Abstract

STAMP1 is predicted to encode a six-transmembrane protein whose expression is highly prostate enriched and is deregulated in prostate cancer. However, the biological role of STAMP1 in prostate cancer cells, or its expression profile at the protein level, is unknown. Here, we find that ectopic expression of STAMP1 significantly increased proliferation of DU145 prostate cancer cells as well as COS-7 cells in vitro; conversely, small interfering RNA–mediated knockdown of STAMP1 expression in LNCaP cells inhibited cell growth and, at least partially, induced cell cycle arrest. In parallel, there were alterations in cell cycle–regulatory gene expression. Knockdown of STAMP1 expression in LNCaP cells also induced significant apoptosis under basal conditions as well as in response to tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) alone, or TRAIL + AKT inhibitor LY294002, previously established apoptotic agents in LNCaP cells. Consistently, LNCaP cells with short hairpin RNA–mediated knockdown of STAMP1 were dramatically retarded in their ability to grow as xenografts in nude mice. Interestingly, activation of extracellular signal-regulated kinase, which has previously been implicated in prostate cancer progression, was significantly increased on ectopic expression of STAMP1 in DU145 cells and, conversely, was strongly downregulated on STAMP1 knockdown in LNCaP cells. In the normal prostate, STAMP1 protein is localized to the cytosol and the cell membrane of the prostate epithelial cells; furthermore, its expression is increased in prostate cancer compared with normal prostate. Taken together, these data suggest that STAMP1 is required for prostate cancer growth, which may be a useful target in prostate cancer treatment. Cancer Res; 70(14); 5818–28. ©2010 AACR.

Introduction

Prostate cancer is the most common noncutaneous cancer among men and is the second leading cause of cancer deaths in men in Western countries (1). Initially, prostate cancer depends on circulating androgens for its growth but later becomes androgen independent in most cases at which stage no curative therapy is available. The specific cause(s) of prostate cancer progression is still unknown (2, 3). An approach to address this question is to find genes that are prostate enriched and differentially expressed during cancer progression. Recent studies have identified several candidate genes whose products may be involved in prostate cancer development and which may therefore be used as markers of prostate cancer (e.g., refs. 4–10).

One of the prostate-specific genes upregulated in prostate cancer is STAMP1 (also known as STEAP2; refs. 6, 11). STAMP1 expression is androgen independent but mainly occurs in androgen receptor (AR)–positive cells (6), suggesting that AR signaling may have a role in its expression. Furthermore, STAMP1 expression is significantly increased in prostate cancer compared with normal prostate. STAMP1 was found to localize to the Golgi, trans-Golgi network, and the plasma membrane and may have a role in endocytic/secretory trafficking pathways (6).

STAMP1 belongs to a recently discovered six-transmembrane protein family. STAMP2 (also known as STEAP4 and TIARP) is another member of this family whose expression is increased in prostate cancer compared with matched normal prostate epithelial cells (6), which may also have a role in metabolic disease (12). Other members of the STAMP family include pHyde, a rat protein that has been implicated in apoptosis of prostate cancer cells (13), and its human homologue TSAP6 (also known as STEAP3), a p53-inducible gene involved in apoptosis and the cell cycle in prostate cancer and HeLa cells (13, 14).

Recent reports indicate that STAMP family members have ferrireductase and cupric reductase activities in HEK-293T cells (15). It is also suggested that these reductase activities are associated with uptake of iron and copper into these...
cells (15). It is currently unclear if STAMP proteins have these activities in prostate cancer cells.

In this study, we assess the consequence of STAMP1 ectopic expression or its knockdown on prostate cancer cell growth in vitro and in vivo. We show that STAMP1 increases prostate cancer cell growth in vitro and in vivo involving both proliferative and antiapoptotic pathways. Furthermore, we find that STAMP1 is significantly overexpressed in prostate cancer compared with normal prostate.

**Materials and Methods**

**Cell culture**

LNCaP, DU145, and COS-7 cells were obtained from the American Type Culture Collection (LGC Standards) and routinely maintained in standard conditions. Their response, or lack thereof, to androgen treatment for growth as well as in reporter assays was determined as a way to authenticate the cell lines.

**Immunohistochemistry**

An antiserum against STAMP1 was raised against the COOH-terminal peptide YLEEGIGGTIPHVSPERVTVM in rabbits. Formalin-fixed, paraffin-embedded tissue microarray was immunostained, scored, and analyzed as described previously (16).

**Small interfering RNA–mediated knockdown**

LNCaP cells were transfected with either luciferase or STAMP1 small interfering RNA (siRNA) using Oligofectamine (Invitrogen) according to the manufacturer’s recommendations (sequences available on request). At indicated time points, cell numbers were counted and cells were harvested for RNA and protein extraction.

**Short hairpin RNA–mediated knockdown**

Lentiviral pGIPZ short hairpin RNA (shRNA) vectors targeting human STAMP1 and nonsilencing pGIPZ control vector were purchased from Open Biosystems (Thermo Fisher Scientific, Inc.). Lentivirus particles were produced in 293T cells according to the manufacturer’s instructions. LNCaP cells were transfected by the lentiviral particles followed by puromycin selection (1 μg/mL) for 10 days. The cells stably expressing shRNA were pooled and maintained in puromycin (0.2 μg/mL).

**Ectopic expression of STAMP1**

For ectopic expression of STAMP1, the green fluorescent protein (GFP) coding sequence of pGIPZ plasmid (Open Bio-systems, Thermo Scientific) was replaced with that of STAMP1 and lentivirus was produced by a packaging system (Open Bio-systems, Thermo Scientific) according to the manufacturer’s recommendations. DU145 cells were then transduced with lentivirus and selected with 500 ng/mL puromycin for 10 days. GFP-expressing parental pGIPZ vector was used as control.

**Cell proliferation assays**

COS-7 cells were transfected with DNA (pcDNA3 or pcDNA3-STAMP1 together with pEGFP-C1 at a ratio of 4:1) using Fugene 6 (Roche Diagnostics). The ratio of the number of GFP-positive cells was measured by flow cytometry at the indicated times. A total of 200,000 single cells were analyzed per sample.

DU145 cells expressing STAMP1 or vector control were seeded in 24-well plates at a density of 10,000 per well. After culturing for various times, cell numbers were measured by WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] assay as described by the manufacturer (Sigma-Aldrich).

**Cell cycle analysis**

Cells were synchronized at the G1/G0 phase by serum starvation for 48 hours and then released into cell cycle by readdition of 10% fetal bovine serum (FBS). After 24 hours, cells were trypsinized, washed with PBS, and then fixed in 70% ice-cold ethanol for 2 hours at 4°C. To assess the cell cycle profile, fixed cells were treated with RNase, stained with propidium iodide (PI), and analyzed by flow cytometry using CellQuest software.

**Apoptosis assays**

Apoptosis and the treatments for its induction and detection were as described previously (17). To detect proapoptotic factor-induced apoptosis, cells were treated with either 50 ng/mL tumor necrosis factor–related apoptosis-inducing ligand (TRAIL; Enzo Life Sciences) or 20 μmol/L LY294002 (Invitrogen) for 24 hours or both agents for 8 hours. Extent of apoptosis was then detected by terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) and flow cytometry.

**Quantitative reverse transcription-PCR**

Quantitative reverse transcription-PCR (RT-PCR) was done as described previously (17).

**Western blot analysis**

Western blot analyses were performed as described previously (18). Antisera specific for HA-tag (Sigma), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Sigma), total extracellular signal-regulated kinase (ERK) 1/2, phosphorylated ERK1/2 (Cell Signaling), and STAMP1 were used.

**Xenografts in nude mice**

For injection into nude mice, 3 million cells expressing STAMP1 shRNA or the control shRNA were suspended in 50 μL RPMI 1640 and mixed with 50 μL Matrigel (BD Biosciences). The mixture was then subcutaneously implanted into male severe combined immunodeficient (SCID) mice in both hind flanks. Tumor size was measured weekly in three dimensions with calipers.

**Cell cycle–specific oligonucleotide array**

A cell cycle–specific oligo array (Oligo GEArray, SuperArray) representing 96 genes involved in cell cycle regulation and progression was used according to the manufacturer’s recommendation and as previously described (19).
Statistics
Comparisons were made with the Student’s t test. A value of \( P < 0.05 \) indicated statistical significance.

Oncomine data analysis
Oncomine, a publicly available database of gene expression microarray (19), was explored for STAMP1 expression in benign prostate and prostate cancer. Data obtained from cDNA microarray analysis from two independent studies (20, 21) were used.

Results
STAMP1 increases cell proliferation
Our previous findings showed that STAMP1 expression is increased in prostate cancer compared with normal prostate (6). We therefore assessed whether STAMP1 expression may influence growth characteristics of prostate cancer cells. Cell growth of DU145 cells stably expressing HA-tagged STAMP1 (two independent pools; Fig. 1A) was significantly faster by 5 days in culture compared with control cells (Fig. 1B).

To assess the effect of ectopic expression of STAMP1 in other cell lines, COS-7 cells were cotransfected with an expression vector for GFP and either an expression vector specifying STAMP1 or an empty vector as control, and STAMP1 expression was confirmed (Fig. 1C). The cells were grown for various times and collected, and the GFP-containing cells were counted using fluorescence-activated cell sorter (FACS). Because GFP was transfected in a significantly smaller amount compared with STAMP1 or the empty expression plasmid, the ratio of GFP-containing cells to the whole-cell population is a measure of cell proliferation. As shown in Fig. 1D, STAMP1-expressing COS-7 cells grew significantly faster compared with cells transfected with the empty expression plasmid. These data show that STAMP1 increases cell proliferation in COS-7 cells.

If STAMP1 is involved in cell proliferation, then its decrease in a cell that normally expresses it should decrease cell growth. To assess this possibility, we used siRNA to specifically knock down STAMP1 expression in LNCaP cells, an androgen-responsive prostate cancer cell line that constitutively expresses STAMP1 (6). LNCaP cells were transfected with either siRNA for luciferase as control or siRNA that is specific to STAMP1. As shown in Fig. 2A, STAMP1-specific siRNA decreased its expression by \( \sim 90\% \) at the RNA level and completely abolished expression at the protein level compared with control siRNA. LNCaP cells transfected with the two siRNAs were then compared for cell growth up to 10 days. As shown in Fig. 2B, LNCaP cell proliferation was significantly decreased in the presence of STAMP1 siRNA compared with luciferase siRNA. Examination of cells under the microscope corroborated these results and indicated no significant change in cell morphology (data not shown).

When cells were allowed to grow to form colonies, there...
was a 60% drop in colony formation in the presence of STAMP1 siRNA compared with luciferase siRNA (Fig. 2C), consistent with changes in cellular proliferation. We also checked possible changes in the cell cycle distribution of LNCaP cells transfected with STAMP1 siRNA compared with luciferase siRNA. As shown in Fig. 2D, there was a significant increase in the percentage of cells in G1 and a corresponding decrease in cells in the S phase, indicating that loss of STAMP1 resulted in partial cell cycle arrest in G0-G1. Taken together, these findings show that STAMP1 directly influences proliferation of prostate cancer cells.

**STAMP1 affects cell cycle–related gene expression**

To gain insight into the proliferative effect of STAMP1 on cell growth, we used a cell cycle–specific oligonucleotide array containing 96 different genes involved in cell cycle

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**Figure 2.** STAMP1 knockdown decreases growth and colony formation of LNCaP cells. LNCaP cells were transfected with either siRNA against luciferase or STAMP1. A, cells were harvested at the indicated time points, RNA was isolated, and quantitative RT-PCR was used to determine STAMP1 mRNA levels. Right, Western blot confirming STAMP1 protein levels. B, after transfection with the siRNAs as in A, cell number was determined at indicated times. C, LNCaP cells expressing either control siRNA or siRNA against STAMP1 were cultured for 2 wk. The colonies formed were stained with crystal violet and photographed. Right, quantification of the data. D, 2 d after transfection with the indicated siRNAs, LNCaP cells were synchronized by serum starvation for 48 h and then released into cell cycle progression by readdition of 10% FBS. After 24 h, cells were subjected to PI staining and flow cytometry analysis. *, P < 0.05.
regulation to screen for genes whose expression may be regulated when STAMP1 expression is knocked down (Fig. 3A). The genes that were upregulated or downregulated >1.5-fold were identified as shown in the scatter plot analysis in Fig. 3B, and some were validated by quantitative PCR (Fig. 3C). One of these was cyclin-dependent kinase inhibitor (CDKI) p21 (Cdki 2B), which was clearly upregulated at 4 or 7 days. E2F5 mRNA level was increased at 4 days and decreased back to basal levels at 7 days. Cyclin H and the proliferation marker Ki67 were downregulated at 4 days, but their expression was increased back to basal levels by 7 days after transfection. These results suggest that the proliferative effect of STAMP1 expression on prostate cancer cells is, at least in part, through the regulation of cell cycle–related gene expression, consistent with findings presented above (Fig. 2D).

**Downregulation of STAMP1 significantly increases apoptosis**

In addition to its effects on proliferation, we investigated the possible involvement of STAMP1 in regulating apoptosis in prostate cancer cells. LNCaP cells were transfected with STAMP1 siRNA or luciferase siRNA as control. At 5 and 7 days after transfection, cells were fixed, nuclei were visualized by 4′,6-diamidino-2-phenylindole staining, and TUNEL assay was used to assess apoptosis. As shown in Fig. 4A, a significant increase in apoptosis was observed for STAMP1 siRNA-transfected cells compared with control cells at both time points. These data indicate that STAMP1 is a prosurvival factor for prostate cancer cells.

**STAMP1 expression is decreased in LNCaP cells undergoing apoptosis**

Because knockdown of STAMP1 increased cell death, it was of interest to investigate if there were changes in STAMP1 levels in prostate cancer cells that are undergoing apoptosis. Previous studies have established that apoptosis in LNCaP cells can be induced by various agents (e.g., refs. 17, 22). We have used three independent inducers of apoptosis that may have differences in their mechanism of action: the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA), thapsigargin, and UV light (17, 22, 23). After treatment of cells for different time points to achieve maximum apoptosis, cells were harvested and proteins were extracted and subjected to Western blot analysis. As shown in Fig. 4B, and its quantification in Fig. 4C, in response to all three treatments, there was a 50% to 60% reduction in STAMP1 protein accumulation compared with vehicle-treated cells. These data support the hypothesis that STAMP1 is a survival factor in prostate cancer cells.

**Downregulation of STAMP1 significantly increases apoptosis induced by TRAIL or combination of TRAIL and AKT inhibitor**

If STAMP1 is a survival factor for prostate cancer cells, then its knockdown should increase their susceptibility to inducers of apoptosis. To check this possibility, we used TRAIL, which induces apoptosis in prostate cancer cells, either alone or in combination with the AKT inhibitor LY294002 (e.g., ref. 24). STAMP1 knockdown significantly increased cell death induced by TRAIL alone or TRAIL plus LY294002 (Fig. 4D). These data indicate that STAMP1 is a survival factor in prostate cancer cells and its absence may increase the susceptibility of prostate cancer cells to some proapoptotic factors.

**STAMP1 knockdown inhibits growth of human prostate cancer xenografts**

The data described above suggested that STAMP1 may have similar effects on prostate cancer cell growth in vivo. To check this possibility, we made stable LNCaP cell lines that had either a shRNA that is specific for luciferase as control or a shRNA directed against STAMP1. STAMP1-specific shRNA, but not luciferase shRNA, almost completely blocked STAMP1 protein accumulation in LNCaP cells (Fig. 5A). These two cell lines were then injected subcutaneously into SCID mice and allowed to grow as xenografts, and the extent of tumor growth was monitored over time. There was a significant decline in tumor growth at all time points tested in STAMP1 knockdown cells compared with control cells (Fig. 5B). These data support the findings above and show that STAMP1 has a role in prostate cancer growth in vivo.

**STAMP1-regulated ERK activation in human prostate cancer cells**

One of the signaling pathways that have been implicated in prostate carcinogenesis is the mitogen-activated protein kinase (MAPK) pathway (for a review, see refs. 25, 26). We therefore assessed whether there were any alterations in the major arms of this pathway under conditions where STAMP1 expression was manipulated. To that end, we ectopically expressed STAMP1, and as control the family member STAMP2 (5), in the prostate cancer cell line DU145 and then assessed ERK activity in response to epidermal growth factor (EGF). As shown in Fig. 5C, there was a robust ERK activation in response to EGF in control cells, which was significantly increased in response to STAMP1, but not STAMP2, expression. To assess this correlation further, we used LNCaP cells in which STAMP1 has been knocked down by shRNA and determined ERK activation in response to EGF. Whereas at the earlier EGF treatment time point of 5 minutes the extent of ERK activation was similar in control and STAMP1 knockdown cells, at 15 minutes ERK activity was almost completely lost in STAMP1 knockdown cells but only modestly decreased in control cells (Fig. 5D). In parallel experiments, there was no significant effect of STAMP1 ectopic expression or its knockdown on c-Jun NH2-terminal kinase or AKT activation (data not shown). Taken together, these data show that STAMP1 expression is required for optimal ERK activity in prostate cancer cells.

**STAMP1 expression is upregulated in human prostate cancer specimens**

Previous work has shown that STAMP1 mRNA expression is increased in prostate cancer specimens compared with
Figure 3. Knockdown of STAMP1 results in the regulation of cell cycle–regulatory genes. A, LNCaP cells were transfected with either luciferase (Luc) siRNA (left) or STAMP1 siRNA (right). At different time points after transfection, the cells were harvested and RNA was used to probe cell cycle oligonucleotide arrays. Spots containing oligonucleotides for β-actin, which was used as loading control, and some of the genes showing different expression in the two arrays are indicated. B, GEArray Analysis Suite software (SuperArray) was used to analyze the data in A, and a boundary of 1.5 was set in a scatter plot analysis. C, luciferase or STAMP1 siRNA-transfected LNCaP cells were harvested at the indicated time points and used in quantitative PCR analysis of the genes that are indicated. GAPDH was used as loading control.

* P < 0.05.
benign prostate (6, 11). We have confirmed the reproducibility of these findings in two independent data sets from the Oncomine database (19) with larger number of subjects (Fig. 6A).

To evaluate the validity of these findings at the protein level, we examined STAMP1 expression by immunohistochemical analysis on a tissue microarray that contained benign (n = 17) and malignant prostate tissue (n = 67). STAMP1 protein was expressed in the normal prostate, solely in epithelial cells, with predominantly cytosolic and cell membrane localization (Fig. 6B). Furthermore, its expression was significantly increased in cancer tissue compared with normal cells, with the membrane localization increasing more significantly compared with the cytosolic fraction (Fig. 6C; Supplementary Table S1). Intensity scoring among different tumor grades (Gleason score) indicated no significant differences (Supplementary Table S1). Together, these data show that STAMP1 expression is increased at both the mRNA and protein levels in prostate cancer compared with normal prostate.

Discussion

Increased expression of STAMP1 mRNA in prostate cancer cells compared with normal prostate has previously suggested that it may have a role in prostate cancer cell growth and progression (5, 11). The data we have presented here significantly strengthen this hypothesis. The results of various
assays on cell proliferation, colony formation, and apoptosis, \textit{in vitro} and \textit{in vivo}, clearly show that STAMP1 is involved in prostate cancer cell growth.

How does STAMP1 affect growth of prostate cancer cells? Cell cycle–specific oligonucleotide array analysis indicated that some cell cycle inhibitors, such as several CDKIs, were upregulated in LNCaP cells with decreased STAMP1 expression, both \textit{in vitro} (Fig. 3) and \textit{in vivo}, when cells were grown as xenografts.\textsuperscript{7} For example, p21, a CDKI (27), was upregulated in cells where STAMP1 was downregulated. Similarly, expression of E2F5, a transcription factor that is involved in negative regulation of the cell cycle (27), was also increased. In contrast, Ki67, which is a commonly used marker for cell proliferation, was significantly downregulated, confirming the proliferative effect of STAMP1. These data are consistent with the partial cell cycle arrest of LNCaP cells in G0-G1 on STAMP1 knockdown (Fig. 2D). Because STAMP1 is not a transcription factor, how it affects the expression of cell cycle–regulated genes is at present not clear and needs to be investigated further.

In addition to cell proliferative effects, downregulation of STAMP1 strongly increased apoptosis. It is at present not clear through which of the apoptotic pathways STAMP1 exerts its inhibitive actions. Given its cell membrane localization, it is tempting to speculate that STAMP1 may primarily affect the extrinsic apoptosis pathways (28) where signals in the cell exterior activate cell surface receptors, which initiate a signaling cascade resulting in the formation of the death-inducing signaling complex (DISC), which results in cell death. It is conceivable that STAMP1 may inhibit this pathway, for example, by decreasing the stability of the DISC. STAMP1 may also affect the intrinsic apoptosis pathway because the cells with STAMP1 knockdown underwent apoptosis in the absence of extrinsic apoptosis inducers (Fig. 4). It is possible that STAMP1 interacts with and affects the function of an antiapoptotic protein, for example, Golgi antiapoptotic protein, which is localized to the Golgi similar to STAMP1, which has recently been reported to inhibit apoptosis by modulating intracellular calcium fluxes (29). Furthermore, it is possible that STAMP1 is involved in endocytic trafficking of cell surface receptors and influences receptor signaling, which mediates cell growth or apoptosis. In addition, the fact that knockdown of STAMP1 expression increases the sensitivity of LNCaP cells to TRAIL-induced apoptosis suggests that it is a survival factor in prostate cancer and could be useful in the clinic in combination with other treatment modalities. Further studies are required to assess these possibilities.

Expression of STAMP1 in the androgen-responsive LNCaP cells, but not DU145 or PC3 cells (6), is of interest. The latter two cell lines were originally derived from prostate cancer metastases and are considered more advanced and highly aggressive when grown as xenografts compared with LNCaP cells (e.g., ref. 30). This presents a puzzle: If STAMP1 is

\textsuperscript{7} Unpublished data.

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\caption{STAMP1 knockdown inhibits LNCaP tumor growth. A, Western blot analysis confirming STAMP1 knockdown by shRNA in LNCaP cells. B, growth curve of xenografted LNCaP tumors in SCID mice. LNCaP cells expressing shRNA against STAMP1 or nonsilencing control shRNA were subcutaneously implanted into male SCID mice (n = 9 for each group). Tumor size was measured weekly. *, P < 0.005. C, ectopic expression of STAMP1 increases ERK activity. DU145 cells were transduced with lentivirus expressing either GFP, STAMP1, or STAMP2. Cells were then either left untreated or treated with EGF for 30 min, and cell lysates were prepared and subjected to Western blot analysis with the indicated antisera. The data presented are representative of three independent experiments. D, shRNA-mediated knockdown of STAMP1 decreases ERK expression. LNCaP cells were transduced with lentivirus either expressing a scrambled shRNA (Scr) or targeting STAMP1 and subjected to EGF induction for the indicated time points. Western blot analyses were performed as above. Data are representative of three independent experiments.}
\end{figure}
indeed required for proliferation of prostate cancer cells and has relevance for disease progression, then one would expect high expression in these aggressive cell types. In contrast to these in vitro findings, STAMP1 expression is increased in relapsed derivatives of the androgen-dependent human xenograft model CWR22 (data not shown; ref. 6). These data suggest that, indeed, STAMP1 expression is increased in advanced disease. It should be pointed out that PC3 and DU145 cells lack a functional AR that is present in the majority of advanced, relapsed prostate cancers (31); thus, these two cell lines are not the best models for advanced, androgen-independent prostate cancer. Further work is required to assess whether AR may have a role on STAMP1 expression.

Our results suggest that the proliferative and antiapoptotic activities of STAMP1 may be mediated by ERK signaling. ERK has been associated with every major aspect of cell physiology, including cell proliferation, cell transformation, and protection against apoptosis (for a review, see ref. 32). Previous studies have implicated ERK in prostate cancer cells (for a review, see ref. 26). Active ERK in epithelial cells increased significantly from normal prostate tissue to benign prostatic hyperplasia to prostate cancer (33). Elevated levels of active ERK in high grade and advanced stage of prostate tumors were linked to

Figure 6. STAMP1 expression is increased in prostate cancer compared with benign prostate. A, expression of STAMP1 mRNA in benign and malignant prostate tissue. Large-scale analysis of the human transcriptome by gene expression profiling from two different studies was used to determine relative changes in STAMP1 expression (19). In study 1 (left), prostate carcinomas (n = 59) versus normal prostates (n = 6) and benign prostates (n = 16) were analyzed for STAMP1 expression. The differences were statistically significant. *, P < 0.003. In another study (right), STAMP1 expression levels in 62 prostate carcinoma tissue samples were compared with that in 41 normal prostate tissues. The differences were significant. **, P < 0.008. B, STAMP1 protein expression is increased in prostate cancer compared with normal prostate. Immunohistochemistry was used to assess STAMP1 expression in normal and malignant prostate tissues. Tissue microarrays with normal prostate or early-stage (Gleason score < 7) and late-stage prostate tumors (Gleason score ≥ 7) were subjected to immunohistochemistry as described in Materials and Methods. The areas within the black squares were enlarged and are presented to the right of each image. C, quantification of STAMP1 expression in normal prostates (n = 17), prostate cancer with Gleason score of <7 (n = 22), and high-grade prostate cancer with Gleason score of ≥7 (n = 45). For statistical significance of differences, please see Supplementary Table S1.
increased cell proliferation that directly related to poor prognostic features (34, 35). ERK activation at later stages of prostate cancer is decreased when cells are treated with different ERK inhibitors, leading to decreased proliferation and an increase in apoptosis (36, 37). Furthermore, ERK is required for sustaining DNA synthesis in various prostate cancer cell lines (38). Supporting these studies, LNCaP cells that were treated with a specific MAPK/ERK kinase 1 inhibitor, PD98059, had a 60% inhibition of EGF-induced proliferation, indicating a role for ERK in this process (39). Furthermore, EGF has been shown to activate the transcriptional activity of the AR, an important driver of prostate cancer growth (for a review, see ref. 25), by increasing the expression or activity of AR coactivators in prostate cancer cells, and in this way thought to promote malignant progression and metastasis of advanced prostate cancer (40). HER receptor tyrosine kinases 1 to 4 of the EGF receptor family are also expressed in prostate cancer cells, and their stimulation by EGF and heregulin activates the MAPK and phosphatidylinositol 3-kinase/AKT pathways (41). In addition, HER2 and HER3, in response to heregulin binding, increase ligand-dependent AR transactivation of reporter genes (41). Taken together with data presented in Fig. 5, STAMP1 may affect prostate cancer progression through activation of ERK signaling.

Previous work using a limited number of tissue specimens indicated that STAMP1 mRNA is overexpressed in prostate cancer compared with benign prostate (6, 11). In addition to extending this to larger numbers of specimens, immunohistochemical analysis indicated that STAMP1 was localized in the cytosol and the cell membrane of human prostate cancer cells in situ. Furthermore, STAMP1 expression was increased, especially the fraction localized to the plasma membrane, in cancer cells compared with normal prostate. These data are consistent with the in vitro and in vivo studies and indicate that STAMP1 is involved in prostate cancer growth.

In summary, the data presented show that STAMP1 is involved in a signaling pathway that is linked to cell cycle progression as well as inhibition of apoptosis. Consistent with its increased expression in prostate cancer, these data suggest that STAMP1 may play important roles in prostate cancer development and progression.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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STAMP1 Is Both a Proliferative and an Antiapoptotic Factor in Prostate Cancer

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