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ARTICLES

Colocalization of the p185^{HER2} Oncoprotein and Integrin α6β4 in Calu-3 Lung Carcinoma Cells

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Abstract Anti-p185^{HER2} monoclonal antibodies often show intense reactivity with the basement membrane of tumor cells that overexpress the HER2/neu gene product (p185^{HER2}). To evaluate a possible interaction between p185^{HER2} and adhesion molecules or their receptors, the polarity of p185^{HER2} was tested in lung carcinoma cell line Calu-3, which overexpresses this protein, in cultures grown as confluent monolayers or as aggregates. MAb immunostaining patterns indicated that p185^{HER2} is concentrated on the baso-lateral membrane of cells and that it colocalizes with the integrin α6β4 at the cell-cell junctions where laminin is also found. The same membrane region showed intense reactivity with antiphosphotyrosine antibodies. Furthermore, integrin clustering induced by the specific antibody was accompanied by the clustering of p185^{HER2}, as indicated by immunoelectron microscopy, and by a subsequent increase in p185^{HER2} tyrosine phosphorylation. Treatment with exogenous laminin also resulted in increased basal levels of p185^{HER2} phosphorylation. These data suggest a physical interaction between the integrin and the oncoprotein that might be functionally relevant in directly controlling the tyrosine phosphorylation of the catalytic domain of p185^{HER2}.

Key words: HER2/neu, integrins, laminin, tyrosine phosphorylation, oncoprotein

The product of the HER2/neu protooncogene is a transmembrane glycoprotein of 185 kDa (p185^{HER2}) with tyrosine kinase activity and close homology with the epidermal growth factor receptor (EGF receptor) [Coussens et al., 1985]. These properties have suggested that p185^{HER2} might act as a receptor for as yet unidentified growth factors [Yarden and Peles, 1991; Lupu et al., 1990, 1992; Tarakhovsky et al., 1991; Plowman et al., 1993]. Previous studies indicated a high incidence of amplified HER2/neu expression in different tumors of epithelial origin and a correlation between the overexpression of its product and a poor prognosis [Slamon et al., 1989; Rilke et al., 1991]. The mechanism by which the overexpressed oncoprotein increases tumor aggressiveness has not yet been identified; however, the increased metastatic potential of 3T3 or other low-metastatic-potential cells transfected with neu-oncogene suggests its involvement in the metastatic process [Yusa et al., 1990; Yu and Hung, 1991]. Immunohistochemical tests using monoclonal antibodies (MAbs) directed against the ectodomain of the p185^{HER2} molecule on tumors that overexpress the oncogene product have often shown a strong reactivity at the basement membrane level [Centis et al., 1992; Natali et al., 1990]. This finding has suggested that p185^{HER2} defines membrane domains involved in cellular adhesion to the extracellular matrix and possibly regulated by the polar topography of epithelial cell membranes.

Cell-matrix and cell-cell adhesion are mediated by integrins which are cell surface heterodimers formed by two noncovalently associated subunits, the α and β chains. Changes in integrin expression and in the extracellular matrix organization have been implicated in many biological functions and are frequently observed in cancer cells [Ruoslahti, 1991]. In the present study we have investigated the membrane association of the p185^{HER2} oncoprotein with structural components of the extracellular matrix and their integrin receptors in a lung carcinoma

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cell line, Calu-3, which overexpresses the oncoprotein. Our results suggest a structural interaction between $p185^{HER2}$ and the integrin receptor $\alpha6\beta4$, which colocalize on the plasma membrane of Calu-3 cells; clustering of the integrin receptor enhances the tyrosine phosphorylating activity of the oncoprotein, suggesting that the two molecules are involved in the same signal transduction pathway.

METHODS

Cells

The human lung adenocarcinoma cell line Calu-3 (provided by ATCC, Rockville, MD) was maintained in RPMI-1640 medium (Irvine Scientific, Santa Ana, CA) with or without 10% fetal calf serum (FCS) and supplemented with penicillin (100 μg/ml) and streptomycin (100 μg/ml).

Antibodies

The purified MAbs used were MGR9 and MGR4 [Centis et al., 1992], directed against the extracellular domain of $p185^{HER2}$; c-neu Ab3 (Oncogene Science, Inc., Manhasset, NY), directed against the intracellular domain of $p185^{HER2}$; MGR1, directed against the extracellular domain of the EGF receptor [Pellegrini et al., 1991]; W6/32, directed against a monomorphic epitope on the 45 KDa polypeptide products of the human leukocyte antigen (HLA) A, B, and C *loci* (*DAKO*, Glostrup, Denmark); 4B4, directed against the ectodomain of the β1 integrin subunit (Coulter Immunology, Hialeah, FL); and anti-α2, 3, 4, 5, 6 and β4 integrin chain MAbs (Telios, San Diego, CA).

Three rabbit antisera, directed, respectively, against $p185^{HER2}$ (DAKO), laminin (Telios), and phosphotyrosine (Upstate Biotechnology, Inc., Lake Placid, NY), were also used.

Binding Assays

For direct binding, Calu-3 cells were seeded in 96 well plates and grown as monolayers. Plates were fixed with 0.1% glutaraldehyde and rinsed in phosphate-buffered saline (PBS), and cells were either permeabilized or not for 5 min at room temperature in HEPES–Triton X-100 buffer (20 mM HEPES, pH 7.4, 300 mM sucrose, 50 mM NaCl, 3 mM $MgCl_2$, and 0.5% Triton X-100). After washing, cells were incubated for 45 min at 37°C with 50 μl of different dilutions of purified MAb MGR9 labeled with ^{125}I by lactoperoxidase-catalyzed iodination [Tagliabue et al., 1991].

For indirect binding assay, unlabeled antibodies were detected by sheep ^{125}I-labeled anti-mouse Ig (Amersham, Little Chalfont, UK). The test was carried out in 96 well plates using cell monolayers, as described above.

Fluorescence-Activated Cell Sorter (FACS) Analysis

Reactivity of MAbs directed against different integrin α and β subunits with cells was quantitated by indirect immunofluorescence with fluorescein isothiocyanate-conjugated goat anti-mouse Ig (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). Immunofluorescence was measured using a FACScan flow cytometer with LISYS™II software (Becton Dickinson, Mountain View, CA).

Immunostaining

Indirect immunoperoxidase assay was performed with an avidin-biotin peroxidase complex (ABC) kit (Vector, Burlingame, CA) on a clump of Calu-3 cells grown in the peritoneal cavity of nude mice. Cells were attached on glass slides by Cell-tak (BioPolimers, Inc., Farmington, CT) and fixed in 3% paraformaldehyde in PBS, pH 7.6, containing 2% sucrose for 5 min at room temperature. After rinsing in PBS, cells were permeabilized by soaking the slides in HEPES–Triton X-100 buffer for 3–5 min at 0°C. This procedure of fixation or fixation-permeabilization permits immunostaining of either membrane molecules or cytoskeletal and adhesion components respectively [Marchisio et al., 1984; Dejana et al., 1988a,b].

Indirect immunofluorescence experiments were performed as described [Marchisio et al., 1984] on Calu-3 cells grown on glass coverslips, fixed, and permeabilized. Briefly, primary antibody (Ig concentration of 10–30 μg/ml) was layered on fixed and permeabilized cells and incubated in a humidified chamber for 30 min. After rinsing in PBS–0.2% bovine serum albumin (BSA), coverslips were incubated in the appropriate rhodamine-tagged second antibody (DAKO) for 30 min at 37°C in the presence of 2 μg/ml of fluorescein-labeled phalloidin (F-PHD; Sigma, St. Louis, MO). Indirect double-labeling immunofluorescence experiments were performed essentially as described [Dejana et al., 1988a]. Coverslips were mounted in Mowiol 4-88 (Hoechst AG, Frankfurt, Germany). Routine observations were carried out in a Zeiss Axiophot

photomicroscope equipped for epifluorescence and photographed with a 63×/NA 1.4 plan-apochromatic lens. Fluorescence images were recorded on Kodak T-Max 400 film exposed at 1000 ISO and developed in a T-Max developer for 10 min at 20°C.

Immunoelectron Microscopy

Calu-3 cells suspended in calcium- and magnesium-free PBS buffer (GIBCO, Renfrewshire, Scotland) were incubated with anti-α6 MAb for 1 h at 4°C or 37°C, followed by antimouse IgG labeled with 15 nm gold particles (GAR G15; Janssen-Amersham, Little Chalfont, U.K.) for 40 min at room temperature. Cells were fixed in a 2% paraformaldehyde, 0.05% glutaraldehyde solution and then incubated with $NaBH_4$ (0.5 mg/ml) for 15 min. Cells were then incubated with MAb MGR4 for 1 h at 4°C for 40 min and, after washing with antimouse IgM reagent, labeled with 5 nm gold particles (GAR G5) for 40 min at room temperature. In control experiments, Calu-3 cells were incubated with anti-α6 or anti-p185^{HER2} MAbs and with both gold-labeled antimouse IgM and IgG MAb. After labeling, pelleted cells were postfixed in 2.5% glutaraldehyde in PBS for 15 min, followed by 1% osmium tetroxide in the same buffer for 60 min, and embedded in Epon.

Radioimmunoprecipitation

Adherent confluent Calu-3 cells were ^{125}I-labeled by membrane lactoperoxidase-catalyzed iodination as described [Tagliabue et al., 1991]. Solubilization was carried out at 0°C for 2–3 min by adding 1.5 ml of lysing buffer (10 mM PIPES, pH 6.8, 300 mM sucrose, 100 mM KCl, 3 mM $MgCl_2$, 10 mM EGTA, 2 mM phenyl methyl sulfonyl fluoride (PMSF), 0.5% Triton X-100). Supernatants were collected and the insoluble structure, which remained on the flask, was incubated at 0°C for 15 min with 20 mM Tris-HCl containing 10 mM NaCl, 3 mM $MgCl_2$, 2 mM PMSF, 1% Tween 80, and 0.5% sodium deoxycholate (DOC). Both lysates were cleared by centrifugation (15 min at 10,000*g*), and the soluble extracts were immunoprecipitated with trichloroacetic acid (TCA). Aliquots of the two precleared extracts containing equal TCA-precipitable radioactivity were incubated with 50 μl of MAbs, bound to protein A–Sepharose CL4B coated with rabbit antimouse IgG heavy and light chains (100 μg/ml) after overnight incubation at 0°C with 500 μl of appropriately diluted MAbs, and immunoprecipitated using established procedures [Tagliabue et al., 1991].

Adherent Calu-3 cells previously treated or untreated with laminin (50 μg/ml) (Sigma) for 5 min at room temperature were incubated with rabbit antiphosphotyrosine serum, solubilized in sample buffer [Laemmli, 1970], and immunoprecipitated as described above. Precipitated proteins were electrophoresed in a 7.5% polyacrylamide gel under reducing conditions, transferred to nitrocellulose, and probed with anti-p185^{HER2} MAb Ab3. Autoradiography was performed after labeling the antigen-antibody complex with 250,000 cpm/ml of ^{125}I-labeled antimouse Ig (Amersham International, Buckinghamshire, England).

Immunoblotting

Calu-3 cells in suspension were incubated with anti-6 integrin subunit and anti-EGF receptor MAbs (20 ng/ml) for 30 min at room temperature. Cells were lysed in sample buffer (4% SDS, 18% glycerol, 50 ng/ml leupeptin, 2 mM PMSF, 2 mM orthovanadate), and protein content of each sample was determined using a bicinchoninic acid (BCA) protein assay.

Samples (15 and 150 ng of each) were electrophoresed on a 7.5% polyacrylamide gel under reducing conditions, and proteins were electrophoretically transferred to nitrocellulose; the p185^{HER2} and the phosphotyrosine-containing protein were detected using MAb Ab3 (3 ng/ml) and rabbit antiphosphotyrosine antibodies (5 ng/ml) (UBI, Lake Placid, NY), respectively.

Antigen-antibody complexes were incubated with 250,000 cpm/ml of ^{125}I-labeled antimouse Ig or ^{125}I-protein A (Amersham) and autoradiographed.

RESULTS

Integrin Expression Pattern of Calu-3 Cells

Figure 1 shows the results of cytofluorometric analysis on suspended Calu-3 cells using MAbs directed against α and β subunits of integrins involved in cell-matrix adhesion; α2, α3 and α6, as well as β1 and β4 subunits were expressed by these cells, whereas α4 and α5 were either absent or expressed at low levels.

Localization of p185^{HER2} on Calu-3 Cells Grown as Monolayers

Binding experiments with ^{125}I-labeled MAb MGR9, directed against the extracellular domain of p185^{HER2}, were performed on a mono-

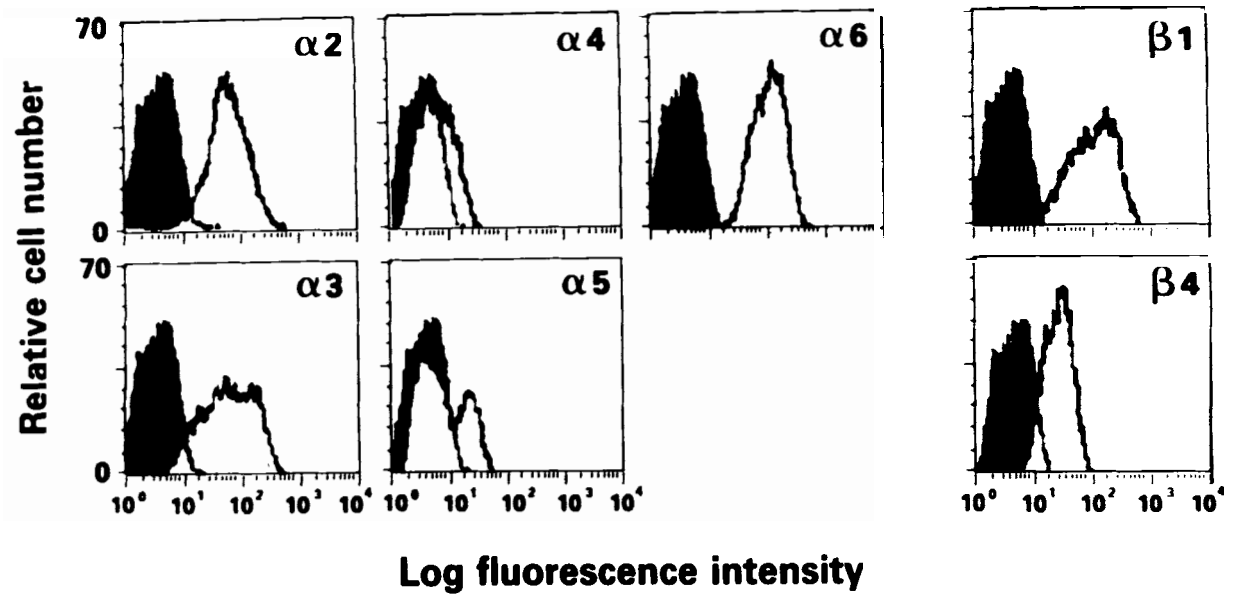

Fig. 1. Fluorescence profiles of antiintegrin MAbs on Calu-3 cells. Cells were treated with different MAbs direct against α and β integrin subunits. Solid histogram in each panel shows control values.

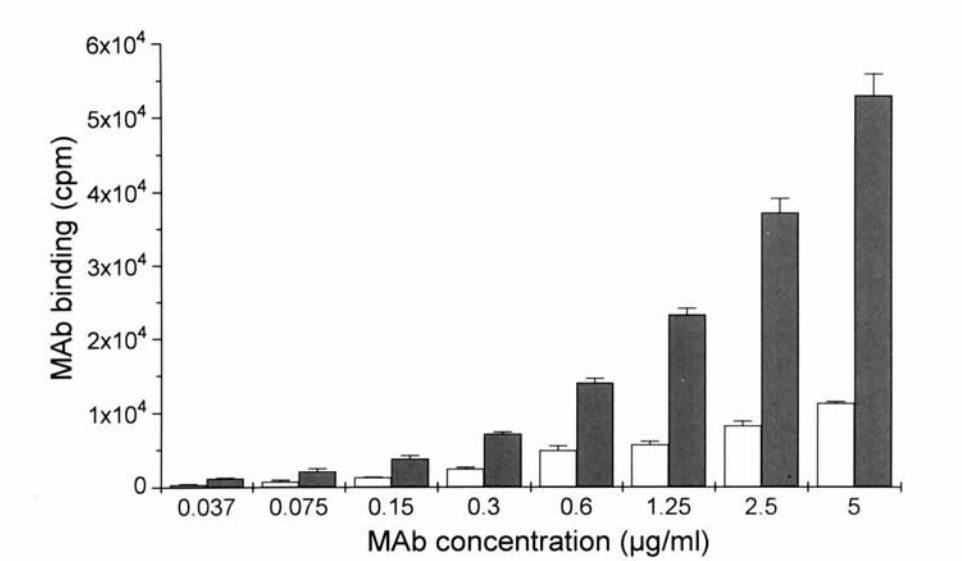

Fig. 2. Binding of ^{125}I-labeled MGR9 on living and permeabilized Calu-3 adherent cells. Cells were permeabilized (shaded bar) or not (white bar) in HEPES–Triton X-100 buffer and incubated with the MAb at various dilutions.

layer of Calu-3 cells. Tests were performed on living and permeabilized cells in order to allow access of the antibody to the cytoplasm and to the basal and lateral surface. As shown in Figure 2, MAb binding was significantly higher in permeabilized cells than in untreated cells, suggesting that most of the p185^{HER2} receptor is located on the basolateral membrane. As a control for specificity, the binding test was carried out using 3 MAbs directed against extracellular domain epitopes (MGR9, anti-β4 MAb, anti-HLA MAb) and an antiintracellular domain of the p185^{HER2} MAb. As shown in Figure 3C, the intracellular domain of p185^{HER2} was detected only in permeabilized cells. Reactivity of the anti-β4 and MGR9 MAbs, even though directed against extracellular domain sites, was also detected mainly on permeabilized cells (Fig. 3B,D). By contrast, HLA was detected on both living and permeabilized cells (Fig. 3A).

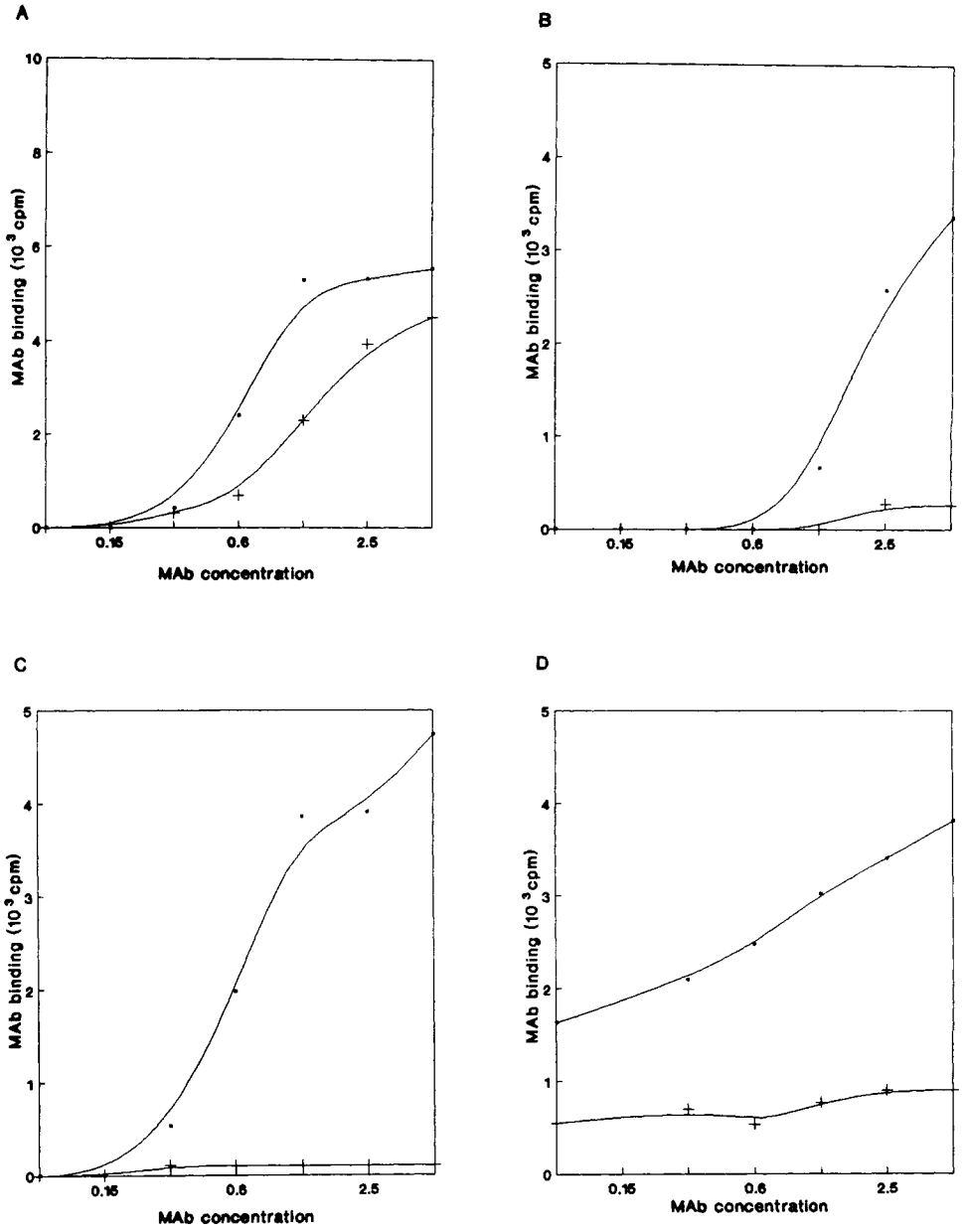

Fig. 3. Indirect binding assay with anti-β4, anti-HLA, and two anti-p185^{HER2} MAbs on Calu-3 cells. Cells permeabilized (--) or not (-+-) with HEPES–Triton X-100 buffer were incubated with antibodies directed to HLA (A), β4 (B), p185^{HER2} intracellular (C), or extracellular domain (D).

Polarization of p185^{HER2} and Integrins on Clumps of Calu-3 Cells

Calu-3 cells, grown in the peritoneal cavity of nude mice, formed clumps that could not be disaggregated by pipetting. Immunohistochemistry performed on these cells, fixed on slides, with MGR9 MAb, indicated that p185^{HER2} was clustered in those areas of the plasma membrane involved in cell-matrix and cell-cell contact (Fig. 4a), as previously observed on monolayers of Calu-3 cells. The same pattern of localization was found with the MAbs directed against the α6 and β4 integrin subunits, as well as with the polyclonal antibodies detecting laminin and phosphotyrosine moieties. By contrast, antiintegrin MAbs directed against the β1 (Fig. 4b), α2, and α3 subunits, as well as the control anti-HLA MAb, displayed homogeneous staining throughout the membrane of the same cells (Table I). This pattern was also found on

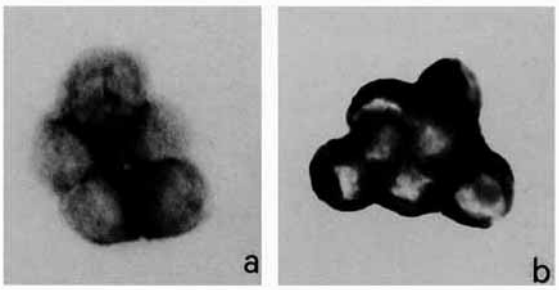

Fig. 4. Localization of p185^{HER2} on Calu-3 cell aggregates grown in mice. Cells were fixed on slides and permeabilized, incubated with 10 μg/ml of anti-p185 MGR9 MAb (**a**) or anti-β1 MAb (**b**), and the reaction developed with immunoperoxidase.

TABLE I. Expression and Distribution of Integrin Subunits and p185^{HER2} Oncoprotein in Calu-3 Cells

Analyzed molecule	Expression evaluated by I.F.	Distribution
α2	+	Uniform on cell membrane
α3	+	Uniform on cell membrane
α6	+	Polarized in cell-cell contacts
β1	+	Uniform on cell membrane
β4	+	Polarized in cell-cell contacts
p185^{HER2}	+	Polarized in cell-cell contacts
HLA	+	Uniform on cell membrane
Laminin	+	Polarized in cell-cell contacts
Phospho-tyrosine	+	Polarized in cell-cell contacts

perpendicular cryosections of acetate filters containing cells grown to near confluence (not shown).

Double fluorochrome immunofluorescence assay to simultaneously detect β4 and p185^{HER2} on Calu-3 cell clusters cultured on coverslips showed that these two proteins were colocalized on cellular adhesion domains (Fig. 5). A MAb directed against α6 gave a nearly identical staining pattern (not shown), suggesting that the heterodimer α6β4 coincides topographically with p185^{HER2}. Laminin and phosphotyrosine-containing molecules also appeared to codistribute with p185^{HER2}, consistent with the reactivity patterns obtained from cells grown in the peritoneal cavity of nude mice.

Solubilization of p185^{HER2} From the Membrane

To determine whether p185^{HER2}, like the integrins, is associated with cytoskeletal molecules, cells were solubilized with different detergents (ionic and nonionic) that differ in their ability to extract membrane proteins depending on their linkage to the cytoskeleton [Sonnenberg et al., 1991; Elices et al., 1989]. The ionic detergent DOC preferentially extracts cytoskeleton-bound components. Indeed, the two integrin chains α6 and β4 were revealed in DOC-treated but not in Triton X-100–treated cells (cf. Fig. 6B, lanes 7, 8 with A, lanes 2, 3). The band corresponding to the HLA molecule, used as the control for noncytoskeleton bound molecules, was more intense in the nonionic detergent extract (cf. Fig. 6A, lane 5 with B, lane 10). By contrast, p185^{HER2} was extracted by both detergents (Fig. 6A, lane 4 and B, lane 9), although the band was more intense in the DOC-extracted material. This suggests a partial or indirect linkage of the p185^{HER2} with the cytoskeleton.

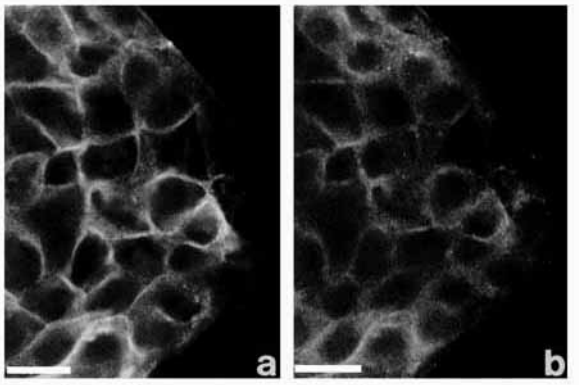

Fig. 5. Double immunofluorescence photograph showing the colocalization of β4 (**a**) and p185^{HER2} (**b**) in a cluster of fixed and permeabilized Calu-3 cells. Bars denote 10 μm.

Coclustering of the p185^{HER2} Oncoprotein and the α6β4 Integrin

To further verify the association between p185^{HER2} and the α6β4 integrin molecules, the coclustering of these molecules was investigated by immunoelectron microscopy. Double-immunogold labeling with the anti-p185^{HER2} or anti-α6 MAbs and both gold-labeled anti-IgM and anti-IgG MAbs was performed on suspended Calu-3 to control labeling specificity. As shown in Figure 7A,B, anti-p185^{HER2} was labeled only with 5 nm gold particle and anti-α6 only with 15 nm gold particle. The use of anti-α6 and anti-p185^{HER2} in the same sample revealed clusters consisting of both small and large gold particles (Fig. 7C,D), indicating that the clustering of the integrin mediated by the specific antibody also induced a redistribution of p185^{HER2} which ap-

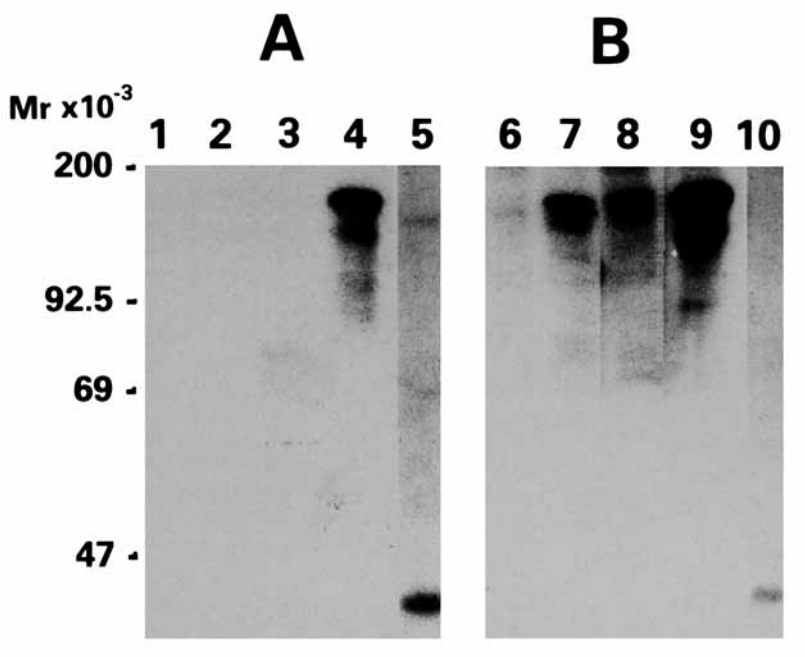

Fig. 6. Autoradiographic analysis of ^{125}I-labeled Calu-3 cell extract after treatment with Triton X-100 nonionic detergent **(A)** or DOC ionic detergent **(B)** and immunoprecipitation with normal serum **(lanes 1,6)**, anti-α6 MAb **(lanes 2,7)**, anti-β4 MAb **(lanes 3,8)**, anti-p185^{HER2} MAb **(lanes 4,9)**, or anti-HLA MAb **(lanes 5,10)**.

peared coclustered with α6β4 integrin receptors.

Functional Interaction Between α6β4 and the Oncoprotein p185^{HER2}

To determine whether coclustering of the oncogene product with α6β4 integrin might induce p185^{HER2} activation, tyrosine phosphorylation of the oncoprotein was analyzed in cells treated with the anti-α6 MAb. As shown in Figure 8, the same amounts of p185^{HER2} were found in the three samples (Fig. 8A) but were associated with different levels of phosphotyrosine (Fig. 8B). Indeed, as compared with control basal levels (lane 1), p185^{HER2} phosphorylation was higher after treatment with the anti-α6 MAb (lane 3), whereas no changes were observed after treatment with the control anti-EGF receptor MAb (lane 2).

Since laminin is a potential ligand of the α6β4 integrin subunit, we investigated whether laminin itself could induce the tyrosine phosphorylation of the oncoprotein in Calu-3 cells. Protein was immunoprecipitated using antiphosphotyrosine polyclonal antibodies, blotted on nitrocellulose and immunostained with the Ab3 MAb. As shown in Figure 9, the amount of tyrosine phosphorylation of p185^{HER2} molecules immunoprecipitated from cells pretreated with exogenous laminin (lane 1) was greater than in the untreated cells (lane 2).

DISCUSSION

Clustering or association among integrins, adhesion molecules, and growth factor receptors has recently been reported in cell-cell junctions [Lotz et al., 1990; Sonnenberg et al., 1990]. We now present evidence that the transmembrane growth factor receptor p185^{HER2}, which is highly expressed in Calu-3 cells, is codistributed with the integrin heterodimer α6β4 on the plasma membrane, notably at cell-matrix and cell-cell contacts. Together with the two receptors, laminin produced by Calu-3 cells is also present, whereas other molecules such as very late antigen (VLA)-2 and VLA3 receptors have a different distribution on the plasma membrane. Consistent with previous data [Tsukita et al., 1991], phosphotyrosine was found to be mostly polarized at the cell-cell contact. The observation that the oncoprotein was more easily extracted by detergents generally used for molecules associated with the cytoskeleton supports the notion of a linkage between the p185^{HER2} and adhesion molecules. However, more striking is the observation that the clustering of the integrin induced by an artificial ligand such as the anti-α6 MAb causes the coclustering of the oncoprotein,

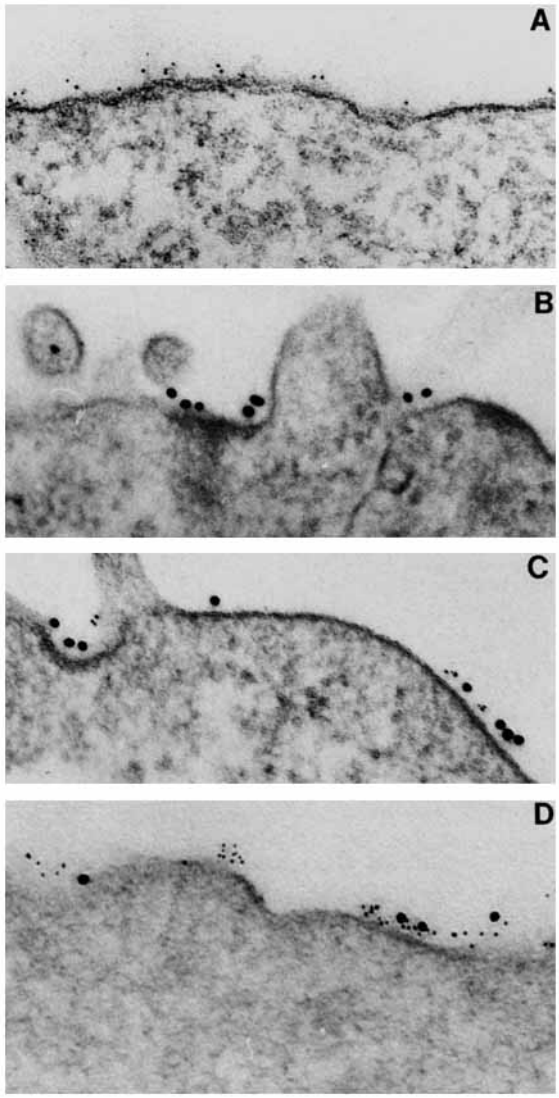

Fig. 7. Immunoelectron micrograph of Calu-3 cells double-labeled with anti-p185 and anti-α6 MAbs. Double-labeling with anti-IgM (5 nm gold particles) and anti-IgG (15 nm gold particles) MAbs of cells treated with anti-p185^{HER2} MAb (**A**), anti-α6 MAb (**B**), anti-α6 (37°C) and anti-p185^{HER2} MAbs (**C**), and anti-α6 (4°C) and anti-p185^{HER2} MAbs (**D**). ×84,000.

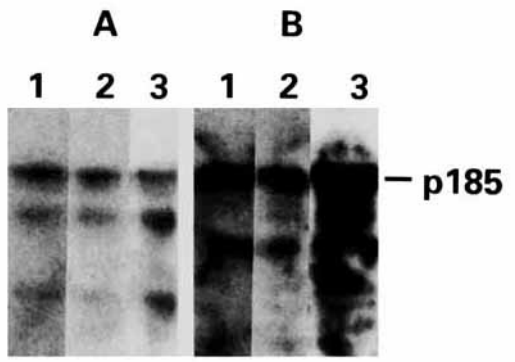

Fig. 8. Tyrosine phosphorylation of p185^{HER2} in Calu-3 cells treated with anti-α6 MAb. Samples were evaluated for the amount of p185^{HER2} (**A**) and for the corresponding level of tyrosine phosphorylation of p185^{HER2} (**B**) after treatment with anti-α6 MAb (**lanes 3**), treatment with anti-EGF receptor MAb (**lanes 2**), and no treatment (**lane 1**).

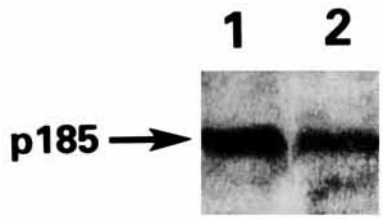

Fig. 9. Tyrosine phosphorylation of p185^{HER2} as a function of laminin treatment. Calu-3 cells, treated (**lane 1**) or not (**lane 2**) with soluble laminin, were solubilized and immunoprecipitated using antiphosphotyrosine polyclonal antibody. Proteins resolved by gel electrophoresis and transferred to nitrocellulose were probed using the anti-p185^{HER2} MAb.

as shown by immunoelectron microscopy and by analysis of its tyrosine phosphorylation. The simple dimerization of receptors with tyrosine kinase activity has been shown to be sufficient to induce tyrosine phosphorylation. Clustering of the p185^{HER2} can be mediated by ligand [Rommerskirch and von Figura, 1992; Peles et al., 1992, 1993], by bivalent antibodies [Hinds and Levitt, 1992], or, as shown here, by integrin capping or polarization.

Our experiments using laminin suggest that this phenomenon may also occur in physiological conditions, since laminin was also able to increase basal levels of p185^{HER2} phosphorylation, although we cannot yet exclude the effect of possible contaminants in our laminin preparation. Confirmation that laminin specifically activates p185^{HER2} would, however, suggest at least two hypotheses: if laminin were a ligand for α6β4, a possibility that has been suggested [Lotz et al., 1990; Lee et al., 1992] but is still controversial [Sonnenberg et al., 1990], then clustering of the integrin complex may occur, as with the artificial ligand, and lead to the same increase in oncoprotein phosphorylation. If laminin is not a ligand for α6β4, the latter might interact with a complex of basement membrane molecules and indirectly induce integrin clustering, followed by upregulation of p185^{HER2} phosphorylation.

Recently, it has been suggested that integrins are involved in transducing messages involved in the control of gene expression [Werb et al., 1989; Sporn et al., 1990] and ionic pumps [Ingber et al., 1990], although the specific role of the

integrin in signal transduction awaits clarification. Integrins may act by clustering and trapping other molecules within the plane of the plasma membrane. Indeed, upregulation of membrane protein tyrosine phosphorylation by integrin β1 clustering has been reported [Kornberg et al., 1991]. Similarly, the α6β4 integrin might induce signal transduction through phosphorylation of the colocalized p185^{HER2} receptor, although other mechanisms that might be regulated during integrin clustering, such as the involvement of phosphatases, cannot be excluded.

The relevance of the association between p185^{HER2} and adhesion receptors must still be established. Laminin and other adhesion molecules produced by carcinomas have been suggested to be associated with the metastatic potential and aggressiveness of tumors [Liotta, 1989]. It has been suggested that p185^{HER2} may also be involved in the metastatic process. In particular, the oncoprotein was found in a plasma membrane protrusion necessary for cell motility [De Potter and Quatacker, 1993], which is an essential step in the process of tumor invasiveness and metastasis. In this work, we suggest that integrins, in addition to their role in tumor cell migration, are also involved in tyrosine kinase activation of growth factor receptor. This finding, together with the involvement of p185^{HER2} and adhesion molecules in tumor metastasis, could explain the aggressiveness of p185^{HER2}-overexpressing tumors.

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