Differential Suppression of Mammary and Prostate Cancer Metastasis by Human Chromosomes 17 and 11

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ABSTRACT

Metastasis suppressor activities have previously been mapped to human chromosomes 17 and 11. Decreased expression of the metastasis suppressor gene NM23, which is located on chromosome 17, has been correlated with increased metastatic potential in mammary cancers. A region on human chromosome 11, from 11p11.2-p13, has been shown to suppress metastasis in rat prostatic carcinoma cells. In both cases the metastasis suppressor activity had no effect on tumorigenicity or tumor growth rate, demonstrating that the encoded activities are distinct from effects of tumor suppression. To determine whether these human chromosomes encode general or tissue-specific metastasis suppressor activities, a truncated human chromosome 17 (i.e., pter-q23) and a full-length human chromosome 11 were separately transferred into highly metastatic rat mammary and prostate cancer cell lines and tested for their ability to suppress spontaneous metastasis in vivo. These studies demonstrated that when the pter-q23 region of human chromosome 17 is retained by the microcell hybrids, the metastatic ability of both mammary and prostatic cancer cells is suppressed. In contrast, when the pter-q14 region of human chromosome 11 is retained, only the metastatic ability of prostatic cancer cells is suppressed. Additional studies demonstrated that the metastasis suppressor activity encoded by the chromosome 17 pter-q23 region is p53-independent and not due to enhanced expression of NM23 protein.

INTRODUCTION

Over the last 60 years, prostate cancer has become the most common cancer in the American male, with 165,000 new cases diagnosed each year (1). During this time period the annual death rate due to prostate cancer (35,000 deaths per year) has doubled (1). These changes are due in part to increased life span, to a larger proportion of American males above the age of 50 years, and to the fact that metastatic prostate cancer is a lethal disease for which there is no curative therapy (2). This has led to the question of whether more aggressive screening for prostatic cancer in aging men should be undertaken to allow more of these cancers to be diagnosed at a localized stage when they are still potentially curable by definitive local therapy.

Although such screening is logical, its implementation is complicated by the biological characteristics of histological and clinical prostatic cancers. Necropsy studies indicate that 10% of men 50–60 years old and 50% of those 70–80 years old have histological cancers in their prostates (3). These histologically detected, localized prostatic cancers are heterogeneous, with only a small subset having undergone all of the malignant changes required to produce clinically aggressive tumors (3). The majority of these histologically detected, localized prostatic tumors never become fully malignant, despite host longevity (3). At present, it is not possible to predict which localized cancers will progress to clinical disease and which will not. In addition, approximately 30% of clinically localized prostatic cancers that are presently being detected without aggressive screening have already established micrometastatic disease at the time of definitive local therapy (2). Thus, these patients require additional systemic therapy which should be initiated as early as possible to maximize the therapeutic response (4). At this time there are no diagnostic methods to individually substage patients with histologically detected, localized prostatic cancer as to those requiring no therapy, versus those requiring definitive local therapy, versus those requiring definitive local therapy plus systemic therapy.

Acquisition of metastatic ability is a definitive criterion by which to substage histologically localized prostatic cancers. Identification of molecular and cellular markers for the metastatic ability of prostate cancer, therefore, should be useful in developing diagnostic methods for substaging histologically localized prostatic cancers on an individual patient basis. Toward this end, we are constructing a molecular map of general and specific suppressors of prostate cancer metastasis. This study examines the ability of human chromosomes 17 and 11 to specifically suppress metastasis of highly metastatic rat prostate and mammary cancer cell lines. Chromosome 17 encodes the metastasis suppressor genes NM23 H1 and H2 at 17q21.3 (5) while the 11p11.2-p13 region has been shown to encode a gene or genes which suppress the metastatic ability of highly metastatic rat prostatic cancer cells (6). In both cases this suppression had no effect on the tumorigenicity or tumor growth rate in vivo, indicating that this effect is due to metastasis suppressor, and not tumor suppressor, gene or genes.

To determine whether chromosome 17 and 11 encode prostate-specific metastasis suppressor gene or genes or general suppressor or suppressors of metastasis, we have developed and characterized a highly metastatic rodent mammary cancer cell line, designated R1564, and used it, along with rat R3327 AT6.1 (AT6.1) prostatic cancer cells, as the recipient for microcell transfer of human chromosomes 17 and 11. In this study we report the characterization of the new rat mammary cancer cell line R1564 and demonstrate that chromosome 17pter-q24 encodes gene or genes which suppress both R1564 and AT6.1 metastasis while the 11p11.2-p13 region has been shown to encode a gene or genes which suppress the metastatic ability of highly metastatic rat prostatic cancer cells (6). In both cases this suppression had no effect on the tumorigenicity or tumor growth rate in vivo, indicating that the observed suppression was due to metastasis suppressor, and not tumor suppressor, gene or genes.

MATERIALS AND METHODS

Establishment and Characterization of the R1564 Mammary Cancer Cell Line. The R1564 cell line is a highly metastatic, estrogen-independent rat mammary cancer cell line. It was obtained from a BD IV rat that originated...
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from an inbred colony maintained at Texas A & M University. Microbiological and serological screens for common infectious agents were negative (performed by Microbiological Associates, Bethesda, MD). The original tumor, a mammary adenocarcinoma designated R1564, occurred in a female BD-IV rat inoculated i.p. at 30 days of age with 90 mg/kg of a solution of direct acting alkylating carcinogen, N-ethyl-N-nitrosourea. At 250 days postinoculation the exposed rat developed a small (0.5-cm) s.c. nodule at the right axillary region. This nodule slowly increased in size, became ulcerated, and centrally necrotic. The animal was sacrificed at 370 days postinoculation, and a complete necropsy was performed. Metastases into the right axillary lymph node, lung, and right ovary were grossly detected. Continuous cell lines were established from the primary mammary gland, lymph node, lung, and ovarian metastases. The R1564 cell line was isolated from the ovarian metastasis by mammary fat pad inoculation of cultured tumor cells into a syngeneic female rat, reculturing cells from the resulting tumor, and eliminating mesenchymal cells mainly by differential trypsinization. Briefly, tissues were collected aseptically at necropsy, placed in warm Dulbecco’s modified Eagle’s medium, finely minced, incubated with 0.1% collagenase solution for 40 min, centrifuged, resuspended, and transferred into 20-mm² culture flasks with fresh medium. Host stromal cells were rapidly overgrown by the neoplastic epithelial cells and essentially pure cultures were obtained after 1 or 2 passages. Adherent cells were detached for subculture or experimentation by trypsinization. Culture medium consisted of Dulbecco’s modified Eagle’s medium supplemented with 5% fetal bovine serum, 2 mM L-glutamine, penicillin (100 units/ml), and streptomycin (100 mg/ml).

Characteristics of the AT6.1 Prostatic Cancer Cell Line. The AT6.1 cell line is a highly metastatic, anaplastic, androgen-independent rat prostatic cancer cell line developed spontaneously during the serial passage of the nonmetastatic, well-differentiated, androgen-responsive Dunning R3327H rat prostatic cancer. The AT6.1 cell line was established from a lung metastasis as described previously (6). AT6.1—11 clones 1–3 were established by microcell transfer of human chromosome 11 into the AT6.1 cells (6). As a control for the effects of expression and selection of the neomycin-resistant gene, AT6.1 cells were calcium phosphate transfected with the pZipNeoSV(X) plasmid as described previously (6) and the AT6.1-neo clones 1–3 were selected.

Cell Culture Conditions. All of the cell lines were grown in a standard RPMI 1640 (M. A. Bioproducts, Walkersville, MD) containing 10% fetal calf serum (HyClone, Logan, UT), 1 mM glutamine, streptomycin (100 µg/ml), penicillin (100 units/ml) (antibiotic and glutamine from M. A. Bioproducts), and 250 nM dexamethasone (Sigma Chemical Co., St. Louis, MO). The cells were grown at 37°C in 5% CO₂ and 95% air.

Microcell Transfer of Human Chromosomes 11 and 17 into Recipient Cells. Microcell-mediated chromosome transfer was performed as described previously (6). AT6.1(1-1) and A9(neo 17) cells were the donors of human chromosomes 11 and 17 to the recipient cell line. The A9(neo 11-1) cells contain a single intact human chromosome 11 with an integrated neomycin-resistant gene at position 11p11.2 (6, 8). The A9(neo 17) contains a truncated human chromosome 17 (i.e., pter-q23) with an integrated neomycin-resistant gene which has not yet been mapped. Human chromosome 11- and 17-containing microcell hybrids were selected and maintained by culturing in standard RPMI 1640 containing 500 µg/ml of G418.

Measurement of DNA Synthesis after γ-Irradiation. Logarithmically growing cells were exposed to either 0 or 4 Gy of γ-irradiation and 20 h following irradiation pulsed for 1 h at 37°C with [³H]thymidine in Hanks’ balanced salt solution containing magnesium and calcium (Gibco/BRL), 10 µM thymidine, and 4 µCi/ml [methy1-³H]thymidine (Amersham). Incorporation of radioactivity was stopped by washing the cells with Hanks’ balanced salt solution containing 0.1% unlabeled thymidine. Cells were trypsinized, and an aliquot was centrifuged for 5 min at 1000 X g. The cell pellet was washed with 0.2 M perchloric acid, and the DNA was hydrolyzed with 0.8 M perchloric acid for 30 min at 70°C and the hydrolysate was counted. Incorporation of [³H]thymidine is expressed as cpm/10⁶ cells.

Molecular Analysis and Cyogenetic Characterization of the Parental R1564 Mammary Cancer Cell Line and Microcell Clones. Cells were harvested for cytogenetic analysis and incubated in 0.01 µg/ml Colcemid for 2 h. Chromosomal spreads were prepared and banded using the trypsin-Giemsa technique as described previously (6). To accurately karyotype the R1564 cell line, 20 metaphases were examined and 5 karyotypes were prepared. The number of copies of human chromosomes 17 and 11 in the AT6.1 and R1564 microcell clones was determined by FISH² analysis using biotin-labeled total human genomic and centromere-specific DNA probes (Oncor Inc., Gaithersburg, MD) which use avidin-fluorescein isothiocyanate as the fluorescent tag and propidium iodide as a counterstain as described previously (6).

Southern blot analysis was used to detect the presence of the SV2-neo sequence located on human chromosome 11 at 11p11.2 (6). Briefly, genomic DNA was isolated as described previously (6) and 10 µg were digested with the restriction enzyme HindIII (New England Biolabs, Beverly MA), fractionated on a 1% agarose gel, transferred onto nylon membranes (Schleicher and Schuell, Keene, NH), and probed with the 5.7-kilobase BamHI-linearized SV2-neo plasmid (6). The probe was labeled with 32P-dCTP by random hexanucleotide priming (Multiprime DNA labeling system; Amersham Corp., Arlington Heights, IL). Blots were hybridized at 65°C for 2 h with 32P-labeled plasmid in QuickHyb hybridization solution (Stratagene, La Jolla, CA) containing 200 µg/ml salmon sperm DNA (Sigma). The filters were sequentially washed with 1× SSPE-0.5% sodium dodecyl sulfate (37°C, 20 min) and 0.1× SSPE-0.5% sodium dodecyl sulfate (65°C, 30 min) prior to autoradiography.

PCR was used to determine the number of copies of human chromosome 17 present in the AT6.1—17 and R1564—17 microcell hybrids and to document the region of human chromosome 11p arm retained by the R1564—11 microcell hybrids. PCR primers for sequences which have been physically mapped to the p arm [DI7575B (17p13), DI75200 (17p12), CHRN1 (17pter-p12)] and to the q arm [THRA (17q11.2-q12), DI75201 (17q24-d23)] were used for analysis of human chromosome 17. In addition, additional primers for the 11p12-13 region (DI15534, DI15578) were specific for microsatellite sequences in this region and their sequences and map positions are as described by Weissbach et al. (9). Additional primers for the 11p11.2-1p3 region (DI15534, DI15578) were as described and mapped by Miwa et al. (10). All primers were either purchased from Research Genetics, Inc. (Huntsville, AL) or synthesized by Bio-Synthesis, Inc. (Lewisville, TX). Fifty-100 ng genomic DNA was used in 25-µl reactions under the following conditions: 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1–2.5 mM MgCl₂, 0.4 µM primers, 5% dimethyl sulfoxide, and 0.65 units Taq polymerase (Perkin Elmer/Cetus, Norwalk, CT). PCR products were amplified in a M. J. Research Thermocycler for 35 cycles with annealing temperatures ranging from 45 to 65°C. PCR products were fractionated by electrophoresis on a 3.5% Metaphor agarose gel (FMC, Rockland, ME) in ×1 Tris-borate-EDTA buffer and visualized by ethidium bromide staining. Msp I digested λ-DNA (New England Biolabs) was used for molecular weight markers.

Immunoblotting with Anti-NM23 and Antihuman p53 Antibodies. Logarithmically growing cells were harvested and lysed in 300–500 µl lysis buffer (0.5% v/v Nonidet P-40, 10 µg/ml aprotinin in 0.9% NaCl, and 20 µM Tris, pH 7.5). Cells and debris were collected and sonicated for 1 min, cellular debris was pelleted by centrifugation for 5 min at 12,000 × g, and the supernatant fraction was saved for analysis. Equal amounts of total soluble protein (25 µg) from each of the cell lines were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 13% polyacrylamide gel. The separated proteins were transferred to Immobilon (polyvinylidene difluoride) membrane and stained with fast green (0.1%) to ensure equal loading of samples. The blots were blocked in Blotto, incubated with the primary antibody [anti-NM23 polyclonal antibody (11); gift of Dr. Patricia Siegel or antihuman p53 antibody (Oncogene Science) gift of Dr. Bert Vogelstein], and incubated with the peroxidase-labeled secondary mouse anti-rabbit antibody. The immune complexes were visualized with the ECL Detection System (Amersham Corp.).

Characterization of in Vivo Growth and Metastasis. To characterize the in vivo growth rate and metastatic ability of the microcell clones, SCID mice (Taconic Lab Animals and Services, Germantown, NY) received s.c. injections in the flank of 5 × 10⁶ cells. Five animals were used per microcell clone and parental line. Tumor volume was used as an index of growth rate and was determined as described previously (6). Approximately 60 days postinocula-

3 The abbreviations used are: FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction.
RESULTS

Characterization of the Mammary Cancer Line R1564. The mammary cancer cell line R1564 was established from a mammary adenocarcinoma induced by i.p. injection of N-ethyl-N-nitrosourea into female BD IV rats. In culture, R1564 cells form epithelial colonies which assume cobblestone monolayers with "dome" formation. Near confluence the cells begin to overlap and grow in three dimensions, forming spheroids. The epithelial appearance remained stable over 50 in vitro passages. Ultrastructurally, cultured cells have large irregularly shaped nuclei, numerous surface microvillous projections, desmosomes, and rare intracellular lumina. Cytofluorometric analysis of tumor cell DNA content revealed a single diploid G0 + G1 peak at 5, 10, and 40 passages.4

Cytogenetic analysis of the R1564 cells using G banding demonstrated a diploid karyotype with a modal chromosome number of 42 and no obvious clonal structural abnormalities. There were numerical aberrations however, in that the cells were trisomic for chromosome 4 and monosomic for chromosome 16 in all analyzable cells (Fig. 1). The in vivo doubling time and metastatic ability of the R1564 cells were examined by injecting $5 \times 10^5$ cells s.c. into 4–6-wk-old SCID mice. The in vivo growth rate was found to be $7.27 \pm 1.2$ days until the tumors reached approximately $1.3 \text{ cm}^3$ in volume. After this time the tumor volume remained relatively constant. The primary R1564 tumors exhibited a consistent phenotype in that they became ulcerated when they reached a volume of $\leq 0.5 \text{ cm}^3$ and never exceeded a volume of $1.5–2.0 \text{ cm}^3$ even when left untreated until the death of the animal (approximately 60–80 days). At death, numerous macroscopic metastases ranging from 1–5 mm in diameter were detectable (Fig. 2A). The arrows in Fig. 2B indicate large and small encapsulated metastatic nodules in a histological section of lung ($\times 16$). Microscopic analysis of such lung metastasis demonstrated that histologically these lesions were composed of medullary cords of cancer cells (Fig. 2C).

Effect of Transferring Human Chromosome 17 into Metastatic Breast and Prostate Cancer Cells. Microcell-mediated chromosomal transfer was used to introduce a truncated human chromosome 17 (i.e., pter-q23) into both the newly characterized, highly metastatic, estrogen-independent R1564 rat mammary cancer cells and the previously characterized, highly metastatic, androgen-independent AT6.1 rat prostatic cancer cells. The portion of human chromosome 17 present in the R1564–17 and AT6.1–17 microcell clones was determined by a combination of cytogenetic, FISH, and PCR mapping techniques. Both the R1564 (Fig. 1) and AT6.1 cell lines have pseudodiploid karyotypes, making the identification of transferred human chromosomes straightforward. This is illustrated by Fig. 3 which shows a representative mitotic spread of an AT6.1–17 microcell hybrid. In this case the parental AT6.1 cells have the karyotype of 44XY, +4, +12 del(3p12–36), del(15)p14 simplifying the identification of the transferred human chromosome (i.e., see Fig. 3, inset). These G banding results were confirmed by FISH analysis using total human genomic and chromosome 17 centromere-specific probes. The portion of human chromosome 17 retained in the AT6.1–17 and R1564–17 microcell clones was determined by PCR mapping with chromosome 17-specific primers. The analyses of two AT6.1–17 and three R1564–17 microcell clones are shown in Fig. 4. Markers that have been physically mapped are denoted by circles while those that have been mapped by linkage analysis are indicated by squares. As can be seen, all of the AT6.1–17 and R1564–17 microcell hybrids retained the six 11p markers examined, demonstrating that the p arm is intact in all of the microcell clones. In the case of the q arm, the seven markers proximal to 17q23 were retained in all of the microcell clones except for R1564–17 c6 which was missing the D17S806 marker. The primers for MPO, D17S801, and D17S802 did not yield products, indicating that the distal region (17q24-pter) has been deleted. PCR analysis of this region showed that it was also deleted in the donor A9(17) cells, explaining the absence of these markers in the AT6.1–17 and R1564–17 microcell hybrids (data not shown). The results of PCR mapping of both the p and q arms are in agreement with the cytogenetic analyses which showed that the microcell clones had a morphologically normal p arm and a deletion of the distal region of the q arm (Fig. 3).

The effect of the introduction of human chromosome 17pter-q23 on the tumorigenicity and metastatic ability of R1564 and AT6.1 cells was tested in SCID mice. Five R1564–17 microcell clones that were

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4 D. G. Hall and G. Stoica, unpublished data.

Fig. 1. G-banded karyotype of the R1564 mammary cancer parental cell line. This line has a chromosome complement of 42, XX, +4, −16.
day 40 was used as an index of tumorigenicity. The presence of chromosome 17pter-q23 did not suppress tumorigenicity of the R1564 or the AT6.1 cells as is shown in Tables 1 and 2. There were no macroscopic metastases observed in the lungs from the R1564—17 or AT6.1—17 tumor-bearing animals. These results demonstrate that the

shown to contain intact copies of human chromosome 17pter-q23 by the aforementioned methods were examined for their tumorigenicity and metastatic ability in vivo. Because of the growth characteristics of R1564 cells, the tumor volume of the R1564—17 microcell hybrids on
presence of human chromosome 17pter-q23 completely suppressed the metastatic ability of both R1564 and AT6.1 cells. Interestingly, all of the R1564-17 clones examined exhibited a 3- to 6-fold increase in their tumor volumes (4.8–9.7 cm³) as compared to the R1564 control cells (1.6 cm³). This does not appear to be due to the loss of the p53 gene (17p13) since no cytogenetic or molecular losses were detected on 17p (Fig. 4).

To evaluate any potential role of p53 in our findings, experiments were conducted to determine whether the AT6.1 and R1564 cell lines have functional wild-type p53 genes and whether the microcell hybrids express human p53. The approach used to determine whether the AT6.1 and R1564 cell lines have a functional wild-type p53 is based on the finding by Slebos et al. (12) that cells with functional wild-type p53 arrest in G1 20 h after sublethal DNA damage. Based on this observation, logarithmically growing cells were exposed to 4 Gy γ-irradiation and pulsed with [³H]thymidine 20-h postirradiation as a measure of entry into S phase (Fig. 5). As a control, RKO cells, which have a functional wild-type p53 gene were likewise irradiated. These data demonstrated that, as expected, there is a >70% reduction in the number of RKO cells entering S phase (i.e., the majority of cells are arrested in G1). In contrast, there is a <20% reduction in the number of AT6.1 or R1564 cells entering S phase (Fig. 5). Neither the AT6.1 nor R1564 arrest in G1 after sublethal DNA damage, demonstrating that they do not have functional wild-type p53 genes. Previous studies by Goyette et al. (13) demonstrated that microcell mediated chromosomal transfer of a single copy of human chromosome 17 containing a wtp53 gene into SW480 cells (a human colon cancer cell line which contains two copies of mutant p53) resulted in growth arrest. Similarly, Isaacs et al. (14) demonstrated that transfection of wtp53 into human prostate cancer cell lines containing mutant p53 alleles resulted in decreased colony formation. In our experiments transfer of human chromosome 17pter-q23 had no effect on the growth microcell hybrids, indicating that either there was no expression of human p53 or the human p53 expressed was the mutant form. To determine which of these possibilities was the case, Western blot analysis of the parental cell lines and microcell hybrids was conducted using human-specific p53 antibodies. The results of these experiments demonstrated that there is no human p53 protein produced in the human chromosome 17 AT6.1 or R1564 microcell hybrids.5 These results demonstrate that chromosome 17pter-q23-mediated metastasis suppression in prostate and mammary cancer cells is independent of p53 function.

Candidates for the chromosome 17-encoded suppressor activity observed in these systems are the NM23 H1 and H2 metastasis suppressor genes located at 17q21.3. To determine whether chromosome 17-mediated metastasis suppression is correlated with increased NM23 protein expression, the level of NM23 expressed in the chromosome 17pter-q23 microcell hybrids was examined by Western blotting with an anti-NM23 antibody. The level of NM23 protein detected in AT6.1–17 clones 9 and 10 was compared to AT6.1 transfection controls (AT6.1-neo-2, 3) and the MCF7-positive control. The results of these experiments demonstrated that there is no human NM23 protein produced in the human chromosome 17 AT6.1 or R1564 microcell hybrids.5 These results demonstrate that chromosome 17pter-q23-mediated metastasis suppression in prostate and mammary cancer cells is independent of p53 function.

Table 1 In vivo characteristics of parental R1564 and R1564-17 microcell hybrid clones

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tumor volumea,b (cm³ on day 40)</th>
<th>Metastatic abilityc (no. of lung metastases/animal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1564 (parental)</td>
<td>1.6 ± 0.2</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>R1564-17-c1</td>
<td>4.8 ± 1.7</td>
<td>0</td>
</tr>
<tr>
<td>R1564-17-c6</td>
<td>8.0 ± 2.1</td>
<td>0</td>
</tr>
<tr>
<td>R1564-17-c7</td>
<td>5.8</td>
<td>0</td>
</tr>
<tr>
<td>R1564-17-c8</td>
<td>9.7 ± 1.2</td>
<td>0</td>
</tr>
<tr>
<td>R1564-17-c11</td>
<td>5.3 ± 0.5</td>
<td>0</td>
</tr>
</tbody>
</table>

a Mean ± SE.

b cm³ on day 40 used as an index of tumorigenicity.

c Number of macroscopic lung metastases, each group with three to five animals.

Table 2 In vivo characteristics of parental AT6.1 and AT6.1-17 microcell hybrid clones

<table>
<thead>
<tr>
<th>Cell line</th>
<th>In vivo tumor doubling timea (days)</th>
<th>Metastatic abilitya,b (no. of lung metastases/animal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT6.1</td>
<td>3.3 ± 0.6</td>
<td>83 ± 5</td>
</tr>
<tr>
<td>AT6.1-17-c9</td>
<td>3.8 ± 0.2</td>
<td>0</td>
</tr>
<tr>
<td>AT6.1-17-c10</td>
<td>4.8 ± 0.4</td>
<td>0</td>
</tr>
</tbody>
</table>

a Mean ± SE.

b Number of macroscopic lung metastases, each group with five animals.

5 J. Pietenpol and B. Vogelstein, unpublished data.
also no correlation between metastasis suppression in the R1564—17 microcell hybrids and the level of NM23 expression (data not shown).

Effect of Transferring Human Chromosome 11 into Metastatic Breast and Prostatic Cancer Cells. We have previously demonstrated that the 11p11.2-p13 region of chromosome 11 can suppress the metastasis of highly metastatic, androgen-independent Dunning R3327 rat prostatic cell lines (6). To determine whether the metastasis suppressor activity on 11p11.2-p13 was specific for prostatic cancer or had a more generalized effect, human chromosome 11 was transferred into the highly metastatic, estrogen-independent, rat mammary cancer cell line R1564. The resulting R1564 microcell hybrids were characterized using two methods. Initially, four clonal lines, in which 80–90% of the cells contained human chromosomal material, were identified by FISH analysis using total human chromosome painting. The number of copies of human chromosome 11 transferred into the recipient R1564 cells was determined by FISH analysis. The FISH analysis of one clone (R1564—11-clone 5) is shown in Fig. 7. In each of the clones >95% of the cells contained a single copy of human chromosome 11.

Using G banding and cytogenetic analysis, all of the R1564—11 clones were shown to have a morphologically normal p arm and a deletion of the q arm from q14-qter (Fig. 8). Results from the FISH analysis (Fig. 7) demonstrate that the deletion of the q arm is not the result of a rearrangement between human and rat chromosomes (i.e., transfer of this region onto a rat chromosome). The region of 11p retained in the R1564—11 clonal lines was determined in detail using microsatellite primers mapped to distal regions of human chromosome 11p and primers (D11S554, D11S578) which have been mapped to the 11p11.2–13 region (10) (Fig. 8). All of the R1564—11 clones analyzed retained the markers within (D11S554, D11S578) and proximal to (pSV2-neo, D11S905) the region of metastasis suppressor activity (Fig. 8). R1564—11 clones 5 and 6 retained all of their distal markers (D11S915, D11S926, D11S909), whereas R1564—11 clone 4 had lost one marker (D11S915), indicating that it may have undergone small interstitial deletions or mutations that prevent PCR product formation (Fig. 8).

R1564—11 clones 4, 5, and 6 retained the prostate cancer metastasis suppressor region; thus, each clone was assayed for its metastatic ability and growth rate in SCID mice. The data in Table 3 demonstrate that the presence of human chromosome 11pter-q14 in the R1564 cells did not inhibit the tumorigenicity of the microcell hybrids cells in vivo. The growth rate of one clone, R1564—11—4, was actually increased. In addition, metastatic lung nodules were observed in 100% of the animals that received injections of the parental R1564 cells and R1564—11 microcell clones. Each animal had 15–40 lung metastases ranging from 2–6 mm in diameter. The average size and number of R1564 lung metastases was not affected by the presence of human chromosome 11p11.2-p13.

The lack of metastasis suppression in the R1564 microcell clones could have been due to the loss of the 11p11.2-p13 chromosomal region during in vivo growth. To eliminate this possibility, DNA was isolated from metastatic lung nodules and analyzed by PCR using the primers described in Fig. 9. All of the metastatic tissues retained the DNA sequences which were present in the original cell lines. An example of three such PCR products (D11S909, D11S578, and D11S905) is shown in Fig. 9. The fact that the metastatic tissue retained markers for the 11p11.2-p13 region indicates that 11p11.2-p13 encoded activities which suppress the metastatic ability of the rat prostatic cancer cells are insufficient for suppression of rat mammary cancer metastasis.

<table>
<thead>
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<th>Tumor volume (cm³ on day 40)</th>
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<tr>
<td>R1564 (parental)</td>
<td>1.6 ± 0.2</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>R1564-11-4</td>
<td>3.2 ± 0.3</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>R1564-11-5</td>
<td>1.7 ± 0.8</td>
<td>27 ± 4</td>
</tr>
<tr>
<td>R1564-11-6</td>
<td>1.5 ± 0.2</td>
<td>40 ± 3</td>
</tr>
</tbody>
</table>

a Mean ± SE.

b cm³ on day 40 used as an index of tumorigenicity.

c Number of macroscopic lung metastases, each group with five animals.
DISCUSSION

This study is a continuation of work designed to construct a molecular map of prostate-specific and general suppressors of metastasis within the human genome. The Dunning system of rat prostatic cancers has proven to be an excellent model for studies of the malignant progression of prostate cancer (15). Metastatic Dunning sublines, such as AT6.1, have the advantages of being well-characterized in vitro and in vivo and producing 80–150 metastases in spontaneous lung metastasis assays in SCID and nude mice (6). At this time there is no analogous human xenograft system which is sufficiently metastatic to allow such quantitative metastasis suppression assays. Chromosome 17q21.3 encodes NM23 H1 and H2, metastasis suppressor genes whose decreased expression has been correlated with increased metastatic potential in breast cancer (16). Previously, we have identified a region of human chromosome 11p11.2-p13 (not including the Wilms’ tumor-1 locus at 11p13) which is capable of suppressing metastasis in highly metastatic rat prostatic cancer sublines (6). The genes encoded by the 11p11.2-p13 region do not alter the growth rate of prostatic cancer cells, demonstrating that this suppression is distinct from effects on proliferation (6).

To determine whether chromosomes 17 and 11 encode tissue-specific or general suppressors of metastasis, we characterized a highly metastatic rat mammary cancer cell line R1564. Like the androgen-independent rat prostatic cancer cell line AT6.1, the estrogen-independent R1564 cell line is also highly metastatic and has a stable pseudodiploid karyotype. The R1564 cell line was used, along with the highly metastatic AT6.1 rat prostatic cell line, as a recipient for chromosomes 17 and 11. In vivo characterization of the AT6.1-17 and R1564-17 microcell hybrids showed that chromosome 17pter-q23 is capable of completely suppressing spontaneous metastasis of both cell lines in SCID mice without suppressing the tumorigenicity of the microcell hybrids. In the case of the R1564, the presence of chromosome 17pter-q23 actually increased the tumorigenicity of the microcell hybrids. The lack of tumor suppression could be due to the fact that in these human/rodent microcell hybrids, no human p53 protein is expressed. Therefore, human 17pter-q23 suppresses mammary and prostate cancer cell metastasis via a p53-independent process. Since the NM23 H1 and H2 genes are located at 17q21.3 (5), the observed metastasis suppression could have been due to modulation of NM23 protein expression. Cytogenetic and molecular analyses of the AT6.1-17 and R1564-17 microcell hybrids demonstrate that this region of the q arm is retained in the microcell hybrids. Therefore the level of NM23 protein was examined as a measure of NM23 gene expression. In these studies there was no correlation between the level of NM23 protein expression and the metastatic ability in the AT6.1-17 or R1564-17 microcell hybrids and control cells examined. This suggests that there are additional metastasis suppressor activities encoded by human chromosome 17pter-q23.

To further characterize the activity encoded by human chromosome 11p11.2-p13, chromosome 11 was transferred into the rat mammary cancer cell line R1564. Loss of heterozygosity on another region of human chromosome 11 (11p15.5) is a relatively common event in some cancers, suggesting that it may encode tumor or metastasis suppressor genes (17). Specific data on loss of heterozygosity analysis for 11p11.2-p13 have not been reported, possibly due to the relatively small number of genetic markers for this region. In the current study, we found that transfer of human chromosome 11pter-q14 had no effect on the growth rate or metastatic ability of the R1564 rat mammary cancer cell line. These results indicate that the expression or activity of metastasis suppressor genes encoded by 11p11.2-p13 may be dependent on the genetic context of the recipient cells. This is similar to the finding of Negrini et al. (18) who demonstrated that the transfer of chromosome 11 could suppress the tumorigenicity of estrogen-dependent MCF-7 cells, but had no effect on estrogen-independent MDA-MB-231 cells. Since the R1564 cell line is also estrogen independent it is possible that, in the case of mammary cancer cells, the progression to hormone independence is concomitant with an inability to express chromosome 11-encoded functions. In this case, tumor suppressor or metastasis suppressor gene or genes may fail to be transcribed or translated, or perhaps once synthesized, their gene product or products might be nonfunctional in the context of the estrogen-independent cell. Alternatively, our results could simply represent a tissue-specific phenomenon. This would be similar to the findings of Oshimura et al. (7) who demonstrated that the ability of human chromosome 11 to function as a tumor suppressor varied widely among organ-specific cancer cell types. It is possible that the regulatory sequences or tissue-specific transcription factors which are responsible for transcription of the metastasis suppressor gene or genes encoded by 11p11.2-p13 are not functional in mammary cancer cells. We have evidence for such a mechanism in the prostate-specific expression of the newly identified prostate-specific membrane antigen gene located on chromosome 11 (i.e., when human chromosome 11 is transferred via microcells into rat prostate cancer cells, but not mammary cancer cells, recipient cells express the human prostate-specific membrane antigen protein). Alternatively, the metastasis suppressor genes may be expressed, but may not be able to block the pathways which lead to metastasis in the mammary cancer system.

This work provides evidence for a general metastasis suppressor activity on human chromosome 17pter-q23 and a prostate-specific metastasis suppressor activity on human 11p11.2-p13. We are presently using deletion mapping to identify the metastasis suppressor region on chromosome 17pter-q23 and positional cloning of the 11p11.2-p13 region to identify the gene or genes involved in suppression of prostatic cancer metastasis.

ACKNOWLEDGMENTS

We would like to thank Barbara Lee for her assistance in preparation of this manuscript and John Lamb and Sue Dairymple for their technical assistance. We also thank Dr. Jennifer Pietenpol for conducting the p53 Western blotting of our cell lines.

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Differential Suppression of Mammary and Prostate Cancer Metastasis by Human Chromosomes 17 and 11

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