Elevated Expression of Caveolin Is Associated with Prostate and Breast Cancer

Guang Yang, Luan D. Truong, Terry L. Timme, Chengzhen Ren, Thomas M. Wheeler, Sang Hee Park, Yasutomo Nasu, Chris H. Bangma, Michael W. Kattan, Peter T. Scardino, and Timothy C. Thompson


ABSTRACT

To identify genes associated with prostate cancer progression, we developed a strategy involving the use of differential display-PCR with a panel of genetically matched primary tumor- and metastasis-derived mouse prostate cancer cell lines. We isolated a cDNA fragment with homology to the mouse caveolin-1 gene. Northern blotting with this fragment revealed increased caveolin expression in metastasis-derived cell lines relative to primary tumor-derived cell lines. Western blotting with a polyclonal caveolin antibody confirmed increased caveolin protein in metastasis-derived mouse cell lines and expression in three of four human prostate cancer cell lines. Immunohistochemical analysis of a human prostate cancer cell line demonstrated a prominent granular pattern of caveolin accumulation. Subsequent analysis of mouse and human prostate specimens revealed minimal caveolin expression in normal epithelium with abundant staining of smooth muscle and endothelium. The frequency of caveolin-positive cells was increased in prostate cancer with markedly increased accumulation of caveolin and a granular staining pattern in lymph node metastatic deposits. In human breast cancer specimens, increased caveolin staining was detected in intraductal and infiltrating ductal carcinoma as well as nodal disease. Caveolin therefore appears to be associated with human prostate cancer progression and is also present in primary and metastatic human breast cancer.

INTRODUCTION

Prostate and breast cancers are the most commonly diagnosed cancers in men and women, respectively (1), and are similar in that they arise in hormonally regulated secretory tissues. The ability to treat both cancers relates to the stage of the disease, with those cancers that are metastatic having a much poorer prognosis. To better understand the molecular pathways associated with metastasis, we developed a strategy to isolate genes related to metastasis by using DD-PCR to compare mRNA expression in a panel of genetically matched cell lines derived from primary and metastatic mouse prostate tumors. The malignant tissues were produced using the MPR model system, which involves the induction of metastatic prostate cancer in vivo by the transduction of the ras and myc oncogenes in fetal prostate tissues from p53 knock-out mice (2). Multiple sets of clonal cell lines from both primary and metastatic tumor foci recovered from the same inbred, experimental animal were established and analyzed for differential gene expression using a modified DD-PCR protocol (3, 4). Subsequent screening of mRNAs derived from these panels of matched primary and metastatic cell lines served to further resolve the validity of the DD-PCR results. One of the cDNA fragments identified using this approach encoded a portion of caveolin. Caveolin is a major structural protein of caveolae, lipid-based organelles that are part of the trans-Golgi network and involved in many cellular processes, such as signal transduction and transport of small molecules into cells (5–7). Formation of a multimeric complex involving glycosylphosphatidylinositol-linked uPAR, integrins, and caveolin has been shown to correlate with uPAR-mediated extracellular matrix adhesion in 293 cells (8). We analyzed caveolin expression in both mouse and human prostate cancer cell lines by Western blotting and confirmed elevated levels in metastasis-derived cells. Immunohistochemical studies demonstrated a granular staining pattern specific for caveolin in a high percentage of metastatic human prostate cancer specimens and in primary and metastatic human breast cancer. Overall, our results establish a significant association between caveolin expression and prostate cancer progression. In addition, increased accumulation of caveolin was demonstrated in primary and metastatic breast cancer relative to normal epithelium.

MATERIALS AND METHODS

Cell Lines. Mouse prostate cancer cell lines were derived from primary tumors (PA or PB) or metastatic deposits (LMA, LMB, LMC, LMD, LM1, LM2, and MMA) in the same host animal implanted with a ras + myc initiated p53 nullizygous MPR (2). The cell lines were analyzed for retroviral integration

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2 To whom requests for reprints should be addressed, at Scott Department of Urology, Baylor College of Medicine, 6560 Fannin, Suite 2100, Houston, TX 77030. Phone: (713) 799-8718; Fax: (713) 799-8712; E-mail: timothyt@bcm.tmc.edu.

3 The abbreviations used are: DD-PCR, differential display-PCR; MPR, mouse prostate reconstitution; uPAR, urokinase plasminogen activator receptor; dNTP, deoxynucleotide triphosphate.
RNA Isolation and Northern Blot Analysis. RNA was isolated from cell lines as described previously (2) or with commercially available RNA isolation reagents. mRNA was purified from total RNA with PolyATtract mRNA Isolation System (Promega Corp.).

For Northern blot analysis, 20 µg of total RNA were fractionated under denaturing condition on a 1% agarose, 6.7% formaldehyde gel and transferred onto Hybond-N Nylon membrane (Amersham). The membrane was baked at 80°C for 2 h. Blots were prehybridized for 2 h at 65°C in 7.5% SDS, 0.5 M sodium phosphate buffer (pH 7.2), 1 mM EDTA, 4X Denhardt’s solution (50X = 1% Ficoll, 1% polyvinylpyrrolidone, and 1% BSA), and 50 µg/ml salmon testes DNA. Hybridization was carried out by adding a 32P-labeled probe that had been purified with a QIA quick spin column (QIAGEN) and incubating overnight at 65°C. Blots were washed at 65°C for 20 min with 40 mM sodium phosphate (pH 7.2), 5% SDS, followed by another wash at 65°C for 20 min with 40 mM sodium phosphate buffer (pH 7.2), 1% SDS.

DD-PCR. One (primer 3, TCTGCGATCC) of a set of unique 10-mer deoxoygugonucleotide primers with an arbitrary sequence was used for reverse transcription and as both a 5’ and 3’ primer for amplification by PCR. The primers were selected based on having approximately the same ratio of G+C to A+T with no unintended self-complementaryity of more than two nucleotides (12). Reverse transcription of mRNA was with the Perkin-Elmer Cetus GeneAmp RNA PCR kit. A reaction volume of 10 µl contained 5 mM MgCl2, 1X PCR buffer II, 1 mM of each dNTP (dATP, dCTP, dGTP, and dTTP), 1 unit/µl RNAsin inhibitor, 2.5 µl/µl reverse transcriptase, 250 ng of primer, and 60 ng of mRNA. The reaction mixture was covered with 50 µl of mineral oil and incubated at 22°C for 10 min and 42°C for 15 min and terminated by incubation at 99°C for 5 min. The reaction was immediately diluted to 50 µl and adjusted such that it contained 2 mM MgCl2, 1X PCR buffer II, 1.25 units of AmpliTaq DNA polymerase, and 20 µCi of [3P]dATP (3000 Ci/mM). No additional dNTPs or primers were added so that the final concentration was 0.2 µM of each dNTP and 250 ng of primer. The PCR consisted of 40 cycles at 94°C for 40 s, 40°C for 2 min, and 72°C for 35 s with a final extension period of 72°C for 4 min.

Samples from the PCR were separated on a nondenaturing 5% polyacrylamide gel (29:1) with 5% glycerol at 9 W for 18 h. The gel was transferred to Whatman 3MM paper, dried, and exposed to X-ray film overnight. The differentially displayed bands were excised from the dried polyacrylamide gel and soaked in 500 µl of H2O for 15 min at room temperature to remove the filter paper, and the gel slice was transferred to 20 µl of TE buffer, smashed, and incubated at room temperature 2 h to overnight. A 5-µl aliquot was reamplified in a 50-µl PCR mixture containing 1X PCR buffer II, 2 mM MgCl2, 0.25 mM dNTP, 1.25 units of AmpliTaq DNA polymerase, and 1 µg of primer. The PCR was 45 cycles with the same parameters as above. The reamplified cDNA fragments were purified on 2% NuSieve agarose (FMC) by gel electrophoresis. The bands were excised and used to make a 32P-labeled probe for Northern blot analysis as described above or cloned into a TA cloning vector (pCR II vector from Invitrogen). The cloned DD-PCR fragment was sequenced with Sequenase version 2.0 (USB).

Protein Isolation and Western Blot Analysis. All cell lines were grown to subconfluence and lysed with Laemmli sample buffer [125 mM Tris-HCl (pH 6.8), 2% SDS, 5% glycerol, and 1% 2-mercaptoethanol]. Lysates were separated on a 12.5% polyacrylamide–SDS gel and electrophoretically transferred to a nitrocellulose membrane. The membrane was reacted with a polyclonal caveolin antibody (C13630; Transduction Labs) at 1:2000 or a monoclonal β-actin antibody (A5441; Sigma) at 1:5000. The immunoblots were stained, using an avidin-biotin-complex (ABC) kit (Vector). Preabsorption of caveolin antiserum with specific antigen in excess resulted in a negative reaction against a positive control human endothelial lyase (not shown). This caveolin antiserum was raised against a 97-amino acid NH2-terminal fragment of human caveolin-1. The mouse caveolin-1 protein is 96.9% identical to human caveolin-1 in this region, whereas the human caveolin-2 protein is 15.8% identical, although this region does contain an eight-amino acid conserved domain shared by caveolin-1 and -2 (13). Because of the possibility that this polyclonal antibody could recognize caveolin-2 as well as caveolin-1 protein, we also performed Western blotting with a rabbit polyclonal antibody specific for amino acids 2–21 of human caveolin-1 (sc-894; Santa Cruz Biotechnology) and a caveolin-2 specific monoclonal (C57820, Transduction Labs). Although caveolin-2 was detected in most specimens, the results confirmed that caveolin-1 was the predominant species expressed in the mouse and human cell lines (not shown).

Human Tissues Used for Immunolocalization of Caveolin. Formalin-fixed, paraffin-embedded prostatic tissues including normal epithelium (13 cases), hyperplastic epithelium (17 cases), and primary adenocarcinomas (46 cases) were used. Prostate tissue was obtained from radical prostatectomy specimens. All prostate cancers were staged using the American Joint Committee on Cancer Tumor-Node-Meta- satasis classification (14) as either T1a/T2bN0 (n = 29) or T2N1 (n = 17), and all tissues were examined and assigned a grade by a single pathologist (T. M. W.). For the T2/T2aN0 patients, clinical follow-up was for at least 5 years, and patients with increased prostate-specific antigen level to >0.4 ng/ml were assumed to have a clinical recurrence. In addition, 25 lymph node metastatic deposits (of which, 8 were derived from the set of 17 T2N1 patients used in this study) were also examined. The breast tissues included benign epithelium (24 cases), intraductal carcinoma (15 cases), invasive ductal carcinoma (15 cases), and lymph node metastasis (9 cases) and were obtained from the Pathology Department of The Methodist Hospital (Houston, TX).

Immunohistochemistry. Formalin-fixed, paraffin-embedded mouse and human tissue sections were reacted with a polyclonal antiserum against caveolin (C13630; Transduction Labs) at 1:400 dilution and visualized with the ABC detection system (Vector Labs). Control incubations were done using either normal rabbit
sis-related Genes: Identification of Caveolin. To investigate responses to specific growth factors such as transforming growth factor-β1 (9) as well as differential metastatic activities \textit{in vivo} (15). Because these cells have identical metastatic background, originate from primary and metastatic tumors from the same animal, are of low passage, and have been derived in a similar fashion, we used these cells to demonstrate differential gene activities and isolate corresponding cDNA fragments for further analysis (Fig. 1A). One of the fragments detected as differentially expressed in the initial comparison is shown in Fig. 1B. Using primer 3, increased levels of this fragment were detected in 151-2 LMB, a clonal cell line derived from a lung metastasis, relative to that detected in 151-2 PA, a cell line derived from the primary MPR tumor in the same animal. This fragment was isolated and cloned, and 230 bp were sequenced: upon comparison with GenBank version 86.0, the sequence was similar at the nucleotide level to human caveolin-1 cDNA (16). Subsequent comparison with the National Center for Biotechnology Information BLAST WWW server revealed that the 5' end of the DD-PCR fragment was 100% identical over 75 bases of the 3' end of the mouse \textit{caveolin-1} gene (17) and 85% identical over 82 bases of the human \textit{caveolin-1} gene but was not similar to the human \textit{caveolin-2} gene (13).

The cloned DD-PCR fragment was used for Northern blotting analysis to screen an extended panel of primary and metastatic cell lines derived from three different animals, and in every case increased steady-state levels of caveolin mRNA were demonstrated for the metastasis derived cell lines relative to their matched primary cell lines (Fig. 1C). Interestingly, in a comparison of NIH 3T3 cells with polyclonal Zipras/myc 9-infected NIH 3T3 cells, caveolin mRNA levels were reduced in the transformed cells relative to their nontransformed and non-tumorigenic partner, in general agreement with results obtained previously using NIH 3T3 cells with single oncogenes including \textit{ras} (18).

**RESULTS**

MPR Differential Display Cloning System for Metastasis-related Genes: Identification of Caveolin. To investigate the genetic basis of metastasis, we developed a strategy to compare and isolate the species of mRNA expressed by genetically matched pairs of primary and metastatic mouse prostate cancer cell lines, using DD-PCR. These cell lines have been characterized and shown to demonstrate differential responses to specific growth factors such as transforming growth factor-β1 (9) as well as differential metastatic activities \textit{in vivo} (15). Because these cells have identical metastatic background, originate from primary and metastatic tumors from the same animal, are of low passage, and have been derived in a similar fashion, we used these cells to demonstrate differential gene activities and isolate corresponding cDNA fragments for further analysis (Fig. 1A). One of the fragments detected as differentially expressed in the initial comparison is shown in Fig. 1B. Using primer 3, increased levels of this fragment were detected in 151-2 LMB, a clonal cell line derived from a lung metastasis, relative to that detected in 151-2 PA, a cell line derived from the primary MPR tumor in the same animal. This fragment was isolated and cloned, and 230 bp were sequenced: upon comparison with GenBank version 86.0, the sequence was similar at the nucleotide level to human caveolin-1 cDNA (16). Subsequent comparison with the National Center for Biotechnology Information BLAST WWW server revealed that the 5' end of the DD-PCR fragment was 100% identical over 75 bases of the 3' end of the mouse \textit{caveolin-1} gene (17) and 85% identical over 82 bases of the human \textit{caveolin-1} gene but was not similar to the human \textit{caveolin-2} gene (13).

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Elevated Caveolin Protein Levels in Mouse and Human Prostate Cancer Cell Lines Derived from Metastases. To evaluate expression of caveolin at the protein level, pairs of primary as well as metastatic cell lines from three different animals (148-1, 151-1, and 151-2) were compared by Western blotting using a commercially obtained antibody to caveolin. The results (Fig. 2A) demonstrate an overall 2–3-fold increase of caveolin protein in metastatic-derived cell lines relative to their matched primary cell line counterparts. To extend these results to human prostate cancer, similar Western blotting experiments were performed on four human prostate cancer cell lines and a human endothelial cell line (Hum Endo), as a positive control. Three of four of the human prostate cancer cell lines were derived from metastases, and one (ND-1) was derived from a high Gleason grade primary carcinoma. In three of four cases, caveolin protein was abundant (Fig. 2A). The exception was metastasis-derived, androgen-sensitive LNCaP cells. The pattern of localization of caveolin within human prostate cancer cells in vitro was evaluated by immunohistochemistry. In some cells (e.g., ND-1 cells), caveolin was detected within the cytoplasm of cells in a granular pattern (Fig. 2B). Interestingly, in many cases accumulation of caveolin appeared to be localized to substrate attachment sites (Fig. 2C).

Association of Caveolin Expression with Tumor Progression Using Immunohistochemical Staining of Prostate and Breast Cancer Specimens. To validate the in vitro studies using cell lines, a series of immunohistochemical studies were undertaken to assess the pattern and amount of caveolin expression in tissue specimens of both primary and metastatic prostate carcinoma. Initially, specimens derived from normal mouse prostate and primary and metastatic mouse prostate carcinoma generated by the MPR model were analyzed. The results demonstrated only minimal caveolin expression in normal mouse prostate epithelial cells within the prostate gland; however, abundant caveolin staining was observed in smooth muscle cells, which uniformly surround mouse prostate acini as well as endothelial cells in the stromal compartment (Fig. 3A). A diffuse, increased accumulation of caveolin was seen in primary prostate cancer (Fig. 3B), and in the corresponding metastatic cancer cells within the mesentry, higher levels of caveolin appearing as a granular pattern localized near the plasma membrane were seen (Fig. 3C). In normal human prostate, as in the mouse, accumulation was seen in smooth muscle cells as well as endothelial cells with minimal or no staining of ductal or acinar epithelial cells (Fig. 3D). In primary prostate cancer, detectable accumulation of caveolin in malignant cells was occasionally observed (Fig. 3E), whereas in metastatic cancer within lymph nodes, an obvious granular accumulation of caveolin was seen in the carcinoma cells (Fig. 3F).

A semiquantitative scoring system for caveolin staining based on the frequency of caveolin-positive cells was applied to areas of normal human prostate, benign prostatic hyperplasia, and primary and metastatic prostate carcinomas (Table 1). The results indicated a very low frequency (7.7%) of positivity in cases of normal glandular epithelium and low but increased frequency of caveolin-positive epithelium in cases of hyperplasia (17.6%) and primary cancer of low stage (T1/T2,N0; 13.8%).
Fig. 3  Immunostaining with a polyclonal caveolin antibody in mouse (A–C) and human (D–F) prostate and human breast (G–I) tissues. In A, caveolin reactivity was confined to the smooth muscles immediately adjacent to glandular epithelium of normal mouse prostate. In B, punctate immunoreaction product appeared in primary mouse prostate cancer. Increased immunoreactivity localized adjacent to the cell membrane, was present in cancer cells located in the mesentery (C) of the same animal as in B. In normal human prostate, caveolin reactivity was localized in the stroma. Most primary human prostate (E) cancers revealed weak or no (D) caveolin immunoreactivity compared with the granular immunoreaction products seen frequently in metastatic cancer cells located in lymph nodes (F). Caveolin was detected in the myoepithelial cells of normal human breast tissue and not detected in the luminal epithelial cells (G). Prominent granular immunoreaction products accumulated in cancer cells of intraductal carcinoma (H) and infiltrating ductal carcinoma (I).

The T1/T2aN0 patients were subdivided into those who remained cancer free for 5 years after radical prostatectomy and those whose prostate cancer recurred. No statistical difference was seen between the recurrent and nonrecurrent group. Although no association was found between caveolin and prostate cancer recurrence, our sample size was small, which limited our ability to detect any difference. Thus, our lack of a difference may reflect the limited statistical power of our test rather than a true lack of association. Increased frequency of caveolin staining was seen in T3N1 primary cancers of high stage and with nodal metastasis (T3N1; 29.4%), and markedly increased levels of caveolin staining were detected in cancer cells metastatic to lymph nodes (56% $P < 0.01$; Fisher’s Exact test).

To determine whether this phenomenon was shared by other hormone-sensitive adenocarcinomas in humans, a series of breast carcinomas and nonneoplastic breast tissues was also evaluated for caveolin expression using the same staining technique. As in prostate, caveolin staining in normal ductal or lobular epithelial cells was minimal, whereas prominent caveolin staining was observed in the adjacent myoepithelial cells (Fig. 3G). However, intraductal carcinomas stained positive with a similar granular pattern as that observed in metastatic prostate cancer, yet more striking (Fig. 3H). As in prostate cancer, increased levels of caveolin staining were detected in breast cancer cells metastatic to lymph nodes (Fig. 3I). Careful quantitative analysis using the described scoring system confirmed that significantly higher expression of caveolin was detected in intraductal carcinomas relative to normal breast epithelium ($P < 0.001$; Fisher’s Exact test) and a statistically significant increase was demonstrated for infiltrating ductal carcinoma as well as nodal metastases ($P < 0.001$; Fisher’s Exact test for both comparisons; Table 2).
Elevated Caveolin in Prostate and Breast Cancer

Table 1  Caveolin immunostaining in human prostate tissues

<table>
<thead>
<tr>
<th>Prostate specimens</th>
<th>Caveolin</th>
<th>Positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal glandular epithelia</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Hyperplastic epithelia</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Pathological stage of cancers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T N0</td>
<td>29</td>
<td>25</td>
</tr>
<tr>
<td>Recurrent</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>No recurrence</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>T N1</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>Primary cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic site in lymph node</td>
<td>25</td>
<td>11</td>
</tr>
</tbody>
</table>

^ n values denote the number of patients in each group.
^ Positivity was defined as over one measuring field showing granular immunostaining in cancer.
^ Tissues from metastases included eight cases of T N1 stage, for which the primary cancer was also stained.
^ p < 0.01 (Fisher's Exact test) as compared with both normal and hyperplastic epithelia as well as the T N0 cancers.

Table 2  Caveolin immunostaining in human breast tissues

<table>
<thead>
<tr>
<th>Breast specimens</th>
<th>Caveolin</th>
<th>Positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign epithelia</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>Intraductal carcinoma</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Infiltrating ductal carcinoma</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

^ These values are significantly higher than that in the benign breast epithelia (P < 0.001: Fisher's Exact test).

DISCUSSION

We have previously established sets of early-passage cell lines from primary and metastatic mouse prostate cancer that were initiated in the same animal by transduction of ras and myc oncogenes into fetal prostate tissues from p53 knock-out mice, i.e., MPRs (2). These sets of cell lines are clonally tagged by the initiating retrovirus, are early passage, and are genetically and biologically matched, such that the predominant genetic differences between the primary versus metastasis-derived cell lines should be related to the metastatic process. Using this system together with DD-PCR techniques, we identified the caveolin gene as being up-regulated in both mouse and human prostate cancer metastases and interestingly in primary and metastatic breast cancer.

Previous studies of caveolin function in normal cells have revealed its involvement in many biological activities that are germane to cancer progression. Caveolin is a major protein constituent of caveolae, a recognized subcompartment of the plasma membrane and Golgi network (19). Caveolae are strategically positioned to sequester glycosylphosphatidylinositol-linked proteins and apparently organize their interaction with downstream cytoplasmic signal transduction complexes (5, 6, 20). Caveolae identified in nonmalignant cells play important roles in signal transduction (20–22), molecular transport (23), and cellular motility and adhesion (8). In regard to signal transduction, specific molecules involved in transformation have been associated with caveolae including members of the ras family (24), c-src (25, 26), as well as the endothelin receptor (21). Although the specific roles for these molecules in prostate and breast cancer progression remain to be fully elucidated, mutations or aberrant expression of these molecules have been identified in these malignancies (27–29). Caveolae are also involved in the molecular transport of ceramide and cholesterol. Because ceramide has been clearly demonstrated to be involved with apoptotic activities, inappropriate transport of this molecule could perturb the apoptotic pathway and play a role in cancer progression (30). Recently, it was demonstrated that caveolin mRNA levels are up-regulated by free cholesterol in human skin fibroblasts (31), and increased expression of caveolin-1 has been reported in both mouse (32) and human (33) cells with impaired ability to metabolize low density lipoprotein-derived cholesterol. Interestingly, a prospective study of dietary fat and risk of prostate cancer reached the conclusion that advanced prostate cancer was associated with high fat intake, especially fat derived from red meat (34). In addition, caveolin, together with β-1 integrin and uPAR, was identified as components of a functional complex involved in matrix attachment and motility (8), two biological activities that are highly relevant to the metastatic cascade (8, 35, 36).

Although caveolin is involved in numerous biological activities relevant to malignant progression, direct evidence of a role for caveolin in progression of human carcinoma has not been reported. A recent study demonstrated that overexpression of selected dominantly acting oncogenes resulted in suppression of caveolin mRNA and protein levels and fewer caveolae in fibroblastic NIH3T3 cells (18). In this report, it was further shown that caveolin levels were inversely correlated with the size but not the number of colonies produced in soft agar by oncogene-transfected clones. In our study, transformation of NIH3T3 cells with both ras and myc also led to reduced caveolin mRNA (Fig. 1C). The down-regulation of caveolin in transformed NIH3T3 cells appears to contradict the major observations of our present study; however, this may only reflect differences in transformation and selection of immortalized fibroblasts in vitro compared with the malignant progression of cancer cells within prostate or breast tissue in vivo. Previous studies have demonstrated that caveolin expression is associated with the differentiated phenotype in some simple squamous epithelium including capillary endothelial cells, type 1 pneumocytes, and specific mesenchymal cells, including fibroblasts, smooth muscle cells, and adipocytes (37–41). Our analysis demonstrates that caveolin is barely detectable in normal prostate and breast glandular epithelium in vivo, whereas adjacent smooth muscle, endothelial, and breast myoepithelial cells had abundant staining.

The results of this study clearly associate increased accumulation of caveolin with progression of human prostate cancer and with primary and metastatic breast cancer relative to normal epithelium. Prostate cancer cell lines derived from high-grade localized disease (ND-1), as well as metastases, expressed high...
levels of caveolin in vitro. Further analysis using immunohis-
tochemistry indicated extensive accumulation in metastatic dis-
ease. These results were closely mimicked in breast cancer tissues. However, our analyses also demonstrated widespread expression of caveolin in localized breast disease, suggesting that increased caveolin expression occurs earlier in progression of breast cancer relative to prostatic cancer. Alternatively, me-
tastasis without extensive local growth may occur more fre-
quently in caveolin-positive prostate cancer relative to caveolin-
positive breast cancer. Regardless of the timing of elevated caveolin during the progression of these important malignan-
cies, metastatic lesions of both prostate and breast cancer are characterized by significantly increased levels of this protein. Further studies should reveal additional information regarding a potential role of caveolin in prostate and breast cancer progression.

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