86 and Antigens Associated with Transforming Genes of Mouse Mammary Carcinomas. The transforming gene detected by transfection of MCF-7 DNA is closely related to the transforming genes of mouse mammary carcinomas induced by either MMTV or dimethylbenzanthracene (7). Therefore, sera from mice bearing tumors induced by
NIH cells transformed by DNA of a MMTV-induced mouse mammary carcinoma [NIH(MMTV-Tu2) cells] and by NIH cells transformed by DNA of a chemically induced mouse mammary carcinoma [NIH(D1-DMBA-4) cells] were assayed by immunoprecipitation of extracts of NIH(MCF-7) cells. Both of these antisera precipitated gp86 from extracts of [35S]methionine-labeled NIH(MCF-7) cl 2 cells (Fig. 6). In addition, gp86 was precipitated from extracts of NIH(MCF-7) cl 2 cells by sera from C3H mice bearing primary MMTV-induced mammary carcinomas.

These results indicate that gp86 is related to antigens expressed in NIH cells transformed by DNA of both virally and chemically induced mouse mammary carcinomas and to antigens expressed in primary mouse mammary carcinomas. On the other hand, gp86 was not detected in extracts of NIH(MMTV-Tu2) cells, NIH(D1-DMBA-4) cells, primary MMTV-induced mouse mammary carcinoma cells, or MCF-7 cells by immunoprecipitation with sera from mice bearing primary MMTV-induced mammary carcinomas or from mice bearing tumors induced by NIH(MCF-7) cells, NIH(MMTV-Tu2) cells, or NIH(D1-DMBA-4) cells. The observation that sera from mice bearing tumors induced by these cells immunoprecipitated gp86 from NIH(MCF-7) cells but not from the homologous cells may relate to differences in the amount, stability, or modification of this antigen in extracts of NIH(MCF-7) cells and in extracts of NIH cells transformed by mouse mammary carcinoma DNAs or in extracts of primary mouse mammary carcinomas.

DISCUSSION

The use of sera from tumor-bearing mice has allowed the detection of gp86, an 86,000-dalton glycoprotein, in extracts of NIH cells transformed by DNA of the MCF-7 human mammary carcinoma cell line. This protein was detected by immunoprecipitation assays of three independent lines of NIH cells transformed by MCF-7 DNA and of four independent lines of NIH cells transformed by DNA of NIH(MCF-7) cells in secondary transfection assays. However, gp86 was not detected by immunoprecipitation assays of NIH cells transformed spontaneously or by normal human DNA, human bladder carcinoma DNA, or Rous sarcoma virus DNA. In addition, sera from mice bearing tumors induced by these cells did not precipitate gp86 from NIH(MCF-7) cell extracts. Therefore, gp86 appears to represent an antigen that is specifically associated with expression of the transmissible transforming gene activated in the MCF-7 human mammary carcinoma cell line.

Nonglycosylated proteins of approximately 70,000 and 19,000 daltons and a glycoprotein of 72,000–76,000 daltons were also detected in some immunoprecipitates of NIH(MCF-7) cells.

Sera from mice bearing tumors induced by NIH cells transformed by DNAs of virally or chemically induced mouse mammary carcinomas also immunoprecipitated gp86 from extracts of NIH(MCF-7) cells. This observation is consistent with pre-

FIG. 4. Lack of expression of gp86 in NIH cells transformed by DNAs not from mammary carcinoma. [35S]Methionine-labeled extracts of NIH 3T3 cells (lanes a and b), NIH (MCF-7) cl 2 cells (lanes c and d), spontaneously transformed NIH cells (lanes e and f), NIH cells transformed by normal human DNA (lanes g and h), NIH cells transformed by human bladder carcinoma DNA (lane i), and NIH cells transformed by Rous sarcoma virus DNA (lanes k and l) were immunoprecipitated with control serum (lanes a, e, g, i, and k) or serum from a NIH(MCF-7) cl 2 tumor-bearing mouse (lanes b, d, f, h, j, and l).

FIG. 5. Lack of immunoprecipitation of gp86 by sera from mouse bearing tumors induced by NIH cells transformed by DNAs not from mammary carcinoma. [35S]Methionine-labeled extracts of NIH 3T3 cells (lanes a and b) and NIH(MCF-7) cl 2 cells (lanes c–f) were immunoprecipitated with control serum (lanes a and c), serum from a NIH(MCF-7) cl 2 tumor-bearing mouse (lanes b and d), serum from a mouse bearing tumors induced by NIH cells transformed by normal human DNA (lane e), or serum from a mouse bearing tumors induced by NIH cells transformed by human bladder carcinoma DNA (lane f).
Fig. 6. Immunoprecipitation of gp86 from NIH(MCF-7) cells by sera from mice bearing tumors induced by NIH cells transformed by mouse mammary carcinoma DNAs and by sera from mice bearing primary mammary carcinomas. [35S]Methionine-labeled extracts of NIH 3T3 cells were immunoprecipitated with control serum from a BALB/c mouse immunized with NIH 3T3 cells (lane a) or with serum from a BALB/c mouse bearing NIH(MCF-7) cl 2 induced tumors (lane b). Extracts of NIH(MCF-7) cl 2 cells were immunoprecipitated with control serum from a BALB/c mouse immunized with NIH 3T3 cells (lane c) or with sera from BALB/c mice bearing tumors induced by NIH(MCF-7) cl 2 cells (lane d), NIH cells transformed by DNA of a MMTV-induced mouse mammary carcinoma (lane e), and NIH cells transformed by DNA of a dimethylbenzanthracene-induced mouse mammary carcinoma (lane f). Extracts of NIH(MCF-7) cl 2 cells were also immunoprecipitated with serum of a normal C3H mouse (lane g) or serum of a C3H mouse bearing a primary MMTV-induced mammary carcinoma (lane h).

vious results indicating that closely related transforming genes were activated in human and mouse mammary carcinomas and further supports the hypothesis that specific transforming genes are involved in neoplasms of specific differentiated cell types (7). In addition, precipitation of gp86 by sera from mammary carcinoma-bearing mice indicates that this antigen is expressed in primary mouse mammary carcinomas as well as in NIH cells transformed by mammary carcinoma DNAs.

Expression of this antigen thus appears to be characteristic of both primary mouse mammary carcinomas and NIH cells transformed by either human or mouse mammary carcinoma DNAs. These results may be accounted for by either of two possibilities. First, this antigen might be encoded by a transforming gene that is expressed in primary mammary carcinomas and is transferred to NIH 3T3 cells upon transfection with mammary carcinoma DNAs. Alternatively, expression of the gene encoding this antigen might be induced as a consequence of expression of the mammary carcinoma transforming gene in both primary mammary carcinomas and in NIH cells transformed by mammary carcinoma DNAs. In either case, identification of an antigen specifically associated with a mammary carcinoma transforming gene may contribute to elucidation of the biochemical events involved in this pathway of oncogenesis.

We are grateful to D. M. Livingston for many helpful suggestions and to D. M. Livingston and T. Benjamin for critical comments on the manuscript. This research was supported by National Institutes of Health Grants CA18689, CA29846, and CA26925, by National Institutes of Health Fellowship CA06721 to D.B., and by an American Cancer Society Faculty Research Award to G.M.C.