Structural and Functional Analyses of a Novel Ig-like Cell Adhesion Molecule, hepaCAM, in the Human Breast Carcinoma MCF7 Cells*

Received for publication, January 24, 2005, and in revised form, May 24, 2005
Published, JBC Papers in Press, May 25, 2005, DOI 10.1074/jbc.M500852200

Mei Chung Moh‡, Chunli Zhang‡, Chunli Luo§, Lay Hoon Lee‡, and Shali Shen‡¶

From the §Department of Physiology, Faculty of Medicine, National University of Singapore, 2 Medical Drive, Singapore 117597, Republic of Singapore and ¶Department of Laboratory Diagnosis, Chongqing Medical University, Chongqing 400016, China

We have recently identified a novel gene, hepaCAM, in liver that encodes a cell adhesion molecule of the immunoglobulin superfamily. In this study, we examined the characteristics of hepaCAM protein and the relationship between its structure and function, in particular its adhesive properties. The wild-type and the cytoplasmic domain-truncated mutants of hepaCAM were transfected into the human breast carcinoma MCF7 cells, and their physiological and biological properties were assessed. Biochemical analyses revealed that hepaCAM is a single-pass N-linked glycoprotein phosphorylated in the cytoplasmic domain and that it forms homodimers through cis-interaction on the cell surface. The subcellular localization of hepaCAM appears density-dependent; in well spread cells, hepaCAM is distributed to cell protrusions, whereas in confluent cells, hepaCAM is predominantly accumulated at the sites of cell-cell contacts on the cell membrane. In polarized cells, hepaCAM is recruited to the lateral and basal membranes, and lacking physical interaction, hepaCAM is shown to co-localize with E-cadherin at the lateral membrane. Cell adhesion and motility assays demonstrated that hepaCAM increased cell spreading on the matrices fibronectin and matrigel, delayed cell detachment, and enhanced wound healing. Furthermore, when the cytoplasmic domain was deleted, hepaCAM mutants did not affect cell surface localization and dimer formation. Cell-matrix adhesion, however, was less significantly increased, and cell motility was almost unchanged when compared with the effect of the wild-type hepaCAM. Taken together, the cytoplasmic domain of hepaCAM is essential to its function on cell-matrix interaction and cell motility.

Cell adhesion is a dynamic process essential for the normal development and maintenance of tissues and organs in multicellular organisms. Cell-cell and cell-matrix interactions are mediated by a large and complex number of cell adhesion molecules expressed on the cell surface that interact with each other in a spatially and temporally regulated manner. According to their structural and functional features, cell adhesion molecules are generally classified into at least four major families: the cadherins, integrins, selectins, and members of the immunoglobulin superfamily (1–5). Apart from linking cells to each other or to components of the extracellular matrix, an exciting concept that has emerged from recent cell biological research is that cell adhesion molecules function also as receptors critical in modulating signal transduction (6). Such interactions are vital for the regulation of cellular adhesion, proliferation, apoptosis, migration, and differentiation.

We have recently reported the identification of a novel gene in liver, designated as hepaCAM (GenBank™AY047587), which was differentially expressed in human hepatocellular carcinoma. Located on human chromosome 11q24 and spanning 7 exons, hepaCAM encodes a novel member of the immunoglobulin superfamily. The predicted protein of 416 amino acids displays a typical structure of Ig-like adhesion molecules, including two extracellular Ig-like domains, a transmembrane segment, and a cytoplasmic tail. In addition, when exogenously expressed in the human hepatocellular carcinoma cell line HepG2, hepaCAM accelerates cell spreading and increases cell motility (7).

The mechanism of hepaCAM in mediating cell-matrix interaction is unknown. However, transfection studies with mutant and chimeric constructs of other adhesion molecules have suggested that the structural features of adhesion molecules play important roles in mediating their physiological and biological roles. Structure and function study of E-cadherin reveals that the formation of cis-dimer is fundamental for cell adhesion, and inhibition of cis-dimer formation is correlated with the lack of cell-cell interaction (8). For CEACAM1, it has been proposed that both the first extracellular Ig domain and cytoplasmic domain are required for its adhesion function (9). Thus, defining the molecular organization of hepaCAM may help to elucidate the functional roles of hepaCAM.

In this study, we aimed to characterize the physiological and biological properties of hepaCAM and to investigate the importance of the cytoplasmic domain on hepaCAM functions in the hepaCAM-deficient MCF7 cells. We showed that hepaCAM is a phosphorylated glycoprotein that forms cis-homodimers on the cell surface and mediates cell-matrix interaction. In addition, the cytoplasmic domain is required for cell-matrix modulation but dispensable in subcellular localization and surface dimerization.

EXPERIMENTAL PROCEDURES

Plasmid Construction—The complete coding sequence of hepaCAM and its mutants with truncated cytoplasmic domain were generated by PCR amplification. The cDNAs of hepaCAM residues 1–416 (wild-type), residues 1–320, or residues 1–263 were cloned into pEGFP-N2 vector (Clontech, Palo Alto, CA) or pcDNA6/V5-His vector (Invitrogen), at the HindIII/BamHI restriction sites. For polyclonal antibody generation, hepaCAM (residues 260–416) was cloned into the BglII/SalI restriction sites. For polyclonal antibody generation, hepaCAM-deficient MCF7 cells were transfected with the hepaCAM vector. The sequences of the recombinant plasmids were verified by sequencing.

Cell Culture and Transfection—The MCF7 breast carcinoma cell line obtained from American Type Culture Collection (Manassas, VA) was maintained in the recommended conditions. Transfections of MCF7
cells were carried out using the reagent Lipofectamine Plus (Invitrogen) according to the manufacturer's instructions. Transfected cells were selected for 4 weeks, either in the presence of 600 \( \mu \text{g/ml} \) G418 or 10 \( \mu \text{g/ml} \) blasticidin, and cloned.

**Western Blot Analysis**—Cells were lysed in radioimmunoprecipitation assay buffer to extract the total cell lysate. Immunoprecipitation was carried out by incubating the precleared cell lysate with the appropriate mouse monoclonal antibody and horseradish peroxidase-rec-protein G (Zymed Laboratories Inc., San Francisco, CA) overnight at 4 °C. Protein was resolved by SDS-PAGE, transblotted onto membrane, and detected by either rabbit anti-hepaCAM polyclonal antiserum, mouse anti-V5 antibody (Invitrogen), mouse anti-GFP antibody (Santa Cruz Biotechnology, Santa Cruz, CA), or mouse anti-E-cadherin (Zymed Laboratories Inc.).

**Alkaline Phosphatase Treatment**—Cell lysate was incubated in de-
phosphorylation buffer for 10 min at 30 °C. Calf intestinal alkaline phosphatase (Roche Applied Science) was added and incubated for a further 15 min prior to Western analysis.

N-Linked Glycosylation Analysis—For inhibiting N-linked glycosylation, MCF7 cells were transiently transfected with hepaCAM-GFP and subsequently exposed to tunicamycin (Sigma) at the indicated concentrations for 24 h before lysis. For enzymatic digestion of N-linked oligosaccharides, the cell lysate of MCF7/hepaCAM-V5 was treated with peptide N-glycosidase F (New England Biolabs) according to the manufacturer’s instructions. The samples were then subjected to Western analysis.

Chemical Cross-linking—A monolayer or a single suspension of cells was incubated in phosphate-buffered saline containing 3 mM BS3 \(^{1}\) (Pierce) or DTSSP (Pierce) at room temperature for 30 min. The reaction was quenched with the addition of 20 mM Tris-HCl, pH 7.5, for 15 min. Single cell suspension was assayed by microscopic observation before and after chemical cross-linking reaction. DTSSP-cross-linked proteins were resuspended in Laemmli sample buffer without 50 mM dithiothreitol, unless indicated. Cell lysate was prepared in radioligand immunoprecipitation assay buffer containing 10 mM iodoacetamide (10).

Immunocytochemistry—Cells cultured on coverslips were fixed with 2% paraformaldehyde and permeabilized with 0.2% Triton X-100. Non-specific sites were blocked in 10% normal goat serum (Santa Cruz Biotechnology). Protein expression of V5-tagged hepaCAM was detected using mouse anti-V5 antibody, biotin-conjugated goat anti-mouse IgG antibody, and subsequently streptavidin-fluorescein. For co-localization experiments, cells were grown to confluence on 0.4-μm transwell filters (Costar, Cambridge, MA). Protein expression of E-cadherin was demonstrated by fluorescence microscope (Carl Zeiss) or confocal microscope LSM 510 (Carl Zeiss) with sectioning performed at 0.5 μm.

Cell Spreading—Cells were seeded onto coverslips coated with 40 μg of matrigel basement membrane matrix (Clontech) or 10 μg/ml fibronectin (Santa Cruz Biotechnology) and incubated under standard culture conditions. Morphology was observed by microscopy. Unspread cells were defined as round cells, whereas spread cells were defined as cells with extended processes (11). The percentage of cells demonstrating spread morphology was quantified in 10 randomly selected fields.

Cell Detachment—A confluent monolayer of cells was detached in 1 mM EDTA in phosphate-buffered saline at 37 °C. Cell detachment was evaluated under the inverted microscope at 5 and 15 min of incubation. Concurrently, the dissociated cells were harvested and counted in 10 randomly selected fields.

Wound-healing Assay—A confluent monolayer of cells was wounded with a sterile plastic 200-μl micropipette tip. The wound was observed microscopically at 24 and 48 h. The percentage of wound filling was calculated by measuring the remaining gap space on the pictures.

Bioinformatics and Statistical Analysis—The protein sequence of hepaCAM was analyzed using the NetPhos version 2.0 and Prosite programs. Nonparametric analysis of variance was performed to compare the difference among more than two means. Software InStat version 3.0 (GraphPad) was employed, and \( p < 0.01 \) was considered significant.

RESULTS

Wild-type and COOH-terminal Mutants of hepaCAM—The wild-type hepaCAM encodes a transmembrane Ig-like adhesion molecule of 416 amino acids. To assess the importance of hepaCAM cytoplasmic domain in its physiological and biological functions, we constructed two deletion mutants of hepaCAM, hCAM\_mt1, lacking the entire cytoplasmic tail, was constructed by truncating residues 264–416 of hepaCAM. hCAM\_mt2 was constructed by deleting residues 321–416 of hepaCAM to obtain a partial cleavage of the cytoplasmic tail (Fig. 1A). Wild-type hepaCAM, hCAM\_mt1, and hCAM\_mt2 were fused in-frame at the NH\(_2\)-terminal of the green fluorescent protein (GFP) gene of the expression vector pEGFP-N2, and the resulting plasmids were named hepaCAM-GFP, hCAM\_mt1-GFP, and hCAM\_mt2-GFP, respectively. In addition, wild-type hepaCAM and hCAM\_mt1 were inserted at the NH\(_2\)-terminal of the V5 tag of the pcDNA6/V5-His vector and designated hepaCAM-V5 and hCAM\_mt1-V5, respectively. The constructs, as well as the empty vectors, were transfected into MCF7 cells, and the expressed proteins were analyzed by Western blotting using anti-GFP and anti-V5 antibodies accordingly (Fig. 1, B and C). Subsequently, MCF7 cells stably expressing pEGFP-N2 vector (MCF7/pEGFP-N2), hepaCAM-GFP (MCF7/
hepaCAM-GFP), hCAM_mt1-GFP (MCF7/hCAM_mt1-GFP), hCAM_mt2-GFP (MCF7/hCAM_mt2-GFP), pcDNA6 vector (MCF7/pcDNA6), hepaCAM-V5 (MCF7/hepaCAM-V5) and hCAM_mt1-V5 (MCF7/hCAM_mt1-V5) were generated and cloned.

Phosphorylation of the hepaCAM Cytoplasmic Domain—We generated a polyclonal antiserum that recognizes the hepaCAM cytoplasmic domain but in its dephosphorylated form. The recombinant His bacterial fusion protein used for immunization contained residues 260–416 of hepaCAM. Western analysis showed that the resulting antiserum could specifically detect the bacterial fusion protein, otherwise undetectable by the pre-immune serum. However, when the antiserum was tested on the cell lysate of MCF7/hepaCAM-V5, no specific band was observed (data not shown). We suspected that the antiserum was unable to recognize the cytoplasmic domain of hepaCAM because of the presence of post-translational modifications, e.g. phosphorylation. Evaluation of the region selected for antibody generation by the NetPhos version 2.0 server predicted 28 potential serine-, threonine-, or tyrosine-phosphorylated residues scattered along the cytoplasmic domain of hepaCAM protein, with 20 of them giving a potential phosphorylation >0.5 (Fig. 2A). To verify that the hepaCAM cytoplasmic domain is phosphorylated, we dephosphorylated cell lysates of C3A cells expressing endogenous hepaCAM and MCF7/hepaCAM-V5 cells expressing exogenous hepaCAM with calf intestinal alkaline phosphatase. The untreated cell lysates were included as controls. Indeed, calf intestinal alkaline phosphatase-treated endogenous and exogenous hepaCAM were detected by the rabbit antiserum (Fig. 2B), confirming that the cytoplasmic domain of hepaCAM is phosphorylated.

N-Linked Glycosylation of hepaCAM—Sequence analysis of hepaCAM predicted six N-linked glycosylation sites on its extracellular domain (Fig. 3A). To investigate whether hepaCAM was glycosylated, the MCF7/hepaCAM-GFP cell lysate was enzymatically digested with peptide N-glycosidase F to release putative N-linked oligosaccharides. An untreated sample was included as the control. The molecular mass of hepaCAM-GFP, shown by Western analysis to be ~100 kDa, was shifted to ~75 kDa after deglycosylation. Consistently, when MCF7 cells transfected with hepaCAM-GFP were treated with tunicamycin (an antibiotic that inhibits N-linked glycosylation) at dif-
ferent doses for 24 h, a band at ~75 kDa was also observed (Fig. 3B). The results verified that hepaCAM is a glycoprotein. By subtracting the molecular mass of GFP, i.e., 27 kDa, the deglycosylated form of hepaCAM is 48 kDa.

Dimerization of hepaCAM and Mutant on Plasma Membrane—We evaluated the pre-existing forms of hepaCAM on cell membrane by incubating a monolayer of MCF7/hepaCAM-GFP cells with BS3, a noncleavable membrane-impermeable cross-linker. The cell lysate was prepared in the presence of iodoacetamide to inhibit the formation of nonspecific disulfide bonds (10). An untreated sample was included as the control. The samples were analyzed by Western blotting with anti-GFP. In the presence of BS3, a band of ~200 kDa appeared, which seemed to represent the dimerized form of hepaCAM-GFP, accompanied with the disappearance of the hepaCAM monomers. Similarly, treatment of MCF7/hepaCAM-V5 cells with BS3 resulted in a decrease of the ~75-kDa monomeric form of hepaCAM and an accumulation of the higher molecular weight species at ~150 kDa, although no distinct band was noted (Fig. 4A). It is possible that the anti-V5 antibody did not recognize the higher molecular weight species as efficiently as monomers.

To examine whether tailless hepaCAM proteins form dimers, MCF7/hCAM_mt1-GFP cells were treated with BS3 and analyzed by Western blotting (Fig. 4D). The monomeric form of hCAM_mt1-GFP was diminished and replaced with its dimeric form at ~125 kDa in the BS3-treated sample. Interestingly, in the untreated sample of hepaCAM-GFP and hCAM_mt1-GFP, protein species that seemed to represent the dimeric form of the proteins were observed. This phenomenon could be due to covalent bonding between the dimers of hepaCAM-GFP or hCAM_mt1-GFP. To determine whether hepaCAM-GFP forms cis- or trans-dimers on the cell surface, both adherent monolayer and single cell suspension of MCF7/hepaCAM-GFP cells were treated with BS3 (Fig. 4E). The extent of dimerization was comparable in both adherent and suspension cells, indicating that hepaCAM homodimerization occurs predominantly through cis-interactions rather than trans-interactions within the plane of the membrane of individual cells.

Subcellular Localization of hepaCAM and Mutants in MCF7 Cells—We explored the subcellular distribution of wild-type hepaCAM in MCF7/hepaCAM-GFP cells at low and at high cell densities by fluorescence and inverted microscopy (Fig. 5A). When
cells were well spread, hepaCAM protein was localized to punctuate structures in the perinuclear membrane, cytoplasm, and at the tip of the cell surface protrusions, which were about to make contact with adjacent cell surfaces, forming zipper-like structures. Once the cells became confluent, the protein was localized at a lesser extent in the perinuclear membrane and cytoplasm and predominantly on the plasma membrane, particularly in the areas of cell-cell contacts. The results suggest that the subcellular localization of hepaCAM is cell density-dependent. We also examined the effect of hepaCAM cytoplasmic domain in its plasma membrane localization. hCAM mt1-GFP and hCAM mt2-GFP were both recruited to the plasma membrane of MCF7 cells (Fig. 5B). Similarly, MCF7/hepaCAM-V5 and MCF7/hCAM mt1-V5 cells immunostained with anti-V5 showed that hepaCAM and its mutant were predominantly expressed on cell membranes (Fig. 5C). The results indicate that the cytoplasmic domain is dispensable for membrane localization.

Co-localization of hepaCAM with E-cadherin—The distribution of hepaCAM was further examined in confluent polarized MCF7/hepaCAM-GFP cells by confocal laser scanning microscopy (Fig. 6A). The cells were also stained for E-cadherin, which localizes in the lateral cell surface, to compare its localization with that of hepaCAM. In the X-Y sections, hepaCAM-GFP was distributed to honeycomb-like structures at cell-cell boundaries, which significantly co-localized with E-cadherin. In the X-Z vertical cross-section, the distribution of E-cadherin along the entire lateral cell surface coincided with hepaCAM-GFP. Moreover, hepaCAM was detected at the basal membrane that was in contact with the Transwell membrane. Because hepaCAM and E-cadherin appeared to co-localize, we investigated whether there were any physical interactions between them by co-immunoprecipitation (Fig. 6B). Cell lysate prepared from MCF7/hepaCAM-V5 was precipitated with the anti-V5 antibody and subjected to Western blotting using the anti-E-cadherin (top panel) or anti-V5 antibody (bottom panel). The signals corresponding to E-cadherin and hepaCAM-V5 molecules are marked with arrowheads. Lane 1, cell lysate of MCF7/hepaCAM-V5 before IP; lane 2, cell lysate of MCF7/hepaCAM-V5 after IP; lane 3, cell lysate of MCF7/hCAM-GFP after IP; lane 4, cell lysate of MCF7/hepaCAM-V5 after IP; lane 5, precipitate of MCF7/hepaCAM-V5; lane 6, precipitate of MCF7/hepaCAM-V5.
Moreover, MCF7/hepaCAM cells were enlarged, therefore retarding migration. However, in the wound-healing assay (Fig. 9), we demonstrated that, after 24 h of incubation, MCF7/hepaCAM-V5 cells filled 59.3% of the scratched area \( (p < 0.01) \), compared with 36.3% by MCF7/hCAM_mt1-V5 cells \( (p > 0.05) \) and 33.1% by MCF7/pcDNA6 cells. After 48 h, MCF7/hepaCAM-V5 cells closed 83.7% of the wound \( (p < 0.01) \), compared with 55.2% by MCF7/hCAM_mt1-V5 cells \( (p > 0.05) \) and 49.5% by MCF7/pcDNA6 cells. Hence, the cytoplasmic domain is important for cell motility modulated by hepaCAM.

**DISCUSSION**

In our previous work, we identified a novel Ig-like molecule, hepaCAM, which exhibits typical structural characteristics of adhesion molecules of the immunoglobulin superfamily (7). In this study, we demonstrated physiological and biological characteristics of hepaCAM and the relationship between its structure and function, particularly with respect to the cytoplasmic domain.

Sequence analysis revealed that the cytoplasmic domain of hepaCAM contains a proline-rich region that provides putative binding sites for SH3 domains and potential phosphorylation sites of serine/threonine and tyrosine kinases. Experimentally, we showed that the cytoplasmic domain is phosphorylated, suggesting an important role of the hepaCAM cytoplasmic domain in signaling cascades controlling cellular adhesion, motility, morphology, and all processes depending on the cytoskeleton. To evaluate the significance of the cytoplasmic domain, we transfected wild-type and cytoplasmic domain-truncated constructs of hepaCAM into MCF7 cells and analyzed their effects on hepaCAM functions.

Biochemical analysis revealed that hepaCAM is a glycosylated protein and forms a cis-homodimer on the cell surface. Deletion of the cytoplasmic domain did not interfere with dimer formation, suggesting that dimerization may be stabilized by the extracellular and/or transmembrane domains but not the cytoplasmic domain. Notably, chemical cross-linking of hepaCAM or its mutated protein both showed the presence of high molecular weight proteins, indicating that hepaCAM may form large complexes with other endogenously expressed cellular proteins through its extracellular and/or transmembrane do-

![Cell spreading assay](image_url)
mains. Alternatively, it may represent higher order homo-oligomers of hepCAM or its mutant. It is interesting to observe the seemingly dimeric form of hepCAM and its mutant in their respective un-cross-linked samples. Although the mechanism resulting in such interaction is unknown to us, Hunter et al. (5) and others (12) have observed a similar phenomenon in C-CAM and raise the possibility that C-CAM dimers become covalently linked, perhaps through the action of transglutaminase, an enzyme which catalyzes the formation of γ-glutamyl-ε-lysine bonds in a restricted number of cellular proteins.

**FIG. 8. Cell detachment assay.** MCF7/pDNA6 (left panels), MCF7/hCAM_mt1-V5 (middle panels), and MCF7/hepaCAM-V5 (right panels) cells were detached in 1 mM EDTA for 5 min or 15 min. The microscopic photos were taken under ×200 and ×400 magnifications. At 5 min or 15 min after incubation, the total number of detached cells was counted in ten randomly selected fields, and the percentage of cell detachment was then computed. The data represent means ± S.D. (n = 6), **, p < 0.001 as assessed by analysis of variance.

**FIG. 9. Wound-healing assay.** Wounds were made by pipette tip on confluent MCF7/pDNA6 (left panels), MCF7/hCAM_mt1-V5 (middle panels), and MCF7/hepaCAM-V5 (right panels) cells and allowed to heal for 24 and 48 h. The microscopic photos were taken under ×100 magnification. The diameters of wounds were measured on the microscopic photos at 0, 24, and 48 h after wounding. Changes in wound diameter were computed into percentage (means ± S.D., n = 6) to represent wound closure. *, p < 0.01 as assessed by analysis of variance.
Subcellular localization of hepaCAM in nonpolarized MCF7 cells showed that hepaCAM molecules were recruited to the cytoplasmic membranes at sites of cell-cell attachment. In polarized cells, hepaCAM was preferentially expressed in the lateral and basal membranes. Co-localization analysis demonstrated that hepaCAM co-localized laterally with E-cadherin, but no physical interaction between the two molecules was detected. We also showed that partial truncation and complete deletion of the cytoplasmic domain did not alter the plasma membrane localization. It has been reported that the CEACAM1 cytoplasmic domain regulates its lateral localization. Differing in their cytoplasmic domains, isoform CEACAM1-S distribution is exclusively apical, whereas isoform CEACAM1-L occurs both in apical and lateral cell surfaces (13). However, whether the loss of cytoplasmic domain affects the lateral and basal localization of hepaCAM remains to be determined.

Functionally, hepaCAM is capable of modulating cell-matrix interaction. Cell adhesion to the substratum plays a crucial role in cell migration, which is a key aspect of many normal and abnormal biological processes, including embryonic development, immunity, wound healing, and metastasis of tumor cells (14, 15). The distribution of hepaCAM on the basal membrane of cells, in addition to the spread morphology of MCF7/hepaCAM-V5 cells, hinted at possible trans-interaction between hepaCAM and the substrate. Evidently, cell spreading, cell detachment, and wound-healing assays revealed increased cell-substrate affinity and cell motility mediated by hepaCAM. Deletion of the cytoplasmic domain reduced, but did not completely abrogate, cell-matrix adhesion mediated by the wild-type hepaCAM, implicating that, to a considerable extent, the extracellular and transmembrane domains are able to initiate adhesion. However, the rate of wound healing of cells expressing mutant hepaCAM was close to the level of the control cells, indicating that the cytoplasmic domain is essential for mediating wound recovery. The data implies that cell-matrix adhesion and cell motility are controlled separately, and phosphorylation of the cytoplasmic domain may play a pivotal role in the regulation. Indeed, phosphorylation of CD44 was shown to regulate melanoma cell and fibroblast migration on, but not attachment to, a hyaluronan substratum (16). Additionally, it has been proposed for the cadherins (8, 17, 18) and for CEA (19) that cis-dimerization will lead to strengthened cell adhesion, and cis-homodimer formation of ICAM-1 enhances its binding to a leukocyte β2-integrin (20). However, the functional significance of hepaCAM post-translational modification and dimerization in regulating cell-matrix interaction is still under investigation.

In conclusion, we have shown that hepaCAM is a phosphorylated glycoprotein, forms cis-homodimers on the cell surface, and modulates cell-matrix interaction. The cytoplasmic domain, although unessential for cell surface localization and dimerization, is required to maintain a complete functional form of hepaCAM as a modulator of cell-matrix interaction.

Acknowledgment—We thank Asha Reka Das for her assistance whenever and wherever needed.

REFERENCES
1. Edelman, G. M. (1986) Annu. Rev. Cell Biol. 2, 81–116
2. Edelman, G. M. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 1460–1464
3. Edelman, G. M., and Crossin, K. L. (1991) Annu. Rev. Biochem. 60, 155–190
4. Takeishi, M. (1991) Science 251, 1451–1453
5. Hunter, I., Sawa, H., Edlund, M., and Obrink, B. (1996) Biochem. J. 320, 847–853
6. Rosales, C., O’Brien, V., Kornberg, L., and Juliano, R. (1995) Biochim. Biophys. Acta 1242, 77–98
7. Moh, M. C., Lee, L. H., and Shen, S. (2005) J. Hepatol. 42, 833–841
8. Takeda, H., Shimoyama, Y., Nagafuchi, A., and Hirohashi, S. (1999) Nat. Struct. Biol. 6, 310–312
9. Cheung, P. H., Luo, W., Quo, Y., Zhang, X., Earley, K., Milliron, P., and Lin, S. H. (1995) J. Biol. Chem. 270, 24303–24310
10. Masuda, M., Yageta, M., Fukuhara, H., Kuramoto, M., Maruyama, T., Nomoto, A., and Murakami, Y. (2002) J. Biol. Chem. 277, 30114–30119
11. Richardson, A., Malik, R. K., Hildebrand, J. D., and Parsons, J. T. (1997) Mol. Cell Biol. 17, 6906–6914
12. Greenberg, C. S., Birckbichler, P. J., and Rice, R. H. (1991) PASEB J. 5, 3071–3077
13. Sundberg, U., Beaugenin, N., and Obrink, B. (2004) J. Cell Sci. 117, 1091–1104
14. Dedhar, S., and Hannigan, G. E. (1996) Curr. Opin. Cell Biol. 8, 657–669
15. Xiao, H., Pallero, M. A., Gupta, K., Chang, F., Ware, M. F., Witke, W., Kwiatkowski, D. J., Lauflerburger, D. A., Murphy-Ullrich, J. E., and Wells, A. (1998) J. Cell Sci. 111, 615–624
16. Peck, D., and Isacke, C. M. (1996) Curr. Biol. 6, 884–890
17. Shapiro, L., Fannon, A. M., Kwong, P. D., Thompson, A., Lehmann, M. S., Grubel, G., Legrand, J. F., Als-Nielsen, J., Colman, D. R., and Hendrickson, W. A. (1995) Nature 374, 327–337
18. Nagar, B., Overduin, M., Ikura, M., and Rini, J. M. (1998) Nature 380, 360–364
19. Bates, P. A., Luo, J., and Sternberg, M. J. (1992) FEBS Lett. 301, 207–214
20. Reilly, P. L., Woska, J. R., Jr., Jeanfavre, D. D., McNally, E., Rothlein, R., and Bormann, B. J. (1995) J. Immunol. 155, 529–532
