A JUN N-terminal kinase inhibitor induces ectodomain shedding of the cancer-associated membrane protease Prss14/epithin via protein kinase CβII

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Running title: PKCβII in Prss14/epithin shedding induces cell invasion

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Abstract

Serine protease 14 (Prss14)/epithin is a transmembrane serine protease that plays essential roles in tumor progression and metastasis and therefore represents a promising target for managing cancer. Prss14/epithin shedding may underlie its activity in cancer and may worsen outcomes; accordingly, a detailed understanding of the molecular mechanisms in Prss14/epithin shedding may inform the design of future cancer therapies. On the basis of our previous observation that an activator of protein kinase C (PKC), phorbol 12-myristate 13-acetate (PMA), induces Prss14/epithin shedding, here we further investigated the intracellular signaling pathway involved in this process. While using mitogen-activated protein kinase (MAPK) inhibitors to investigate possible effectors of downstream PKC signaling, we unexpectedly found that an inhibitor of JUN N-terminal kinase (JNK), SP600125, induces Prss14/epithin shedding, even in the absence of PMA. SP600125-induced shedding, like that stimulated by PMA, was mediated by tumor necrosis factor-α–converting enzyme (TACE). In contrast, a JNK activator, anisomycin, partially abolished the effects of SP600125 on Prss14/epithin shedding. Moreover, results from loss-of-function experiments with specific inhibitors, short hairpin RNA–mediated knockdown, and overexpression of dominant-negative PKCβII variants indicated that PKCβII is a major player in both JNK inhibition– and PMA-mediated Prss14/epithin shedding. SP600125 increased phosphorylation of PKCβII and TACE and induced their translocation into the plasma membrane. Finally, in vitro cell invasion experiments and bioinformatics analysis of data in the TCGA breast cancer database revealed that JNK and PKCβII both are important for Prss14/epithin-mediated cancer progression. These results provide important information regarding strategies against tumor metastasis.

Prss14/epithin (also known as matriptase, suppression of tumorigenicity 14, membrane-type protease-1), a typical member of the type II transmembrane serine protease family, plays important roles in cancer progression and metastasis (1,2). Overexpression of Prss14/epithin is found in various epithelial cancer types (2). Particularly, ER- negative (ER’) breast cancer patients with poor prognosis express higher levels of Prss14/epithin (3). In our earlier report involving pathological examinations, we showed that prognosis of post-surgery esophageal cancer patients with higher Prss14/epithin expression is very poor (4). Transgenic expression of Prss14/epithin in
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In mice, reduced Prss14/epithin expression in transgenic mouse models clearly impaired tumor progression and metastasis (6). Moreover, downregulated Prss14/epithin inhibits ErbB2-induced prostate cancer cell invasion (7), and specific inhibitors of Prss14/epithin protease activity impaired tumor growth and metastasis (8). We have reported that knockdown of Prss14/epithin reduces cell migration (9,10) and metastatic ability of breast cancer cells such as 4T1 (9) and EO771 (11). When a specific monoclonal antibody raised against the activation loop portion of Prss14/epithin was injected into a PyMT breast cancer mouse model, metastasis was significantly reduced (11).

Prss14/epithin has a multi-domain structure with a short cytoplasmic N terminus, single transmembrane region, sea domain, four CUB domains, four LDLRA repeats, and C-terminal serine protease domain (12,13), and undergoes the multiple processing steps before it gets fully matured. Its full-length form with molecular weight of 110 kDa molecular weight converts to a 92 kDa form after being processed at Gly149, and this conversion is essential for ectodomain shedding of this protease (14). Previously, we showed that phorbol 12-myristate 13-acetate (PMA) and TGFβ can induce ectodomain shedding of Prss14/epithin (15,16), by inducing actin cytoskeletal remodeling, which results in filamin-dependent translocation of the protease to the cell-cell contact (17). Filamin appears essential for the translocation and shedding of Prss14/epithin (17). Prss14/epithin shedding events induced by PMA or TGFβ occur mainly through the action of tumor necrosis factor-α converting enzyme (TACE) (15,16). Although there is still debate about whether shedding occurs prior to activation; in the case of TGFβ-induced shedding, shedding precedes activation of the protease (10,16) suggesting that the shedding is possibly required for activation of the protease. Because PMA, diacylglycerol (DAG) mimic acts as activator of the protein kinase C (PKC) family, and some PKC isoforms are already recognized for being involved in ectodomain shedding of other transmembrane proteins (18,19), there is no doubt regarding the involvement of PKCs in ectodomain shedding of Prss14/epithin. However, it is still a mystery as to which PKC isoforms and other intracellular signaling pathways are involved in shedding of Prss14/epithin. Considering that ectodomain shedding of the protease can profoundly affect the extracellular environment in favor of cancer metastasis (20-22), identification of such signaling pathways will be useful for establishing therapeutic approaches. Moreover, because of the broad biological effects of PKC family members, pinpointing the PKC isoforms responsible for shedding may also be critical for specific targeting of Prss14/epithin ectodomain shedding.

In this study, we show that PKCβII is a key molecule required for shedding of Prss14/epithin induced by PMA and JNK inhibitor. Both PMA treatment and JNK inhibition can increase the phosphorylation and translocation of PKCβII to the plasma membrane, which could be the essential step for ectodomain shedding of Prss14/epithin. Indeed, inhibition of PKCβII and JNK reduces in vitro cell invasion. Finally, bioinformatic analysis revealed that levels of signaling molecules are correlated with better or poorer patient survivals. Thus, our finding can provide important information on new therapeutic approaches for cancer patients with high expression of Prss14/epithin.

Results

JNK inhibition increases Prss14/epithin shedding.

In order to investigate signaling pathways involved in PMA-induced Prss14/epithin ectodomain shedding, we first sought to test three main MAPK pathways (ERK, p38, JNK) (23) employing commonly used specific inhibitors in the absence or presence of PMA in 427.1.86 cells. As seen in Figs. 1A and 1B (and Fig. S1 for full size blot), in the absence of any pathway-specific inhibitors, PMA slightly increased the shed form of Prss14/epithin (Epi-S') in the conditioned medium, but decreased the amount of protein (Epi-S) remaining in the cell lysate. When three inhibitors, PD98059 for ERK, SB203580 for p38, and SP600125 for JNK, were used, SP600125, significantly increased levels of Epi-S' regardless of the presence of PMA in 427.1.86 cells. As seen in Figs. 1A and 1B (and Fig. S1 for full size blot), in the absence of any pathway-specific inhibitors, PMA slightly increased the shed form of Prss14/epithin (Epi-S') in the conditioned medium, but decreased the amount of protein (Epi-S) remaining in the cell lysate. When three inhibitors, PD98059 for ERK, SB203580 for p38, and SP600125 for JNK, were used, SP600125, significantly increased levels of Epi-S' regardless of the presence of PMA (Fig. 1B). Effects of SP600125 on Epi-S' appearance in the medium (and disappearance of Epi-S in the cell lysate) were in a dose- and time-dependent fashion. (Fig. 1C).
Because we already know that PMA-induced Prss14/epithin shedding is mediated by TACE (15), we tested if SP600125-induced shedding is also mediated by TACE (Figs. 1D and E). Use of a TACE-specific inhibitor and knocking down of TACE message significantly reduced the amount of Epi-S' in the conditional medium while retaining the amount of cellular Epi-S. In the experiments with 427.1.86 cell line, we rarely observed the smaller activated form in the same blot (data not shown).

We also tested SP600125-induced shedding in other cell types: T47D human breast cancer and 4T1 mouse breast cancer cell lines. T47D cells showed that SP600125 induced Epi-S' and aEpi-S (30 kDa activated form) in a dose-dependent manner (Fig 1F). The time- and dose-dependent increase of Epi-S' and aEpi-S in 4T1 cells are shown in Fig. 1G. Both T47D and 4T1 breast cancer cell lines clearly revealed the active form, aEpi-S (with enhanced exposure of the same blot) following the appearance of Epi-S, suggesting that shedding is a general phenomenon and may precede the activation event.

To investigate whether SP600125-induced Prss14/epithin shedding is indeed due to its inhibitory effect on JNK activity, the downstream effects of SP600125 were tested (Fig. 2). SP600125 abolished phosphorylation of p-c-jun, a target of JNK. It is generally considered that anisomycin is a JNK activator involved in protein stability and/or transcription (24). To confirm the effect of SP600125 is specifically mediated by JNK inhibition not by possible off-target effects of the inhibitor, we attempted to suppress the inhibitory effect by pretreatment of anisomycin and test the effects of SP600125. When anisomycin was used together with SP600125, shedding of Prss14/epithin and phosphorylation of c-jun were partially affected, suggesting that anisomycin can interfere with SP600125-induced shedding (Fig 2A). In addition, when expression of JNK1 and JNK2 were reduced by specific siRNAs (relative reductions to control samples with nontargeting siRNA were 63.9% for JNK1 and 48.4% for JNK2 respectively), ectodomain shedding of Prss14/epithin to the medium was increase to 3.0 fold for JNK1 knockdown and 4.8 fold for JNK2 knockdown respectively) (Fig 2B).

These results indicated that JNK activity inhibits Prss14/epithin shedding.

To navigate the mechanisms of SP600125-induced Prss14/epithin shedding in more detail, we applied cycloheximide, which is generally considered as an inhibitor of protein synthesis. As seen in Fig. 2C, cycloheximide abolished SP600125-induced Prss14/epithin shedding. This strongly suggested that Prss14/epithin shedding requires de novo synthesis of labile protein(s). When new transcription was interfered with by either actinomycin D or α-amanitin pretreatment, SP600125-induced Prss14/epithin shedding was slightly reduced, but was severely affected by treatment with both reagents together, suggesting that at least some new transcription is required (Fig. 2D).

Previously, we showed that actin rearrangement induced by PMA is essential for the translocation and shedding of Prss14/epithin (17). SP600125, similar to PMA, induced actin to rearrange to form cortical actin filaments. This rearrangement was abolished by additional anisomycin treatment (Fig. S2).

**PKCβII is involved in PMA- and SP600125-induced Prss14/epithin shedding**

We tried to identify the PKC isoforms that are involved in PMA- and/or SP600125-induced Prss14/epithin shedding, using PKC inhibitors, siRNAs, and dominant negative (DN) forms (Fig. 3). Treatment with a broad-spectrum PKC inhibitor, Go6976 (Go), and a PKCβ selective inhibitor (βi) significantly inhibited both PMA- and SP600125-induced shedding of Prss14/epithin in 427.1.86 cell (Fig. 3A). Both inhibitors decreased Epi-S’ in the medium, whereas Epi-S levels in the cell lysate were complementary to those of Epi-S’. Knockdown studies using PKCα and β siRNAs were then carried out (Fig. 3B). Only PKCβ, but not PKCα siRNA, affected SP600125- and PMA-induced shedding. Because PKCβ1 and PKCβII use the same message, the levels of both PKCβ1 and PKCβII proteins were decreased when cells were treated with PKCβ siRNA. PMA treatment also reduced PKC levels, which is known as PMA-induced downregulation following activation (25). However, treatment with 5 μM SP600125 for 2 h did not severely affect the expression
levels of these PKC isoforms. From these results, it was concluded that SP600125- and PMA-induced Prss14/epithin shedding was primarily mediated by PKCβ, not by PKCa (Fig. 3B). Next, DN forms of PKCβ isoforms were tested. Over-expression of dnPKCβII cDNA inhibited PMA- or SP600125-induced Prss14/epithin shedding, whereas over-expression of dnPKCβI cDNA did not (Fig. 3C). PKCβ inhibitor also reduced SP600125-induced Prss14/epithin shedding in PC3 and MCF7 cells (Fig. 3D). These results indicated that PKCβII is critical for PMA- and SP600125-induced Prss14/epithin shedding.

SP600125 induces activation of PKCβII and membrane translocation

Signaling further downstream of PKCβII, such as activation and translocation of PKCβII, was then investigated with SP600125 in 427.1.86 cells (Fig. 4). SP600125 increased phosphorylation of PKCβII at serine residue position 660 (a hallmark of the active form), while additional anisomycin pretreatment did not (Fig. 4A). In all samples, total amounts of PKCβII were not changed upon treatments. In addition, activity of PKCβII in immunoprecipitated samples treated with SP600125 peaked after 1 h (Fig. 4B) and was reduced by anisomycin (Fig. 4C).

The location of PKCβII after SP600125 treatment was in the plasma membrane (Figs. 4D and E). Because levels of endogenous PKCβII protein were insufficient to investigate cellular localization on a small scale, cellular location was investigated using overexpression of PKCβII. Thus, cells were transfected with PKCβII cDNA, and then cellular localization of PKCβII was determined by specific antibody staining using immunofluorescence microscopic technique and stylized image transformation. Indeed, SP600125 induced translocation of PKCβII to the membrane (Fig. 4D). PKCβII in SP600125 treated cells clearly located in the cell to cell contact among clustered cells or in the plasma membrane of an isolated cell. When the fractions of cells with PKCβII appearing in the membrane were calculated, almost all the cells, if not all cells, in PMA treated samples showed complete membrane localization, but only 20% of cells in SP600125 treated samples showed the clear membrane localization. Using subcellular fractionation as measured by the ratio of phosphorylated PKCβII over E-cadherin of each fraction, the peak of PKCβII in the membrane fraction were obtained at 30 min, (Fig. 4E).

In Fig. 4F, we show the appearance of phosphorylated TACE upon PMA or SP600125 treatment. Phosphorylation at threonine 735 of TACE, which reflects activation of the enzyme, was increased by PMA and SP600125. From these results, we concluded that TACE phosphorylation is common downstream of PMA and SP600125.

PKCβII plays critical role in PMA- or SP600125-induced cell invasion

Previously, we and others showed that Prss14/epithin is important for cell invasion toward high serum concentration (10,26,27). Serum is also an important factor for inducing Prss14/epithin shedding. Consistently, the invasiveness of 427.1.86 cells were decreased when TACE inhibitor was treated as well as when Prss14/epithin was knocked down (Fig. 5A). In order to study roles of PKCβII in cell’s invasiveness, we generated PKCβII-specific knockdown cell lines (Fig. 5B). As seen figure 5C, the degrees of 427.1.86 cell invasion induced by serum gradient was increased upon SP5200125 or PMA treatment. However, the invasive nature of PKCβII knockdown cells in the same condition was abolished even in the presence of SP5200125 or PMA (Fig. 5C).

Taken together, from these results, we established a model how SP600125 induces ectodomain shedding of Prss14/epithin. SP600125 suppresses the inhibitory effect of JNK on PKCβII, and the SP600125-mediated increase in PKCβII activity may require a labile not-yet-identified regulator of PKCβII (Fig. 5D). PKCβII can be activated after PMA or JNK inhibition, either directly or indirectly, affecting TACE activation. PKCβII, TACE, and Prss14/epithin, are all translocated to the membrane; then, cleavage of Prss14/epithin occurs at the membrane, increasing cell invasion.

PKCβII and JNKs as prognostic markers in metastatic breast cancer
In order to evaluate the clinical significance of PKCβII and JNKs in cancer patients, we utilized the publicly available TCGA breast cancer RNAseq database (Fig. 6). Expression levels of Prss14/epithin (ST14), PKCβ (PKCB), and three JNKs (MAPK 8, 9, 10) in more aggressive ER-negative (ER−) and less aggressive ER-positive (ER+) breast cancer patients were compared individually in the plot (Fig. 6A). ST14 and PKCB levels were higher in ER+ patients, as expected. Among the MAPKs, MAPK9 (JNK2) exhibited significant differences between two groups. MAPK9 levels were higher in ER+ patients.

In survival curve analysis (Fig. 6B), using a combination of ST14 and PKCB, both ST14<sup>high</sup> and PKCB<sup>high</sup> group patients showed significantly lower survival compared to ST14<sup>low</sup> PKCB<sup>low</sup> and ST14<sup>low</sup> PKCB<sup>high</sup>, suggesting that ST14 is a stronger parameter, but PKCB collaborates. MAPK9 did not affect patient’s survival in a statistically significant way, although ST14<sup>low</sup> MAPK9<sup>low</sup> appeared to have the best survival.

Discussion

In this study, we showed the upstream signaling of induced Prss14/epithin shedding, and the significance and application of resulting biology. Prss14/epithin shedding was induced by JNK inhibition by SP600125 as well as PMA, which shares downstream molecules such as PKCβII and TACE through phosphorylation and translocation (summary model in Fig. 5C).

**JNK inhibition in addition to PMA can mediate Prss14/epithin shedding through PKCβII and TACE**

SP600125- or PMA-induced shedding is mediated by TACE. We already showed that PMA- and TGFβ-induced Prss14/epithin shedding depends on TACE (15,16). Although Prss14/epithin shedding can be blocked by serine protease inhibitors such as ecotin (17,28), we still believe that shedding is mediated by TACE, as tested by specific inhibition and knockdown experiments (Figs. 1D and E). It is likely that shedding precedes activation, as shown in Figs. 1F and G. The activated form of 30 kDa appeared more slowly in the medium than the shed form. In addition, we hardly observed the 30 kDa form in cell lysates (data not shown). In the case of TGFβ–induced Prss14/epithin shedding, we showed that the kinetics of Prss14/epithin activity were slower than those of Prss14/epithin shedding (16).

JNK activity, as revealed by p-c-Jun, appeared to indirectly affect Prss14/epithin shedding (Fig. 2). JNK inhibition by SP6200125 was partially affected by anisomycin, a known JNK activator, and a general inhibitor of protein synthesis. Knocking down JNK1 or JNK2 expression using siRNAs also induced Prss14/epithin shedding (Fig. 2B). SP6200125-induced Prss14/epithin shedding is also affected by cycloheximide, in addition to the transcription inhibitors α-amanitin and actinomycin D. These observations suggested that JNK plays a role in Prss14/epithin shedding through either labile protein degradation or diminished protein stability.

Among conventional PKC isotypes that are known for being activated by PMA, PKCβII was found to be involved in both PMA- and SP600125-induced shedding of Prss14/epithin (Fig. 3). Experiments with isotype-specific inhibitors, specific knockdown, and DN forms revealed that PKCβII is the key player in Prss14/epithin shedding induced either by JNK inhibition or by PMA exposure. JNK inhibition alone is sufficient to induce translocation of PKCβII to the membrane. Inhibition of JNKs increases phosphorylation of PKCβII at serine 660 (Fig. 4A). Serine 660 of PKCβII resides in a C-terminal hydrophobic region, and controls PKCβII folding and stability (29), thereby activating PKCβII. SP600125, in fact, increased the activity of PKCβII (Figs. 4B and C) and induced TACE activation similar to PMA (Fig. 4D). These results are incorporated in the model presented in Fig. 5C.

In our earlier studies, we showed that actin rearrangement from stress fibers to the cortical actin form is essential for Prss14/epithin shedding (17). This actin rearrangement involves filamin that can function as a vessel to bring proteins together (30). Prss14/epithin and TACE are brought to membranes by filamin upon PMA activation and interact together in complexes (17). Thus, TACE can cleave Prss14/epithin at the membrane. Because SP600125 induces cortical...
actin formation, and PKCβII translocates to the membrane, we suspect that PKCβII may also interact with filamin. However, to date, there are no data to support this idea, although PKCα and PKCε isoforms have been shown to bind to filamin (31,32). There are evidences that actin cytoskeleton remodeling is associated with JNKs activity. Actin stress fibers and associated shear stress mediates inhibition of JNKs in vascular endothelium (33). TGFβ-induced actin stress fibers promote activity of JNK in human mesangial cells (34). Inhibition of JNK induces rearrangement of actin stress fibers to cortical actin filaments (Fig. S2). Therefore, it is conceivable that inhibition of JNK may lead to interactions to form complexes of F-actin, filamin, and PKCβII.

Implication of PKCβ and JNK as prognosis markers as well as therapeutic target for metastatic cancer

Biological significances of Prss14/epithin shedding is intriguing at this point; although activity is clearly take important parts in tumorigenesis and homeostasis of normal epidermal barrier (6,35-37). From the results obtained using PKCβII knockdown cell lines that had lost the ability of induced invasion to higher serum gradient (Fig. 5A and B), we now understand that PKCβII-mediated shedding is necessary for cell invasion.

In our earlier careful analysis of TCGA breast cancer patients, we showed that Prss14/epithin (ST14) is an excellent prognostic marker for highly metastatic ER- breast cancer (3). In this study, we have shown that expression profiles of PKCB (PKCβ) and ST14 are in reasonably well-correlated (Fig. 6A). The levels of PKCβ are higher as those of ST14 in ER- patients, and MAPK9 (JNK2) is levels were lower in the same group. In survival analysis (Fig. 6B), PKCβ collaborated with ST14 in terms of the poorest survival of patients. ST14 low PKCB low patients are the group with the best survival. Taking advantage of these analyses, we can propose that PKCB and MAPK9 can be additional prognosis markers and can be used in precision medicine for breast cancer patients.

JNK has previously been considered a target for cancer therapy using kinase inhibitors (38). However, there are complications and complexities in application of such applications (39). It is important to note that JNK negatively regulates PKCβ downstream of Prss14/epithin shedding, and that JNK2 expression is lower in more aggressive cancer. Therefore, these observations represent a critical point for consideration of JNK inhibition as a therapeutic approach, at least in breast cancer.

Experimental procedures

Cell culture

Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 µg/mL streptomycin. For drug treatments, 90% confluent cells were serum-starved for 4 h and then treated with 0.5 μM PMA (Sigma, St. Louis, MO, USA) or SP600125 (Merck Millipore, Billerica, MA, USA). PKCβ selective inhibitor (539654, Merck Millipore), anisomycin (Sigma), and TAPI-0 (Merck Millipore) were pretreated for 30 min before drug treatment. Cycloheximide, α-amanetin, and actinomycin D were purchased from Sigma. For overexpression or knockdown of specific proteins, transfection of cDNA or siRNA was performed. When cells reached 30~40% confluence, cells were transfected with JNK1 siRNA, JNK2 siRNA, PKCα siRNA, PKCβ siRNA, and TACE siRNA (Santacruz Biotechnology, Dallas, TX, USA), wild type (wt) PKCβII cDNA, dominant negative (DN) PKCβ (K371R), and DN PKCβII (K371R) cDNA (Addgene, Cambridge, MA, USA) using Lipofectamine2000 (Invitrogen, Carlsbad, CA, USA) for 48-72 h. Control siRNA (Catalog Number: sc-37007, Santacruz biotechnology, Dallas, Texas, USA) designed not to target any gene was used as a negative control in knockdown experiments.

Western blot analysis

Total cell lysates were extracted with RIPA buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% Nonidet P-40, and 0.1% SDS supplemented with protease and phosphatase inhibitors. Soluble Prss14/epithin released into cell culture medium was obtained through protein precipitation using trichloroacetic acid (TCA) solution (10%, final concentration). Protein lysates were separated by SDS-PAGE.
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and transferred to nitrocellulose or PVDF membrane. The membrane was blocked with 5% non-fat dry milk in Tris-buffered saline, and proteins were detected using specific antibodies: mAb5 (40), anti PKCβII polyclonal antibody (Catalog Number: sc-13149, Santa Cruz biotechnology), anti-phosphospecific PKCβII (Ser 660) polyclonal antibody (Catalog Number: sc-365463, Santa Cruz biotechnology), anti-E-cadherin polyclonal antibody (Catalog Number: sc-8426, Santa Cruz biotechnology), anti-TACE polyclonal antibody (Catalog Number: ab13535, Abcam), and anti-phospho-specific TACE (Thr 735) polyclonal antibody (Catalog Number: A1978, Sigma).

**Subcellular fractionation**

Cells were plated in a 100 mm dish at 2.5 × 10^6 cells, and harvested with Tris buffer containing 10 mM Tris-HCl (pH 8.0), 10 mM NaCl, 1 mM KH₂PO₄, 5 mM NaHCO₃, 1 mM CaCl₂, and 0.5 mM MgCl₂ supplemented with inhibitors of proteases and phosphatases. After sonication on ice, nuclei and debris were eliminated by centrifugation at 7,500 rpm for 5 min. Supernatants containing cytosol and plasma membrane were centrifuged at 34,000 rpm for 60 min (Sorvall ultracentrifuge OTD-combi). Cytosol supernatants were collected, and crude plasma membrane pellets were resuspended with RIPA buffer, and then centrifuged at 34,000 rpm for 15 min. Plasma membrane supernatants were collected. To verify cross contamination, β-actin and E-cadherin were detected by western blot analysis.

**Immunocytochemistry**

Immunocytochemistry was performed as described previously (16). F-actin and PKCβII were detected with rhodamine-conjugated phalloidin (Molecular Probes) and anti-PKCβII polyclonal antibody, respectively. Cells labeled with anti-PKCβII polyclonal antibody were incubated with FITC-conjugated anti-rabbit IgG (Santa Cruz Biotechnology). Cells were observed with a fluorescence microscope (Axio-observer Z1m, Zeiss, Germany); fluorescence images were captured and processed with Axio-vision imaging software for microscopy.

**Immunoprecipitation, PKCβII activity assay, and TACE phosphorylation**

PKCβII activity was determined by modifying an immune complex kinase assay method described elsewhere (41). Briefly, cells were lysed with Tris lysis buffer containing 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerol phosphate, protease inhibitors, and phosphatase inhibitors. The cell lysates were precipitated with anti-PKCβII polyclonal antibody and protein A Sepharose beads (GE Healthcare). PKCβII kinase activity was determined by ADP-Glo™ kinase assay kit (Promega). Luciferase values were detected using a Glomax 96 microplate luminometer (Promega). Statistical analyses were performed using Sigma plot software. Comparisons between two groups were performed by Student’s t-test and results are expressed as means ± SD. To investigate phosphorylation of TACE, cell lysates were analyzed by western blot analysis using anti-phospho-TACE antibody (Thr 735) and anti-total TACE antibody.

**Cell invasion assay**

Invasion assay was performed using BioCoat Matrigel invasion chambers (Corning) according to the manufacturer’s instructions. 427.1.86, 427-PKCβII-KD4, and 427-EpiKD (9) cells were incubated with serum free medium for 12 h. Cells (2 × 10⁵) were seeded on the upper side of BioCoat Matrigel invasion chambers. The lower chamber was filled with DMEM containing 2% FBS with PMA (1 μM), SP600125 (5 μM), or TAPI-0 (20 μM). After 24 h, cells on the lower surface of the membrane were fixed with 100% methanol for 10 min and stained with 0.2% crystal violet for 5 min. Invading cells were counted under Axioimager M1 of five random fields. The total number of cells was divided by the number of counted fields in each assay.

**Analysis of TCGA datasets**

The Cancer Genome Atlas (TCGA) breast cancer patient data were downloaded using the
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Broad Institute TCGA Genome Data Analysis Center (2016), web portal site that has been developed for automated analyses of TCGA data to general users. For comparison of gene expression between ER+ and ER- groups, box plots were generated using GraphPad Prism 7. Comparisons were analyzed by unpaired two-tailed Student’s t-test. For 5 year survival rate, Kaplan-Meier survival analysis was performed using TCGA breast cancer data, including those of patients not 5 years. P values were calculated using a Log-rank (Mantel-Cox) test and the hazard ratio (HR) was determined by the Mantel-Haenszel method.

Data availability

TCGA datasets used in survival curve analysis were Level 3 normalized RNA-seq data (Broad GDAC Firehose 2016_01_28 standard data run, https://doi.org/10.7908/C11G0KM9) from Broad Institute TCGA Genome Data Analysis Center (2016). All other data are contained in this manuscript.
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Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.
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References

1. Uhland, K. (2006) Matriptase and its putative role in cancer. Cell Mol Life Sci 63, 2968-2978
2. List, K. (2009) Matriptase: a culprit in cancer? Future Oncol 5, 97-104
3. Kim, S., Yang, J. W., Kim, C., and Kim, M. G. (2016) Impact of suppression of tumorigenicity 14 (ST14)/serine protease 14 (Prss14) expression analysis on the prognosis and management of estrogen receptor negative breast cancer. Oncotarget 7, 34643-34663
4. Ha, S. Y., Kim, K. Y., Lee, N. K., Kim, M. G., and Kim, S. H. (2014) Overexpression of matriptase correlates with poor prognosis in esophageal squamous cell carcinoma. Virchows Arch 464, 19-27
5. List, K., Szabo, R., Molinolo, A., Sriuranpong, V., Redeye, V., Murdock, T., Burke, B., Nielsen, B. S., Gutkind, J. S., and Bugge, T. H. (2005) Deregulated matriptase causes ras-independent multistage carcinogenesis and promotes ras-mediated malignant transformation. Genes Dev 19, 1934-1950
6. Zoratti, G. L., Tanabe, L. M., Varela, F. A., Murray, A. S., Bergum, C., Colombo, E., Lang, J. E., Molinolo, A. A., Leduc, R., Marsault, E., Boerner, J., and List, K. (2015) Targeting matriptase in breast cancer abrogates tumour progression via impairment of stromal-epithelial growth factor signalling. Nat Commun 6, 6776
7. Wu, S. R., Cheng, T. S., Chen, W. C., Shyu, H. Y., Ko, C. J., Huang, H. P., Teng, C. H., Lin, C. H., Johnson, M. D., Lin, C. Y., and Lee, M. S. (2010) Matriptase is involved in ErbB-2-induced prostate cancer cell invasion. Am J Pathol 177, 3145-3158
8. Galkin, A. V., Mullen, L., Fox, W. D., Brown, J., Duncan, D., Moreno, O., Madison, E. L., and Agus, D. B. (2004) CVS-3983, a selective matriptase inhibitor, suppresses the growth of androgen independent prostate tumor xenografts. Prostate 61, 228-235
9. Kim, C., Lee, H. S., Lee, D., Lee, S. D., Cho, E. G., Yang, S. J., Kim, S. B., Park, D., and Kim, M. G. (2011) Epithin/PRSS14 proteolytically regulates angiopoietin receptor Tie2 during transendothelial migration. Blood 117, 1415-1424
10. Lee, H. S., Kim, C., Kim, S. B., Kim, M. G., and Park, D. (2010) Epithin, a target of transforming growth factor-beta signaling, mediates epithelial-mesenchymal transition. Biochem Biophys Res Commun 395, 553-559
11. Kim, K. Y., Yoon, M., Cho, Y., Lee, K.-H., Park, S., Lee, S.-r., Choi, S.-Y., Lee, D., Yang, C., Cho, E. H., Jeon, S. D., Kim, S.-H., Kim, C., and Kim, M. G. (2019) Targeting metastatic breast cancer with peptide epitopes derived from autocatalytic loop of Prss14/ST14 membrane serine protease and with monoclonal antibodies. Journal of Experimental & Clinical Cancer Research 38, 363
12. Kim, M. G., Chen, C., Lyu, M. S., Cho, E. G., Park, D., Kozak, C., and Schwartz, R. H. (1999) Cloning and chromosomal mapping of a gene isolated from thymic stromal cells encoding a new mouse type II membrane serine protease, epithin, containing four LDL receptor modules and two CUB domains. Immunogenetics 49, 420-428
13. Bugge, T. H., Antalis, T. M., and Wu, Q. (2009) Type II transmembrane serine proteases. J Biol Chem 284, 23177-23181
14. Cho, E. G., Kim, M. G., Kim, C., Kim, S. R., Seong, I. S., Chung, C., Schwartz, R. H., and Park, D. (2001) N-terminal processing is essential for release of epithin, a mouse type II membrane serine protease. J Biol Chem 276, 44581-44589
15. Cho, Y., Park, D., and Kim, C. (2017) Disruption of TACE-filamin interaction can inhibit TACE-mediated ectodomain shedding. Biochem Biophys Res Commun 490, 997-1003
16. Lee, H. S., Park, B. M., Cho, Y., Kim, S., Kim, C., Kim, M. G., and Park, D. (2014) Shedding of epithin/PRSS14 is induced by TGF-beta and mediated by tumor necrosis factor-alpha converting enzyme. Biochem Biophys Res Commun 452, 1084-1090
17. Kim, C., Cho, Y., Kang, C. H., Kim, M. G., Lee, H., Cho, E. G., and Park, D. (2005) Filamin is essential for shedding of the transmembrane serine protease, epithin. EMBO Rep 6, 1045-1051
18. Kveiborg, M., Instrell, R., Rowlands, C., Howell, M., and Parker, P. J. (2011) PKCdelta and PKCdelta regulate ADAM17-mediated ectodomain shedding of heparin binding-EGF through...
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separate pathways. PLoS One 6, e17168

19. Lemjabbar-Alaoui, H., Sidhu, S. S., Mengistab, A., Gallup, M., and Basbaum, C. (2011) TACE/ADAM-17 phosphorylation by PKC-epsilon mediates premalignant changes in tobacco smoke-exposed lung cells. PLoS One 6, e17489

20. Kim, S. B., Lee, D., Jeong, J. W., Kim, C., Park, D., and Kim, M. G. (2010) Soluble epithin/PRSS14 secreted from cancer cells contains active angiogenic potential. Mol Cells 29, 617-623

21. Chou, F. P., Chen, Y. W., Zhao, X. F., Xu-Monette, Z. Y., Young, K. H., Gartenhaus, R. B., Wang, J. K., Kataoka, H., Zuo, A. H., Barndt, R. J., Johnson, M., and Lin, C. Y. (2013) Imbalanced matriptase pericellular proteolysis contributes to the pathogenesis of malignant B-cell lymphomas. Am J Pathol 183, 1306-1317

22. Chu, L. L., Xu, Y., Yang, J. R., Hu, Y. A., Chang, H. H., Lai, H. Y., Tseng, C. C., Wang, H. Y., Johnson, M. D., Wang, J. K., and Lin, C. Y. (2014) Human cancer cells retain modest levels of enzymatically active matriptase only in extracellular milieu following induction of zymogen activation. PLoS One 9, e92244

23. Johnson, G. L., and Lapadat, R. (2002) Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science 298, 1911-1912

24. Hazzalin, C. A., Le Panse, R., Cano, E., and Mahadevan, L. C. (1998) Anisomycin selectively desensitizes signalling components involved in stress kinase activation and fos and jun induction. Mol Cell Biol 18, 1844-1854

25. Favaron, M., Manev, H., Siman, R., Bertolino, M., Szekely, A. M., DeErausquin, G., Guidotti, A., and Costa, E. (1990) Down-regulation of protein kinase C protects cerebellar granule neurons in primary culture from glutamate-induced neuronal death. Proc Natl Acad Sci USA 87, 1983-1987

26. Zoratti, G. L., Tanabe, L. M., Hyland, T. E., Duhaime, M. J., Colombo, E., Leduc, R., Marsault, E., Johnson, M. D., Lin, C. Y., Boerner, J., Lang, J. E., and List, K. (2016) Matriptase regulates c-Met mediated proliferation and invasion in inflammatory breast cancer. Oncotarget 7, 58162-58173

27. Tsai, C. H., Teng, C. H., Tu, Y. T., Cheng, T. S., Wu, S. R., Ko, C. J., Shyu, H. Y., Lan, S. W., Huang, H. P., Tseng, S. F., Johnson, M. D., Lin, C. Y., Hsiao, P. W., and Lee, M. S. (2014) HAI-2 suppresses the invasive growth and metastasis of prostate cancer through regulation of matriptase. Oncogene 33, 4643-4652

28. Tseng, C. C., Jia, B., Barndt, R., Gu, Y., Chen, C. Y., Tseng, I. C., Su, S. F., Wang, J. K., Johnson, M. D., and Lin, C. Y. (2017) Matriptase shedding is closely coupled with matriptase zymogen activation and requires de novo proteolytic cleavage likely involving its own activity. PLoS One 12, e0183507

29. Facchinetti, V., Ouyang, W., Wei, H., Soto, N., Lazorchak, A., Gould, C., Lowry, C., Newton, A. C., Mao, Y., Miao, R. Q., Sessa, W. C., Qin, J., Zhang, P., Su, B., and Jacinto, E. (2008) The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. EMBO J 27, 1932-1943

30. Zhou, A. X., Hartwig, J. H., and Akyurek, L. M. (2010) Filamins in cell signaling, transcription and organ development. Trends Cell Biol 20, 113-123

31. Tigges, U., Koch, B., Wissing, J., Jockusch, B. M., and Ziegler, W. H. (2003) The F-actin crossing-linking and focal adhesion protein filamin A is a ligand and in vivo substrate for protein kinase C alpha. Journal of Biological Chemistry 278, 23561-23569

32. Kim, H., Nakamura, F., Lee, W., Hong, C., Perez-Sala, D., and McCulloch, C. A. (2010) Regulation of cell adhesion to collagen via beta 1 integrins is dependent on interactions of filamin A with vimentin and protein kinase C epsilon. Experimental Cell Research 316, 1829-1844

33. Boon, R. A., Leyen, T. A., Fontijn, R. D., Fledderus, J. O., Baggen, J. M. C., Volger, O. L., Amerongen, G. P. V., and Horrevoets, A. J. G. (2010) KLF2-induced actin shear fibers control both alignment to flow and JNK signaling in vascular endothelium. Blood 115, 2533-2542

34. Yang, C., Patel, K., Harding, P., Sorokin, A., and Glass, W. F. (2007) Regulation of TGF-beta
PKCβII in Prss14/epithin shedding induces cell invasion

1/MAPK-mediated PAI-1 gene expression by the actin cytoskeleton in human mesangial cells. *Experimental Cell Research* **313**, 1240-1250

35. Szabo, R., Rasmussen, A. L., Moyer, A. B., Kosa, P., Schafer, J. M., Molinolo, A. A., Gutkind, J. S., and Bugge, T. H. (2011) c-Met-induced epithelial carcinogenesis is initiated by the serine protease matriptase. *Oncogene* **30**, 2003-2016

36. Sales, K. U., Friis, S., Abusleme, L., Moutsopoulos, N. M., and Bugge, T. H. (2015) Matriptase promotes inflammatory cell accumulation and progression of established epidermal tumors. *Oncogene* **34**, 4664-4672

37. Buzza, M. S., Netzel-Arnett, S., Shea-Donohue, T., Zhao, A., Lin, C. Y., List, K., Szabo, R., Fasano, A., Bugge, T. H., and Antalis, T. M. (2010) Membrane-anchored serine protease matriptase regulates epithelial barrier formation and permeability in the intestine. *Proc Natl Acad Sci U S A* **107**, 4200-4205

38. Wagner, E. F., and Nebreda, A. R. (2009) Signal integration by JNK and p38 MAPK pathways in cancer development. *Nature Reviews Cancer* **9**, 537-549

39. Bubici, C., and Papa, S. (2014) JNK signalling in cancer: in need of new, smarter therapeutic targets. *Br J Pharmacol* **171**, 24-37

40. Cho, E. G., Schwartz, R. H., and Kim, M. G. (2005) Shedding of membrane epithin is blocked without LDLRA4 and its protease activation site. *Biochem Biophys Res Commun* **327**, 328-334

41. Yoon, J. B., Kim, S. J., Hwang, S. G., Chang, S., Kang, S. S., and Chun, J. S. (2003) Non-steroidal anti-inflammatory drugs inhibit nitric oxide-induced apoptosis and dedifferentiation of articular chondrocytes independent of cyclooxygenase activity. *Journal of Biological Chemistry* **278**, 15319-15325
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Figure 1. SP600125 or PMA induce Prss14/epithin ectodomain shedding. (A) Diagram of Prss14/epithin domain structure and processed forms. Epi-S’, Epi-S, aEpi-S are indicated. (B) Effects of MAP kinase inhibitors in Prss14/epithin shedding. 427.1.86 cells were treated with 10 μM of PD98059, 20 μM of SB203580, and 5 μM of SP600125 for 30 min, and then treated with, or without 0.5 μM of PMA for additional 2 h. (C) Dose- and time-dependent profiles of Epi-S’ and Epi-S. 427.1.86 cells were treated with the indicated concentration of SP600125 for 2 h (Left panel), and with 5 μM of SP600125 up to 2 h (Right panel). (D) 427.1.86 cells were pretreated with 10 μM of TAPI-0 (TPI) for 30 min, and then cells were treated with 5 μM of SP600125 or 0.5 μM of PMA for additional 2 h. TACE inhibitor abolished the appearance of Epi-S’ while retaining Epi-S in the cell, regardless of shedding induction methods, PMA, and SP600125. (E) Removal of TACE with siRNA abolished shedding of Prss14/epithin. 427.1.86 cells were transfected with 200 nM of TACE siRNA for 48 h, starved for serum for 4 h, and then treated with 5 μM of SP600125 or 0.5 μM of PMA for 2 h. The control samples were treated exactly the same way except transfection with non-targeting control siRNA. (F) SP600125 dose-
dependently induced Epi-S’ and aEpi-S in T47D cells. SP600125 was treated for 2 hours. (G) SP600125 time (with 5 µM) and dose (for 2 h) dependently induced Epi-S’ and aEpi-S in 4T1 cells. In all panels, soluble form of Prss14/Epithin (Epi-S’) collected from culture medium and other proteins including Prss14/Epithin (Epi-S) from cell lysates were detected by western blot analysis. Tubulin or β-actin was used for normalization.
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Figure 2. SP600125-induced Prss14/epithin shedding involves JNK activity as well as de novo synthesis of labile protein. (A) 427.1.86 cells were pretreated with 10 μM of anisomycin (AN) for 30 min, and then treated with 5 μM of SP600125 (SP) for additional 2 h. Soluble form of Prss14/Epithin (Epi-S') collected from culture medium and Prss14/epithin (Epi-S) in the cells were detected by western blot analysis. (B) Suppression of JNKs by transfection with JNK1- or JNK2-specific siRNA induced Prss14/epithin shedding. 427.1.86 cells were transfected with 100 nM of JNK siRNA or non-targeting control siRNA. After 48 h, medium was replaced and incubated for additional 2 h before harvesting medium and cells. Control siRNA designed not to target any genes was used as a negative control in knockdown experiments. The band intensities were scanned and estimated the degree of % reduction or fold increase relative to control samples as described in the text. (C) Effects of cycloheximide (CHX) in SP600125-induced shedding. 427.1.86 cells were pretreated with 10 μM cyclohexamide, and then treated with 5 mM SP600125 for additional 2h before harvesting the samples. (D) Effects of pretreatment of actinomycin D (AD; 5 μM for 30 min) and/or α-amanitin (α-A; 5 μM for 12 h) on the SP600125-induced shedding was analyzed as in (A).
Figure 3. PKCβII is responsible for PMA- and SP600125-induced shedding of Prss14/epithin. (A) 427.1.86 cells were pretreated with 5 μM of Go6976 (Go) or 1 μM of PKCβ selective inhibitor (βi) before 5 μM of SP600125 or 0.5 μM of PMA treatment for 2 h. (B) PKCα or β knockdown effects on SP600125 or PMA induced Prss14/epithin shedding. 427.1.86 cells were transfected with 200 nM of PKCα or PKCβ siRNA or nontargeting control siRNA for 48 h, and then treated with 5 μM of SP600125 (SP) or 0.5 μM of PMA for additional 2 h. PKCβ knockdown abolished the SP600125- and PMA-induced shedding of Prss14/epithin. (C) 427.1.86 cells were transfected with 1 μg/mL of dominant negative form (DN) of PKCβII and PKC PKCβII for 72 h. Control cells were transfected with empty vector. DN forms of PKCβII inhibited shedding. (D) PKCβ inhibition to PRSS14 shedding in two human cell lines. PC3 prostate cancer cells and MCF7 breast cancer cells were maintained in serum free medium for overnight, and then treated with 1 μM of PKCβ inhibitor for additional 6 h before testing.
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Figure 4. JNK inhibition increases PKCβII activity, translocation into membrane, and TACE phosphorylation. (A) Phosphorylation of PKCβII by JNK inhibitor. 427.1.86 cells were pretreated with 10 μM of anisomycin for 30 min, and then treated with 5 μM of SP600125 for additional 1 h. SP600125 treatment induced PKCβII phosphorylation. Relative values of band intensity are expressed as means ± SD for 4 independent experiments. (B) Kinetics of SP600125- or PMA-induced PKCβII activity is shown. 427.1.86 cells were incubated with 5 μM of SP600125 or 0.5 μM of PMA for 0, 30, 60, 120 min. (B) Kinetics of PKCβII activity. After immunoprecipitation, PKCβII activities were determined by ADP-GloTM kinase assay kit. All values are expressed as means ± SD (**p < 0.01, #p < 0.05, ###p < 0.001, n = 3). (C) Enzymatic activity of PKCβII induced by SP600125 alone not by anisomycin combination to SP600125. 427.1.86 cells were pretreated with 1 μM of anisomycin for 30 min, and then treated with 5 μM of SP600125 for additional 1 h. (D) Immunofluorescent staining of PKCβII overexpressed 427.1.86 cells. Cells were transfected with 1 μg/mL of PKCβII wild-type cDNA for 48 h, and then treated with 5 μM of SP600125 or 0.5 μM of PMA for 1 h. Immunofluorescence staining was performed with anti PKCβII polyclonal antibody (1:50) followed by FITC conjugated anti rabbit IgG antibody (1:200). For nucleus staining, cells were incubated with DAPI for 10 min. Membrane localization of PKCβII was indicated with arrows. The images of two cells treated with SP600125 were stylized by embossing appearance of the signal intensities using Adobe Photoshop. The graph indicates the percent of cells with PKCβII localized in the membrane from 4 independent experiments. (E) Membrane localization of PKCβII by cellular fractionation. 427.1.86 cells were treated...
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with 5 μM of SP600125 up to 60 min and the PKCβII in cytosolic and membrane fractions were examined. Relative values of band intensity are expressed as means ± SD for 3 independent experiments. Arrowhead indicates PKCβII. *p < 0.05. (F) Phosphorylation of TACE by shedding inducers. In the cells treated with 0.5 μM of PMA or 5 μM of SP600125 for 1 h, the phosphorylation of TACE was analyzed by western blot analysis using antibody specific for phosphorylated TACE (Thr 735).
Figure 5. PKCβII is critical for PMA- or SP600125-induced cell invasion. (A) Schematic diagram of serum induced invasion assay (left panel). Invasion of 4271.86 cells depends on TACE activity and Prss14/epithin expression. After serum starvation for 12h, cells were plated on upper chamber and DMSO or 20 μM of TAPI-0 was added in lower chamber. Invaded cells to the underside of the membrane for 24 h were stained with crystal violet and counted. Scatter plot shows fold change of the average number of invaded cells in five randomly selected fields. (B) PKCβII was knocked down in 427.1.86 cells using PKCβII siRNA (KD2, 3, and 4). Expression of PKCβII was reduced in KD3 and KD4. (C) Invasive activity in PMA- and SP600125-treated 427.1.86 and PKCβII knockdown cells. The graph shows fold change of the average number of invaded cells on the underside of the membrane after 24 h in the presence or absence of PMA or SP600125. Cells in five different microscopic fields were counted. All statistical analyses were performed using unpaired two-tailed Student’s t-test. *p < 0.05, ns for not significant. Error bars, mean ± SD (n=3). (D) Model of intracellular signaling events and modulation by SP600125 and PMA during ectodomain shedding of Prss14/epithin.
Figure 6. PKCβII and JNKs are good prognostic markers in metastatic breast cancer together with ST14. (A) Box plots presented mRNA expression of indicated genes in ER- and ER+ breast cancer patients from TCGA datasets. Comparisons were analyzed by unpaired two-tailed Student’s t-test. (B) Survival analysis of four breast cancer patient groups divided by expression levels of ST14 and PKCB (left) or MAPK9 (right). P-values were calculated using log-rank statistics. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
A JUN N-terminal kinase inhibitor induces ectodomain shedding of the cancer-associated membrane protease Prss14/epithin via protein kinase C βII
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