Identification of a 110-kDa glycoprotein involved in cell-substratum adhesion

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Plasma membrane glycoproteins are involved in cell-matrix interactions. For identification of such glycoproteins a recently developed cell system was used. Two cell populations differing in their adhesion properties were selected from Morris hepatoma 7777. One population was able to grow as a monolayer, while the other proliferated in suspension. From both cell lines spontaneous revertants were selected. By using antibodies raised against plasma membranes of the hepatoma and of rat liver for sequential immunoprecipitation a glycoprotein of M_r 110000 was identified. This glycoprotein was only expressed in adherent hepatoma cells as well as in normal rat liver, but was absent in non-adherent hepatoma cells and in the in vivo growing Morris hepatoma 7777. This suggests that the glycoprotein is involved in cell-substratum adhesion of hepatocytes and adherent hepatoma cells.

Cell-substratum adhesion (Hepatoma cell) Plasma membrane Glycoprotein

1. INTRODUCTION

The interaction of cells with substances of the extracellular matrix is a complex process in which several membrane components of different functions are involved. Membrane glycoproteins mediating adhesion to extracellular factors such as fibronectin [1–4], laminin [5,6], vitronectin [7] and collagen [8] have been identified. Other membrane components are thought to be responsible for motility [9] or matrix assembly [10]. Recently we have shown that hepatocytes express at least three glycoproteins, which are involved in cell-substratum adhesion. Two of these glycoproteins were separated by size-exclusion HPLC and blocked the adhesion-inhibiting activity of antibodies raised against the plasma membranes of liver and hepatomas [11]. Antibodies raised against a third glycoprotein inhibited the initial adhesion of hepatocytes [12]. This glycoprotein was identified as dipeptidyl peptidase IV (DPP IV). We have also shown that an adhesion-involved glycoprotein with an apparent M_r of 105000 as well as the DPP IV were lost or markedly reduced in Morris hepatoma 7777 [11,13].

For further identification and characterization of adhesion-involved molecules a cell system was established which allowed the determination of molecular alterations associated with the transition of an adherent to a non-adherent phenotype [14]. By propagation of cells isolated directly from the solid Morris hepatoma 7777 an adherent and a non-adherent cell variant have been obtained [15,16]. To provide a conclusive relation between a molecular alteration and adhesiveness, additionally spontaneous revertants were selected from the two directly isolated cell lines [14]. Thus, two adherent and two non-adherent cell lines of the same cellular origin were compared with their normal counterparts, i.e. rat hepatocytes. It can be assumed that glycoproteins expressed in hepatocytes and adherent variants of Morris hepatoma 7777, but not expressed in both non-adherent variants, are involved in cell-substratum

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adhesion. Here, a 110 kDa glycoprotein is described which is not identical with DPP IV and is expressed only in adherent cell variants and in normal hepatocytes. It is therefore suggested, that this glycoprotein is involved in cell-substratum adhesion.

2. MATERIALS AND METHODS

2.1. *Cells and cell culture conditions*

The cultivation of an adherent and a non-adherent cell line from Morris hepatoma 7777 isolated from the solid carcinoma has been described [15,16]. The establishment of the revertant cell lines is described in [14]. All cell lines were propagated in Dulbecco's modification of Eagle's medium (DMEM) containing 10% serum, 10^{-8} M insulin and 10^{-6} M dexamethasone.

2.2. *Adhesion-inhibiting assay*

The preparation of broad spectrum antisera raised against plasma membranes of rat liver (anti-liver antibodies) and Morris hepatoma 7777 (anti-hepatoma antibodies) has been described [15]. The adhesion-inhibiting assay was performed essentially as in [17]. Briefly, various concentrations of an IgG preparation isolated from anti-liver or anti-hepatoma antiserum were added to 10^4 freshly passaged cells. The cells were seeded on coated plates in serum-free DMEM for collagen-, vitronectin- and fibronectin-mediated adhesion, or in DMEM containing 10% fetal calf serum for serum-mediated adhesion on uncoated plates. Fibronectin and collagen I isolation and the coating of tissue culture plates were carried out by standard methods [18,19]. Collagen IV isolated from human placenta was purchased from Sigma. Vitronectin (serum spreading factor) was a gift from Dr D. Barnes, Corvallis, OR. Inhibition of cell-substratum adhesion was evaluated after complete spreading of untreated control cultures, i.e. after 3 h for adhesion on fibronectin or collagen I and IV, and after overnight incubation for serum- and vitronectin-mediated adhesion.

2.3. *Immunoprecipitation and immunodepletion*

For metabolic labeling 10^6 cells were seeded in 10 ml serum-free medium containing 50 μCi [^{3}H]glucosamine (D-[6-^{3}H]glucosamine hydrochloride, 30 Ci/mmol, Amersham). After 8 h incubation the cells were washed with phosphate-buffered saline (0.9% NaCl, pH 7.2) and solubilized in 1 ml buffer A (10 mM Tris-HCl, pH 7.8, containing 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride and 1% Nonidet P40). The cells were homogenized in a Potter-type homogenizer (800 rpm, 8 strokes) and then ultracentrifuged at 100000 $\times$ g for 30 min. The supernatant (500 μl) was diluted with 4.5 ml buffer A without Nonidet P40 and 50 μl antiserum were added. After an overnight incubation with 20 mg protein A-Sepharose (Sigma) the pellet was washed 3 times with buffer A, twice with phosphate-buffered saline and once with water. Specifically bound glycoproteins were solubilized in sample buffer according to Laemmli [20] and subjected to SDS gel electrophoresis. Fluorographic analysis was carried out as described by Laskey and Mills [21]. For the immunodepletion experiment anti-hepatoma antibodies were added to the cell solubilisates. After the first immunoprecipitation again anti-hepatoma antibodies were added to the supernatant. The procedure was repeated until practically no radioactivity was precipitated by the anti-hepatoma antibodies. In the last step anti-liver antibodies were added instead of anti-hepatoma antibodies.

3. RESULTS AND DISCUSSION

Plasma membrane glycoconjugates are assumed to mediate cell-substratum adhesion. Such glycoproteins present in adherent hepatoma cells would be altered or not expressed in non-adherent hepatoma cells. It can be assumed that these adhesion-involved molecules are also present in the normal counterparts of rat hepatoma cells, i.e. rat hepatocytes. Recently we have shown that antibodies against plasma membranes of rat liver (anti-liver antibodies) are able to inhibit serum-mediated adhesion of hepatocytes or adhesion on culture plates coated with vitronectin (serum spreading factors), fibronectin or collagen [11,15,17]. On the other hand, antibodies against plasma membranes of Morris hepatoma 7777 (anti-hepatoma antibodies) only inhibited adhesion of hepatocytes on fibronectin and collagen. They did not inhibit serum-mediated adhesion or adhesion on vitronectin. Similar results were ob-

tained when adherent hepatoma cells were used instead of hepatocytes (table 1). Thus, the anti-hepatoma antibodies do not inhibit vitronectin- or serum-mediated adhesion of adherent hepatoma cells. This suggests that adherent hepatoma cells express a membrane component involved in serum-mediated adhesion, which is absent in the hepatoma in vivo. However, since anti-liver antibodies inhibit this type of adhesion of the hepatoma cells, this membrane component seems to be present in normal rat liver.

The different inhibitory specificities of the antibodies raised the possibility of identifying those membrane components which are expressed in normal rat liver plasma membrane, but are lost in the plasma membrane of Morris hepatoma 7777. Consequently, they would be re-expressed in the adherent populations of the hepatoma cells. The anti-hepatoma antibodies were used to immunoprecipitate all proteins from adherent cells, which were common for this hepatoma and for in vivo growing hepatoma cells. Proteins present in adherent hepatoma cells, but absent in the in vivo growing hepatoma should remain in the supernatant of the immunoprecipitation. Subsequently, immunoprecipitation with the anti-liver antibodies was used to precipitate membrane proteins common to liver cells and adherent hepatoma cells. By this procedure only one glycoprotein of 110 kDa was immunoprecipitated by the anti-liver antibodies (fig.1). Analogous experiments with non-adherent cells precipitated no protein in the last step. Thus, a 110 kDa protein was identified which is only expressed in normal hepatocytes and the adherent hepatoma cells, but not in the non-adherent and in vivo growing hepatoma cells. This suggests that this glycoprotein is associated with the adherent phenotype. Beside this difference in the expression of a 110 kDa plasma membrane glycoprotein other alterations of the membrane composition of the four cell lines have been

Table 1

Inhibition of adhesion by antibodies raised against plasma membranes of rat liver (anti-liver antibodies) or Morris hepatoma 7777 (anti-hepatoma antibodies)

Substratum	Anti-liver antibodies	Anti-hepatoma antibodies
Fibronectin	+	+
Collagen I	+	+
Collagen IV	+	+
Serum spreading factor vitronectin	+	–
Serum	+	–

Adherent hepatoma cells were seeded on plates coated with different substrata or in the presence of 10% fetal calf serum. The concentration of the antibodies was 200 μg/ml. Complete inhibition of adhesion (+) or lack of any effect (–) was observed after 3 h on collagens and fibronectin or 16 h on vitronectin and in the presence of fetal calf serum. Non-adherent hepatoma cells were unable to adhere to any of these substrata

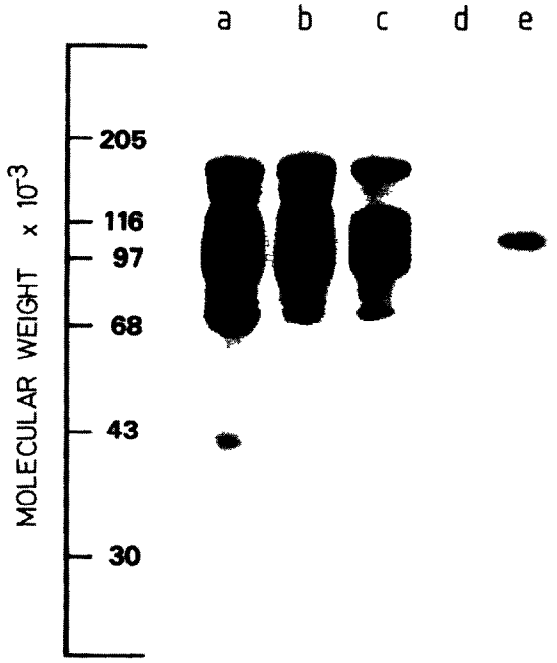

Fig.1. Polyacrylamide gel electrophoresis of precipitates obtained in the immunodepletion experiment using [^{3}H]glucosamine-labeled adherent hepatoma cells. Anti-hepatoma antiserum was added to a cell solubilisate together with protein A-Sepharose. After the first immunoprecipitation (lane a) anti-hepatoma antiserum and protein A-Sepharose were again added to the supernatant. This was repeated until no radioactivity was precipitated by anti-hepatoma antiserum (lanes b–d). In the last step anti-liver antiserum was added instead of anti-hepatoma antiserum (lane e). No labeled protein was precipitated in the last step when non-adherent cells were used.

detected ([14] and unpublished). However, most of them cannot be related to cell-substratum adhesiveness.

Since we have shown that the ectoenzyme DPP IV is involved in cell-substratum adhesion [12] and expressed in a higher concentration in adherent hepatoma cells compared to non-adherent hepatoma cells [14], it was of interest to exclude or to prove the identity of the 110 kDa glycoprotein with DPP IV, which has the same molecular mass as a monomer. Two lines of evidence showed that the 110 kDa glycoprotein and DPP IV are distinct entities: (i) anti-hepatoma antibodies precipitated the enzymatic activity of DPP IV, and (ii) additionally a monoclonal antibody to DPP IV [22] did not yield any precipitated material after immunodepletion with anti-hepatoma antibodies. However, from this supernatant the 110 kDa glycoprotein could be precipitated with anti-liver antibodies.

On the other hand, it seems likely that the 110 kDa glycoprotein described here is identical with the adhesion-involved molecule of M_r 105000 identified previously in the liver by size-exclusion HPLC [11]. This glycoprotein was also not detected in the plasma membrane of the in vivo growing Morris hepatoma. It was suggested that the glycoprotein is involved in serum-mediated adhesion [17]. We have shown that the initial adhesion of hepatocytes in the presence of serum is mediated by serum factors of 60–80 kDa, probably vitronectin or 'serum spreading factors' [17]. A receptor for vitronectin has been identified recently as a pair of glycoproteins of M_r 115000 and 125000 [7]. The isolation and further characterization of the 110 kDa glycoprotein identified by the immunodepletion experiment will reveal whether it is related to the vitronectin receptor glycoproteins.

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