Immunochemical characterization of two isoforms of rat liver ecto-ATPase that show an immunological and structural identity with a glycoprotein cell-adhesion molecule with M_r 105000

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One of the cell-adhesion molecules (CAMs) responsible for rat hepatocyte aggregation has been described as a glycoprotein having an M_r of 105000 (cell-CAM105). The M_r and localization of cell-CAM105 in liver membranes are very similar to those of liver ecto-ATPase, an ATPase with its nucleotide-hydrolysing site localized on the outside of the cell membrane. The protein sequence of the ecto-ATPase has been deduced from cDNA cloning. Structural analysis of the sequence indicates that the ecto-ATPase has immunoglobulin-like domains and is a member of the immunoglobulin superfamily. Since a group of proteins in the immunoglobulin superfamily has been shown to have functions related to cell adhesion, the structural characteristics of the ecto-ATPase further led to the possibility that the ecto-ATPase may have functions related to cell adhesion. In this paper, using the cDNA for the ecto-ATPase, the anti-peptide antibodies produced against peptides derived from the ecto-ATPase cDNA sequence and monoclonal antibodies against the cell-CAM105, we present evidence of identity between cell-CAM105 and ecto-ATPase. First, in Western immunoblots, two anti-cell-CAM105 monoclonal antibodies cross-reacted with the purified ecto-ATPase. Secondly, in immunodepletion experiments, antibodies against the ecto-ATPase depleted the same protein recognized by the anti-cell-CAM105 antibodies. Thirdly, in two-dimensional gel-electrophoretic analysis, anti-peptide antibodies generated against an extracellular *N*-terminal peptide and the intracellular *C*-terminal peptides of the ecto-ATPase immunoprecipitated proteins of similar isoelectric points and M_r values to those of the cell-CAM105. Fourthly, proteins immunoprecipitated by anti-ecto-ATPase antibodies and anti-cell-CAM105 antibodies have similar V8-proteinase-digest peptide maps. Finally, monoclonal antibodies against the cell-CAM105 specifically recognized the protein expressed in COS cells transfected with the ecto-ATPase cDNA. These results indicate that the ecto-ATPase cDNA codes for a protein that is identical with the cell-CAM105. Since the ecto-ATPase has structural features of immunoglobulin domains, the identity of cell-CAM105 with ecto-ATPase leads to the conclusion that this liver CAM, similarly to neuronal CAM, is also a member of the immunoglobulin supergene family. Furthermore, immunological studies indicate that the cell-CAM105/ecto-ATPase is composed of two isoforms of different *C*-terminal sequences. The association of ATPase activity with cell-CAM105 raises the possibility that extracellular nucleotides may play important roles in regulating cell adhesion.

INTRODUCTION

Recognition and binding between cells are of fundamental importance for proper functions of multicellular organisms, both during embryonic development and in the adult stage. Cell-adhesion molecules (CAMs) are a group of cell-surface glycoproteins with functions related to cell recognition (Edelman & Thiery, 1985). A cell-surface molecule involved in intercellular adhesion between rat hepatocytes *in vitro* was first identified by monitoring the ability of papain-solubilized plasma-membrane components to neutralize the inhibition of cell aggregation caused by antibodies directed against the cell surface (Ocklind & Öbrink, 1982). Using this assay, Ocklind & Öbrink (1982) purified this aggregation-responsible protein from rat liver plasma membrane. This molecule, which was designated cell-CAM105, is an integral plasma-membrane glycoprotein with an apparent M_r of 105000. Hixson *et al.* (1985) later established by two-dimensional gel analysis that cell-CAM105 is composed of a pair of acidic glycoproteins with isoelectric points of 4.1 and 4.3. In examining the changes in normal cell-surface protein expression in hepatomas, Hixson *et al.* (1985) observed that there was an apparent loss of cell-CAM105 on the cell surface of hepatocellular

carcinoma lines examined. Antibodies against purified cell-CAM105, which strongly inhibited re-aggregation of normal hepatocytes, displayed no reactivity in immunoprecipitation assay with hepatocellular carcinoma cells (Hixson & McEntire, 1989; McEntire *et al.*, 1989). These observations suggest that expression of cell-CAM105 is altered during the development of the malignant phenotype of hepatocytes.

We have found that the biochemical properties of the cell-CAM105 and the localization of the cell-CAM105 in the liver membranes are very similar to those previously described for ecto-ATPase (Lin, 1989). The ecto-ATPase is a plasma-membrane nucleotidase with its nucleotide-hydrolysing site localized on the outside of the cell surface (Lin & Russell, 1988; Lin, 1989; Lin & Guidotti, 1989). The characteristics of this ecto-ATPase include its activation to a similar extent by either Ca^{2+} or Mg^{2+} and its ability to hydrolyse non-specifically several different nucleotides. The ecto-ATPase was purified from rat liver plasma membrane (Lin & Fain, 1984; Lin, 1989), and antibodies against the protein were prepared. The amino acid sequence of the ecto-ATPase from rat liver was deduced from analysis of cDNA clones and a genomic clone. The deduced sequence predicts a 519-amino acid protein with a calculated M_r of 57388. There are

Abbreviations used: CAM, cell-adhesion molecule; cell-CAM105, a glycoprotein CAM with M_r 105000; mAb, monoclonal antibody; CEA, carcinoembryonic antigen; BGPI, biliary glycoprotein; NCA, non-specific cross-reacting antigen; PSGB1, pregnancy-specific β -1 glycoprotein.

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16 potential asparagine-linked glycosylation sites in the protein. Hydropathy analysis of the deduced amino acid sequence indicates that the protein has two hydrophobic stretches. One is located at the *N*-terminus and has properties of a membrane signal sequence (von Heijne, 1983), and the other is near the *C*-terminus. The structural arrangement predicts that most of the protein mass is on the outside of the cell and that the *C*-terminus of the protein is intracellular. In the extracellular domain, there are two stretches of sequences similar to the consensus sequences for the nucleotide-binding domains of other nucleotide-binding proteins (Lin & Guidotti, 1989). This structural information is consistent with the fact that the protein is an ecto-ATPase with its active site localized on the outside of the cell. In the present paper we provide immunochemical evidence showing that rat liver ecto-ATPase cDNA codes for a protein identical with the cell-CAM105. We also establish that ecto-ATPase/cell-CAM105 has two isoforms that differ in the sequence of their *C*-terminal domain.

EXPERIMENTAL

Materials

Peptides were synthesized according to the deduced amino acid sequence at an institutional protein core facility (University of Texas M. D. Anderson Cancer Center). Horseradish-peroxidase-conjugated anti-(rabbit immunoglobulin) antibodies were purchased from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Keyhole-limpet haemocyanin was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Preparation of antibodies

Polyclonal antibodies 669 and 708, which were against purified rat liver ecto-ATPase, were prepared as described previously (Lin, 1989). Anti-peptide antibodies were prepared by injecting rabbits with peptides coupled to keyhole-limpet haemocyanin as described in Lin (1989). Anti-*N* anti-peptide antibody was prepared against a peptide sequence derived from the *N*-terminal portion of the ecto-ATPase, i.e. SVLLLAHNLPQEFQVC (amino acid positions 50–64 of the ecto-ATPase). Anti-C1, anti-C2 and anti-C3 antibodies were prepared against peptide sequences within the *C*-terminal portion of the ecto-ATPase. Anti-C1 is against peptide sequence CDLTEHKPSTSSHNLGP (amino acid positions 460–475), anti-C2 is against peptide sequence CDSPNKVDDVSYSVLNFAQ (amino acid positions 478–496) and anti-C3 is against peptide sequence CQSKRPTSASSPTETVYSVVKKK (amino acid positions 497–519). Monoclonal antibodies mAb 362.50 and mAb 5.4 were prepared as described in Hixson & McEntire (1989). Rabbit antibody against cell-CAM105, anti-gp105-2, was prepared as described in Hixson & McEntire (1989).

Immunoprecipitation and SDS/PAGE analysis

Normal adult rat hepatocytes were isolated by a modification of the collagenase perfusion technique of Bonney *et al.* (1974). Viability of isolated cells was greater than 80% as determined by Trypan Blue dye exclusion. Surface labelling with ¹²⁵I was performed by the lactoperoxidase/glucose oxidase procedure of Keski-Oja *et al.* (1977). Procedures for immunoprecipitation analysis of radiolabelled antigens were described in Hixson *et al.* (1983). Comparison of the reactivity of two different antibodies was determined by immunodepletion analysis as described in Hixson *et al.* (1983). One-dimensional SDS/PAGE was performed as described by Laemmli (1970). Two-dimensional gel electrophoresis was performed according to the procedure of O'Farrell (1975). Staining, destaining, drying of gels and autoradiography were carried out as described in Hixson *et al.* (1983).

One-dimensional maps of peptides generated by digestion with *Staphylococcus aureus* V8 proteinase were prepared by the method of Cleveland *et al.* (1982).

Expression of recombinant ecto-ATPase in COS cells

Expression plasmid cdm8 (Seed, 1987) was obtained from T. Maniatis (Harvard University, Cambridge, MA, U.S.A.). Plasmid containing the full-length ecto-ATPase cDNA was constructed as described by Lin & Guidotti (1989). COS-M6 cells were transfected with 25 µg of plasmid DNA per 100 mm plate by electroporation in a Cell-Porator (Bethesda Research Laboratory, Bethesda, MD, U.S.A.). In brief, COS-M6 cells (2 × 10⁶/ml) were suspended in Hepes-buffered saline containing 21 mM-Hepes (pH 7.05), 137 mM-NaCl, 5 mM-KCl, 0.7 mM-Na₂HPO₄ and 6 mM-glucose. For electroporation, COS-M6 cells in a volume of 0.5 ml were placed in the electroporation chamber, and the electroporation was performed at 250 V at 330 µF. After electroporation, the cells were removed from the electroporation chamber and placed in 10 ml of Dulbecco's minimum essential medium (GIBCO, Gaithersburg, MD, U.S.A.) containing 10% fetal-calf serum. After incubation for 48 h, the culture medium was aspirated and the cells were scraped off dishes with a rubber 'policeman'. The cells were disrupted by mixing with 0.5 ml of distilled water followed by freezing and thawing to obtain total cell lysate.

Other procedures

The liver ecto-ATPase was purified according to the procedures described by Lin & Fain (1984) with modifications as described in Lin (1989). Western immunoblotting was performed as described in Lin (1989). Protein concentrations were determined by the micro BCA method according to the procedures described by the manufacturer (Pierce Chemical Co., Rockford, IL, U.S.A.), with BSA as a standard.

RESULTS

Identification of the cell-CAM105 as the ecto-ATPase

Immunological identity. To test the identity of the ecto-ATPase against that of the cell-CAM105, Western immunoblots were performed on the purified ecto-ATPase with monoclonal antibodies against cell-CAM105 (mAb 362.50 and mAb 5.4) (Hixson & McEntire, 1989; McEntire *et al.*, 1989). As shown in Fig. 1 (lanes 1 and 1'), both monoclonal antibodies cross-reacted with the purified ecto-ATPase. We have previously reported that the purified ecto-ATPase has different mobilities in SDS/PAGE depending on whether the sample is heated or not (Lin, 1989). The apparent *M_r* was in the region of 85 000 if the sample was not heated before being loaded on to the gel. The apparent *M_r* of the protein changed to around 100 000 if the sample was heated before being loaded on to the gel. As shown in Fig. 1, monoclonal antibodies against cell-CAM105 also recognized a protein with mobility shifts similar to those of the ecto-ATPase (lanes 2 and 3, lanes 2' and 3'). The weak reactivity of mAb 362.50 with the reduced form of ecto-ATPase may be due to the conformational dependence of antibody recognition, since we have observed that an anti-peptide antibody against the *N*-terminal sequence of the ecto-ATPase (anti-*N*) (Lin & Guidotti, 1989) recognizes the reduced but not the non-reduced form of the ecto-ATPase (results not shown). These results indicate that the ecto-ATPase and the cell-CAM105 are either immunologically related or the same protein. One-dimensional SDS/PAGE analysis showed that antiserum 708 raised against purified native ecto-ATPase and antiserum 669 against SDS/PAGE-purified denatured enzyme were reactive with a 105 000-*M_r* component with a mobility

Fig. 1. Immunoblots of purified ecto-ATPase and liver plasma-membrane proteins with antibodies against cell-CAM105

(a) Immunoblot of purified ecto-ATPase and liver plasma-membrane proteins with monoclonal antibody against cell-CAM105 (mAb 362.50). Purified ecto-ATPase (2.5 μ g) (lane 1) and plasma membrane (46 μ g of protein) (lane 2) treated with SDS were loaded on to the gel. In lane 3, plasma membrane (46 μ g of protein) was treated with SDS and 2-mercaptoethanol (0.14 M) and heated at 100 °C for 5 min before being loaded on to the gel. Monoclonal antibody mAb 362.50 (1:50 dilution) was used. (b) Immunoblot of purified ecto-ATPase and liver plasma membrane with monoclonal antibody against cell-CAM105 (mAb 5.4). Liver ecto-ATPase (2.5 μ g) purified according to the procedure of Lin (1989) (lane 1') and plasma membrane (46 μ g of protein) (lane 2') treated with SDS were loaded on to the gel. In lane 3', plasma membrane (46 μ g of protein) was treated with SDS and 2-mercaptoethanol and heated at 100 °C for 5 min before being loaded on to the gel. Monoclonal antibody mAb 5.4 (1:50 dilution) was used.

Fig. 2. SDS/PAGE analysis of components immunoprecipitated from detergent extracts of radioiodinated hepatocytes with (a) antiserum 669, (b) antiserum 708 and (c) mAb 362.50

Both polyclonal antibodies showed reactivity with two components of M_r identical with the high- M_r and low- M_r forms of cell-CAM105 (arrows).

Fig. 3. Immunodepletion analysis of extracts from radioiodinated hepatocytes

Radioiodinated hepatocytes were solubilized by detergent Triton X-100. The detergent extracts were sequentially immunoprecipitated with antisera. Sequential depletion with antiserum 708 three times (708 no. 1, 708 no. 2, 708 no. 3) or antiserum 669 three times (669 no. 1, 669 no. 2, 669 no. 3) removed all reactivity with mAb 362.50 (362.50 no. 4). STD, standard proteins.

identical with that of the cell-CAM105 immunoprecipitated with mAb 362.50 (Fig. 2).

Two-dimensional gel-electrophoretic analysis showed cell-CAM105 to be composed of a pair of acidic glycoproteins different in pI (4.1 versus 4.3) and in apparent M_r (i.e. 110000 versus 105000) (Hixson & McEntire, 1989; McEntire *et al.*, 1989). One-dimensional peptide maps of fragments produced with V8-proteinase digestion indicated that these acidic glycoproteins were closely related in structure (McEntire *et al.*, 1989). Two-dimensional PAGE showed that both antisera 708 and 669 were reactive with high- M_r and low- M_r forms (results not shown). That these two components were identical with cell-CAM105 was confirmed by the ability of the two antisera to deplete the solubilized cell lysates of both forms reactive with mAb 362.50 (Fig. 3). In addition, both forms that were reactive with antiserum 669 were depleted by anti-gp105-2, an antiserum against cell-CAM105 that has previously been shown to block re-aggregation of isolated hepatocytes (results not shown).

Sequence-specific anti-peptide antibodies. Anti-peptide antibodies against sequences derived from ecto-ATPase cDNA (anti-N, anti-C1, anti-C2 and anti-C3) were prepared. Anti-N antibody was generated against a peptide sequence derived from the *N*-terminus of ecto-ATPase, and anti-C1, anti-C2 and anti-C3 antibodies were generated against three different *C*-terminal peptide sequences. In the two-dimensional gel, antibodies against the three *C*-terminal peptides were reactive with only the higher- M_r form of cell-CAM105, which is also recognized by mAb 362.50 (Fig. 4). On the other hand, antibodies raised against an *N*-terminal peptide (anti-N) immunoprecipitated both the higher- M_r and lower- M_r forms (Fig. 4), albeit less efficiently (approx. 10%) than mAb 362.50. This lower efficiency of anti-N most probably resulted from the low affinity of this antiserum for the non-reduced form of the protein. These results together suggest that at least one of the differences between the higher- M_r and lower- M_r forms lies in the *C*-terminal portion of the proteins. Furthermore, all four anti-peptide antibodies against peptides derived from *N*-terminal and *C*-terminal portions of the ecto-ATPase immunoprecipitated cell-CAM105. This result indicates that these sequences are present in cell-CAM105.

Fig. 4. Two-dimensional gel analysis of components immunoprecipitated with anti-peptide antisera from detergent extracts of radiiodinated hepatocytes

Anti-C1, anti-C2 and anti-C3 antisera were only reactive with the high- M_r form, whereas anti-N recognized both the high- M_r and low- M_r forms. The dotted line indicates the position of the lower- M_r form.

Fig. 5. Immunoblots of protein expressed in COS cells transfected with the ecto-ATPase cDNA

COS cells were transfected with the ecto-ATPase cDNA as described in the Experimental section. The total cell lysate from each sample was used for SDS/PAGE analysis. All the samples were boiled in SDS sample buffer containing 2-mercaptoethanol (Laemmli, 1970). (a) Immunoblot with monoclonal antibody mAb 362.50 (1:50 dilution). Lane 1, cell lysate (351 μ g of protein) from COS cells transfected with the ecto-ATPase cDNA; lane 2, cell lysate (403 μ g of protein) from control COS cells. (b) Immunoblot with antiserum 708 (1:1000 dilution). Lane 3, cell lysate (27 μ g of protein) from COS cells transfected with the ecto-ATPase cDNA; lane 4, cell lysate (31 μ g of protein) from control COS cells.

Peptide mapping. To provide additional evidence of the structural identity of cell-CAM105 with the ecto-ATPase, V8-proteinase-digest peptide maps were prepared from the following proteins: (a) the higher- M_r and lower- M_r forms of cell-CAM105 immunoprecipitated with mAb 362.50 (which has been shown to be specific for cell-CAM105); (b) the higher- M_r and lower- M_r components immunoprecipitated from whole extracts with antibody 669 (which can precipitate the ecto-ATPase); (c) the higher- M_r form immunoprecipitated with anti-C1 antiserum (which was prepared against the C-terminal peptide derived from the

sequence information of the ecto-ATPase cDNA clone). The peptide maps of mAb 362.50 and 669 immunoprecipitates were essentially identical (results not shown). There was also identity between the maps of the high- M_r forms immunoprecipitated with mAb 362.50 and anti-C1 antisera (results not shown).

Protein expressed from the ecto-ATPase cDNA. To demonstrate that the ecto-ATPase cDNA codes for a protein identical with cell-CAM105, COS cells were transfected by electroporation with ecto-ATPase cDNA in expression vector cdm8. As shown in Fig. 5, transfected COS cells expressed a protein that is specifically recognized by both antiserum 708 and mAb 362.50. These results establish unequivocally that the cDNA clone for the liver ecto-ATPase codes for a protein identical with cell-CAM105.

DISCUSSION

This paper reports the immunological and structural identity between the ecto-ATPase cloned by Lin & Guidotti (1989) and rat liver cell-CAM105. Evidence supporting this identity includes: (1) cross-reactivity of two monoclonal antibodies against cell-CAM105 with purified ecto-ATPase, (2) ability of antibodies against ecto-ATPase to deplete proteins recognized by the anti-cell-CAM105 antibodies, (3) immunoprecipitation of proteins of similar isoelectric points and M_r values to those of the cell-CAM105 by anti-peptide antibodies against the N-terminal and C-terminal sequences of the ecto-ATPase, (4) close similarity in the V8-proteinase-digest peptide maps of proteins immunoprecipitated by anti-ecto-ATPase and anti-cell-CAM105 antibodies and (5) recognition of the protein expressed in COS cells transfected with the ecto-ATPase cDNA by monoclonal antibodies against the cell-CAM105.

In addition, we have shown the presence of at least two isoforms of cell-CAM105/ecto-ATPase: a long form that has a complete C-terminal domain as described in the published ecto-ATPase sequence and a short form that lacks reactivity with all three C-terminal antipeptide antibodies. This short form, however, still reacts with anti-peptide antibodies generated against an N-terminal peptide and all monoclonal and polyclonal antibodies generated against cell-CAM105 or ecto-ATPase. These results support an overall structural homology between these two forms. On the basis of the intensities of the iodinated proteins, the relative abundance of these two forms in rat hepatocytes is about 30% and 70% for long form and short form respectively.

The identity of cell-CAM105 and ecto-ATPase was also reported by Aurivillius *et al.* (1990). In their studies they obtained five peptide sequences from cell-CAM105 purified by anti-cell-CAM105 antibody affinity column chromatography. Four out of these five peptides were found in the ecto-ATPase sequence. Two of these peptides were also found in mouse mmCGM2 (Turbide *et al.*, 1991) and thus could not distinguish between ecto-ATPase and rat CEA-related proteins. Two of the five sequences, however, were unique to ecto-ATPase. Thus their results, obtained by a different approach, agree with our conclusion. The fifth peptide was not found in the sequence of ecto-ATPase, a finding these investigators attributed to the presence of this peptide in the long form of cell-CAM105. On the basis of this conclusion it was speculated that the published sequence must correspond to the short form of cell-CAM105, the more abundant of the two forms. Furthermore, they also proposed that alternative splicing may account for the formation of these isoforms. On the basis of the results reported here, several corrections need to be made regarding this hypothesis. First, contrary to what they have proposed, the results presented in this study, i.e. all three anti-C-terminal antibodies recognize the high- M_r form, clearly indicate that the previously published ecto-ATPase sequence represents the long form of the cell-CAM105/ecto-

Fig. 6. Comparison of the sequence similarity of the three immunoglobulin-like domains of the liver cell-CAM105/ecto-ATPase

Each immunoglobulin-like domain is approx. 90 amino acid residues in length. Domain 1 includes amino acid residues 140–232, domain 2 includes amino acid residues 233–317 and domain 3 includes amino acid residues 318–408.

ATPase. In addition, we have recently cloned the short isoform of the ecto-ATPase (S.-H. Lin, O. Culic, D. Flanagan & D. C. Hixson, unpublished work). Compared with the previous sequence, the short-form ecto-ATPase cDNA has quite a few nucleotide substitutions scattered over the entire sequence. This sequence information clearly argues against alternative splicing as the mechanism of isoform generation. The extra peptide sequence that Aurivillius *et al.* (1990) obtained may come from protein contaminated in their purified cell-CAM105 preparation.

Studies by Margolis *et al.* (1990) have also shown that the protein coded by the ecto-ATPase cDNA is identical with pp120/HA4, a membrane protein found in hepatocyte plasma membranes and a substrate for the insulin-receptor tyrosine kinase. This result raises an interesting aspect that the function of cell-CAM105/ecto-ATPase may be regulated by tyrosine kinase. This possibility requires further investigation.

Since we have cloned and sequenced the ecto-ATPase, the demonstration that the ecto-ATPase is identical with cell-CAM105 has provided the sequence information of cell-CAM105 for the first time. Furthermore, our studies also lead to the conclusion that cell-CAM105 is associated with an ATPase activity. For the first time, one CAM is shown to be associated with an ATPase activity.

Cell-CAM105/ecto-ATPase is a member of the immunoglobulin superfamily

The structure of ecto-ATPase contains three immunoglobulin-like domains of the C2 class (Williams & Barclay, 1988), all of which share sequence similarities to each other (Fig. 6). Although the *N*-terminal domain does not contain the typical cysteine

Table 1. Sequence similarity of ecto-ATPase to CEA-related cell-surface proteins

Protein	Sequence similarity
BGP1	54.7% identity in 318 amino acid residues
NCA	54.7% identity in 307 amino acid residues
CEA	49.8% identity in 293 amino acid residues
PSGB1	44.2% identity in 181 amino acid residues

residues, this domain has characteristics of the immunoglobulin V class (Williams & Barclay, 1988). Such a sequence feature suggests that the ecto-ATPase/cell-CAM105 is a member of the immunoglobulin superfamily.

Cell-CAM105/ecto-ATPase is highly similar in sequence to CEA

The predicted amino acid sequence of the liver ecto-ATPase/cell-CAM105 is highly similar to that of CEA (Oikawa *et al.*, 1987; Beauchemin *et al.*, 1987) and other related cell-surface glycoproteins, i.e. biliary glycoprotein (BGP1) (Hinoda *et al.*, 1988), non-specific cross-reacting antigen (NCA) (Neumaier *et al.*, 1988) and pregnancy-specific β -1 glycoprotein (PSGB1) (Watanabe & Chou, 1988) (Table 1). The immunoglobulin-like domains of ecto-ATPase/cell-CAM105 are similar to the immunoglobulin-like domains of CEA with similarity scores around 50% (Table 1). Sequences similar to the possible ATP-binding domains of the ecto-ATPase can also be identified

in the primary sequences of BGP1, PSGB1 and CEA. Therefore it is possible that these proteins also have an ATPase (nucleotidase) activity. The structural similarity between the ecto-ATPase/cell-CAM105 and CEA, also a member of the immunoglobulin superfamily, predicts that the ecto-ATPase and CEA may have similar functions. The overproduction of CEA or proteins related to the CEA family is a common cellular phenotypic change in human tumours. This observation has led to the development of immunochemical assays of colon cancers. However, the function and significance of these antigens in malignant transformation are not clear. CEA is a highly glycosylated cell-surface glycoprotein with an M_r of 180000 (the unglycosylated CEA has an M_r of 72800). Consistent with our result, Benchimol *et al.* (1989) have reported that CEA functions as an intercellular-adhesion molecule.

The similarities in structure and function of cell-CAM105/ecto-ATPase and CEA raise an interesting question concerning their roles during normal cell development and carcinogenesis. Several observations make it likely that the adhesive events mediated by CEA and ecto-ATPase/cell-CAM105 play vastly different roles during histotypic interactions. In normal tissues, CEA is expressed at highest levels during early fetal development, whereas cell-CAM105 appears late and shows a major increase at the fetal/neonatal interface (Thompson *et al.*, 1990; Odin & Öbrink, 1986, 1988). The major role of cell-CAM105 was postulated to be in mediating the formation and maturation of the bile canalicular domain (Mowery & Hixson, 1991). In contrast with CEA, which shows an increased expression in tumours, cell-CAM105 expression is greatly decreased or disappears on primary hepatocellular carcinomas (Hixson *et al.*, 1985; McEntire *et al.*, 1989; Hixson & McEntire, 1989). When considered in the context of fetal/neonatal expression, these differences are consistent with selection for a less differentiated phenotype during carcinogenesis. Alternatively, it is possible that the gene(s) coding for cell-CAM105/ecto-ATPase have been deleted or inactivated during carcinogenesis, analogously to the deletion of neuronal CAM-like sequences in colorectal cancers (Fearon *et al.*, 1990).

The association of ATPase activity with cell-CAM105 raises questions concerning the role of ATPase activity in the regulation of cell adhesion. It is possible that ATP binding and ATP hydrolysis by cell-CAM105 may contribute to the mechanism of hepatocyte adhesion mediated by this molecule. In this model, hydrolysis of ATP may be directly involved in the conformational change of cell-CAM105. This conformational change then affects cell adhesion. However, it is also possible that the ATPase activity associated with cell-CAM105 is unrelated to the molecule's cell-adhesion function.

Ecto-ATPase/cell-CAM105 is composed of a family of isoforms

The cell-CAM105 was shown to be composed of a pair of acidic glycoproteins that had slight differences in their apparent M_r in SDS/PAGE and in their isoelectric points. These different forms could have resulted from proteolysis during sample preparation, from variations in glycosylation or from expression of different genes. By pulse-chase analysis, we have found no evidence for significant proteolytic conversion and have observed a similar ratio of the higher- M_r to the lower- M_r form in all experiments performed (results not shown). These observations make it unlikely that the lower- M_r form results from random degradation during isolation or immunoprecipitation. There are also no significant differences in glycosylation or phosphorylation that could explain the difference in isoelectric points and M_r values (McEntire *et al.*, 1989). In the studies with antibodies against C-terminal peptide sequences, we have found that they only immunoprecipitated the higher- M_r form. This result

suggests that the major difference between the higher- M_r and lower- M_r forms might be in the C-terminal portion of the proteins. Using probes derived from the ecto-ATPase clone to screen a rat liver cDNA library further, we have obtained an additional clone coding for an ecto-ATPase isoform. Besides a cluster of substitutions in the N-terminal domain, this ecto-ATPase isoform has a shorter C-terminal intracellular domain than that of the previously published sequence (S.-H. Lin, O. Culic, D. Flanagan & D. C. Hixson, unpublished work). This information indicates that the two forms of cell-CAM105 observed in the two-dimensional gel-electrophoretic analysis may arise from different gene products.

The presence of an ecto-ATPase activity on the surface of plasma membrane has been observed for many years. Several possible functions for the ecto-ATPase have been proposed (Lin, 1989) but there was no direct evidence. Our studies on the structure of the ecto-ATPase leads to the finding that the liver ecto-ATPase has structural features of the immunoglobulin superfamily and is identical with the rat liver cell-adhesion molecule, cell-CAM105. Further studies on the relationships between ATPase activity and cell adhesion should be able to provide information on the mechanism and regulation of cell adhesion.

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