

Vav3 oncogene is involved in regulation of secretory phospholipase A2-IIa expression in prostate cancer

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Abstract. Our previous study revealed that Vav3 oncogene and secretory phospholipase A2-IIa (sPLA2-IIa) are overexpressed in androgen-independent prostate cancer cells relative to their androgen-dependent counterparts and contribute to development of hormone refractory prostate cancer. Vav3 is a multiple function protein with both signaling molecule and coactivator activities. sPLA2-IIa is a downstream effector of HER/HER2-PI3K-Akt-NF- κ B signaling and involved in inflammatory response and tumorigenesis. The aim of the current study was to determine whether Vav3 is involved in up-regulation of sPLA2-IIa expression, given that Vav3 signals in the HER/HER2-elicited pathway. Among 46 prostate cancer specimens examined, Vav3 and sPLA2-IIa are overexpressed in 48 and 83% human prostate cancers, respectively. Vav3 overexpression is significantly associated with a high level expression of sPLA2-IIa. In addition, significant Vav3 nuclear localization is observed in two prostate cancer specimens, supporting a coactivator activity in prostate cancer cells. Further analysis revealed that Vav3 up-regulates expression of the sPLA2-IIa gene at the transcriptional level via HER/HER2-PI3K-Akt-NF- κ B signaling. These data revealed that Vav3 overexpression as an additional underlying mechanism contributes to elevated sPLA2-IIa expression in prostate cancer.

Introduction

Vav proteins are a family of proteins with multiple function motifs and involved in various cellular signaling processes, including cytoskeleton organization, calcium influx, phagocytosis, and cell transformation (1). Vav3 functions as a signaling molecule, which is directly or indirectly activated

by receptor protein tyrosine kinase (RPTK) in various signal transduction pathways, such as EphA receptor and EGFR (2,3). Vav3 binds to several partners, including Rac1, Cdc42, PI3K, Grb2, and PLC- γ , leading to alteration in cell morphology and cell transformation (4). Overexpression of Vav3 leads to PI3K activation and focus formation in NH3T3 cells, and blocking PI3K activity by PTEN and LY294002 efficiently inhibits Vav3-induced cell transformation activity (5). Recently, we and others found that Vav3 oncogene is overexpressed in several lines of prostate cancer cells (6,7). Vav3 enhances androgen receptor (AR) activity and stimulates androgen-independent growth in prostate cancer cells partially via PI3K-Akt signaling (7). Vav3 functions as a signaling molecule in the HER/HER2-elicited pathway and coactivator for AR. We further showed that the targeted overexpression of a constitutive active Vav3 in the epithelium of the prostate gland induced prostatic intraepithelial neoplasia (mPIN) and prostate cancer as a result of an elevated AR signaling axis and PI3K-Akt signaling in the prostate gland (8). Vav3 overexpression also led to significant chronic non-bacterial inflammation in the prostate gland, which was associated with elevated incidence of prostate cancer. Vav3 up-regulated NF- κ B activity in prostate cancer cells partially via PI3K-Akt signaling. These data suggest that Vav3 overexpression enhances both AR signaling axis and NF- κ B-mediated pathway contributing to the development of non-bacterial prostatitis and prostate cancer.

Phospholipases A2 (PLA2s) are phospholipid hydrolase enzymes that mediate the release of biologically active fatty acids and lysophospholipids such as arachidonic acid (AA) and lysophosphatidylcholine, which are the precursors of eicosanoids and platelet-activating factor, respectively (9,10). It was reported that secretory phospholipase A2-IIa (sPLA2-IIa) is overexpressed in almost all human prostate cancer specimens and elevated levels correlate with tumor grade (11-13). sPLA2-IIa remains elevated in androgen-independent prostate cancers failing hormonal treatment (14). sPLA2-IIa stimulates tumor cell growth (14,15). Elevated sPLA2-IIa expression is associated with androgen-independence and a more aggressive cancer phenotype in the spontaneous TRAMP prostate cancer model (16). sPLA2-IIa is a NF- κ B target gene (17,18). We recently showed that sPLA2-IIa is overexpressed in androgen-independent prostate cancer LNCaP-AI cells relative to their parental androgen-dependent cell line LNCaP,

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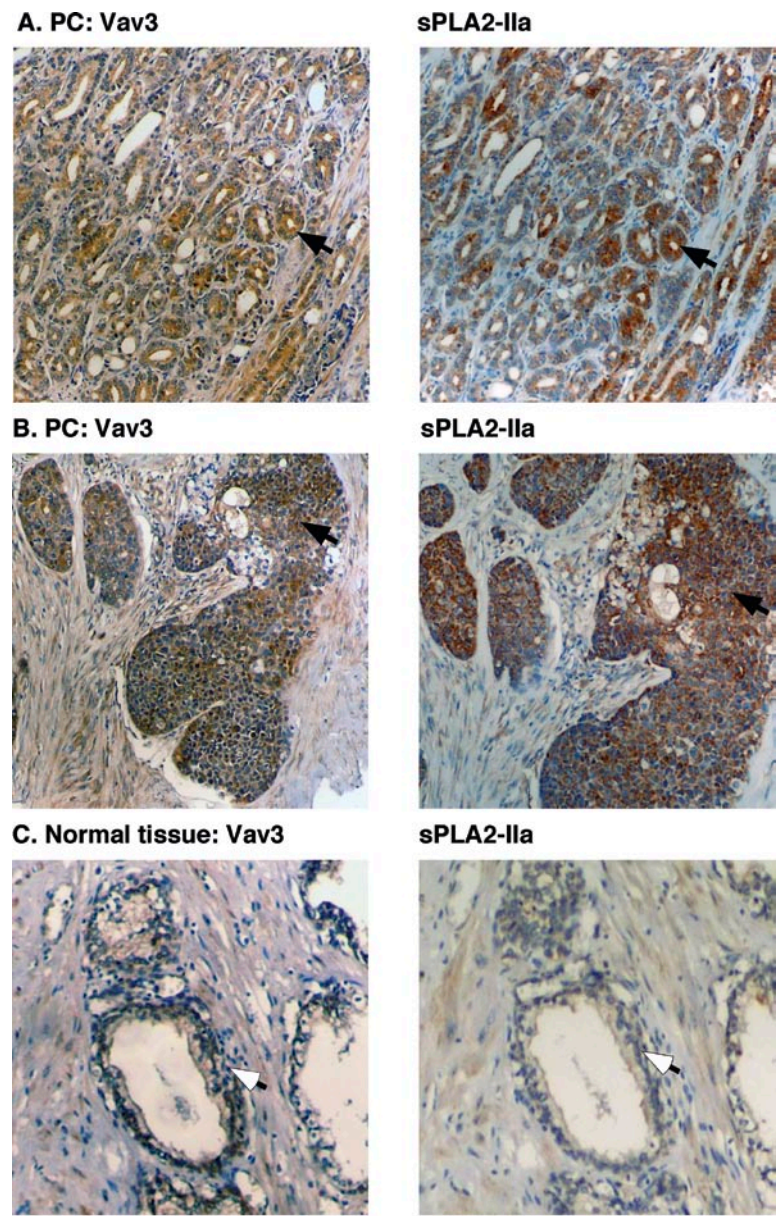


Figure 1. Expression analysis of Vav3 and sPLA2-IIa in prostate cancer specimens. Solid arrow indicates positive staining in corresponding section of prostate cancer tissue, while open arrow indicates negative staining in normal prostate tissue. A significant cytoplasmic staining of both Vav3 and sPLA2-IIa is shown in prostate cancer specimen from the same patients.

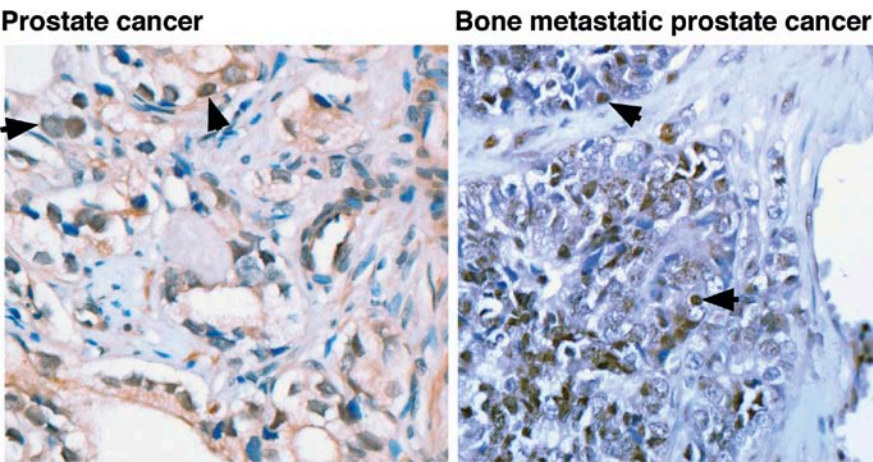


Figure 2. A significant nuclear localization of Vav3 in prostate cancer specimens. Solid arrow indicates positive staining in nucleus. A significant nuclear localization of Vav3 was identified in a prostate cancer specimen and a bone metastatic prostate cancer.

Table I. Vav3 overexpression is associated with an increased expression of the sPLA2-IIa gene by IHC analysis.

	sPLA2-IIa staining intensity		Total cases
	0, 1 (%)	2, 3, 4 (%)	
Vav3 staining intensity			
0,1	14 (48)	5 (52)	29
2, 3, 4	2 (12)	15 (88)	17

Pearson χ^2 test, P=0.01.

which may contribute to androgen-independent growth (18). Elevated signaling of the HER/HER2-PI3K-Akt-NF- κ B pathway contributes to sPLA2-IIa overexpression and secretion in prostate cancer cells (18). We are the first to show that there is an elevated level of serum sPLA2-IIa in prostate cancer patients and serum sPLA2-IIa is a potential biomarker for prostate cancer.

The current study is to determine whether Vav3 overexpression contributes to an elevated level of sPLA2-IIa in prostate cancer. We found that Vav3 overexpression is significantly associated with a high level expression of sPLA2-IIa in prostate cancer specimens examined. A significant nuclear localization of Vav3 was found in two prostate cancer specimens, although Vav3 is mainly localized in cytoplasm in majority of prostate cancer specimens and prostate cancer cell lines. Vav3 up-regulates expression of the sPLA2-IIa gene at the transcriptional level. These data suggest that Vav3 overexpression as an additional underlying mechanism contributes to the elevated sPLA2-IIa expression in prostate cancer.

Materials and methods

Reagents. Plasmid sPLA2-IIa(-800)-Luc was generated by insertion of the PCR promoter fragment into pGL5-Luc vector, which has been described previously (18). Anti-Vav3 antibodies were obtained from Upstate Biotechnology (Charlottesville, VA). Anti-sPLA2-IIa antibody was obtained from Cayman Chemical (Ann Arbor, MI). Prostate disease spectrum tissue array was purchased from Biomatrix US (Rockville, MD). Some of the prostate cancer specimens were provided by the Cancer Center Tissue Bank, University of Cincinnati.

Cell culture. The human prostate adenocarcinoma cell line LNCaP was obtained from ATCC (Rockville, MD) and maintained in RPMI-1640 medium supplemented with 10% FBS (complete medium) at 37°C in 5% CO₂. LNCaP-AI cells were maintained in RPMI-1640 medium supplemented with 10% charcoal/dextran-treated FBS (stripped medium). LAPC-4 cells, which express wild-type AR, were maintained in Iscove's modified Dulbecco's medium supplemented with 10% FBS and 10 nmol/l DHT. Transient transfection experiments were performed in stripped medium.

Reporter assay. Cells (10⁵/well) were seeded in 12-well tissue culture plates. Next day, Optifect-mediated transfection was used for the transient transfection assay according to the protocol provided by Invitrogen/Life Technologies, Inc. The cells were then treated with drugs in stripped medium for 24 h. Subsequently, the cell extracts were prepared and luciferase activity was assessed in a Berthold Detection System (Pforzheim, Germany) using a kit (Promega, Madison, WI) following the manufacturer's instruction. For each assay, cell extract (20 μ l) was used and the reaction was started by injection of 50 μ l of luciferase substrate. Each reaction was measured for 10 sec in the Luminometer. Luciferase activity was defined as light units/mg protein.

Immunohistochemical (IHC) staining. IHC staining was performed as detailed in our previous studies (7). Briefly, paraffin-embedded tissue sections were deparaffinized in xylene, rehydrated in graded alcohol, and transferred to PBS. The slides were treated with a citric acid-based antigen-retrieval buffer (Dako Co., Carpinteria, CA), followed by 3% H₂O₂ in methanol, incubated in blocking buffer (5% BSA and 5% horse serum in PBS) and then in the blocking buffer containing first antibody. After washing, the slides were incubated with a biotinylated secondary antibody (Bio-Genex Laboratories, San Ramon, CA), followed by washing and incubation with the streptavidin-conjugated peroxidase (BioGenex). A positive reaction was visualized by incubating the slides with stable diaminobenzidine and counterstaining with Gill's hematoxylin (BioGenex) and mounted with Universal Mount mounting medium (Fisher Scientific, Pittsburgh, PA). The intensity and extent of cytoplasm-positive labeling for sPLA2-IIa in tissue arrays were assessed semiquantitatively and scored as 0 (no staining), 1+ (weak and focal staining in <25% of tissue), 2+ (moderate intensity in 25-50% of tissue), 3+ (moderate intensity in >50% of tissue), and 4+ (strong and diffused staining in >50% of tissue).

Statistical analysis. Pearson χ^2 test was used to determine whether Vav3 overexpression is significantly associated with an elevated level of sPLA2-IIa expression in prostate cancer specimens.

Results

Vav3 overexpression is associated with an elevated level of sPLA2-IIa in prostate cancer. We determined expression of Vav3 and sPLA2-IIa genes in prostate cancer specimens. Among 46 biopsies examined by IHC, sPLA2-IIa is overexpressed in 83% of prostate cancer specimens, while Vav3 is overexpressed in 48% of prostate cancer specimens (Fig. 1). Low positive rate of sPLA2-IIa staining relative to previous reports (11-13) may be due to the nature of heterogeneous gene expression in cancer and tissue array specimens in which insufficient specimens were provided. Further analysis revealed that many prostate cancer specimens overexpressed both Vav3 and sPLA2-IIa. Vav3 overexpression is significantly associated with a high level expression of sPLA2-IIa (Pearson χ^2 test, P=0.01) (Table I). Our previous studies showed that Vav3 is mainly localized in cytoplasm in cell lines and

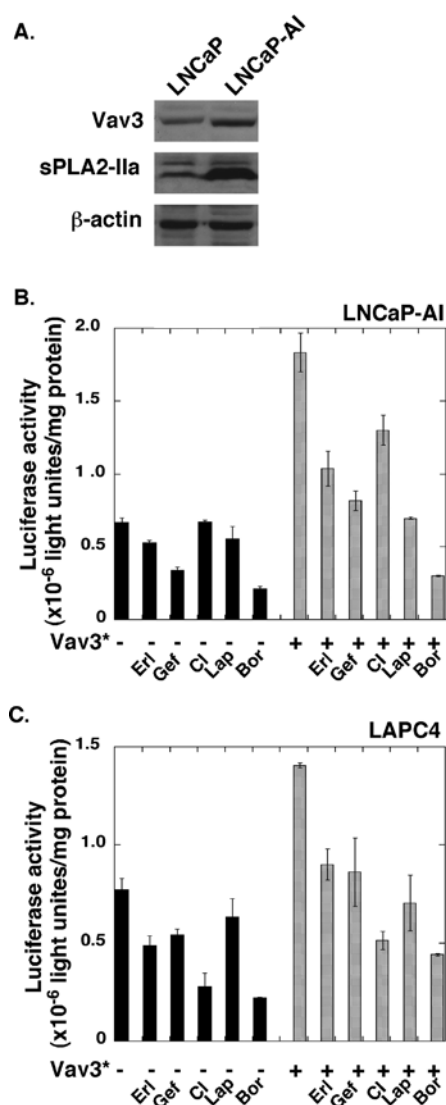


Figure 3. HER/HER2-PI3K-Akt-NF- κ B signaling regulates promoter activity of the sPLA2-IIa gene. (A) Expression of Vav3 and sPLA2-IIa in LNCaP and LNCaP-AI cells. (B and C) LNCaP-AI (B) and LAPC4 (C) cells were transiently transfected with sPLA2-IIa(-800)-Luc (0.5 μ g) and a expression vector (0.25 μ g) encoding a constitutively active Vav3* overnight. The cells were then treated with erlotinib (Erl, 10 μ M), gefitinib (Gef, 10 μ M), lapatinib (Lap, 10 μ M), CI-1033 (CI, 4 μ M), and bortezomib (Bor, 5 μ M) for 24 h. Luciferase assay was performed according to a standard protocol with Renilla luciferase as an internal control. Data are presented as the mean \pm SD of duplicate values of a representative experiment that was independently repeated five times.

specimens of both prostate and breast cancer, although it is a nuclear protein with the nuclear localization signal (Fig. 1) (7,19). Here, we identified a significant nuclear localization of Vav3 protein in two prostate cancer specimens, supporting its preference to regulate gene expression at the transcriptional level rather than as signal molecule in some cancer cells (Fig. 2).

Vav3 up-regulates sPLA2-IIa gene expression at the transcriptional level. We have shown that both Vav3 and sPLA2-IIa are overexpressed in androgen-independent LNCaP-AI cells relative to their androgen-dependent counterpart LNCaP cells (Fig. 3A) (7,18). The HER/HER2-PI3K-Akt-NF- κ B pathway

contributes to sPLA2-IIa overexpression and secretion in prostate cancer cells (18). Given that Vav3 is a signaling molecule in the HER/HER2-elicited pathway, we determined whether Vav3 regulates sPLA2-IIa expression and whether EGFR inhibitors erlotinib and gefitinib, EGFR and HER2 dual inhibitors lapatinib and CI-1033, and NF- κ B inhibitor bortezomib could suppress basal and Vav3-mediated expression of the sPLA2-IIa gene. LNCaP-AI and LAPC4 cells were transiently cotransfected with sPLA2-IIa luciferase reporter sPLA2-IIa(-800)-Luc and expression vector including a constitutively active Vav3*. As shown in Fig. 3B and C, overexpression of Vav3* enhanced the basal promoter activity of the sPLA2-IIa gene by 1.7-fold in LNCaP-AI and 0.8-fold LAPC4 cells. Vav3-elevated activity of the sPLA2-IIa promoter was inhibited by erlotinib, gefitinib, lapatinib, CI-1033, and bortezomib. Among these inhibitors, bortezomib is the most potent inhibitor for the promoter of the sPLA2-IIa gene in both prostate cancer cells. This finding is consistent with the observation that high level expression of Vav3 is associated with an increased expression of sPLA2-IIa in prostate cancer specimens (Fig. 1 and Table I). These data indicate that Vav3 overexpression contributes to an enhanced signaling of the HER/HER2-PI3K-Akt-NF- κ B pathway leading to an increased expression of the sPLA2-IIa gene.

Discussion

Our previous study showed that Vav3 is overexpressed in human prostate cancer, stimulates prostate cancer cell growth, and enhances AR signaling axis partially via the PI3K-Akt pathway (7). Vav3 transgenic mice, a genetically engineered mouse prostatitis and prostate cancer model, showed that elevated Vav3 function leads to enhanced signaling levels in both NF- κ B- and AR-mediated pathways, partially via the PI3K-Akt signaling and induces non-bacterial chronic inflammatory response and cancer development in the prostate gland. Furthermore, we found that elevated signaling of the HER/HER2-PI3K-Akt-NF- κ B pathway contributes to sPLA2-IIa overexpression and secretion (18). Given the role of Vav3 in HER/HER2-elicited signaling pathway and downstream effector nature of sPLA2-IIa in the pathway, we investigated whether Vav3 regulates sPLA2-IIa expression. We found that Vav3 overexpression is significantly associated with a high level expression of sPLA2-IIa in prostate cancer. Vav3 up-regulates expression of the sPLA2-IIa gene at the transcriptional level. A significant nuclear localization of Vav3 in two prostate cancer specimens further supports its role as coactivator in regulation of gene expression. These data suggest that Vav3 overexpression as an additional underlying mechanism contributes to the elevated sPLA2-IIa expression in prostate cancer.

It was widely accepted that inflammation contributes to cancer development (20,21). Many cancers, such as hepatocellular carcinoma, stomach cancer, and colorectal carcinoma, arise from chronic infection and inflammation. Compelling data indicate that inflammation is also associated with prostate cancer (22). The source of intraprostatic inflammation is, however, largely unknown, although multiple factors, such as infectious agents, dietary carcinogens, hormone change, and physical trauma, may contribute to inflammation in the pros-

tate gland. Chronic inflammation results in a sustained innate immune response, which creates a microenvironment rich in cytokines, chemokines, growth factors, and angiogenesis factors and fosters proliferation and survival, a critical step for carcinogenesis (20,21). The nuclear factor- κ B (NF- κ B) is a key linking molecule in inflammation and immunity to cancer development and progression (23,24). Various carcinogens, oncogenes, and cell signaling pathways, such as PI3K-Akt signaling (25-27), activate NF- κ B, which in turn leads to expression of inflammatory cytokines and growth factors, blocking of apoptosis, promotion of proliferation, angiogenesis, and tumor invasion process. Previously, we demonstrated that Vav3 overexpression enhances activity of NF- κ B in prostate cancer cells, suggesting a role in inflammatory response in cancer (8,28).

Eicosanoids are products of both sPLA2-IIa and cyclooxygenase-2 (Cox-2) and exert control over many physiologic processes, such as inflammation and immunity. Recent findings implicate an elevated eicosanoid signaling in the pathogenesis of prostate cancer and its progression. Multiple key genes in the eicosanoid biosynthetic pathway, e.g. NF- κ B (29,30), Cox-2 (31-33), and sPLA2-IIa (11-13), are overexpressed in prostate cancer and associated with disease progression. A few early studies have demonstrated that sPLA2-IIa is overexpressed in almost all human prostate cancer specimens and elevated levels of sPLA2-IIa are associated with tumor grade (11-13). sPLA2-IIa remains elevated in androgen-independent prostate cancers failing hormonal therapy (14). Recently, we showed that an elevated signaling of the HER/HER2-PI3K-Akt-NF- κ B pathway contributes to sPLA2-IIa overexpression and secretion in prostate cancer cells (18). The current study revealed that Vav3 is involved in up-regulation of sPLA2-IIa gene expression. This finding reveals that Vav3 overexpression as a novel underlying molecular mechanism leads to an aberrant activation of sPLA2-IIa gene expression in prostate cancer cells.

In summary, we found that both Vav3 and sPLA2-IIa are overexpressed in prostate cancer. Vav3 significantly stimulates sPLA2-IIa expression via the HER/HER2-PI3K-Akt-NF- κ B pathway. The up-regulation of sPLA2-IIa gene expression by Vav3 is mediated at the transcriptional level. Our finding supports the notion that altered function of multiple genes in the HER/HER2-elicited signaling pathway contributes to overexpression of effector gene sPLA2-IIa in prostate cancer cells.

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