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Hepsin colocalizes with desmosomes and induces progression of ovarian cancer in a mouse model

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Abstract

Hepsin is a serine protease that is widely expressed in different tissues and cell types, most prominently in the normal liver and kidney. Overexpression of hepsin has been associated with prostate cancers, ovarian cancers and renal cell carcinomas. The physiological functions of hepsin in normal tissues and tumors are poorly understood. To gain insight into its function in ovarian cancer, we analyzed the expression and subcellular localization of hepsin protein in ovarian cancer cell lines and tumors. We showed that the membrane-associated hepsin protein is present at desmosomal junctions, where it colocalizes with its putative proteolytic substrate hepatocyte growth factor. Consistent with the growing evidence that desmosomal junctions and their constituents play a role in cancer progression, we demonstrated that overexpression of hepsin promotes ovarian tumor growth in a mouse model. The ability of ectopic hepsin to induce tumor growth in mice is abrogated by the mutation of 3 critical residues in the catalytic domain, thus implicating the enzymatic activity of hepsin in promoting tumor progression.

Keywords

desmosome; hepsin; ovarian cancer; serine protease

Hepsin is a type II transmembrane serine protease that was originally cloned from cDNA libraries of human liver and hepatoma cells.[1] The human hepsin gene localizes to chromosome 19q11-13.2 and encodes a glycoprotein of 417 amino acids with a predicted molecular mass of 51 kDa.[2,3] Hepsin is likely to be synthesized as a single chain zymogen and cleaved by an unknown enzyme to generate the mature, disulfide-linked 2-chain form.[4] In addition to the catalytic domain, which includes the active site triad residues of His, Asp and Ser, hepsin protein is characterized by a macrophage scavenger receptor-like domain of unknown function, a transmembrane domain and a short cytoplasmic domain.[1–7] An alternatively-spliced, nontransmembrane isoform of human hepsin was recently identified.[8]

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Hepsin is frequently overexpressed in prostate cancers,[9–14] renal cell carcinomas and ovarian cancer.[15–18] The overexpression of hepsin mRNA was found in 60% of low-grade ovarian tumors and 80% of ovarian carcinomas,[17] whereas it was not expressed in normal ovarian tissues.[15] The biological functions of hepsin in normal tissues and cancers are not well understood. *In vitro* data implicate hepsin in the maintenance of cell morphology and cell growth,[19] blood coagulation through human factor VII activation[20] and developmental processes.[7] The evidence for these functions remains inconclusive since, aside from a profound hearing loss,[21] hepsin knockout mice develop normally and do not show differences in various measures of blood coagulation compared to wild-type lit-termates.[22,23]

In vitro assays using neutralizing antibodies demonstrated that, while hepsin does not play a role in the proliferation of prostate, ovarian and hepatoma cell lines in culture, it plays a role in the invasion of ovarian and prostate cells in transwell-based invasion assays.[24] Recently, Klezovitch *et al.* provided *in vivo* evidence that overexpression of hepsin in a mouse model of nonmetastatic prostate cancer has no impact on cell proliferation, but it causes disorganization of the basement membrane and promotes primary prostate cancer progression and metastasis.[25] These results are consistent with the notion that malignant cells depend on a group of proteolytic enzymes that are synthesized and secreted by tumor cells to disrupt basement membranes, invade neighboring tissues and metastasize.[26]

Material and methods

Antibodies

Antibodies were obtained from the following sources: rabbit polyclonal anti-hepsin antibody raised against a synthetic peptide (aa 241–260) of human hepsin (Cayman Chemical); rabbit polyclonal anti-hepsin antibody raised against a synthetic peptide corresponding to C-terminus (aa 397–416) of rat hepsin (obtained from Dr. Yoko Aniya); monoclonal anti-desmoplakin and anti-α-tubulin (Sigma-Aldrich); monoclonal anti-connexin-43, anti-E-Cadherin, anti-β-Catenin and anti-γ-Catenin (BD Transduction Laboratories); monoclonal anti-occludin (Zymed Laboratories); rabbit polyclonal anti-HGF-α raised against the N-terminal domain of human HGF-α (American Research Products); rabbit polyclonal anti-HGF-α raised against amino acids 32–176 of human HGF-α and monoclonal anti-pan-cytokeratin (Santa Cruz Biotechnology).

Cell lines and culture

The mouse ovarian cancer cell line C11 was generated by infecting ovaries from K5-TVA/p53$^{-/-}$ mice with retroviral RCAS vectors carrying genes that encode mouse *K-ras*^{G12D} and human *c-myc*. The human ovarian cancer cell lines OVCAR5 and OVCAR5-TR were obtained from Dr. Michael Seiden. The OVCAR5 cell line was exposed to incrementally increasing concentrations of paclitaxel for 3 months to generate the OVCAR5-TR subline that exhibits resistance to paclitaxel. Fortuitously, the OVCAR5-TR cells lost expression of the desmosomal protein des-moplakin. To render the human cell lines susceptible to avian RCAS virus infection, the avian retroviral receptor TVA was introduced into the OVCAR5 and OVCAR5-TR cells by stable transfection using the FuGENE 6 kit (Roche). SKOV3 cell line was obtained from ATCC. The mouse and human cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% glutamine and 1% penicillin/streptomycin (Mediatech).

Retroviral vectors and green fluorescent protein fusion constructs

Wild-type human *hepsin* full-length cDNA was amplified from a commercial universal human cDNA library (Clontech) using the following PCR primers: ATGGCGCAGAAGGAGGGTGGCCGGACTGTG and

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TCAGAGCTGGGTCACCATGCCGCTGGCTTCG. The resulting cDNA was sequenced and verified to be identical to the known *hepsin* cDNA sequence (accession number M18930). The 3 active-site residues H203, D257 and S353 constituting the catalytic triad[7] were sequentially mutated into Alanine by PCR-based mutagenesis to create the $hepsin^{mp}$ triple mutant bearing point mutations of H203A, D257A and S353A. Both the wt-*hepsin* and $hepsin^{mp}$ cDNA were originally carried in the pCEP4 vector (Invitrogen). To isolate the human *hepsin* and $hepsin^{mp}$ cDNAs, the pCEP4-based plasmids were digested with KpnI and NotI. Gateway technology (Invitrogen) was used to clone *hepsin* into a destination vector. Purified DNAs were ligated to the pENTR™ 1A entry cloning vector and transformed into TOP10 cells according to the manufacturer's instructions (Invitrogen). The BP cloning reactions were carried out using the RCAS-Y DV destination vector[27] with specific sites (*attL* x *attR*). pEGFP-N1 and pEGFP-C3 (Clontech Laboratories) were used to generate N-terminal and C-terminal hepsin-GFP fusion proteins, respectively. Hind III and EcoR I were used to subclone *hepsin* from pENTR™ 1A into pEGFP-N1 vector. Hind III and Sac II were used to subclone *hepsin* from pENTR™ 1A into pEGFP-C3 vector.

Virus preparation and cell infection

SKOV3 cells were transduced with hepsin or $hepsin^{mp}$ *via* retro-virus-mediated gene transfer and subsequent drug selection as previously described.[28] To produce retroviruses carrying the RCAS-*hepsin*, RCAS-$hepsin^{mp}$ and RCAS-*GFP* plasmids, immortalized DF-1 chicken cells were transfected using the FuGENE 6 kit (Roche) and grown in DMEM supplemented with 10% FBS, 30 g/L Tryptose Phosphate Broth, 1% chicken serum, 1% gluta-mine and 1% penicillin/streptomycin (Mediatech). The viruses were concentrated as previously described.[29,30] TVA-expressing mouse and human ovarian cancer cell lines were infected daily using frozen concentrated virus or fresh viral supernatants. Infection efficiencies were determined by green fluorescent protein (GFP) expression in infected cells.

Mouse injections

Parental SKOV3 cells and SKOV3 cells that were transduced with *hepsin* or $hepsin^{mp}$ were injected subcutaneously at the left flank of female athymic nude mice (Crl: NU/NU-nuBR) that were irradiated with 400 rad of γ-rays prior to injection. Each transfectant was injected into a group of 5 mice. The mice were monitored weekly for tumor formation, and tumor size was measured using a caliper. Tumor weight was determined by excising the tumors from mice at the end of experiment. C11 cells that were infected with RCAS-*GFP*, RCAS-*hepsin* or RCAS-$hepsin^{mp}$, were injected intraperitoneally into adult female FVB mice. The mice were sacrificed 6 weeks after cell injection and tumor dimensions, volume of ascites and metastatic pattern for each mouse were recorded.

Immunohistochemistry

Two tissue microarrays were constructed from paraffin-embedded blocks, which included 26 serous, 17 endometrioid, 17 clear cell, 6 mucinous and 4 transitional cell carcinomas. Tissue micro-array slides were deparaffinized with xylene and alcohol and microwaved in citrate buffer (pH 6.0) to unmask the epitopes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide and the slides were then incubated at room temperature for 20 min in 1.5% bovine serum albumin (BSA)/1 × phosphate-buffered saline (PBS) to reduce nonspecific background staining. Primary polyclonal hepsin antibody (Cayman Chemical) was applied to the slides at a 1:400 dilution and incubated at room temperature for 30 min. Bound antibody was detected by anti-rabbit horseradish peroxidase-labeled secondary antibody (Vector Laboratories) for 60 min. The slides were stained for 5 min with 0.05% 3′, 3-diaminobenzidine tetrahydrochloride and then counterstained with hematoxylin, dehydrated and mounted. The percentage of positive cells, localization and intensity (1+, 2+, 3+) of staining were recorded.

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Immunofluorescence microscopy

Cells grown in slide chambers (Nalgene) were fixed with methanol at −20°C for 20 min. Slides were then washed with PBS and permeabilized for 4 min with 0.1% Triton X-100 in PBS. Nonspecific staining was blocked with 3% goat serum at room temperature for 30 min. Cells were then incubated with specific primary antibodies in PBS/1.5% BSA overnight at 4°C. Secondary antibodies were labeled with fluorescein isothiocyanate (FITC) (Molecular Probes) or Cy3 (Sigma-Aldrich) at room temperature for 60 min. Slides were washed and embedded in mounting medium with 4′, 6′-diamidino-2-phenylindole (DAPI) (Vector Laboratories), and then analyzed with a Carl ZEISS fluorescence microscope and photographed using the MicroMAX (Princeton Instruments) digital system.

Western blotting

Cells were lysed in the CytoBuster™ protein extraction buffer (Novagen) and then centrifuged for 10 min at 4°C to clear the supernatant. The concentration of the extracted protein was determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories). Ten micrograms/lane of the extracted protein were loaded onto 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransferred to the nitrocellulose membrane (Bio-Rad Laboratories). The membrane was blocked with 5% nonfat dry milk in TBS (10 mM Tris, pH 7.5, 150 mM NaCl) for 1 hr at room temperature, followed by overnight incubation with polyclonal hepsin antibody (Cayman Chemical) in the blocking buffer at 4°C. After washing with TBS-Tween/Triton buffer (20 mM Tris, pH 7.5, 500 mM NaCl, 0.05% Tween-20, 0.2% Triton X-100), the membrane was incubated with horseradish peroxidase-conjugated anti-rabbit IgG antibody (Abcam) at a 1:5,000 dilution in blocking buffer for 1 hr at room temperature. The proteins were visualized using an enhanced chemiluminescence (ECL) system (Pierce Biotechnology).

Results

Hepsin expression and cellular localization in human ovarian carcinomas

Normal ovaries with epithelial inclusion cysts and the ovarian cancer tissue microarray, consisting of 70 ovarian carcinoma cores flanked with normal liver cores, were immunostained with a rabbit polyclonal antibody raised against a synthetic peptide (aa 241–260) that corresponds to the extracellular domain of human hepsin. Consistent with reports of high levels of hepsin expression in the normal liver,[6,31] hepsin protein was detected in the cytoplasm and membrane of hepatocytes (Fig. 1*a*). Weak expression of cytoplasmic hepsin was detected in the normal epithelium that lines the ovarian inclusion cyst (Fig. 1*b*).

To examine the expression and localization of hepsin protein in human ovarian tumors, the percentage of positive cells and the localization and intensity of staining were recorded for 26 serous, 17 endometrioid, 17 clear cell, 6 mucinous and 4 transitional cell carcinomas. Hepsin expression was mainly confined to the epithelial cells and not the adjacent stroma (Figs. 1*c* and 1*d*). The majority of tumors displayed cytoplasmic and/or membrane staining. The representative staining patterns in different ovarian carcinoma subtypes are shown in Supplementary Figure 1 All mucinous and transitional cell carcinomas showed cytoplasmic staining. Sixteen of 17 endometrioid carcinomas showed cytoplasmic staining and 1 showed membranous staining. Twelve of 15 clear cell carcinomas showed strong membranous staining. Fourteen serous carcinomas showed predominant membranous staining and 12 had predominant cytoplasmic staining. Overall, cytoplasmic localization of hepsin was prevalent in mucinous, endometrioid and transitional cell carcinomas, while the majority of serous and clear cell carcinomas displayed membranous localization.

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Membrane-associated hepsin is present at desmosomal junctions

To gain insight into the function of hepsin in ovarian cancer, we studied its subcellular distribution. The localization of hepsin was determined by immunofluorescence in the methanol-fixed OVCAR5 human ovarian cancer cell line using a rabbit polyclonal antibody raised against a synthetic peptide (aa 241–260) of human hepsin. Immunodetection revealed a granular staining in the cytoplasm as well as punctate staining at the sites of cell–cell contacts (Fig. 2*a*). The specificity of the membrane staining was demonstrated by complete inhibition of the staining upon preincubation of the antibody with its blocking peptide (not shown). The cyto-plasmic and membrane localization of hepsin was confirmed (not shown) with a rabbit polyclonal antibody raised against a synthetic peptide (aa 397–416) corresponding to the C-terminus of rat hepsin.[32] Hepsin was present in the cytoplasm and along cell–cell contacts in several other ovarian and prostate cancer cell lines that we examined (not shown).

To determine the precise localization of hepsin at the cell membrane, we performed 2-color immunofluorescence detection with polyclonal antibodies against hepsin and monoclonal antibodies against proteins specific for cellular junction complexes: con-nexin-43 for gap junctions, occludin for tight junctions, E-cadherin and β-catenin for adherens junctions and desmoplakin and γ-catenin for desmosomes. Hepsin did not precisely colocalize with connexin-43, occludin, E-cadherin or β-catenin. However, antibodies against desmoplakin and γ-catenin showed overlapping membrane staining to that of antibodies against hepsin, resulting in a punctate yellow line in merged photographs (Figs. 2*a* and 2*b*).

These results indicate that hepsin most likely colocalizes with desmosomes. Desmosomes (maculae adherents) are specialized junctions between adjacent epithelial cells that provide mechanical integrity and participate in dynamic processes such as cell adhesion and motility, tissue morphogenesis and differentiation and cell signaling.[33] Desmosomes also anchor intermediate filaments through desmoplakin.[34,35] To determine the spatial relationship between hepsin and intermediate filaments, we immunostained subconfluent OVCAR5 cells with hepsin and pan-cytokeratin antibodies (Fig. 2*c*). The pan-cytokeratin antibody revealed an extensive filamentous network that radiated into the sites of desmosomal cell junctions. Hepsin was localized at the ends of cytokeratin filaments at the sites of cell–cell contacts (Fig. 2*c*).

To determine whether intact desmosomal structure is necessary for the membrane localization of hepsin, we performed 2-color immunofluorescence for hepsin and desmoplakin in OVCAR5-TR cells. OVCAR5-TR cells display relatively normal adherens junction proteins E-cadherin and β-catenin (not shown), but lack the desmosomal junction protein desmoplakin (Fig. 3*a*). Hepsin was not present at the cell membrane of OVCAR5-TR cells (Fig. 3*a*), indicating that intact desmosomes are required for the membrane localization of hepsin.

Finally, exogenously expressed, GFP-tagged full-length hepsin was detected in the cell cytoplasm as well as in the punctate pattern along the cell membrane of OVCAR5 cells (Fig. 3*b*). Exogenous GFP-hepsin in these punctate structures colocalized with endogenous desmoplakin (Fig. 3*c*). Thus, GFP-tagged hepsin displays cytoplasmic and membrane localization, which is very similar to the endogenous hepsin detected by polyclonal antihepsin antibodies. At present, the functional importance of hepsin localization in the desmosomes is unclear.

Hepsin proteolytically processes pro-hepatocyte growth factor (pro-HGF) into a biologically active α/β-heterodimeric HGF that is capable of activating the hepatocyte growth factor receptor c-Met.[36,37] Using immunofluorescence detection, we demonstrated that HGF-α is present at the cell membrane in structures that resemble desmosomal junctions (Fig. 3*d*). Similar to hepsin, HGF-α colocalizes with desmoplakin (Fig. 3*e*), which is consistent with the

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notion that desmosomes are the primary sites for the catalytic activity of membrane-associated hepsin.

Ectopic expression of hepsin promotes ovarian cancer progression in a mouse model

To elucidate the function of overexpressed hepsin in ovarian cancer, we utilized the human ovarian cancer cell line SKOV3 and the mouse ovarian cancer cell line C11. Neither of these cell lines has detectable levels of endogenous hepsin as determined by Western blotting (Fig. 4*a*). The SKOV3 cells were transfected with wild type *hepsin* or *hepsin* with 3 mutated residues in the catalytic domain (*hepsin*^{mp}). The expression of hepsin protein was confirmed by Western blotting (Fig. 4*a*). Parental SKOV3 cells and SKOV3 cells that were transduced with *hepsin* (SKOV3-hep-sin) or *hepsin*^{mp} (SKOV3-hepsin^{mp}) were injected subcutaneously into nude mice and tumor volume was measured over a period of 2 months. The results of 3 independent injection experiments indicate that wild-type hepsin induces slight, but reproducible, tumor growth advantage in nude mice (Fig. 4*b*). This growth advantage was not observed with the catalytically mutant form of hepsin (Fig. 4*b*). Mice were also sacrificed for tumor weight determination. The results were consistent with the tumor size measurements with the SKOV3-hepsin cells producing the heaviest tumor mass (859±163 mg) *vs*. SKOV3-hepsin^{mp} (141 ± 25 mg) and SKOV3 (121 ± 25 mg).

As a second model system we used the mouse ovarian cancer cell line C11, which was generated by infecting ovarian surface epithelial cells from K5-TVA/p53$^{-/-}$ mice with RCAS-*c-myc* and RCAS-*K-ras*. These cells proliferate rapidly in culture and form tumors 4–6 weeks upon subcutaneous or intraperitoneal injection into nude mice. C11 cells were infected with RCAS vectors expressing wild-type *hepsin* (C11-hepsin) or catalytically mutant *hepsin* (C11-hepsin^{mp}). C11 cells infected with an RCAS vector expressing *GFP* (C11-GFP) were used as a negative control. The expression of hepsin constructs was confirmed by Western blotting (Fig. 4*a*) and immunofluorescence with antibodies against hepsin (Fig. 4*c*). Immunofluorescent detection of ectopically expressed hepsin in C11-hepsin cells revealed cytoplasmic as well as patchy membrane localization (Fig. 4*c*). However, in C11-hepsin^{mp} cells only the cytoplasmic localization of hepsin^{mp} was observed, indicating that the proteolytic function of hepsin may be required for its localization at the membrane.

The C11 cells were derived from mice on a mixed FVB/ C57BL/6J background which are not capable of forming tumors in immunocompetent FVB mice. However, tumor formation can be induced in FVB mice if a potent oncogene, such as polyoma middle T, is introduced into the C11 cells (not shown). To determine whether overexpression of hepsin can also modify the growth properties of C11 cells in FVB mice, the mice were injected intra-peritoneally with C11-GFP, C11-hepsin and C11-hepsin^{mp} cells. Six weeks after cell injection, hemorrhagic ascites (Fig. 4*c*, upper panel) and intraperitoneal tumors (Fig. 4*c*, lower panel) developed in 14 out of 29 mice that were injected with C11-hepsin cells. Tumor metastatic pattern for each mouse is shown in Supplementary Table I. No tumors were observed in the 29 mice injected with C11-GFP cells or in the 29 mice injected with C11-hepsin^{mp} cells, indicating that enzymatic activity of hepsin may be required for its function in tumor progression.

Discussion

Many of the membrane-anchored serine proteases have restricted distribution in normal cells, while their expression is frequently dysregulated during cancer progression.[17,38–40] Recent *in vitro* data support the role for hepsin in prostate and ovarian tumor cell progression.[24] Additionally, hepsin overexpression is associated with disorganization of the basement membrane and promotion of metastasis in a mouse model of prostate cancer.[25] However, the mechanism by which hepsin contributes to tumori-genesis is unclear. Studies of the functional importance of hepsin overexpression in cell culture systems have resulted in conflicting results.

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While antihepsin antibodies or antisense oligonucleotides targeting hepsin significantly blocked hepatoma cell growth[19] and inhibited invasive characteristics of ovarian and prostate cancer cell lines,[24] stable transfection of hepsin cDNA in prostate and ovarian cancer cell lines promoted apoptosis and inhibited growth in soft agar and in nude mice.[41,42]

To gain insight into its function, we studied subcellular localization and expression of endogenous hepsin in human ovarian tumors. We showed that the majority of ovarian tumors display cytoplasmic and/or membrane staining and that the membrane staining was predominantly associated with clear cell and serous carcinomas, which may be related to the more intrinsic aggressive nature of these tumor types. Additionally, in patients with serous ovarian carcinomas, membranous expression of hepsin was associated with decreased survival (HR 5 7.7, $p = 0.003$) and shorter mean time to recurrence (1.0 years *vs*. 2.8 years; $p = 0.008$) (Elisa Lopez-Varela and Esther Oliva, unpublished), supporting the hypothesis that hepsin is involved in ovarian cancer progression through its interaction with the cell membrane.

The functional role of hepsin overexpression in ovarian cancer progression was evaluated in a mouse model of ovarian cancer. We demonstrated by using human and mouse ovarian cancer cell lines that overexpression of hepsin can promote cancer progression *in vivo* and that proteolytic activity of hepsin is necessary for this function. The mechanism by which the proteolytic activity of hepsin promotes cancer progression is currently unknown. The catalytic domain of hepsin has a high degree of similarity to the catalytic domain of the *Drosophila* type II serine protease, stubble-stubbloid, which is known to be involved in signal transduction, cytoskeletal reorganization and changes in cell shape during mor-phogenesis.[43] As a transmembrane protein, hepsin is ideally suited for interaction with cell membrane proteins and proteins on adjacent cells. Additionally, the presence of a short cytoplasmic N-ter-minal domain suggests a role in intracellular signal transduction. However, evidence that these domains support interactions with extracellular, membrane, or intracellular proteins is still lacking.

We demonstrated that hepsin is localized in the cytoplasm and at desmosomal junctions in ovarian cancer cell lines. Desmosomes represent intracellular adhesive junctions that are involved in cell adhesion, differentiation and signal transduction.[33] They also anchor intermediate filaments, thus providing structural integrity. There is cumulative evidence that functional desmosomes are involved in preventing cancer invasion and metastasis.[44] The functional significance of hepsins localization at the desmosomal plaque is currently unclear. The only known proteolytic substrate for hepsin is pro-HGF. Hepsin can cleave pro-HGF to generate active HGF, the ligand for the c-Met receptor.[36,37] It has been shown that c-Met overexpression is a prognostic factor in ovarian cancer and that targeting c-Met inhibits ovarian cancer peritoneal dissemination and invasion in a mouse model.[45] c-Met is also known to form a complex with the desmosomal protein desmoglein 1.[46] In turn, desmosome dissociation is known to be one of the first steps during HGF-induced epithelial-mesenchymal transition.[47,48] Taken together, we propose that, in addition to the demonstrated role of hepsin in extracellular matrix degradation, hepsin and its substrate HGF may play a role in ovarian cancer progression through their interaction with desmosomes. The mechanism and functional significance of this interaction warrants further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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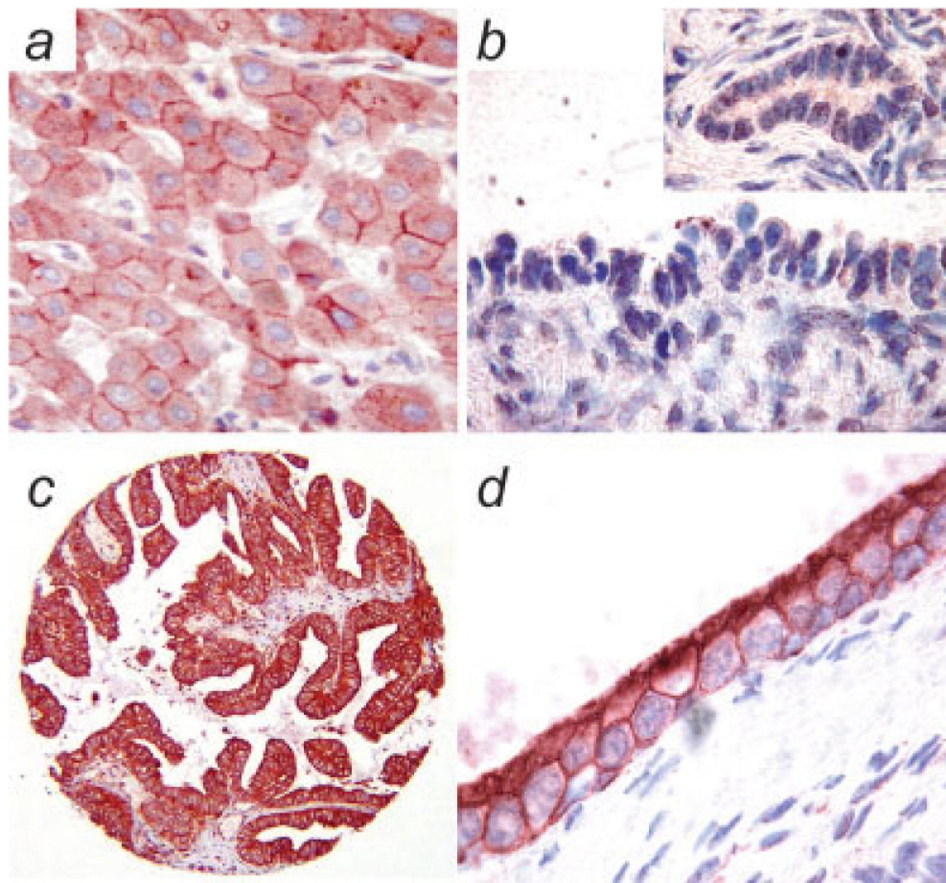

FIGURE 1.

Immunohistochemical detection of hepsin in a normal ovary and in a tissue microarray consisting of normal liver and primary ovarian carcinoma samples. (*a*) Normal human liver. (*b*) Ovarian epithelium that lines the inclusion cyst in a normal ovary. (*c*) Ovarian carcinoma of the serous subtype. (*d*) Higher magnification shows the localization of hepsin at the sites of cell–cell contact in serous ovarian carcinoma.

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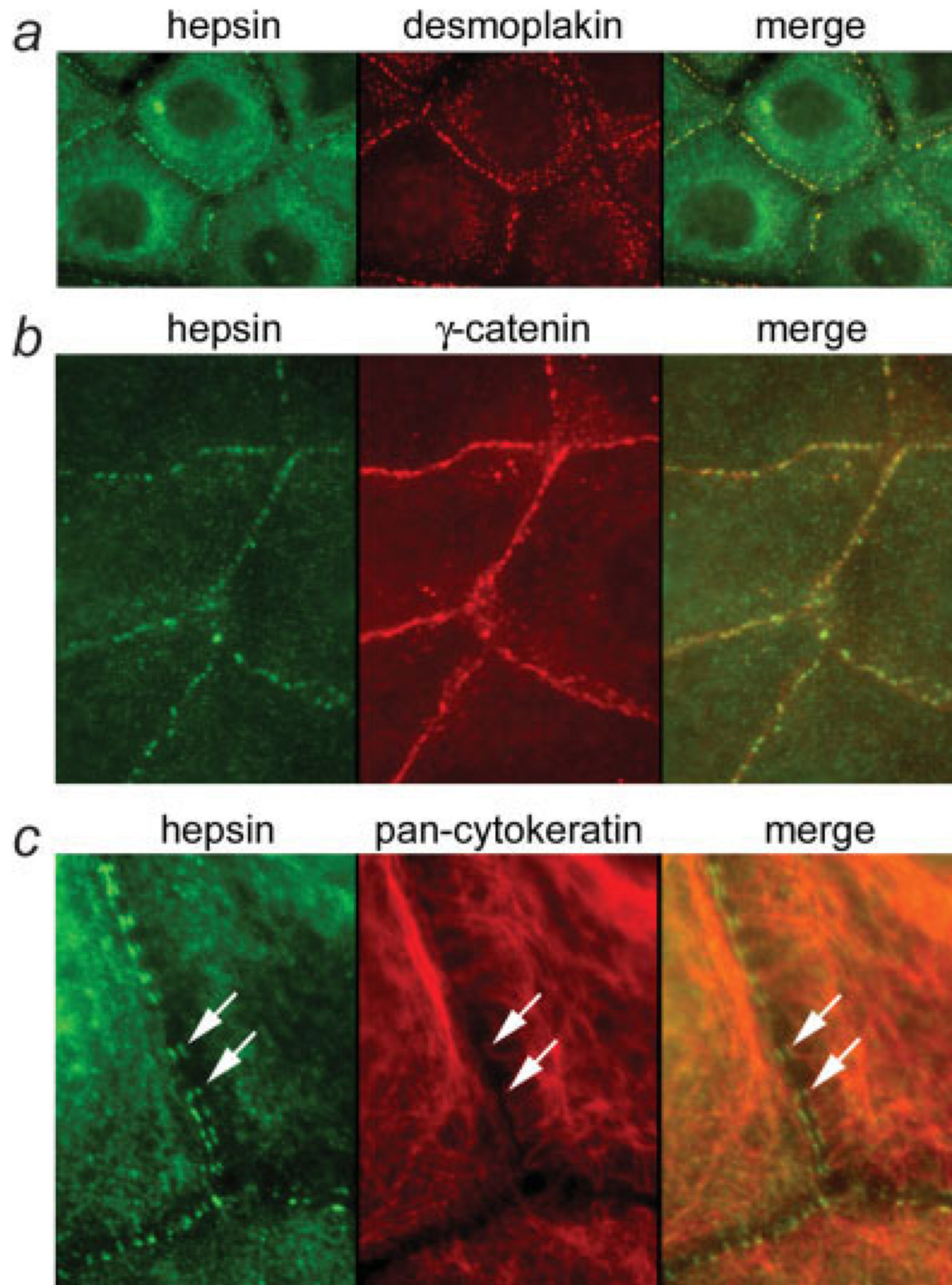

FIGURE 2.

Hepsin colocalizes with desmosomal proteins at the cell membrane. (*a*) Immunofluorescence detection of hepsin in OVCAR5 cells shows that hepsin protein is present in the cytoplasm and linear punctate structures along cell–cell contacts. Two-color immunofluo-rescence detection of hepsin and desmoplakin in OVCAR5 cells shows that these 2 proteins colocalize in desmosomal junctions. (*b*) Two-color immunofluorescence detection of hepsin and γ-catenin in OVCAR5 cells shows a partial overlap of these 2 proteins, which is particularly prominent at the membrane. (*c*) OVCAR5 cells immuno-labeled with hepsin (in green) and pan-cytokeratin (in red). In the merged image, hepsin is present at the sites where keratin filaments contact desmosomal junctions (arrows).

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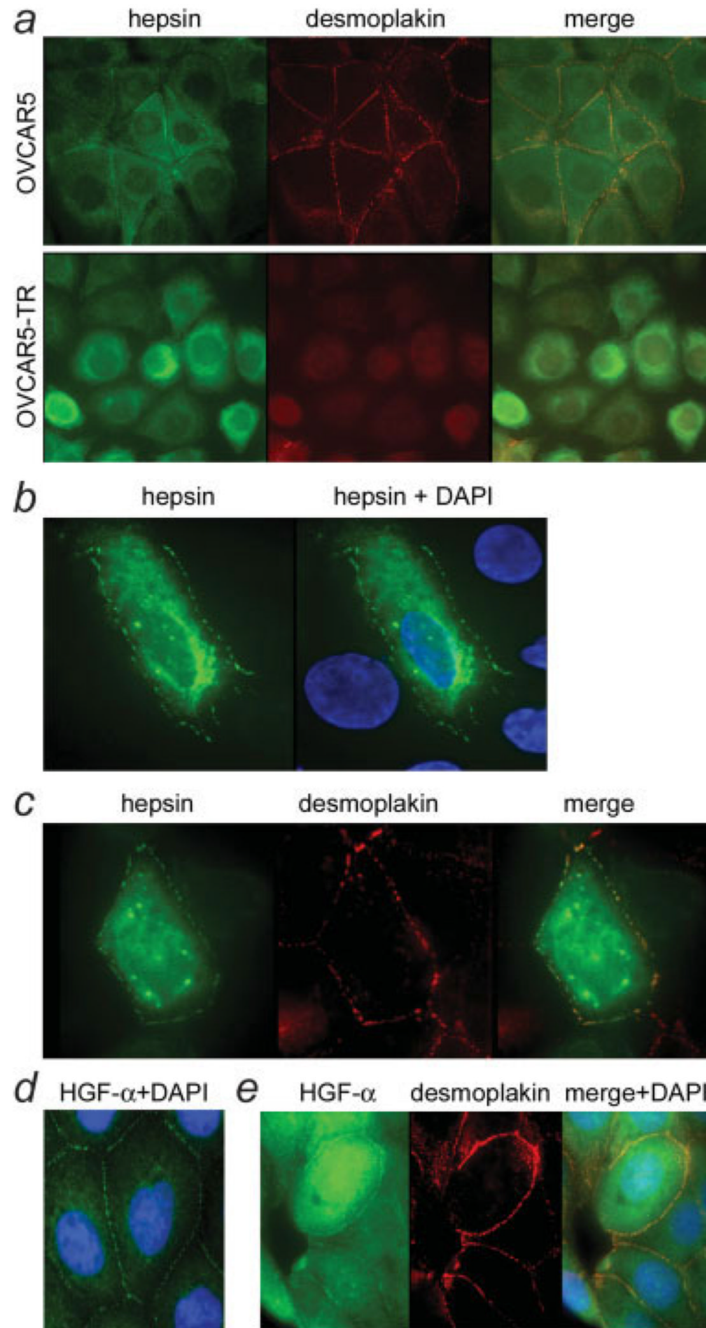

FIGURE 3.

Hepsin and HGF-α are present in desmosomes. (*a*) Immunofluorescence detection of hepsin in OVCAR5 cells and des-moplakin-deficient OVCAR5-TR cells. (*b*) Immunofluorescence detection of GFP-tagged exogenous hepsin OVCAR5 transfected cells. (*c*) Two-color immunofluorescence detection of GFP and des-moplakin shows that GFP-tagged hepsin colocalizes with endogenous desmoplakin at the sites of desmosomal junctions. (*d*) Immunofluorescence detection of HGF-α in OVCAR5 cells. (*e*) Two-color immuno-fluorescence detection of HGF-α and desmoplakin shows that these 2 proteins are present at the cell membrane in desmosomes.

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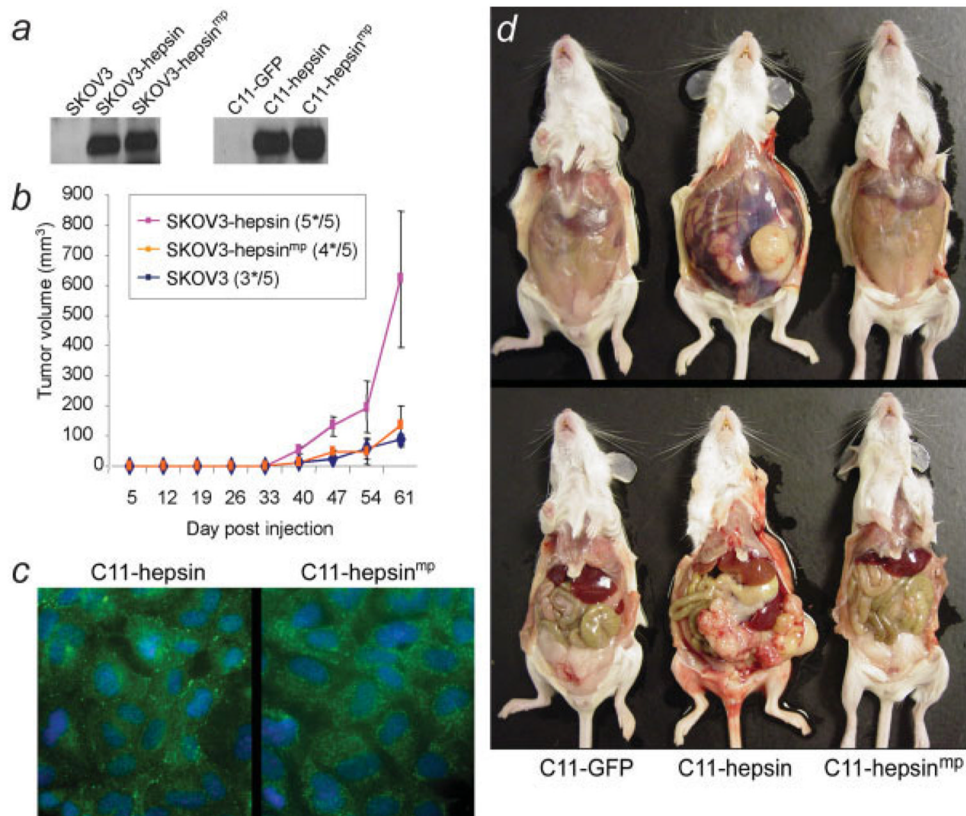

FIGURE 4.

Overexpression of hepsin in mouse ovarian cancer cells promotes tumor growth. (*a*) Western blot analysis of hepsin protein expression in the human ovarian cancer cell line SKOV3 and the mouse ovarian cancer cell line C11. (*b*) Tumor growth in mice injected with parental SKOV3 cells and SKOV3 cells transduced with *hepsin* (SKOV3-hepsin) or *hepsin^{mp}* with 3 mutated residues in the catalytic domain (SKOV3-hepsin^{mp}). Equal numbers of cells were injected subcutaneously into nude mice. Each cell line was injected into a group of 5 mice. The mice were monitored weekly for tumor formation and tumor size was measured using a caliper. An asterisk (*) indicates the number of mice that developed tumors out of the 5 injected mice. Only the mice with tumors were included in the analysis. The graph is representative of 3 independent experiments. T-test was used to compare differential tumor size in each cell line: SKOV3-hepsin *vs*. SKOV3-hepsin^{mp}, p value = 0.022; SKOV3-hepsin *vs*. SKOV3, p value 5 0.027; SKOV3-hepsin^{mp} *vs*. SKOV3, p value = 0.78. (*c*) Immunofluorescence detection of ectopic wild type hepsin or mutant hepsin in C11 mouse ovarian cell lines. (*d*) Examination of FVB mice 6 weeks after intraperitoneal injection with C11 cells that were infected with an RCAS retrovirus carrying *GFP* (C11-GFP), wild type *hepsin* (C11-hepsin) or mutant catalytically deficient *hepsin* (C11-hepsin^{mp}). Twenty-nine mice were injected in each group.