Nuclear versus Cytoplasmic Localization of Filamin A in Prostate Cancer: Immunohistochemical Correlation with Metastases

Rohit G. Bedolla,1 Yu Wang,2,3 Alfredo Asuncion,2 Karim Chamie,3 Salma Siddiqui,2 Maria M. Mudryj,2,3 Thomas J. Prihoda,1 Javed Siddiqui,4 Arul M. Chinnaiyan,4 Rohit Mehra,4 Ralph W. de Vere White,3 and Paramita M. Ghosh2,3

Abstract

Purpose: We previously showed that nuclear localization of the actin-binding protein, filamin A (FlnA), corresponded to hormone-dependence in prostate cancer. Intact FlnA (280 kDa, cytoplasmic) cleaved to a 90 kDa fragment which translocated to the nucleus in hormone-naïve cells, whereas in hormone-refractory cells, FlnA was phosphorylated, preventing its cleavage and nuclear translocation. We have examined whether FlnA localization determines a propensity to metastasis in advanced androgen-independent prostate cancer.

Experimental Design: We examined, by immunohistochemistry, FlnA localization in paraffin-embedded human prostate tissue representing different stages of progression. Results were correlated with in vitro studies in a cell model of prostate cancer.

Results: Nuclear FlnA was significantly higher in benign prostate (0.6612 ± 0.5888), prostatic intraepithelial neoplasia (PIN; 0.6024 ± 0.4620), and clinically localized cancers (0.69134 ± 0.5688) compared with metastatic prostate cancers (0.3719 ± 0.4992, P = 0.0007). Cytoplasmic FlnA increased from benign prostate (0.0833 ± 0.2677), PIN (0.1409 ± 0.2293), localized cancers (0.3008 ± 0.3762, P = 0.0150), to metastases (0.7632 ± 0.4414, P < 0.00001). Logistic regression of metastatic versus nonmetastatic tissue yielded the area under the receiver operating curve as 0.67 for nuclear-FlnA, 0.79 for cytoplasmic-FlnA, and 0.82 for both, indicating that metastasis correlates with cytoplasmic to nuclear translocation. In vitro studies showed that cytoplasmic localization of FlnA induced cell invasion whereas nuclear translocation of the protein inhibited it. FlnA dephosphorylation with the protein kinase A inhibitor H-89 facilitated FlnA nuclear translocation, resulting in decreased invasiveness and AR transcriptional activity, and induced sensitivity to androgen withdrawal in hormone-refractory cells.

Conclusions: The data presented in this study indicate that in prostate cancer, metastasis correlates with cytoplasmic localization of FlnA and may be prevented by cleavage and subsequent nuclear translocation of this protein.

Filamins are a family of cytoskeletal proteins that organize filamentous actin into networks and stress fibers (1). Filamin A (FlnA) is a 280 kDa non–muscle actin binding protein, the appropriate function of which is essential for development. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Abstract

Purpose: We previously showed that nuclear localization of the actin-binding protein, filamin A (FlnA), corresponded to hormone-dependence in prostate cancer. Intact FlnA (280 kDa, cytoplasmic) cleaved to a 90 kDa fragment which translocated to the nucleus in hormone-naïve cells, whereas in hormone-refractory cells, FlnA was phosphorylated, preventing its cleavage and nuclear translocation. We have examined whether FlnA localization determines a propensity to metastasis in advanced androgen-independent prostate cancer.

Experimental Design: We examined, by immunohistochemistry, FlnA localization in paraffin-embedded human prostate tissue representing different stages of progression. Results were correlated with in vitro studies in a cell model of prostate cancer.

Results: Nuclear FlnA was significantly higher in benign prostate (0.6612 ± 0.5888), prostatic intraepithelial neoplasia (PIN; 0.6024 ± 0.4620), and clinically localized cancers (0.69134 ± 0.5688) compared with metastatic prostate cancers (0.3719 ± 0.4992, P = 0.0007). Cytoplasmic FlnA increased from benign prostate (0.0833 ± 0.2677), PIN (0.1409 ± 0.2293), localized cancers (0.3008 ± 0.3762, P = 0.0150), to metastases (0.7632 ± 0.4414, P < 0.00001). Logistic regression of metastatic versus nonmetastatic tissue yielded the area under the receiver operating curve as 0.67 for nuclear-FlnA, 0.79 for cytoplasmic-FlnA, and 0.82 for both, indicating that metastasis correlates with cytoplasmic to nuclear translocation. In vitro studies showed that cytoplasmic localization of FlnA induced cell invasion whereas nuclear translocation of the protein inhibited it. FlnA dephosphorylation with the protein kinase A inhibitor H-89 facilitated FlnA nuclear translocation, resulting in decreased invasiveness and AR transcriptional activity, and induced sensitivity to androgen withdrawal in hormone-refractory cells.

Conclusions: The data presented in this study indicate that in prostate cancer, metastasis correlates with cytoplasmic localization of FlnA and may be prevented by cleavage and subsequent nuclear translocation of this protein.

Filamins are a family of cytoskeletal proteins that organize filamentous actin into networks and stress fibers (1). Filamin A (FlnA) is a 280 kDa non–muscle actin binding protein, the appropriate function of which is essential for development.
The data presented in this study indicate that cytoplasmic localization of filamin A (FlnA) correlates to androgen-independent metastatic prostate cancer. A major cause of cytoplasmic retention of FlnA seemed to be failure to cleave due to its phosphorylation. Because protein kinase A (PKA) is a putative kinase for FlnA, our results suggest that in patients with metastatic prostate cancer, FlnA nuclear localization may be restored by protein kinase A inhibitors, which could potentially prevent metastasis. These observations indicate that induction of FlnA cleavage may be of significant clinical relevance in the treatment of patients with advanced prostate cancer.

**Translational Relevance**

Cytoplasmic. Transfection of FlnA16-24 cDNA in C4-2 cells restored the expression and nuclear localization of 90 kDa FlnA. Unlike LNCaP, C4-2 cells proliferate in androgen-reduced medium and in the presence of the AR-antagonist Casodex. Nuclear expression of 90 kDa FlnA in C4-2 cells prevented proliferation in androgen-reduced medium and restored Casodex sensitivity. These results indicated the importance of FlnA localization in prostate cancer cells. However, the relationship between FlnA localization and the metastatic capacity of the cell has not been identified.

FlnA is highly susceptible to proteolysis at the two hinge regions, H1 and H2 (1, 11), which cleaves the protein to a 170 kDa fragment consisting of the ABD and repeats 1 to 15 (FlnA-15) and a 110 kDa protein consisting of repeats 16 to 24 (FlnA16-24) further cleaved to yield a 90 kDa fragment (1, 4). Proteolysis of FlnA is regulated by its phosphorylation on Ser2152 in repeat 20, which renders the protein stable and resistant to cleavage (1). This site is a known substrate for a number of kinases—including protein kinase A (PKA; refs. 12, 13), p90Rsk (14), and protein kinase Cα (15). We previously showed that in C4-2 cells, FlnA is phosphorylated at Ser2152 (10). This resulted in a failure of this molecule to cleave in this model of androgen independence, causing cytoplasmic retention of the protein. Here, we show that these effects are reversed by the PKA inhibitor, H-89.

In this study, we sought to determine FlnA expression across prostate cancer progression and whether localization of FlnA in human prostate cancer corresponded with metastatic potential. We show, by immunohistochemical examination in paraffin-embedded human prostate tissues, that benign prostate, PIN, and localized prostate cancer had predominantly nuclear FlnA expression, whereas in metastatic prostate cancer, FlnA was found to be primarily in the cytoplasm. In *vitro* studies confirmed that cytoplasmic expression of FlnA is required for the ability of cells to invade and migrate. Because FlnA proteolysis is regulated by PKA, we also show that inhibition of PKA activity prevented FlnA-regulated cell invasion. Our data show that FlnA cytoplasmic localization correlated with increased metastatic potential and a hormone-refractory phenotype in prostate cancer.

**Materials and Methods**

**Patients and tissues used.** Tissue microarrays were constructed from benign prostate (*n* = 32), PIN (*n* = 26), localized (*n* = 67), and androgen-independent metastatic (*n* = 69) prostate cancer tissues from the University of Michigan Prostate Cancer Specialized Program of Research Excellence (16, 17). Metastatic tissue were obtained from "rapid" or "warm" autopsies (mean of 3 h lapsed from death to commencement of autopsy) of 26 patients identified with hormone-refractory prostate cancer. The localized tissues consisted of grade 3 (*n* = 10), grade 4 (*n* = 8), grade 6 (*n* = 10), grade 7 (*n* = 4, *n* = 10), grade 7 (*n* = 4, *n* = 9), grade 8 (*n* = 4, *n* = 10), and grade 9 (*n* = 4, *n* = 10) tumors. The metastatic deposits consisted of tissues procured from the following locations: liver, lung, lymph node, seminal vesicle, bone, dura, and bladder. The tissues were formalin-fixed and paraffin-embedded, then the tissue microarrays were constructed with three cores (0.6 mm in diameter) taken from each representative block.

**Immunohistochemistry and tissue microarray evaluation.** We used a mouse monoclonal anti–filamin-1(A) COOH-terminal antibody, clone TI10 (Chemicon), which we validated earlier by Western blotting and immunohistochemistry (10). For details, see supplementary materials. The degree of staining was evaluated blindly in a semiquantitative fashion by a pathologist taking into account both the intensity of staining as well as the percentage of cells exhibiting that intensity. Only the epithelial cells were scored. This is because the stroma, which also stained for FlnA (18), showed uniform staining in all tissues examined. FlnA staining in the epithelial cells was scored separately in the nucleus and the cytoplasm/cytoplasmic membrane and was recorded as a product of the extent and the intensity of the staining. The extent of staining was recorded as positive (score = +1) or negative (score = 0). A specimen was scored +1 in the nucleus if the majority (>80%) of the cells had positive nuclei. Intermediate scores (e.g., 0.5 for 50% of cells staining positively) were also provided. The intensity of the staining was scored 0 to 3, where 0 represented no staining, +1 represented weak, +2 represented intermediate, and +3 represented strong staining. A similar system of scoring was established for cytoplasmic staining and was described earlier (19, 20).

**Statistical analysis.** The subcellular staining expression of FlnA as positive (+1) or negative (0) was tabulated separately for nuclear or cytoplasmic staining according to groups (benign prostate, PIN, localized prostate cancer, and metastatic prostate cancer). Contingency tables with Fisher's exact test were used to compare and assess the differences in the expression of FlnA in the four groups according to subcellular localization (nuclear or cytoplasmic). The interval estimation is given as a 95% confidence interval. The means of staining intensity in the same groups were compared with a two-sample *t* test with Welch approximation for unequal variances when appropriate. The subcellular localization of filamin was further explored with a sensitivity-specificity analysis, using the main effects and a stepwise model including the interaction of the main effects.

**Protein extraction from frozen tissue.** Protein lysates were prepared from flash-frozen tissue obtained from the Cancer Center Specimen Repository of the University of California Davis under an Institutional Review Board–approved protocol. Two normal prostate, nine localized tumors, and two lymph node metastases were used for these studies, as described in the text. For method of extraction, please see Supplemental Materials.

**Cell culture and transfection.** LNCaP cells were purchased from American Type Culture Collection, whereas C4-2 cells were from UroCor. Casodex was kindly provided by Dr. Barry Furr, AstraZeneca, United Kingdom. 4,5-Dihydrotestosterone was obtained from Sigma-Aldrich. pCMV-FlnA (16–24) and pCMV-FlnA (1–15) plasmids were kindly provided by Dr. E.W. Yong, National University of Singapore, Singapore. A human PSA reporter plasmid consisting of the human PSA 5′-flanking region (-631/-1) containing androgen response elements I and II (ARE I and ARE II) tagged to a luciferase construct (hPSA-luc) was kindly provided by Dr. Bandana Chatterjee, University of Texas Health Science Center at San Antonio, San Antonio, TX. FlnA short interfering RNA (siRNA1P; Santa Cruz Biotechnology) with the following sequences: strand no. 1, 5′-CCAUCACUGACACAAAGA-3′;
strand no. 2, 5'-CUGGAGUUGUAU-CAUUGA-3'; and strand no. 3, 5'-GUCACCCCAUCUCUCAAC-3'. Rabbit polyclonal anti-lamin A/C, were from Cell Signaling Technology. Mouse monoclonal anti–COOH-terminal FlnA (MAB1680) was from Chemicon. For in vitro assays, please see Supplementary Materials.

Results

**Differential localization of FlnA across human prostate cancer progression.** To investigate the expression of FlnA in different human prostate tissues, we stained tissue microarrays representing benign prostates, PIN, localized prostate cancer, and metastatic prostate cancer with an anti-FlnA (COOH-terminal) antibody that recognized both the full-length and the 90 kDa FlnA, and counterstained with hematoxylin. Negative controls were stained with a Universal Mouse negative control (Fig. 1A). Normal (nontumor) prostate tissues stained strongly for FlnA in the nucleus and weakly in the cytoplasm (Fig. 1B), as did prostate tissue obtained from radical prostatectomies displaying high-grade PIN (Fig. 1C) and well-differentiated prostate cancer (Fig. 1D). Significantly, the stromal cells also stained strongly for FlnA in the nuclei, but not in the cytoplasm. Epithelial cells from normal prostate, PIN, or localized prostate cancer displayed stronger staining in the nucleus compared with the cytoplasm. On the other hand, metastatic prostate tumor tissues had strong cytoplasmic staining of FlnA but lacked FlnA expression in the nucleus (Fig. 1E and F).

**Decreased nuclear and increased cytoplasmic expression of FlnA in metastatic prostate tumor tissue from patients with hormone-refractory prostate cancer.** Statistical analysis was done to compare the localization and expression of FlnA in prostate epithelial cells from benign prostate (n = 32), PIN (n = 26), and localized (n = 67) and metastatic prostate cancer (n = 69; Fig. 2A). The mean score for normal prostate, PIN, and localized prostate tissues remained at 0.65, whereas that for metastatic prostate tissues decreased to 0.37, a decline of ~43%. In contrast, the corresponding nuclear staining scores for metastatic prostate tissues was significantly lower (P = 0.0007; Table 1), irrespective of the localization of the metastatic deposit. Univariate logistic regression analysis for nuclear FlnA gives an odds ratio of 3.07 (P = 0.001), indicating that decreased nuclear FlnA is a significant protective factor against the development of metastasis. The area under the receiver operating curve for nuclear FlnA alone, which estimates its discriminatory capacity, was 0.67, indicating good discrimination between nonmetastatic and metastatic tissue (Fig. 2B, top).

In contrast to nuclear staining, there was a steady increase in the fraction of prostatic epithelial cells staining positively for FlnA in the cytoplasm from normal tissue (<10%), to PIN (30%), to localized prostate cancer (45%), to metastatic tissue (almost 90%; Fig. 2A). There was a significant increase in cytoplasmic FlnA from PIN to localized tumors (P = 0.0150), although there was no significant correlation between Gleason scores and the degree of staining within the “localized tumor” group (P > 0.05). The increase in cytoplasmic staining from localized to metastasized tumors was highly significant (P < 0.0001; Table 1), and did not depend on the localization of the metastatic deposit. Logistic regression analysis for cytoplasmic FlnA gives an odds ratio of 0.06 (P = 0.0001), indicating that decreased cytoplasmic FlnA is a significant protective factor against the development of metastasis. The area under the receiver operating curve for cytoplasmic FlnA was 0.79, indicating very good discrimination between nonmetastatic and metastatic tissue (Fig. 2B, middle). Multivariate logistical analysis was used to study the joint effect of both nuclear and cytoplasmic FlnA. The area under the receiver operating curve for the interaction of both is 0.82, and the odds ratio increases to 26, indicating excellent discrimination (Fig. 2B, bottom). Taken together, these results indicate that increased FlnA expression in the cytoplasm and decreased expression in the nucleus of prostate epithelial cells correlated with metastatic potential of the cell.

**Prostate tumors express both full-length and cleaved fragments of FlnA.** Previous studies had shown that full-length (280 kDa) FlnA cleaved to a 90 kDa fragment which then translocated to the nucleus (ref. 8; Fig. 3A). The relevance of the 90 kDa FlnA in human tumor is demonstrated in Fig. 3B, which shows differential expression of this fragment in tissues from different patients. Fresh-frozen tissue obtained from patients with prostate cancer were lysed and analyzed by Western blotting (Table 2). All but one metastatic tissue expressed FlnA as determined by the expression of the 280 kDa band, whereas they showed differential expression of the 90 kDa fragment. Significantly, both non–tumor tissues and all but two primary tumors showed significant FlnA cleavage, whereas one of two lymph node metastases lacked FlnA staining altogether (Fig. 3B). There was no correlation between FlnA cleavage and presurgery PSA, Gleason grade, or the percentage of tumor

| Table 1. Analysis of nuclear and cytoplasmic FlnA staining |
|---------------------------------|----------------|----------------|
| **Negative (%)** | **Positive (%)** | **Normal vs. PIN** | **PIN vs. localized** | **Localized vs. metastatic** |
| **Nuclear staining** | | | | |
| Normal | 32 | 8 (25.00) | 24 (75.00) | 0.6716 | 0.4391 | 0.0007* |
| PIN | 26 | 6 (23.08) | 20 (76.92) | | | |
| Localized prostate cancer | 67 | 17 (25.37) | 50 (74.63) | | | |
| Metastatic prostate cancer | 69 | 38 (55.07) | 31 (44.93) | | | |
| **Cytoplasmic staining** | | | | | | |
| Normal | 32 | 29 (90.63) | 3 (9.37) | 0.3811 | 0.0150* | <0.0001* |
| PIN | 26 | 18 (69.23) | 8 (30.77) | | | |
| Localized prostate cancer | 67 | 37 (55.22) | 30 (44.78) | | | |
| Metastatic prostate cancer | 69 | 8 (11.59) | 61 (88.41) | | | |

*Significant.
involvement, suggesting that FlmA was an indicator of response to hormone therapy alone.

Effect of nuclear and cytoplasmic FlmA on cell migration and invasion. Because metastasis correlated with both increased cytoplasmic localization and decreased nuclear localization of FlmA, we investigated whether the presence of FlmA in the cytoplasm or the absence of FlmA from the nucleus promoted metastasis. Androgen-dependent LNCaP cells expressed FlmA in both the nucleus and the cytoplasm, whereas its androgen-independent subline C4-2 expressed FlmA only in the cytoplasm (ref. 10; see also Supplementary Fig. S1). Hence, these cells would allow us to distinguish between the effects of cytoplasmic and nuclear FlmA. Androgen-independent C4-2 cells, which express cytoplasmic (full-length) FlmA only (10), and no nuclear (90 kDa) FlmA, expressed >2-fold increased migration rates compared with parental androgen-dependent LNCaP cells (Fig. 3C). Hence, we investigated whether the increased migration in C4-2 cells was due to the lack of nuclear FlmA. We previously showed that expression of FlmA16-24 in C4-2 cells caused the transfected fragment to localize to the nucleus (10). Expression of FlmA16-24 significantly suppressed cell migration in C4-2 cells ($P = 0.0126$; Fig. 3C). Similarly, the rate of cell invasion also increased >2-fold in C4-2 cells compared with LNCaP ($P < 0.001$), and this effect was significantly inhibited by the expression of FlmA16-24 ($P < 0.001$; Fig. 3D). However, in LNCaP cells, which spontaneously cleaved FlmA and expressed it in the nucleus, overexpression of FlmA16-24 did not affect the rate of cell migration (Fig. 3D), likely indicating that the presence of nuclear FlmA in LNCaP cells was sufficient for suppressing invasion in the first place. In addition, we down-regulated FlmA expression with a pool of three FlmA siRNA described earlier (10), which in C4-2 cells decreased cytoplasmic FlmA only. Transfection with FlmA siRNA, but not control siRNA, resulted in decreased FlmA expression and decreased invasiveness of both LNCaP and C4-2 cells (Fig. 3D). These results indicated that the expression of FlmA in the...
cytoplasm promotes cell invasion whereas its expression in the nucleus suppresses it.

*Increased FlnA cleavage and cytoplasmic to nuclear translocation prevented cell invasion.* The above indicated that FlnA translocation from the cytoplasm to the nucleus inhibits cell invasion. Hence, we designed methods to induce FlnA nuclear translocation. Because in C4-2 cells, FlnA was cytoplasmic due to a failure to cleave and introduction of the cleaved FlnA

![Fig. 2](image_url)

**A.** Mean intensity of tissues staining positively for FlnA in the nucleus (left) and cytoplasm (right) of normal prostate tissue (top; n = 32), PIN (second row; n = 26), localized prostate tumors (third row; n = 67), and distant metastases (bottom; n = 69).

**B.** Area under the receiver operating curve (AUC) to determine the discriminatory capacity of nuclear (top), cytoplasmic (middle), and both nuclear and cytoplasmic FlnA (bottom) between metastatic and nonmetastatic tissue.
fragment induced nuclear translocation (10), we reasoned that if FlnA can be persuaded to undergo cleavage in these cells, then the 90 kDa would translocate to the nucleus and inhibit invasion and migration. Studies have shown that phosphorylation of FlnA at Ser^{2152} rendered the protein resistant to cleavage (1), and we showed that FlnA was phosphorylated at Ser^{2152} in C4-2 but not LNCaP cells (10). Hence, we investigated whether dephosphorylation of FlnA at this site induced FlnA cleavage and nuclear translocation. Ser^{2152} on FlnA is a consensus site for cyclic AMP (cAMP)-dependent

Table 2. Description of frozen prostatic tissue used in Fig. 3B

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Description</th>
<th>Gleason score</th>
<th>Tumor involvement (%)</th>
<th>Preprostatectomy PSA (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal prostate</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Normal prostate</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Localized tumor</td>
<td>6</td>
<td>80</td>
<td>17.3</td>
</tr>
<tr>
<td>4</td>
<td>Localized tumor</td>
<td>7</td>
<td>40</td>
<td>10.3</td>
</tr>
<tr>
<td>5</td>
<td>Localized tumor</td>
<td>6</td>
<td>30</td>
<td>6.3</td>
</tr>
<tr>
<td>6</td>
<td>Localized tumor</td>
<td>7</td>
<td>50</td>
<td>20.6</td>
</tr>
<tr>
<td>7</td>
<td>Localized tumor</td>
<td>7</td>
<td>40</td>
<td>11.3</td>
</tr>
<tr>
<td>8</td>
<td>Localized tumor</td>
<td>9</td>
<td>30</td>
<td>14.0</td>
</tr>
<tr>
<td>9</td>
<td>Localized tumor</td>
<td>6</td>
<td>40</td>
<td>9.5</td>
</tr>
<tr>
<td>10</td>
<td>Localized tumor</td>
<td>7</td>
<td>50</td>
<td>7.4</td>
</tr>
<tr>
<td>11</td>
<td>Localized tumor</td>
<td>7</td>
<td>80</td>
<td>11.9</td>
</tr>
<tr>
<td>12</td>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. A, proteolysis of FlnA. B, to detect FlnA cleavage in human tumors, fresh-frozen sections from 2 normal prostates (lanes 1 and 2), and 11 tumors (primary, lanes 3-11; metastases, lanes 12-13) obtained by laser capture of epithelial cells selectively were examined by Western blotting. All but one sample showed expression of the 280 kDa full-length FlnA, whereas three lacked expression of the 90 kDa band. C, quantification of cell migration assay. LNCaP cells were less migratory compared with C4-2 cells but transfection of the latter with FlnA16-24 completely inhibited cell migration. For expression of transfected FlnA, see Supplementary Fig. 2A. D, invasion assay in LNCaP and C4-2 cells. Transfection of FlnA1-15 (170 kDa) did not affect the ability of these cells to invade, whereas FlnA16-24 inhibited invasiveness in C4-2 cells which lack nuclear FlnA but not in LNCaP cells which express nuclear FlnA. Depletion of cells with a FlnA siRNA prevented invasiveness in both LNCaP and C4-2 cells. For the expression of FlnA down-regulated with siRNA, see Supplementary Fig. 2B.


Research.
PKA activity (12, 13), hence, we examined if stimulation of PKA activity by the cAMP analogue chlorophenylthio-cAMP (CPT-cAMP, 100 μM) or its inhibition by the PKA inhibitor H-89 (1 μM) altered FlnA localization. Figure 4A (top) shows that in LNCaP cells, increased cAMP levels by CPT-cAMP treatment decreased FlnA cleavage to the 90 kDa fragment, whereas H-89 increased the levels of the cleaved fragments. H-89, but not CPT-cAMP, induced nuclear localization of the 90 kDa FlnA fragment, suggesting FlnA cleavage and nuclear translocation (Fig. 4A, bottom; see also Supplementary Fig. S3). These results indicate that C4-2 cells, which lack FlnA nuclear localization due to increased cAMP levels and PKA activation, can be induced to undergo FlnA cleavage and consequent nuclear translocation by the inhibition of PKA activation. Treatment with H-89, but not CPT-cAMP or Casodex, prevented the invasive nature of C4-2 cells (Fig. 4B). In addition, we had previously shown that the cytoplasmic to nuclear translocation of the 90 kDa FlnA fragment promoted androgen sensitivity in androgen-independent C4-2 cells (10). In support of this observation, 8 days of culture in androgen-reduced medium induced growth arrest in LNCaP, but not in C4-2 cells, an effect reversed by treatment with the PKA inhibitor H-89 (1 μM; Fig. 4C). H-89 also prevented AR transcriptional activity (Fig. 4D). Taken together, these results point to an important role for FlnA in cell migration and invasion in prostate cancer cells, which is regulated by its phosphorylation by a PKA-dependent mechanism.

Discussion

The salient feature of the data presented here is that FlnA translocation from the cytoplasm to the nucleus prevents metastasis in prostate cancer. We show by immunohistochemistry in paraffin-embedded human tissue that in normal prostate, PIN, and localized prostate cancer, FlnA is mostly nuclear, whereas in metastatic tissue, it is mostly cytoplasmic. In vitro
localization of nuclear FlnA as well as inhibition of FlnA in the cytoplasm prevented cell migration and invasion. Induction of FlnA cleavage by PKA inhibition promoted cytoplasm-to-nucleus translocation and prevented invasion. This is the first time that alterations in the distribution of FlnA with progression of prostate cancer in humans have been defined.

Filamins are a family of cytoskeletal proteins consisting of three members—filamins A and B are expressed in non–muscle cells whereas filamin C is expressed in muscles (1). FlnA (280 kDa) is highly susceptible to cleavage by calpains and caspases (1, 11), resulting in two fragments—an NH2-terminal 170 kDa fragment and a COOH-terminal 110 kDa fragment which is further cleaved to yield a 90 kDa fragment (1, 4). We previously showed that in androgen-dependent LNCaP prostate cancer cells, the cleaved 90 kDa fragment is localized to the nucleus whereas in its androgen-independent subline, C4-2, FlnA failed to cleave and remained cytoplasmic (10).

In the current study, we sought to examine the localization of FlnA in human prostate tissue. FlnA is expressed in both the stroma and in the epithelia, thus demonstrating the importance of this protein in prostate development. Nuclear localization of FlnA seems to be the normal configuration in the prostatic epithelium. The mean cytoplasmic expression of FlnA was significantly higher in localized prostate cancer, but there was no direct correlation in this cohort with Gleason scores. However, there is a substantial increase in cytoplasmic staining for FlnA in metastatic tissue, suggesting a role for FlnA in prostate cancer metastases, likely involving cell migration.

Significantly, nuclear translocation of the 90 kDa fragment inhibited the invasiveness of the cell. Further studies are needed to determine how the cell transmits signals to prevent invasion by this cytoplasm-to-nucleus translocation. Our previous studies had shown that in androgen-dependent LNCaP cells, FlnA is cleaved and translocated to the nucleus whereas in its androgen-independent subline C4-2, FlnA was phosphorylated at Ser2152, rendering the protein resistant to cleavage and preventing this translocation. Because Ser2152 is a PKA consensus site (12, 13), we investigated whether inhibition of PKA activity affected the ability of the cells to invade. The PKA inhibitor H-89 induced cleavage of FlnA to the 90 kDa fragment, increased the ability of this fragment to translocate to the nucleus in C4-2 cells, and prevented the invasive quality of C4-2 cells. In addition, it also inhibited AR transcriptional activity and the ability of these cells to grow in low-androgen medium.

These results show that FlnA has multiple roles in a cell. In the cytoplasm, FlnA acts as an actin-binding protein, and helps increase cell motility. The role of FlnA in cell motility is well known and has been documented in numerous cell models, although not in prostate cancer. FlnA was shown to be essential in PAK1-mediated cytoskeletal assembly and regulated ruffle-forming, which is part of the response of cells to external stimuli resulting in alterations in cell shape, adhesiveness, and locomotion (21). FlnA has been shown to play an essential role in neuronal migration (22), and has also been shown to mediate cell adhesion and migration stimulated by tissue factor, the protease receptor initiating the coagulation system, functions in vascular development, angiogenesis, and tumor cell metastasis (23). Recent studies also indicate a role for FlnA in heregulin-stimulated cell migration and cell growth in an ovarian cancer cell line (24). Hence, a role for FlnA in prostate cancer metastasis and migration are understandable and are to be expected.

Once its role in cell motility and migration is complete, FlnA undergoes proteolysis and is cleaved to the 90 kDa fragment, which then translocates to the nucleus, where it helps maintain androgen dependence (10). Hence, in the normal adult prostate, which does not require cell motility, FlnA is quickly cleaved and translocated to the nucleus. This is probably the cause for the lack of cytoplasmic FlnA in normal prostate and its low level of expression in PIN. However, in prostate cancer, FlnA is retained in the cytoplasm, which promotes motility in the cell. Because approximately half the localized tumors examined express cytoplasmic FlnA, our results suggest, and it remains to be proven, whether many of the localized tissues have the potential to undergo metastasis if stimulated to do so by other means, such as growth factor stimulation as previously shown in other cell systems (24).

In conclusion, our studies showed that metastatic prostate cancer tissues express high levels of cytoplasmic FlnA and low levels of nuclear FlnA compared with localized cancers. Furthermore, we showed that cell invasion and motility is regulated by the localization of this protein in in vitro studies and also shows that inhibition of PKA is likely an important way of regulating the activity of FlnA. This is an important point because if FlnA cytoplasmic localization is a cause of metastasis, then the presence of FlnA in the cytoplasm may be used as a predictive marker of future metastasis, whereas inhibition of PKA may be used as a therapeutic tool to prevent this effect.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We are grateful to Dr. Regina Gandour-Edwards and Stephanie Soares of the Cancer Center Specimen Repository and Dr. Xu Bao Shi, Department of Urology, University of California Davis for the frozen prostate tumor specimens used in this study, and to Dr. Naveen K. Krishnegowda, Department of Surgery, UT Health Science Center, San Antonio, TX, for technical help.

References


Nuclear versus Cytoplasmic Localization of Filamin A in Prostate Cancer: Immunohistochemical Correlation with Metastases


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/15/3/788

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2009/02/04/15.3.788.DC1

Cited articles
This article cites 24 articles, 12 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/15/3/788.full.html#ref-list-1

Citing articles
This article has been cited by 13 HighWire-hosted articles. Access the articles at:
/content/15/3/788.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.