A novel integrin ($\alpha_E\beta_4$) from human epithelial cells suggests a fourth family of integrin adhesion receptors

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A new member of the integrin superfamily of adhesion receptors was isolated from human epithelial cells. Analogously to other integrins, this molecule is a heterodimer comprised of structurally unrelated subunits, both glycosylated. Unequivocal amino-acid sequence homologies were observed between these subunits and integrin α and β chain sequences, indicating that this epithelial heterodimer is a novel integrin. No obvious serologic cross-reactivities were detected with other integrins. The β chain of the epithelial integrin displayed a mol. wt significantly higher than other integrin β chains, possibly due to a large sialic acid content. Integrin heterodimers are grouped into three families, based on which of three β chains (β_1 , β_2 and β_3) they contain. Therefore, the epithelial integrin may represent the prototype of a fourth integrin family, because it contains a structurally distinct β chain. The designation $\alpha_E\beta_4$ is proposed for this novel human integrin.

Key words: integrin/ $\alpha_E\beta_4$ /epithelium/human

Introduction

The adhesion of cells is critical to many biological processes, including embryonal development, tissue repair, immune response and malignant transformation (Edelman, 1983; Yamada, 1983; Ekblom *et al.*, 1986). Recently, adhesion receptors for extracellular matrix and plasma proteins such as fibronectin and vitronectin, as well as leukocyte adhesion receptors, were characterized biochemically by several laboratories (Damsky *et al.*, 1981; Giancotti *et al.*, 1985; Pytela *et al.*, 1985; Springer *et al.*, 1985; Tamkun *et al.*, 1986; Fitzgerald *et al.*, 1987) and shown to be structurally homologous to each other (Charo *et al.*, 1986; Suzuki *et al.*, 1986; Kishimoto *et al.*, 1987; Takada *et al.*, 1987a). These related molecules are now organized in a protein superfamily, designated 'integrins' after the chicken fibronectin/laminin receptor (Hynes, 1987).

Integrins are heterodimers comprised of noncovalently associated transmembrane glycoproteins (for reviews, see Buck and Horwitz, 1987; Hynes, 1987; Ruoslahti and Pierschbacher, 1987). The α chains, although unique to each integrin, display extensive similarities in size, amino-acid sequence (~45% homology) and functional domains (e.g. divalent cation binding regions). Three β chains have thus far been identified and found to be similar in size, amino-acid sequence (35–45% homology) and high cysteine

content. According to their β chain, integrin heterodimers can be arranged into three families, the VLA/fibronectin receptor (β_1), the leukocyte adhesion protein (β_2) and the IIb–IIIa/vitronectin receptor (β_3) family.

We present biochemical and serological evidence for a structurally novel integrin from human epithelial cells, which apparently defines a fourth family. In accordance with existing integrin nomenclature (Hynes, 1987), we propose that this new integrin molecule be called ' $\alpha_E\beta_4$ '.

Results

Identification of gp205,125, a molecular complex on epithelial cells

We have previously described a monoclonal antibody, S3-41, reactive with normal and malignant human epithelial cell surfaces (Kajiji *et al.*, 1987). In immunoperoxidase staining of epidermal tissue sections, this antibody predominantly labeled basement membranes and basolateral cell surfaces of the germinative layer, while from the prickle-cell layer outward cells were progressively devoid of reactivity (Figure 1A). This distribution is often displayed by molecules involved in cell adhesion (Buck and Horwitz, 1987).

Fig. 1. Immunoperoxidase staining of human skin and immunoprecipitation of carcinoma cell lysates with S3-41. (A) Frozen sections of human plantar skin were immunoperoxidase stained with either monoclonal antibody S3-41 (upper) or a control monoclonal, S3-60, to a keratinocyte membrane antigen (Kajiji *et al.*, 1987) (lower). Arrows point to the dermal–epidermal junction. (B) Cells were either surface-labeled with ^{125}I (left panel) or metabolically labeled with [^{35}S]methionine (right panel), detergent-solubilized, immunoprecipitated with S3-41, and analyzed by SDS–PAGE under reducing (R) or nonreducing (NR) conditions (5% gel). Mol. wt standards were myosin (200 kd), β -galactosidase (116 kd) and phosphorylase B (97 kd). Arrows point to the positions of gp205 and gp125. Open arrowheads indicate bands corresponding to the gp180 and gp150 forms of gp205 (see text).

Antibody S3-41 was used to immunoprecipitate detergent lysates from human epithelial cells (pancreatic carcinoma lines) metabolically labeled with either [35 S]methionine (Figure 1B, right panel) or [6 - 3 H]glucosamine (data not shown). Fluorography of the immunoreactive material after SDS-PAGE under reducing conditions revealed two bands with M_r of 205 kd (gp205) and 125 kd (gp125), along with two minor bands of 180 kd (gp180) and 150 kd (gp150). For convenience, this set of glycoproteins will be referred to as the gp205,125 complex. Each of these glycoproteins exhibited a different mobility under nonreducing conditions. From immunoprecipitates of surface-radioiodinated cells, it was easily distinguished that gp205 shifted to an M_r of 190 kd (Figure 1B, left panel) under nonreducing conditions, since, under the conditions used, gp205 labeled preferentially over the other components. Similarly, the M_r of the gp180 and gp150 species seemed to shift down by ~20–30 kd under nonreducing conditions. The high mol. wt material in the non-reducing lane of the surface-labeled immunoprecipitation is probably due to non-specific aggregates. These data are consistent with gp205, gp180 and gp150 containing significant amounts of intrachain disulfide bonds. In contrast, the M_r of gp125 increased, under nonreducing conditions, to 150 kd (Figure 1B). This finding suggests that the 150 kd species may contain, in addition to gp125, a disulfide-linked smaller peptide, which is lost upon reduction. A band compatible with such a fragment was visualized by long exposures of immunoprecipitates run on high percentage SDS-PAGE gels (not shown). Identical results were obtained with labeled lysates from normal keratinocytes in short-term culture.

Heteromeric structure of gp205,125

The structural relationships between the components of gp205,125 were investigated by limited peptide mapping. Partial digestion of individual chains, metabolically labeled with [35 S]methionine, was carried out with the V8 protease. SDS-PAGE resolved eight peptides for gp205 and 10 for gp125 (Figure 2), which generated distinct profiles, suggesting that gp205 and gp125 are structurally unrelated. The gp180 and gp150 digests resolved into seven and six labeled fragments, respectively (Figure 2). In both cases, all fragments co-migrated with gp205 fragments, except for one gp150 peptide co-migrating with the major fragment of gp125 (due to its significantly greater intensity, this fragment may however derive from minor contamination of gp150 with the closely spaced gp125). These data suggest that gp205, gp180 and gp150 are structurally related, but distinct from gp125.

This conclusion was further supported by investigations with a polyclonal antiserum (5710) raised against antigen purified from carcinoma cells by immunoaffinity chromatography on the monoclonal antibody S3-41. From carcinoma lysates, the 5710 antiserum precipitated a set of proteins identical to those reactive with S3-41 (Figure 3A, lane 1). However, if the cell lysates were previously treated at 100°C in the presence of SDS to disrupt noncovalent associations, then antiserum 5710 precipitated only gp205, gp180 and gp150, and not gp125 (Figure 3A, lane 2). The isolated gp205, gp180 and gp150 displayed their characteristic M_r downshift under nonreducing conditions (Figure 3A, lane 3). These results suggest that at least some of the epitopes present on gp205, gp180 and gp150 are not found on gp125 and

Fig. 2. Peptide mapping of gp205, gp180, gp150 and gp125 by limited proteolysis. The top lane shows a representative autoradiographic pattern of the immunopurified gp205,125 complex. The bottom panel shows the electrophoretic profiles of excised bands after limited digestion with V8 protease. See text for further explanation. The mol. wt standards are phosphorylase B (97 kd), bovine serum albumin (66 kd), ovalbumin (43 kd), carbonic anhydrase (31 kd), soybean trypsin inhibitor (21 kd), lysozyme (14 kd).

that all 5710 epitopes on gp125, if any, are sensitive to denaturation. This conclusion was verified by Western blots of material immunopurified with S3-41 from carcinoma cells and placental tissue (Figure 3B, lanes 1–2), in which 5710 reacted predominantly with gp205, gp180 and gp150 and displayed little, if any, reactivity with gp125 (Figure 3B, lanes 3–4). This antigenic similarity of gp205, gp180 and gp150 was authentic since, in a further refinement, antibodies that were adsorbed and eluted from gp150 reacted with gp180 (in addition to gp150 itself) (Figure 3B, lane 5), and antibodies adsorbed and eluted from gp180 reacted with gp150 (in addition to gp180 itself) (Figure 3B, lane 6). Furthermore, [35 S]methionine-labeled gp205, gp180, gp150 and gp125 purified by immunoaffinity and electroelution were subjected to 'hot' blotting with the gp150-purified antibodies. These antibodies only reacted with gp205, gp180 and gp150, but not with gp125 (Figure 3C, right panel). Subsequent autoradiography of the blot confirmed that the four proteins were present in approximately equal amounts (Figure 3C, left panel). These results provided further evidence for the structural relatedness of gp205, gp180 and gp150, and for the dissimilarity of gp125. Therefore, co-precipitation of gp125 by S3-41 and 5710 antibodies is probably due to noncovalent associations with gp205, gp180 and gp150.

Glycosylation of gp205,125

In order to investigate whether the M_r differences between gp205, gp180 and gp150 were due to glycosylation, S3-41 precipitates of radiolabeled carcinoma cell lysates were digested with glycosidases. Treatment with Endo-F, which cleaves both complex- and high mannose-type N-linked

Fig. 3. Reactivity of rabbit antiserum 5710 with the components of the gp205,125 complex. (A) An $[^{35}\text{S}]$ methionine labeled lysate of FG-met2 cells was immunoprecipitated with antiserum 5710 before (lane 1) and after (lanes 2–3) denaturation by boiling in the presence of 1% SDS, and then analyzed on a 5% gel under reducing (lanes 1–2) and nonreducing (lane 3) conditions. Note the absence of gp125 from immunoprecipitates of denatured lysates (lanes 2–3). (B) Material purified by chromatography on S3-41 affinity columns from pancreatic carcinoma cells FG (lanes marked FG) or human placenta (lanes marked PL) were separated by SDS-PAGE and then subjected to either silver staining or Western blotting. For the latter, antibodies used were rabbit antiserum 5710, raised against pancreatic gp205,125, or antibodies purified from antiserum 5710 by adsorption-elution on either immobilized placental gp150 or placental gp180. (C) $[^{35}\text{S}]$ Methionine-labeled gp205, gp180, gp150 and gp125, purified from pancreatic carcinoma cells by immunoaffinity and electroelution, were run on 5% SDS-PAGE gels and then subjected to Western blotting with antibodies purified from 5710 antiserum by absorption-elution on immobilized placental gp150 (right panel). After immunostaining, the blot was autoradiographed on X-ray film (left panel). The anti-gp150 antibodies react with gp205, gp180 and gp150, but fail to recognize gp125, whose position in the 'hot' blot filter lane is indicated by the autoradiogram on the left.

glycans, decreased the M_r of gp205, gp180 and gp150 by ~10 kD each (Figure 4A, left panel), while digestion with Endo-H, which cleaves only high mannose-type N-linked glycans, did not affect their M_r , indicating that each of these proteins contained one or more complex-type carbohydrates. The M_r of gp125 was instead decreased both by Endo-H and, to a larger extent, by Endo-F (Figure 4A, left panel), indicating a different glycosylation mode which includes high mannose- as well as complex-type carbohydrates.

Treatment with neuraminidase resulted in two bands of 120 and 100 kD (Figure 4A, right panel). Since an explanation of these changes in M_r of gp205,125 was not straightforward, time-course digestions were carried out on immunoprecipitates from surface-iodinated cells, in which gp205 is preferentially labeled (see Figure 1B). After 2 h of digestion, gp205 gave rise to bands of 180, 150, 125, 120 and 100 kD (Figure 4B), which by 7 h were almost completely converted into the 100 kD band (Figure 4B). This course of events indicated that, in the neuraminidase digestions of Figure 4A, it is gp205 that is digested down to 100 kD, while gp125 is digested to 120 kD. Thus, it appears that gp205 may contain a surprisingly large amount of sialic acid. Furthermore, since incremental removal of sialic acid from gp205 generated two major intermediate products of ~180 and ~150 kD, it is probable that the M_r differences between gp205, gp180 and gp150 may be due to sialylation. In these experiments, the neuraminidase preparations used were of the highest purity available commercially. In the same neuraminidase digests, the M_r

Fig. 4. Glycosidase treatments of gp205,125. (A) S3-41 immunoprecipitates from $[^{35}\text{S}]$ methionine-labeled FG cell lysates were treated with the enzymes Endo-H (H), Endo-F (F) or with control buffer (C), or were incubated overnight in the presence (+) or absence (–) of neuraminidase, and then analyzed on 5% acrylamide SDS-PAGE gels. (B) S3-41 immunoprecipitates from ^{125}I -labeled lysates of normal human keratinocytes were treated with PBS or neuraminidase (Exo-N) for 2 or 7 h. The mol. wt standards are as in Figure 1.

of gp125 decreased only by ~5 kD (Figure 4A), suggesting that the neuraminidase preparations did not contain significant levels of contaminating proteases. However, the possibility that trace contaminants may be responsible for the observed digestion pattern of gp205 will have to be rigorously investigated.

Identification of gp205,125 as a novel integrin by amino-acid sequence homology

Preparations of gp125, purified on S3-41 immunoaffinity columns from either carcinoma cells or placental tissues, were subjected to automated microsequencing, yielding

Fig. 5. Amino-terminal sequences of gp125 and gp150. Recovery of PTH-amino acids following automated Edman degradation of gp125 (~65 pmol) and gp150 (~85 pmol). Identified residues are indicated in the one-letter code.

Fig. 6. Amino-acid sequences of gp125 (α_E) and gp150 (β_4). Amino acids are in the one-letter code; italicized residues are of uncertain assignment, and Xs denote no possible assignment. The sequences are optimally aligned, and residues identical with the top sequence are boxed. In the β chain sequences, asterisks denote cysteine residues. Abbreviations and sources are: FNR, fibronectin receptor (Arggraves *et al.*, 1987); VLA-1–VLA-4 (Takada *et al.*, 1987b); Mac-1 (Springer *et al.*, 1985); p150.95 (Miller *et al.*, 1987); LFA-1 (Springer *et al.*, 1985); platelet IIB–IIIa (Poncz *et al.*, 1987); VNR, vitronectin receptor, α (Suzuki *et al.*, 1986), β (Fitzgerald *et al.*, 1987); LAP, leukocyte adhesion proteins, β (Kishimoto *et al.*, 1987). All sequences are from man.

information up to residue 21 (Figure 5). The sequences of gp125 from these two sources were identical, with the exception of position 6 which could not be assigned for carcinoma gp125 in three separate sequencing runs. The gp125 sequence showed several similarities with integrin α chain N-termini, as depicted in Figure 6. The first five residues, FNLDT, are identical in four α chains, and occur with one replacement in four other α chains and with two replacements in the remaining three α chains. Another segment of significant homology among α chains occurs between residues 15 and 21. In this region, gp125 shares one residue with at least one other α chain at five positions, and at three positions has unique residues. Overall, the homology of gp125 to the integrin α chains appears to be equivalent to the homology of the α chains to each other. The highest level of similarity was observed with the VLA-2 α chain, where 11 of 13 residues available for comparison were shared.

Placenta gp150 was sequenced up to residue 19 (Figure 5). When this sequence was compared to the amino-termini of the three human integrin β chains, several similarities were found (Figure 6). Of particular importance is the exact correspondence of the three cysteines, the serine in position 10 and the proline in position 19, as these residues are invariant among integrin β chains. Overall, gp150 showed 11 identities with β_1 , eight with β_2 and seven with β_3 . However, the gp150 sequence was distinct from those of the other β chains since its N-terminus was offset with respect to β_1 (as predicted from cDNAs) and β_3 sequences, and since it contained six unique residues (Figure 6).

To check further the relationship between gp150 and gp180, placental gp180 was also sequenced up to residue 13. This sequence was identical to gp150 (not shown), except for four residues of uncertain assignment, thus providing further evidence for the structural similarity of gp150 and gp180. Since the neuraminidase digestions suggested that the M_r differences between gp205, gp180 and gp150 were due to variations in sialic acid content, gp205 was expected to display an amino-terminal sequence identical to that of gp150 and gp180. However, gp205 is undetectable in placental lysates, and two attempts to sequence carcinoma-derived gp205 failed, presumably due to insufficient quantities and/or N-blocking during purification.

Absence of antigenic cross-reactions between gp205, 125 and other integrins

The amino-acid sequence homologies represented direct evidence that gp205,125 is a novel integrin. Its antigenic relationship to other integrins expressed by epithelial cells was then investigated. Integrins of the β_1 and β_3 families, but not of the β_2 family (which is known to be leukocyte-restricted), were detected in epithelial cell lysates by immunoprecipitation (Figure 7A and B). The β_1 chain, identified both with a polyclonal (R255) and a monoclonal (A-1A5) antibody, displayed a typical SDS–PAGE M_r of 140/110 kd under reducing/nonreducing conditions, and was associated with a protein of higher M_r (170/155 kd reduced/nonreduced) (Figure 7A and B). The latter was identified as a VLA-2 α chain with a specific monoclonal antibody (12F1) (Figure 7A and B). The β_3 integrins, identified with a polyclonal (VNRpoly) and a monoclonal (142) antibody, migrated as a doublet at an M_r of ~140 kd (Figure 7A and B). The SDS–PAGE profiles of these β_1 and β_3 integrins were distinct both under reducing and non-reducing conditions from those of gp205,125 immunoprecipitated with S3-41 and 5710 antibodies (Figure 7A and B), suggesting the absence of antigenic cross-reactions.

Because of the closer sequence homologies of gp205,125 to β_1 integrins, epithelial cell lysates were also pre-treated with the anti- β_1 monoclonal A-1A5 (Figure 7C). This pre-clearing step removed molecules reactive with anti- β_1 integrin antisera (A-1A5 itself and R255), but did not affect the reactivities of anti-gp205,125 antisera (S3-41 and 5710) (Figure 7C). Finally, in Western blots (data not shown), antibodies to β_1 (A-1A5 and R255), β_2 (60.3) and β_3 (VNRpoly and 142) integrins did not react with purified gp205,125.

These results, although necessarily limited to the reagents used, ruled out obvious cross-reactivities with integrins of the other families and substantiated the conclusion that gp205,125 is a novel integrin.

Fig. 7. Antigenic relationship of gp205,125 to other integrins. (A) [35 S]Methionine-labeled lysates of FG-met2 cells were immunoprecipitated with antibodies to gp205,125 (S3-41, 5710), β_1 (R255, A-1A5, 12F1), β_2 (60.3) and β_3 (VNRpoly, 142) integrins, and then analyzed by SDS-PAGE under reducing or non-reducing conditions. 5710-PS, pre-immune serum control of 5710 antiserum. The mol. wt standards are as in Figure 1. See Materials and methods and Results sections for additional details on the antibodies used. (B) An [35 S]methionine-labeled pancreatic carcinoma lysate was pre-cleared with the anti- β_1 monoclonal antibody A-1A5 or control antibody (60.3), immunoprecipitated with the indicated antibodies, and then analyzed by SDS-PAGE on a 5% gel under reducing conditions. Low mol. wt bands in the 5710 and R255 lanes are probably due to degradation of the β chains. The mol. wt standards are as in Figure 1.

Discussion

Biochemical and serological evidence indicated that gp205,125, a molecular complex initially isolated from epithelial cells with the monoclonal antibody S3-41, is a new member of the integrin adhesion receptor superfamily. Like other integrins, gp205,125 comprises two subunits which are structurally dissimilar, as shown by peptide mapping and amino-terminal sequencing data. The two subunits are both glycosylated, as shown by radioactive sugar incorporation and glycosidase digestions. Because they co-precipitate with a monoclonal antibody as well as with an antiserum predominantly directed against gp205, these subunits are probably held together by noncovalent bonds only, as is the case with integrin α and β chains.

The smaller subunit, gp125, may be disulfide-linked to another polypeptide chain, as suggested by its slower electrophoretic migration under nonreducing conditions. This behavior is typical of some of the integrin α chains (Buck and Horwitz, 1987; Hynes, 1987; Ruoslahti and Pierschbacher, 1987), which were shown to consist of a heavy chain of ~ 125 kd disulfide-linked to a light chain of ~ 25 kd. The amino-terminal sequence of gp125 showed unambiguous homologies with integrin α chains. Numerous differences, however, indicated that gp125 is a novel α chain. The closest of the α chains was that of VLA-2 (an integrin of the β_1 family), with 11 identities out of 13 positions examined. However, VLA-2 and gp205,125 are distinct molecules since we have shown here that: (i) a monoclonal antibody to VLA-2 α chain is unreactive with gp205,125; (ii) polyclonal and monoclonal antibodies to β_1 are not reactive with

gp205,125; (iii) both a monoclonal and a polyclonal antibody to gp205,125 do not react with epithelial cell VLA-2; and (iv) the VLA-2 α chain and gp125 display M_r shifts in opposite directions when examined under reduced and nonreduced SDS-PAGE conditions. Thus, since gp125 appears to be a new integrin α chain, we propose α_E as its provisional designation, because of its original isolation from epithelial-type cells.

The larger subunit of gp205,125 was present in three forms (referred to as gp205, gp180 and gp150), distinguished by their migration on SDS-PAGE. That these three glycoproteins may represent three versions of a single polypeptide was indicated by: (i) similar peptide maps; (ii) similar N-linked glycosylation patterns; (iii) identical reactivity with antibodies affinity-purified on gp180 and gp150; and (iv) identical amino-termini for gp180 and gp150. Furthermore, treatment with neuraminidase decreased the M_r of gp205 to ~ 100 kd, with intermediate digestion products of 180 kd and 150 kd, suggesting the possibility that gp180 and gp150 may be partially sialylated forms of gp205. The gp205, gp180 and gp150 all displayed faster migration under nonreducing than under reducing SDS-PAGE conditions, a behavior suggestive of a high cysteine content. This observation, together with a M_r of desialylated gp205 (~ 100 kd), constituted initial indications of similarity with integrin β chains, which are in the range of 100 kd in mol. wt, and contain $\sim 7\%$ cysteine. These similarities were borne out by the amino-terminal sequence of gp150 and gp180, which contained the typical cysteine spacing and the invariant residues of human integrin β chains. Several differences, including six unique positions and offset amino-terminus,

showed unequivocally that the sequence of gp150/gp180 represents a new human β chain. We therefore propose the designation β_4 for gp205/gp180/gp150. Since the integrin families are discriminated on the basis of a shared β chain, we also suggest that β_4 may define a fourth integrin family.

The identification of $\alpha_E\beta_4$ as a novel integrin was corroborated by its lack of reactivity with various antisera to the three previously identified integrin β chains. Likewise, rabbit polyclonal antiserum 5710, which is reactive with β_4 (gp205/gp180/gp150), failed to recognize other integrins. This absence of cross-reactivity is consistent with β_4 defining a fourth integrin family, since anti- β chain antisera are generally not cross-reactive.

The tissue distribution of $\alpha_E\beta_4$, previously described by the use of antibody S3-41 (Kajiji *et al.*, 1987), is limited to normal and malignant epithelial cells. This is in agreement with $\alpha_E\beta_4$ being a new integrin type, since no other integrin with such distribution has been thus far described. Other types of integrins have been found on epithelial-type cell lines before (Buck and Horwitz, 1987; Takada *et al.*, 1987a), as also shown in this report (e.g. VLA-2). However, while those integrins are also expressed on other cell types (Buck and Horwitz, 1987; Takada *et al.*, 1987a) $\alpha_E\beta_4$ is more narrowly distributed (Kajiji *et al.*, 1987), e.g. in placenta it is only found on cytotrophoblast cells.

The fact that all integrins thus far described are heterodimers, suggests a heterodimeric structure for $\alpha_E\beta_4$, which indeed was consistent with immunoprecipitation analyses. However, the exact stoichiometry of α_E and the various forms of β_4 in the complex remains to be determined. For this purpose, the structural basis for the M_r differences of gp205, gp180 and gp150 also needs to be firmly established. Preliminary results in our laboratory indicate that gp205 is sensitive to the enzyme endoneuraminidase (Hallenbeck *et al.*, 1987) (kindly provided by Dr F. Troy), and therefore may contain $\alpha 2-8$ linked polymers of sialic acid. This would explain the unusually large sialic acid content of gp205. However, since there exists only a single other example of polysialylated glycoprotein in higher vertebrates, i.e. the neural adhesion molecule N-CAM (Finne, 1985), this preliminary observation deserves rigorous confirmation. Whatever the case may be, there should be little doubt that gp205, gp180 and gp150 are closely related molecules. In this respect it should be noted that in carcinoma cells the amount of gp180 and gp150 varied relative to gp205, possibly due to experimental manipulations, but was always considerably lower. In placenta, instead, gp150 was the predominant form. This may reflect either the harsher conditions of the purification procedures adopted for the placental tissue material, or, more attractively, tissue-specific variations.

Integrins are cell surface receptors. By surface radio-iodination, gp180, gp150 and gp125 became labeled faintly compared to gp205, raising the possibility that they may be only intracellular components of the gp205,125 complex. However, gp180, gp150 and gp125 were sensitive, like gp205, to Endo-F and neuraminidase, and thus carry complex-type sugars, indicating they are co-transported past at least the *trans*-Golgi compartment, and probably reach the cell surface. We favor therefore the alternative possibility that they are expressed at the cell surface but are inefficiently radio-iodinated due to steric hindrances.

A VLA-like molecule, recently described in platelets

(Sonnenberg *et al.*, 1987; Hemler *et al.*, 1988; Pischel *et al.*, 1988) and mouse mammary tumor cells (Sonnenberg *et al.*, 1988), bears some structural resemblances to gp205,125. A monoclonal antibody specific to this molecule (GOH3) was found to cross-react with gp205,125 (S.K., unpublished data). Further studies are required to clarify the relationship between these molecules, in the context of the integrin superfamily.

Additional work will now be necessary to determine the complete molecular structure of $\alpha_E\beta_4$, particularly the precise relationship of gp205 to gp180 and gp150. Furthermore, since it is reasonable to assume that $\alpha_E\beta_4$ has a function in cell adhesion like other integrins, a search for a potential ligand will be essential to understanding its function.

Materials and methods

Cell lines

The human pancreatic carcinoma cell line FG-met2 (Kajiji *et al.*, 1987) and lung adenocarcinoma UCLA-P3 (Varki *et al.*, 1984) were grown in RPMI 1640 media with 10% fetal calf serum, 2 mM glutamine and 25 μ g/ml gentamicin. Normal human keratinocytes (Clonetics, San Diego, CA) were maintained in short-term cultures in serum-free defined media.

Antibodies

Monoclonal antibody S3-41 has been described (Kajiji *et al.*, 1987). The following antibodies were obtained through the kind generosity of listed investigators: monoclonal antibody 142 and rabbit antiserum VNRpoly to human vitronectin receptor (Cheresh and Harper, 1987), D. Cheresh, Research Institute of Scripps Clinic, La Jolla, CA; rabbit antiserum R255 to fibronectin receptor, E. Ruoslahti, La Jolla Cancer Research Foundation, La Jolla, CA; monoclonal antibody 60.3 (Arfors *et al.*, 1987), to the β chain of leukocyte integrins, C. Lundberg, Pharmacia Experimental Medicine, La Jolla, CA; monoclonal antibody A-1A5 (Hemler *et al.*, 1987) to VLA β chain, M. Hemler, Dana Farber Cancer Institute, Boston, MA; monoclonal antibody 12F1 (Pischel *et al.*, 1987) to VLA-2 α chain, V. Woods, University of California Medical Center, San Diego, CA. Antiserum 5710 was obtained by bleeding of an NZW rabbit, subcutaneously injected with ~ 2 μ g of immunopurified carcinoma $\alpha_E\beta_4$ at day 0 in Freund's complete adjuvant, and at days 30 and 45 with the same amount in incomplete adjuvant.

Immunohistology

Immunoperoxidase staining of tissue sections was performed according to standard procedures, as described (Kajiji *et al.*, 1987).

Cell labeling

For cell surface labeling, a semi-confluent cell monolayer was rinsed several times with phosphate buffered saline, pH 7.4 (PBS), and then radio-iodinated with [125 I]sodium iodide for 15 min at room temperature by the enzyme-bead procedure (Bio-Rad, Burlingame, CA). Cell viability remained $>95\%$ by trypan blue exclusion. For metabolic labeling, monolayers were incubated for 12–16 h in methionine-free culture media with 400 μ Ci/ml of [35 S]-methionine (>1000 Ci/mM, New England Nuclear, Boston, MA). In some cases, metabolic labeling was carried out with [6- 3 H]glucosamine (40 Ci/mM, Amersham) in regular culture media. After labeling, cells were detached with 10 mM EDTA, 0.02% KCl in PBS, pH 7.5, washed three times in Tris-buffered saline (TBS), pH 7.5 and lysed for 30 min on ice with 2% Renex30 (a non-ionic detergent, Accurate Chemicals), 1 mM CaCl_2 , 2 mM phenylmethylsulfonylfluoride, 0.02% NaN_3 in TBS, pH 8.5. Lysates were cleared by the addition of one-tenth the volume of Pansorbin (Calbiochem) followed by centrifugation at 100 000 g for 60 min at 4°C, and were then stored at -70°C .

Immunoprecipitation and electrophoresis

For monoclonal antibody S3-41, an immunosorbent was made on an Affigel 10 matrix (Bio-Rad), according to the manufacturer's instructions. Rabbit antisera and other monoclonals were absorbed onto protein-A Sepharose (Pharmacia, Uppsala, Sweden) according to standard procedures. Immunoprecipitations were carried out as described (Kajiji *et al.*, 1987), by overnight incubation of the immunosorbents with aliquots of the cell lysates, followed by washing and elution by boiling in Laemmli sample buffer

(Laemmli, 1970), with or without reduction/alkylation with 10 mM dithiothreitol and 50 mM iodoacetamide. In some cases, lysates were pre-cleared by two rounds of immunoprecipitation with one antibody, and then reacted with another antibody immunosorbent. For immunoprecipitation of denatured proteins, the cell lysate was brought to 1% sodium dodecyl sulfate (SDS), boiled for 5 min, cooled to room temperature and then diluted 10-fold with the immunoprecipitation buffer. Samples were analyzed by SDS-PAGE on 5% or 7.5% polyacrylamide slab gels (Laemmli, 1970), followed by either fluorography (Bonner and Laskey, 1974) or autoradiography (125 I-labeled material) on Kodak XAR-5 film.

Peptide mapping

[35 S]methionine-labeled lysates were immunoprecipitated with S3-41 and subjected to SDS-PAGE. Relevant bands were located on the dried gel by overnight autoradiography, excised, rehydrated and inserted in contiguous slots of a 15% acrylamide SDS-PAGE gel, containing a 0.5 μ g/ml solution of *Staphylococcus aureus* V8 protease (Cleveland *et al.*, 1977). Electrophoresis was suspended for 30 min after bands were stacked to allow for enzymic digestion. Gels were fluorographed as described above. The concentration of V8 protease was optimized in preliminary experiments.

Glycosidase treatments

For digestions with Endo-H (endo- β -N-acetyl-glucosaminidase H) (Calbiochem, La Jolla, CA) and Endo-F (a preparation containing a mixture of peptide-N-glycosidase F and endo- β -N-acetyl-glucosaminidase F, kindly obtained from J. Elder, Research Institute of Scripps Clinic, La Jolla, CA, immunoprecipitates were boiled for 3 min in 10 μ l 0.5% SDS, 0.05 M β -2-mercaptoethanol, quick-chilled, diluted 10-fold with PBS that, for Endo-F, contained 50 mM EDTA and 1% NP-40, and incubated for 12 h at 37°C with optimal amounts of enzymes. For neuraminidase, immunoprecipitates were resuspended in 50 μ l PBS containing 1.0 U enzyme (from either *Arthrobacter ureafaciens* or *Clostridium perfringens*, Calbiochem, La Jolla, CA) and incubated at 37°C for indicated times. Following addition of 1/4 volume 5 $\times$ Laemmli sample buffer and boiling, digests were analyzed by SDS-PAGE.

Purification of gp205,125

Gram amounts of UCLA-P3 cells or FG-met2 cells were washed and lysed in an equal volume of TBS containing 2% Renex 30, centrifuged at 10 000 g for 30 min at 4°C, and stored at -70°C. The UCLA-P3 lysate was the generous gift of Dr Leslie E. Walker in this Institution. The cell lysates were passed sequentially through a Sepharose column and an S3-41 immunosorbent column, at a flow-rate of 5–10 ml/h. The columns were washed with 0.1% Renex30, TBS, pH 8.5 followed by 1.0% n-octyl β -D-glucopyranoside, TBS, pH 8.5. The bound material was eluted with 150 mM NaCl, 1.0% n-octyl β -D-glucopyranoside, 50 mM diethylamine, pH 11.5, over a cushion of 0.1 M Tris-HCl pH 6.8, 150 mM NaCl, and 1.0% n-octyl β -D-glucopyranoside for rapid neutralization. The peak material was located by silver staining (Oakley *et al.*, 1980) of SDS-PAGE gels, pooled, concentrated and stored at -70°C. Immunopurifications were also performed starting from human placentas at term (kindly provided by the University of California, San Diego Medical Center and Mercy Hospital, San Diego, CA), based on the observation that S3-41 reacts with cytotrophoblast cells by immunoperoxidase staining. Placental tissue was passed through a commercial meat grinder, washed by centrifugation with 2 mM PMSF, TBS, pH 8.5, detergent-lysed and chromatographed over S3-41 immunoaffinity columns as described above.

Automated amino acid sequencing

Material purified from 20–100 g of carcinoma cells or 150–450 g of placental tissue was electrophoresed on a 5% slab gel. To avoid N-blocking, several precautions were taken, including 2 $\times$ recrystallization of SDS from ethanol, addition of 10 mM sodium thioglycolate in the sample buffer, stacking in agarose gels, 0.5 mM sodium thioglycolate in the upper reservoir buffer and the use of HPLC grade water throughout. Proteins were electrophoretically transferred to Immobilon paper (Matsudaira, 1987) (Millipore Co.), visualized by Coomassie blue staining, excised and inserted in the cartridge of an Applied Biosystems gas-liquid solid phase protein sequencer equipped with an on-line HPLC (Hunkapiller *et al.*, 1983). Each protein was sequenced at least twice, with 5–70 pmol of material each time. The average repetitive yields in the various sequencing runs ranged from 94.7 to 90.5%.

Western blots and 'hot' blots (Towbin *et al.*, 1979)

Proteins separated by SDS-PAGE on a 5% gel were transferred overnight at 4°C to Immobilon (Millipore Corp., Bedford, MA) using a 25 mM Tris, 192 mM glycine buffer. The filter was saturated with 3% (w/v) nonfat dry

milk in TBS, pH 8.0 with 0.05% Tween 20 and 0.02% azide, incubated for 3 h with primary antibody, washed, overlaid for 1 h with alkaline phosphatase-conjugated goat anti-rabbit (Promega Biotec, Madison, WI) or anti-mouse (Boehringer Mannheim, Indianapolis, IN) IgG, washed again and then developed with 0.33 mg/ml nitro-blue tetrazolium, 0.165 mg/ml 5-bromo-4-chloro-3-indolyl phosphate in 100 mM Tris-HCl pH 9.5, 100 mM NaCl, 5 mM MgCl₂, followed by 5 mM EDTA in 20 mM Tris-HCl pH 8.0. For affinity purification (Weinberger *et al.*, 1985) of 5710 antibodies, vertical strips cut from the edges of gp205,125 whole-gel blots previously incubated with 5710 were developed to locate reactive bands. Horizontal strips corresponding to these were excised from the undeveloped mid-part of the filter. Bound antibodies were eluted by three 1-min washes with 5 mM glycine-HCl pH 2.3 containing 150 mM NaCl, 0.05% Tween 20, 100 μ g/ml bovine serum albumin, 2.5 mM sodium azide, and quickly neutralized by addition of Tris-HCl pH 8.0. For 'hot' blots, 1×10^7 cells were metabolically labeled with [35 S]methionine, immunoprecipitated with S3-41 and electrophoresed on a 5% SDS-PAGE gel. The wet gel was autoradiographed overnight to locate radioactive bands, which were then excised. Proteins were electroeluted in an ISCO apparatus as described (Hunkapiller *et al.*, 1983), run on 5% SDS-PAGE and transferred to Immobilon filters. The filters were first immunostained as described above and then autoradiographed.

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