Proteolysis of CCN1 by Plasmin: Functional Implications

Usha R. Pendurthi, Tien T. Tran, Marina Post, and L. Vijaya Mohan Rao

Biomedical Research Division, The University of Texas Health Center at Tyler, Tyler, Texas

Abstract

Plasmin is shown to play a crucial role in many pathophysiologic processes primarily through its ability to degrade extracellular matrix (ECM) and/or mobilizing growth factors that are sequestered in the ECM. Cysteine-rich 61 (CCN1) is a matricellular protein of which expression is up-regulated in cancer and various vascular diseases. The present study was undertaken to investigate whether plasmin liberates CCN1 from the ECM and whether the released growth factor modulates endothelial cell migration. Treatment of breast carcinoma cells (MDA-MB-231) with plasmin released a truncated form of CCN1 (28 kDa) into the overlying medium. Experiments with recombinant CCN1 confirmed that plasmin effectively cleaves CCN1. Thrombin and other clotting/fibrinolytic proteases are ineffective in cleaving CCN1. Further studies revealed that the conditioned medium of plasmin-treated carcinoma cells supports endothelial cell migration and that antibodies specific to CCN1 blocked this enhancing effect. These data were the first to show that plasmin can liberate a pluripotent matrix signaling protein, CCN1, from the ECM. Because both CCN1 and the components of the plasmin generation system are present in tumor cells and a variety of other cells, the proteolysis of CCN1 by plasmin may play a role in many pathophysiologic processes, including tumor cell-mediated angiogenesis. (Cancer Res 2005; 65(21): 9705-11)

Introduction

In addition to its role in fibrinolysis, the plasminogen activator (PA)/plasmin system has an important function in many pathophysiologic processes, including cancer, atherosclerosis, vascular remodeling, and wound healing (1-4). Plasmin is shown to affect various cellular processes primarily through the proteolysis of the extracellular matrix (ECM), either directly or indirectly, via activation of matrix metalloproteinases (MMP; refs. 1, 2). Plasmin may also play a role in tissue remodeling and angiogenesis by activating/ liberating growth factors, such as transforming growth factor-β1 (TGF-β1; refs. 5, 6) and basic fibroblast growth factor (bFGF; refs. 7, 8), from the ECM. Recent studies suggest that plasmin also stimulates various signaling pathways and induces expression of several genes that play an important role in inflammation (9, 10). Our recent studies (11) show that plasmin up-regulates the expression of cysteine-rich 61 (CCN1, CYR61) in fibroblasts, a matricellular protein that is capable of regulating various cellular functions (12).

CCN1 belongs to a novel family of growth regulators, often called cysteine-rich 61, connective tissue growth factor, nephroblastoma overexpressed (CCN) family (13). CCN1 is a secreted protein and associates with the ECM and cell surfaces (14). Purified CCN1 is shown to support cell adhesion, migration, and proliferation of endothelial cells and fibroblasts (15-19). CCN1 acts as a ligand to multiple integrin receptors and its cellular activities are shown to be mediated in part through interaction with integrins and cell-surface heparan sulfate proteoglycans in a cell type- and context-specific manner (15, 19, 20). Overexpression of CCN1 in tumor cells is shown to promote tumor growth and vascularization (21). Furthermore, recent studies show that CCN1 is highly expressed in breast carcinoma (22, 23) and gliomas (24, 25) and the level of expression is positively correlated with clinical and pathologic variables of cancer, suggesting that CCN1 plays a role in the pathogenesis of cancer. Although stimulatory effects of CCN1 on cell proliferation, migration, and survival via its interaction with various integrins are thought to be responsible for its role in tumorigenesis (26), at present, it is unclear how CCN1 sequestered in ECM could interact with its potential receptors or regulatory proteins.

CCN proteins are multimodular proteins. Except for CCN5/ WISP-2, all other CCN proteins contain four structural modules (26). The current view is that each of the four modules can act both independently and interdependently to elicit various cellular responses (26). If so, the production of truncated isoforms of the CCN proteins in pathophysiologic conditions could play a critical role in the modulation of their biological activity. Evidence had been provided for the presence of naturally occurring truncated forms of CCN2 and CCN3 that are biologically active (27, 28). The molecular mechanisms underlying the production of these secreted truncated forms are presently unknown.

Because coagulation and fibrinolytic pathways are frequently activated in many pathophysiologic conditions and are thought to contribute to pathogenesis of various diseases, including tumor metastasis (4), we have investigated in the present study whether proteases involved in clotting and fibrinolysis can generate biologically active truncated forms of CCN1. The present study reveals that plasmin rapidly cleaves CCN1 and releases the truncated form of CCN1 from breast carcinoma cells. The truncated form of CCN1 released from the carcinoma cells is shown to promote endothelial cell migration. These studies raise a novel possibility by which plasmin can play a role in angiogenesis and other cellular processes.

Materials and Methods

Cells. MDA-MB-231 cells were obtained from American Type Culture Collection (Rockville, MD) and cultured at 37°C and 5% CO2 in DMEM containing high glucose and supplemented with 1% glutamine, 1% penicillin/streptomycin, and 10% heat-inactivated fetal bovine serum (FBS). Primary cultures of human umbilical vascular endothelial cells (HUVEC) were obtained from Clonetics (San Diego, CA) and cultured in F-12K medium supplemented with 1% glutamine, 1% penicillin/streptomycin, 10% FBS, 15 units/mL heparin, and 30 μg/mL endothelial cell growth supplement. Endothelial cells, at passages 3 to 7, were used in the present study.

Requests for reprints: Usha R. Pendurthi, Biomedical Research Division, The University of Texas Health Center at Tyler, Tyler, TX 75708. Phone: 903-877-7342; Fax: 903-877-7426; E-mail: usha.pendurthi@uthct.edu.

© 2005 American Association for Cancer Research.

doI:10.1158/0008-5472.CAN-05-0982
Materials. Plasmin, thrombin, and other coagulant proteases (purified from human plasma) were obtained from either Enzyme Research Laboratories (South Bend, IN) or Haematologic Technologies, Inc. (Essex Junction, VT). Recombinant human factor VIIa was generously provided by Novo Nordisk (Gentofte, Denmark). D-Phe-t-Phe-L-Arg chloromethyl ketone (PPACK) was obtained from Calbiochem (San Diego, CA). Recombinant human CCN1 was expressed in High Five insect cells that were stably transfected with a CCN1 expression plasmid containing V5 and His tag (constructed in pIZ/V5-His vector, Invitrogen, Carlsbad, CA). The recombinant CCN1 from the conditioned medium was purified essentially as described earlier using SP-Sepharose chromatography (16). The recombinant protein was further purified by affinity chromatography on a nickel column to obtain an apparent homogeneous protein. The purified CCN1 was used to immunize a rabbit to raise polyclonal antibodies against CCN1. The antiserum was subjected to DEAE Affi-Gel Blue (Bio-Rad, Richmond, CA) chromatography to isolate IgG fraction. CCN1 polyclonal antibodies for epitopes corresponding to internal region of CCN1 (amino acids 163-240; H-78) and COOH terminus (C-20) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Cleavage of purified CCN1. Purified recombinant CCN1 (~10 μg/mL) was treated with varying concentrations of plasmin, thrombin, factor VIIa/tissue factor, or other proteases. At different time intervals, aliquots were removed and added to SDS-PAGE sample buffer to stop the reaction. The samples were subjected to SDS-PAGE followed by immunoblot analysis with the CCN1 antibodies H-78 and C-20.

Cleavage of cell-associated CCN1 and collection of conditioned medium. Confluent monolayers of MDA-MB-231 cells cultured in 12-well plates were washed with serum-free DMEM and allowed to stabilize in serum-free DMEM for 2 hours before they were subjected to treatments. The monolayers were treated with control serum-free DMEM (250 μL) or serum-free DMEM containing plasmin or thrombin (10 nmol/mL). At varying times, the overlying cell medium was removed (to which PPACK was added immediately to a final concentration of 1 μmol/L) and the cells were harvested in 200 μL cell lysis buffer [20 mmol/L Tris-HCl, 150 mmol/L NaCl (pH 7.5) containing the inhibitory cocktail, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% Triton X-100, 1 μmol/L PPACK]. Fifteen microliters of samples were loaded on SDS-PAGE followed by immunoblot analysis with CCN1 or β-actin antibodies. To collect conditioned cell medium for cell migration and other studies, cells cultured in T-75 flasks were treated with 4 mL control serum-free DMEM or serum-free DMEM containing 10 nmol/mL plasmin or thrombin. In parallel, plasmin was added to a culture flask containing no cells. At the end of 2-hour treatment, the overlying cell medium was removed and then treated with PPACK (1 μmol/L) for 90 minutes at room temperature to inhibit the proteolytic activity of plasmin and thrombin. The conditioned medium was then dialyzed overnight against buffer A [10 mmol/L HEPES, 0.15 mol/L NaCl (pH 7.5) containing 4 mmol/L KCl and 11 mmol/L glucose] and passed through a 0.22-μm filter before they were used for cell migration studies.

Immunoadsorption and heparin-agarose adsorption. Anti-CCN1 IgG (raised against full-length recombinant CCN1) was coupled to Affi-Gel 15 (5 mg IgG/mL beads) using 0.1 mol/L HEPES (pH 7.5) as a coupling buffer by following a protocol suggested by the vendor (Bio-Rad). The conditioned medium (3 mL) collected from the plasmin-treated cells was incubated with anti-CCN1 agarose beads (0.5 mL) for 4 hours at 4°C. Then, the beads were loaded into a column and the flow-through (unbound material) was collected. The column was washed with 8 mL of 10 mmol/L HEPES, 0.15 mmol/L NaCl (pH 7.5), and the bound material was eluted with 0.1 mol/L glycine (pH 2.4). The pH of eluted fractions was adjusted to 7.5 by adding 1 mol/L Tris-HCl. Immunoblot analysis revealed that the flow-through was devoid of the 28-kDa CCN1 fragment and the fragment was recovered in eluted fractions (two to three fractions, 1 mL each). These fractions were pooled and dialyzed overnight against buffer A. A similar procedure was followed for adsorption with heparin-agarose beads and the bound material was eluted with a high-salt buffer (buffer A containing 1.0 mol/L NaCl). In this case, the CCN1 peptide fragment was present in the flow-through and not in the eluted fractions.

Endothelial cell migration. A Transwell system (polycarbonate filter, 8 μm pore size, 6.5 mm diameter) was used to evaluate cell migration. The top chamber was coated with fibronectin (40 μg/mL) for 2 minutes and then air dried. HUVECs (50,000 in 100 μL serum-free DMEM) were added to the upper well and 100 μL of conditioned medium (plus 400 μL of serum-free DMEM) were added in the bottom chamber. At the end of 6-hour incubation at 37°C/5% CO2, cells on the top side of the membrane were removed by swiping with a damp cotton swab. The membrane was rinsed once with distilled water and fixed/stained with hematoxylin (Hema3 staining kit, Fisher Scientific, Pittsburgh, PA) according to the instructions provided with the kit. The number of cells that had migrated was determined by counting the number of cells on the underside of membrane (in five randomly selected fields) at ×20 magnification using a microscope equipped with a grid in its eyepiece.

Results

Plasmin cleavage of CCN1. Our recent studies on fibroblasts showed that both plasmin and thrombin induced CCN1 mRNA expression (11, 29), but the CCN1 antigen was found only in cell lysates of thrombin-stimulated cells (30). These data suggested that plasmin might be liberating/cleaving newly synthesized CCN1, which associates with cells or ECM. To test this possibility, we investigated whether plasmin cleaves CCN1. Recombinant CCN1 was treated with plasmin, thrombin, or other clotting/fibrinolytic proteases for varying times and the aliquots were analyzed by immunoblot analysis using CCN1 antibodies that identify different epitopes on the CCN1. As shown in Fig. 1, plasmin (1 nmol/L) rapidly cleaved CCN1, initially resulting in two peptide fragments corresponding to molecular weights of 28 and 21 kDa. H-78 antibody, which recognizes an internal epitope of CCN1, detected the 28-kDa fragment (Fig. 1A), whereas C-20 antibody...
Plasmin treatment of breast carcinoma cells releases a potent endothelial cell migratory factor. To determine pathophysiologic relevance of plasmin release of CCN1, we next investigated whether exposure of MDA-MB-231 cells to plasmin releases a soluble factor that is capable of promoting endothelial cell migration. As shown in Fig. 3A, the conditioned medium obtained from the plasmin-treated cells increased endothelial cell migration by 3- to 4-fold compared with the basal endothelial cell migration observed in the absence of a stimulant. In contrast, the conditioned medium from MDA-MB-231 cells that were treated with serum-free medium or thrombin failed to promote endothelial cell migration. The increased endothelial cell migration observed with the conditioned medium of plasmin-treated cells could not be due to traces of plasmin leftover in the conditioned medium because PPACK, an active site inhibitor that was used to inactivate plasmin protease activity, was shown to completely inhibit the protease activity of plasmin (data not shown).

Next, we investigated whether plasmin cleaves and liberates CCN1 that is associated with cells or ECM. For these studies, we used MDA-MB-231 breast carcinoma cells, which constitutively express CCN1. MDA-MB-231 cells were treated with a control serum-free medium or serum-free medium supplemented with plasmin. Immunoblot analysis of cell lysates and overlying conditioned medium obtained at varying times showed that plasmin released the 28-kDa CCN1 fragment into overlying conditioned medium with a corresponding decrease in cell-associated CCN1 (Fig. 2). Similar results were observed with other breast carcinoma cells, MCF-7 and MDA-MB-435 (data not shown). However, in contrast to the data obtained with plasmin cleavage products of recombinant CCN1, C-20 antibody failed to detect the COOH-terminal CCN1 peptide fragment either in the conditioned medium or in cell lysates of tumor cells exposed to plasmin. The failure of C-20 antibodies to detect the COOH-terminal peptide fragment could probably be due to the low avidity of these antibodies to CCN1, particularly to the peptide fragment. Alternatively, the COOH-terminal fragment is proteolytically degraded further by plasmin and thus not detected by C-20 antibodies. Plasmin-induced proteolysis of CCN1 in MDA-MB-231 cells was not mediated through plasmin activation of MMPs because BM6001 and GM1489, potent and broad range inhibitors of MMPs (MMP1, MMP2, MMP3, MMP8, and MMP9), failed to inhibit plasmin cleavage of CCN1 (data not shown).

Figure 2. Plasmin cleavage of cellular CCN1 and the release of CCN1 fragment into the overlying medium. MDA-MB-231 breast carcinoma cells, which constitutively express CCN1, were treated with plasmin or thrombin (10 nmol/L) for varying times. Both the cell lysates and the overlying cell medium were subjected to SDS-PAGE followed by immunoblot analysis with H-78 or anti-β-actin antibodies.
shown). More importantly, plasmin that was subjected to a similar procedure (except exposing it to the cells) failed to promote endothelial cell migration.

Because plasmin has been shown to release or activate growth factors, such as bFGF (7, 8) and TGF-β (5, 6), which could have been sequestered on ECM or cell surfaces, we first investigated whether neutralizing antibodies against bFGF and TGF-β impaired the ability of the conditioned medium of plasmin-treated cells to support endothelial cell migration. Both bFGF and TGF-β neutralizing antibodies had only minimal and insignificant effects on endothelial cell migration supported by the conditioned medium of plasmin-treated cells (Fig. 3B), suggesting that it is unlikely that the release of these growth factors is responsible for the increased endothelial cell migration observed with the conditioned medium. Similarly, anti-interleukin-8 (IL-8) antibodies also had no effect on cell migration.

Next, to determine whether CCN1 released into the conditioned medium on exposure of breast carcinoma cells to plasmin is responsible for endothelial cell migration, we investigated the effect of anti-CCN1 antibodies on endothelial cell migration supported by the conditioned medium of plasmin-treated cells. As shown in Fig. 4A, CCN1 antibodies fully attenuated the increased endothelial cell migration observed with the conditioned medium of plasmin-treated cells. As expected, control IgG had no effect on endothelial cell migration. In additional experiments, removal of CCN1 from the conditioned medium of plasmin-treated cells (by passing through an anti-CCN1 antibody column) abolished the ability of the conditioned medium to promote cell migration (Fig. 4B). Consistent with this observation, the material eluted from the CCN1 antibody column promoted cell migration.

Because we were unable to detect the COOH-terminal fragment of CCN1 by immunoblot analysis either in the conditioned medium or in cell lysates, we cannot completely rule out the presence of the COOH-terminal derived fragment(s) or its contribution to endothelial cell migration in the conditioned medium derived from plasmin-treated cells. Earlier studies showed that CCN1 binds to heparin tightly and the heparin binding sites are localized within the COOH-terminal module of the molecule (19). We have exploited this property to determine whether the 28-kDa fragment of CCN1, which lacks the COOH-terminal module, or the COOH-terminal fragment of CCN1 was responsible for the cell migration observed in the conditioned medium of plasmin-treated cells. The conditioned medium of plasmin-treated cells was incubated with heparin-agarose beads and the beads were washed and eluted with 1.0 mol/L NaCl. Both the unbound and the eluted materials (after the dialysis) were evaluated for their ability to support cell migration and for the presence of the 28-kDa fragment (by immunoblot analysis). The data revealed that the flow-through material, and not the eluted material, supported cell migration (Fig. 4C). A small difference in cell migration observed between the starting material and the flow-through material was not statistically significant (P = 0.42). Immunoblot analysis revealed that the 28-kDa CCN1 fragment was found exclusively in the flow-through material (data not shown). These data suggest that the NH2-terminal fragment (28 kDa) is responsible for the cell migration observed in the conditioned medium.

To further strengthen the data that the CCN1 fragment generated by plasmin is capable of enhancing endothelial cell migration, we compared the migrating enhancing ability of plasmin-cleaved CCN1 with that of full-length recombinant CCN1. As shown in Fig. 5, both full-length recombinant CCN1 and plasmin-cleaved CCN1 increased endothelial cell migration in a dose-dependent manner. The increase was evident with as low as 200 ng/mL CCN1 and reached statistical significance at 1 μg/mL. Small differences between full-length and plasmin-cleaved recombinant CCN1 were not statistically significant. These data show that the migrating ability of CCN1 was retained on proteolysis by plasmin.

**Discussion**

In addition to its role in clot lysis, the PA/plasmin system is thought to have an important function in several pathophysiologic...
processes, such as inflammation, wound healing, vascular remodeling, tumor invasion, and metastasis, by regulating various cellular activities involved in these processes (1, 4, 31, 32). In tumor metastasis, the system seems to be involved not only in tumor invasion but also in tumor angiogenesis (32–34). Although uPA, uPA receptor, and PA inhibitor-1 were shown to regulate tumor cell migration and angiogenesis by plasmin-independent mechanisms (32, 33, 35), plasmin seems to play a crucial role in facilitating these processes by breaking anatomic barriers through degradation of proteins in basement membranes and ECM directly or indirectly via activation of other proteases (36). Hydrolysis of matrix components by plasmin may lead to the release of biologically active fragments of the matrix and/or the release of matrix-bound growth factors. This would facilitate the diffusion of biologically active peptide fragments and growth factors to more distant sites. Earlier studies showed that plasmin released or cleaved growth factors, such as bFGF (7, 8) and TGF-β1 (5, 6), from the ECM. In the present study, we show that plasmin also cleaves a pluripotent matricellular signaling protein, CCN1, and liberates a biologically active peptide fragment of CCN1 from tumor cells/matrix, which could support endothelial cell migration.

CCN1 is a novel ECM-associated signaling protein, which was shown to influence not only tumor angiogenesis but also a myriad of cellular functions that contribute to many pathophysiologic conditions (12, 21). CCN1 is composed of four discrete structural domains: insulin-like growth factor–binding protein–like module, von Willebrand factor type C–like module (VWC), thrombospondin 1–like module (TSP1), and cysteine knot–containing family of growth regulator–like module (CT). A variable region connects domains I and II with domains III and IV. TSP1 repeat (WSxCSxCG) is thought to be involved in binding to matrix macromolecules, particularly to sulfated glycoconjugates (13). CT domain contains two heparin binding motifs (27) that may participate in the interaction with ECM (28). Our present data show that plasmin cleaves CCN1 in the thrombospondin module region, probably at R250 (Val251) or R259 (Lys260), because the ~28-kDa peptide fragment of the cleavage products was detected by the antibody that recognizes the variable region (163-240 amino acids) and not the COOH terminus. Thus, the 28-kDa fragment represents the NH2-terminal portion of the protein. Multiple attempts of NH2-terminal sequencing of the 21-kDa COOH-terminal peptide fragment (using ~100 pmol or more of the fragment) to identify the precise cleavage site were unsuccessful.

The observation that the 28-kDa fragment of CCN1 was found in overlying medium and not in cell lysates suggests that the binding motifs that participate in CCN1 binding to cell surfaces or ECM lie outside of this region (i.e., in the COOH-terminal portion of TSP1 domain and/or in the CT domain). At present, it is unclear whether the COOH-terminal fragment of CCN1 remains attached to the ECM or cell surfaces or is released into the overlying medium because we were unable to detect this band either in the cell lysate or in the overlying medium on immunoblot analysis with the C-20 antibody.

CCN1 is a member of the newly established CCN family that plays an important regulatory role in development, wound healing, vascular diseases, and cancer (12, 26, 37, 38). CCN1 is a growth factor–inducible immediate-early gene expressed at very low levels in various cells but rapidly induced by growth factors, proteases, and other stimuli (11, 29, 39–41). On synthesis, the CCN1 protein is secreted and associates with cell surfaces and ECM (14). CCN1 has been shown to support cell adhesion and cell migration and to augment growth factor–induced cell proliferation in various cell types through interactions with integrins and cell-surface heparan sulfate proteoglycans in a cell type– and context-specific manner (12, 15, 18–20, 42, 43). In endothelial cells, CCN1 was shown to induce cell migration through interactions with integrin αvβ3 (43). Recent studies identified a novel 20-residue sequence in the VWC domain of CCN1 (residues 116-135) as a functional binding site for integrin αvβ3 (44). Our present observation that the NH2-terminal fragment of plasmin-cleaved CCN1 supports endothelial cell migration agrees with the above finding.

Recent studies showed that CCN1 is highly expressed in breast cancers and elevated levels of this protein in primary breast cancer are associated with more advanced disease (23, 24, 45). Tsai et al. (22) examined the expression of CCN1 in many human breast cancer cell lines and found that the expression of CCN1 is strongly correlated with the ability of breast cancer cells to invade in vitro and to metastasize in vivo. Furthermore, overexpression of CCN1 in MCF-12A normal breast cells was shown to induce tumor formation and vascularization in nude mice (46). Similarly, expression of CCN1 cDNA under the regulation of a constitutive promoter in RF-1 gastric adenocarcinoma cells significantly enhanced tumor growth and vascularization (21). Because CCN1 protein has been shown to influence many cellular activities, it is possible that CCN1 could contribute to tumor growth by multiple mechanisms (26). However, the ability of CCN1 to promote angiogenesis is thought to be responsible primarily for tumor growth and vascularization (21). Angiogenesis requires the coordinated execution of a series of cellular processes, starting from the degradation of basement membranes and ECM surrounding the parent vessel to proliferation and migration of endothelial cells from the parent vessel toward an angiogenic signal to form new capillary sprouts. Several studies showed that tumor cells, including breast cancer cells, express uPA and tissue-type PA and specific receptors for uPA and plasminogen (see review in ref. 33). In agreement with this, primary breast cancers were shown to express active enzymes capable of catalyzing

![Figure 5](https://example.com/figure5.png)
Plasmin cleavage of CCN1 may have wider biological implications because it permits the CCN1 partition into the soluble phase, rather than into the insoluble matrix, and therefore allows the CCN1 to diffuse freely within the tissue and interact with its plasma membrane receptors on various cell types. The 28-kDa fragment could act as either an agonist or an antagonist for full-length CCN1 in a cell type- and context-specific fashion because CCN1 interacts with specific integrins in specific cell types, either requiring the CT domain or independent of the CT domain (12, 20, 42, 43). Because several pathologic conditions are associated with up-regulation of CCN1 (45–49,51) and many cell types express the PA/plasmin system, which could be up-regulated further by pathologic conditions (33, 52–54), it is likely that the truncated CCN1 fragments would be generated in vivo. If so, the 28-kDa fragment of CCN1 may serve as a marker for pathogenesis of disease, particularly cancer and cardiovascular diseases. Although shorter isoforms for other proteins in the CCN family have been detected in biological fluids (26, 27, 55), we are not aware of any published studies that examined the presence of the truncated form of CCN1 in biological fluids in either normal or diseased conditions. Thus, it will be interesting to test in the future whether the truncated form of CCN1 can be found in plasma or biological fluids of cancer patients and patients with cardiovascular diseases and whether its levels correlate with disease variables. It is also of interest to see whether plasmin cleaves other members of the CCN family. In this context, it may be relevant to note that there is 60% to 70% protein sequence homology among various members of CCN family in the region where plasmin cleaves the CCN1 (between amino acids 243 and 267 in CCN1) and the putative plasmin cleavage site R250/V251 (of CCN1) is preserved in five of six members of the CCN family (26).

Acknowledgments


Grant support: NIH grant HL 65500.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Veena Pappan for valuable help in the culturing of cells.

References


Proteolysis of CCN1 by Plasmin: Functional Implications

Usha R. Pendurthi, Tien T. Tran, Marina Post, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/21/9705

Cited articles
This article cites 54 articles, 30 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/21/9705.full.html#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/65/21/9705.full.html#related-urls

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

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

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