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CD24 induces localization of β1 integrin to lipid raft domains

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Abstract

The expression of the glycosyl phosphatidylinositol (GPI)-anchored protein CD24 correlates with poor prognosis in a variety of carcinomas. However, little is known about the cellular mechanisms of the CD24-mediated effects. In this study, we present evidence that CD24 affects the lateral localization of β1 integrin. Using stably CD24-transfected A125 and MDA-MB-435S carcinoma cells we show that CD24 augments β1-dependent cell motility and stimulates transmigration and invasion across a monolayer of endothelial cells. Furthermore, as demonstrated by sucrose density gradient centrifugation and Western Blot analysis, CD24 recruits β1 integrin into lipid raft domains. We suggest that CD24 acts as a gate-keeper for lipid rafts, thereby regulating the activity of integrins and other proteins.

Keywords: GPI-anchor; CD24; Cell invasion; Integrins; Invasion; Motility; Membrane rafts; Lipid rafts; Transmigration

CD24 is a highly glycosylated protein that is linked to the membrane via a glycosylphosphatidylinositol (GPI)-anchor. Various studies identified CD24 as a marker for poor prognosis in several tumors, among them were carcinomas of the ovary [1], breast [2], and pancreas [3] as well as non-small cell lung cancer [4], colorectal cancer [5] and diffuse-type gastric adenocarcinoma [6]. However, little is known about the cellular mechanism involved in activity of CD24. In B-cells, antibody-mediated cross-linking of CD24 induced apoptosis in a process involving the B-cell receptor and MAP kinases [7,8]. Due to the missing cytoplasmic domain, the signal transduction capacities of CD24 have remained rather elusive. Due to its localization in detergent-resistant membrane domains (DRM; lipid rafts), CD24 interacts with members of the family of src-kinases [9–11]. Furthermore, cross-linking of CD24 on tonsil B-cells and mononuclear cells obtained from B cell chronic lymphatic leukemia patients led to an increase of free cytoplasmic calcium, while it triggered hydrogen peroxide production in granulocytes [12]. As ligands of CD24, P-selectin [13,14] and the cell adhesion molecule L1-CAM [10] have been established. Whereas the binding to P-selectin supports the interaction of CD24-expressing tumor cells with activated platelets or endothelial cells [13], its association with L1-CAM has regulatory functions in neurite outgrowth [15]. Furthermore, a recent study demonstrated that expression of CD24 in a rat carcinoma system stimulated cell adhesion, migration, and proliferation [16].

In the present study, using stably transfected carcinoma cell lines A125 and MDA-MB-435S, we investigated the influence of CD24 in collagen-dependent transmigration and invasion across a monolayer of endothelial cells. While expression of CD24 stimulates both transmigration and invasion, inhibition of β1 integrin decreases the motility of CD24-transfectants. Furthermore, we provide evidence that CD24 recruits β1 integrin to lipid raft domains. In combination with our previous findings that CD24 can downregulate the activity of the chemokine receptor CXCR4 by expelling it from lipid rafts [17], the present

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results support the notion that CD24 acts as a ''gate-keeper'' for lipid raft domains, and thereby can influence the function and activity of other proteins.

Materials and methods

Chemicals and antibodies. The following antibodies were used: Monoclonal antibody (mAb) SWA-11 to human CD24 [18]. The antibodies against the integrin subunits β1, α2, α3, α4, and α5 were obtained from BD Transduction (Heidelberg, Germany), the mAb specific for β-actin was from Sigma (Taufkirchen, Germany). For detection of Caveolin-1 a pAb was used (Santa Cruz Biotechnologies). Inhibition of β1 integrin was performed using mAb TDM29 (Chemicon, Hofheim, Germany).

Cells. The lung adenoarcinoma cell line A125 and the breast carcinoma cell line MDA-MB-435S were stably transfected with a CD24 expression plasmid using JetPEI (Biomol, Hamburg, Germany) or calcium-phosphate, respectively. Transfectants were enriched by G418 selection and cell sorting by FACS and MACS using mAb SWA-11 against CD24. A125 cells were maintained in RPMI 1640 containing 10% fetal calf serum (FCS), MDA-MB-435S cells in Dulbecco's modified Eagle's medium supplemented with 10% FCS.

3D Collagen assay. A 3D transendothelial collagen invasion assay was used to study transmigration and invasion of tumor cells [19]. Collagen R (Serva, Heidelberg, Germany) and collagen G (Biochrom, Berlin, Germany) were mixed at a ratio 1/1, 25 mM sodium bicarbonate and 0.1 vol% of 10× DMEM was added. Polymerisation started after the solution was neutralized with 1 N sodium hydroxide and incubated at 37 °C, 95% humidity and 5% CO_2. Polymerized collagen gels were normally 500 μm thick. DMEM in gels were replaced with Endothelial Cell Growth Medium 2 after an incubation period of 2 h, and subsequently 6×10^5 HUVEC were seeded. After 24 h endothelial cells formed a closed monolayer and 100,000 tumor cells were seeded on top of the endothelium. To study invasion in the absence of endothelial cells, tumor cells were added directly to collagen gels. Before seeding, tumor cells were stained with carboxyfluorescein diacetate and DNA-intercalating dye Hoechst 33342 to distinguish them from endothelial cells. All assays were performed with Endothelial Cell Growth Medium 2. Coculture and monoculture took place for 72 h and were stopped by fixation with 2.5% glutaraldehyde solution (in PBS). Cell invasion was then analyzed by counting the invaded cells in each Z-position per 12 fields of view (40× magnification). We quantified invasiveness of each tumor cell line as average invasion depths (in mm) times number of invasive tumor cells per mm^2 field of view (invasion score in 1/mm).

Endothelial cell isolation and cell culture. Human endothelial cells were isolated from the vein of human umbilical cords (HUVEC) [20]. After the vein was washed with 1× PBS buffer, the vein was filled with trypsin/EDTA (0.25%/0.2%) in PBS w/o Ca^{2+} and Mg^{2+} solution and digested for 20 min at 37 °C. Endothelial cells were washed out using 1× PBS buffer and enzymatic reaction was stopped by addition of 1 vol FCS to the cell solution. HUVEC were maintained in Endothelial Cell Growth Medium 2 (Promocell, Heidelberg, Germany). Tumor cells were cultured in DMEM with 1 g/l D-glucose supplemented with 10% FCS (low endotoxin) and 100 U/ml penicillin/100 μg/ml streptomycin (DMEM complete). All cells were cultured at 37 °C, 95% humidity, and 5% CO_2. Cells were harvested using Accutase (PAA, Linz, Austria) prior to experiments.

Fluorescence-activated cell sorting. Cells were washed, resuspended in cold PBS containing 5% FCS and then incubated with mAbs to different integrin subunits for 30 min followed by washing and incubation for 20 min with PE-conjugated IgG secondary antibodies (Jackson ImmunoResearch). Cells were analysed with FACS Calibur or sorted with FACS Vantage (Becton Dickinson, Heidelberg, Germany). For data analysis, FlowJo software (Ashland, OR) was used.

Haptotactic cell migration assay. For the haptotactic cell migration assay, collagen-1 (10 μg/ml in PBS; Sigma, Taufkirchen, Germany), FN40 (10 μg/ml or as depicted; Gibco-BRL Life Technologies, Eggenstein, Germany). or 1% BSA (w/v in PBS) were coated for 90 min on the backside of Transwell chambers (6.5 mm diameter, 5 μm pore size; Corning, Wiesbaden, Germany). Chambers were placed in 24-well plates containing RPMI1640 medium supplemented with 0.5% BSA (w/v). For collagen-1 migration, 5×10^4 of the A125 transfectants or 1×10^5 of the MDA-MB-435S cells in RPMI1640 medium supplemented with 0.5% BSA (w/v) were seeded into the upper chamber and allowed to migrate to the lower compartment for 4h (A125) or 16 h (MDA-MB-435S) at 37 °C. For β1 integrin blocking, cells were preincubated with 10 μg/ml mAb TDM29 for 30 min at 37 °C prior to the experiment. Migration on FN40 was tested using 1×10^5 cells or cell number as depicted for 16 h. To quantify the transmigrated cells, the inner chamber was removed and the upper side of the membrane cleaned carefully with a cotton swab to remove the non-migrated cells. Migrated cells adherent to the bottom of the membrane were stained with crystal violet solution. The membranes were extensively washed in water and the remaining stain was eluted with 10% acetic acid. The eluted dye was measured at 590 nm in an ELISA reader.

Lipid raft isolation and Western blot analysis. Equal numbers of cells were detached from tissue culture plastic surface by treatment with PBS/5 mM EDTA and lysed in ice-cold lysis buffer (20 mmol/L Tris/HCl, pH 8.0, containing 50 mmol/L β-octylglycopyranoside (BOG) or 1% Triton X-100, 10 mmol/L NaF, 10 mmol/L orthovanadate, 1 mmol/L PMSF, 1 μg/ml of each leupeptin, aprotenin, and pepstatin) for 30 min on ice. The lysates were mixed with an equal volume of 85% sucrose (w/v in TBS), and a step gradient was prepared by overlaying with 35% sucrose (w/v in TBS) followed by a final layer of 5% sucrose (w/v in TBS). The gradient was centrifuged for 20 h at 200,000*g* using a Beckman SW60 rotor. Fractions of 500 μl were collected from the top of the gradient, precipitated with a tenfold volume of acetone and then washed with a fivefold volume of 50% acetone in H_2O. Samples were dried and mixed with non-reducing SDS-sample buffer (30% sucrose, 80 mmol/L Tris/HCl (pH 8.8), 3% SDS, 0.01 mg/ml bromphenol blue). For the analysis of whole cell lysates, the lysates were cleared by centrifugation and boiled with non-reducing SDS-sample buffer. Samples were separated on SDS–PAGE gels and transferred to Immobilon membranes using semi-dry blotting. After blocking with 5% skimmed milk in TBS, membranes were probed with primary antibodies followed by horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibodies and ECL detection (GE-healthcare, Freiburg, Germany).

Statistical analysis. The data were expressed as mean values ± SE, if not indicated otherwise. Statistical analysis was performed using the *t*-test. A $p < 0.05$ was considered to be statistically significant.

Results

Expression of CD24 stimulates cell invasion and transmigration

In order to investigate the involvement of CD24 in cell migration and invasion, the CD24-negative carcinoma cell lines A125 and MDA-MB-435S were stably transfected with CD24. As a control, transfectants with an empty expression vector were established. The CD24 phenotype of the transfectants was assessed by Western blot analysis of whole cell lysates. Only CD24-transfectants showed a positive reaction to the CD24-specific mAb SWA-11. As a control for equal loading, the lysates were tested for β-actin (Fig. 1A).

Recent reports demonstrate that the expression of CD24 stimulates the motility of tumor cells [16]. To evaluate if the motility of stable transfectants was enhanced, invasion assays into a cushion of mixed collagens forming a collagen fiber network with an average pore-size of 0.6 μm were performed. Both in A125 (Fig. 1B) and in MDA-MB-435S cells (Fig. 1C), stable transfection with CD24 increased

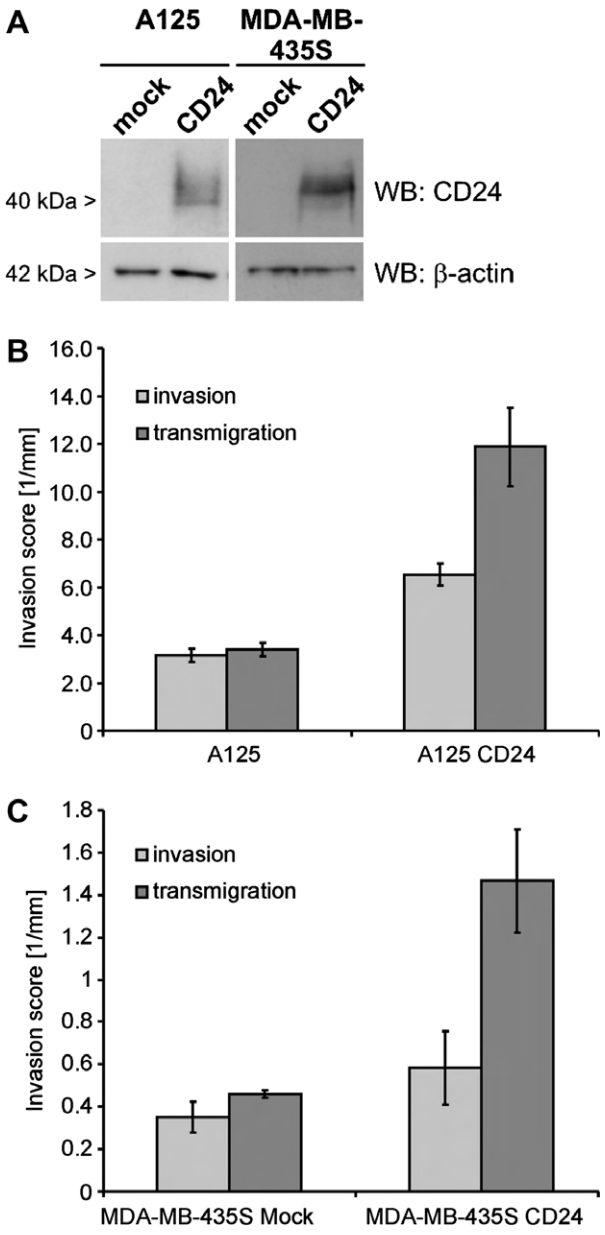

Fig. 1. CD24 stimulates cell invasion and transmigration across an endothelial monolayer. (A) A125 and MDA-MB-435S cells were stably transfected with CD24 or an empty pcDNA3.1 vector (mock). Whole cell lysates were analysed by SDS–polyacrylamide gelelectrophoresis and Western blotting using mAb SWA-11 against CD24. Equal loading was confirmed with a mAb to β-actin. (B and C) Invasiveness of CD24-positive and -negative A125 (B) and MDA-MB-435S (C) cells was analysed in a 3D collagen assay. To evaluate transmigration in dependence of CD24, the collagen matrix was overlaid with a monolayer of endothelial cells. After 72 h of cultivation at 37 °C, the invasion process was stopped by fixation. The invasiveness of each cell line was quantified as average invasion depth multiplied with the number of invaded tumor cells per mm^2 field of view.

the ability of the cells to invade into the collagen cushion. A previous work of our group has established a role for CD24 in the rolling of tumor cells on endothelium and subsequent extravasation [14]. We therefore wanted to know whether the enhanced invasiveness of CD24-transfected cells could also be observed in the presence of a confluent monolayer of endothelial cells overlaying the collagen matrix. Both in A125 and MDA-MB-435S cells, the capability of cells to transmigrate through the endothelial monolayer into the collagen cushion was stimulated in the presence of CD24 (Fig. 1B and C). Taken together, these results confirm an involvement of CD24 in extravasation and tissue invasion of tumor cells.

Antibody-mediated inhibition of β1 integrin reduces motility of CD24-transfectants

As the protein family of integrins plays an important role in cell adhesion and migration processes, we investigated whether integrins are involved in the increased motility of CD24-positive cells. For this, haptotactic transwell migration assays with CD24-positive and -negative A125 cells were performed with collagen-1 as substrate. Fig. 2A confirms the enhanced motility of the CD24-expressing A125 cells. To evaluate the involvement of integrins, cells were pre-incubated with a blocking antibody against the β1 subunit, which is a prominent integrin on tumor cells and forms heterodimers with multiple integrin α-chains. While the inhibition of β1 had no effect on the motility of the CD24-negative control cells, it significantly decreased the migration of the CD24-transfectants.

Binding to collagens is mediated by β1 integrins which have α1, α2, α10, or α11 as dimerization partner [21]. To test whether CD24 also affects β1-mediated cell motility on another substrate, we performed cell migration experiments with FN40 as substrate, which represents a fragment of fibronectin containing the α4β1-specific LDV-motif, but not the RGD-binding site for α5β1 integrin. While both CD24-positive and control cells displayed only minor motility at low substrate concentrations (0.001–0.1 μg/ml), CD24-transfectants migrated significantly more on FN40 at concentrations of 1 or especially 10 μg/ml (Fig. 2B). We could also detect a cell number dependent increase in cell migration. In this case, however, CD24-positive cells showed a higher motility than control cells, irrespective of the cell number (Fig. 2C). This result indicates that the β1 integrin subunit is involved in the CD24-mediated effects on cell migration.

CD24 does not affect the cell surface expression of integrin subunits

CD24 is expressed on a variety of tumors and serves in many cases as an indicator of poor prognosis [1–6]. Similarly, the expression of β1 integrins correlates with decreased patient survival in cancers of breast [22], lung [23], pancreas carcinoma [24], and cutaneous melanoma [25]. In order to analyse, whether CD24 affects the expression of β1 integrins at the cell surface, the presence the β1 integrin subunit and some of its α-subunit binding partners was analysed by flow cytometry. Neither in A125 nor in MDA-MB-435S cells a CD24-dependent difference in the avidity of the β1, α2, α3, α4, and α5 subunits at the plasma membrane was observed (Fig. 3). Therefore, CD24 seems

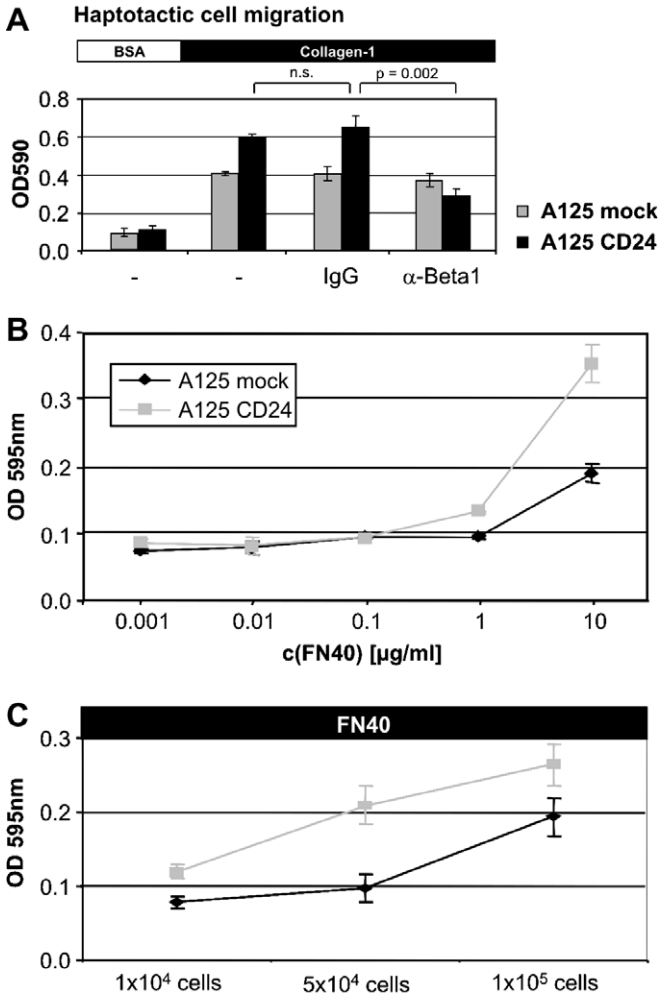

Fig. 2. Inhibition of β1 integrin decreased motility of CD24-transfectants. The motility of CD24-positive and -negative A125 cells was assessed in a transwell migration assay with collagen-1 or the fibronectin fragment FN40 as substrates. (A) To investigate the influence of β1 integrin in cell migration oncollagen-1, cells were incubated with a blocking mAb to β1 integrin (TDM29) for 30 min at 37 °C prior to the onset of the experiment. The cells were seeded into the upper chamber and allowed to migrate for 4 h at 37 °C. (B) Cell migration on FN40 was performed with substrate concentrations as indicated for 16 hours at 37 °C. (C) To test the correlation between cell motility on FN40 and the cell number, the indicated amounts of cells were used for 16 h at 37 °C. (A–C) The migrated cells at the bottom of the membrane were stained with crystal violett solution. The dye was eluted from the filter and measured at 590 nm. The amount of dye is proportional to the number of migrated cells.

to exert an influence on β1 integrin functional activity rather than regulating integrin cell surface expression.

CD24 induces enhanced association of β1 integrin with DRMs

The lateral localization of integrins is of importance for their ligand binding and signalling capacity [26]. We were therefore interested, if CD24 affects the association of β1 integrin with DRMs. Lipid rafts domains of CD24- and control-transfected A125 and MDA-MB-435S cells were isolated by sucrose density gradient centrifugation of Triton X-100 lysates and analysed by Western blot analysis. Fraction 2 of the gradient was identified as lipid raft-fraction using a pAb against Caveolin-1. Furthermore, as reported before, the GPI-anchored protein CD24 completely localized to the DRM fraction 2 (Fig. 4). In order to analyse the distribution of the β1 subunit, the gradients were incubated with a β1-specific mAb. Whereas β1 integrin was exclusively restricted to the non-DRM-fractions 3–9 in control cells, a minor fraction of β1 integrin was associated with lipid raft domains in CD24-transfectants of both cell lines analysed (Fig. 4). This demonstrates that CD24 alters the lipid raft residence of β1 integrin and might thereby influence integrin-mediated cell adhesion, migration and signal transduction.

Discussion

The results presented confirm and extend previous studies on the pro-migratory effects of the GPI-anchored protein CD24 [16]. We show that stable expression of CD24 in the lung adenocarcinoma cell line A125 and in the breast carcinoma cell line MDA-MB-435S stimulates the haptotactic migration on collagen-1. Moreover, CD24-transfectants increased both the invasiveness into a cushion of mixed collagens as well as the transmigration through a monolayer of endothelial cells (Fig. 1B and C). The CD24-mediated cell migration depends on the activity of β1 integrin, as the pretreatment of CD24-positive A125 cells with a blocking antibody against β1 reduces motility, while it has no effect in control cells (Fig. 2).

These findings are in agreement with previous studies concerning the influence of CD24 on integrin function. In mice, the murine homologue of CD24, heat-stable antigen (HSA), regulated the binding of the α4β1 integrin (VLA-4) to VCAM-1. Pre-B-cells isolated from HSA-deficient mice were still able to adhere to activated endothelium. However, this interaction could not be inhibited with antibodies directed against VCAM-1 [26]. Accordingly, these cells were not able to bind to the FN40, a fragment of fibronectin which contains the α4β1-binding motif LDV. Re-expression of HSA rescued the binding capacity of α4β1 to VCAM-1 and FN40 [26]. In a rat carcinoma system, CD24 induced the adhesion to fibronectin, collagen-1, collagen-4, and laminin by stimulating the integrins α3β1 and α4β1 [16].

Integrins are heterodimeric proteins that are involved in cell adhesion and migration processes. The protein family of integrins comprises 18 α- and 8 β-subunits. So far, 24 different α-β-heterodimer-combinations have been identified, that are capable of binding to a large variety of ligands, among them were components of the extracellular matrix as well as binding partners on adjacent cells [27]. The ability of integrins to bind to their corresponding ligand can be regulated by two mechanisms: (i) an increased avidity by enhancing the number of activated integrin heterodimers at the cell surface. This can be

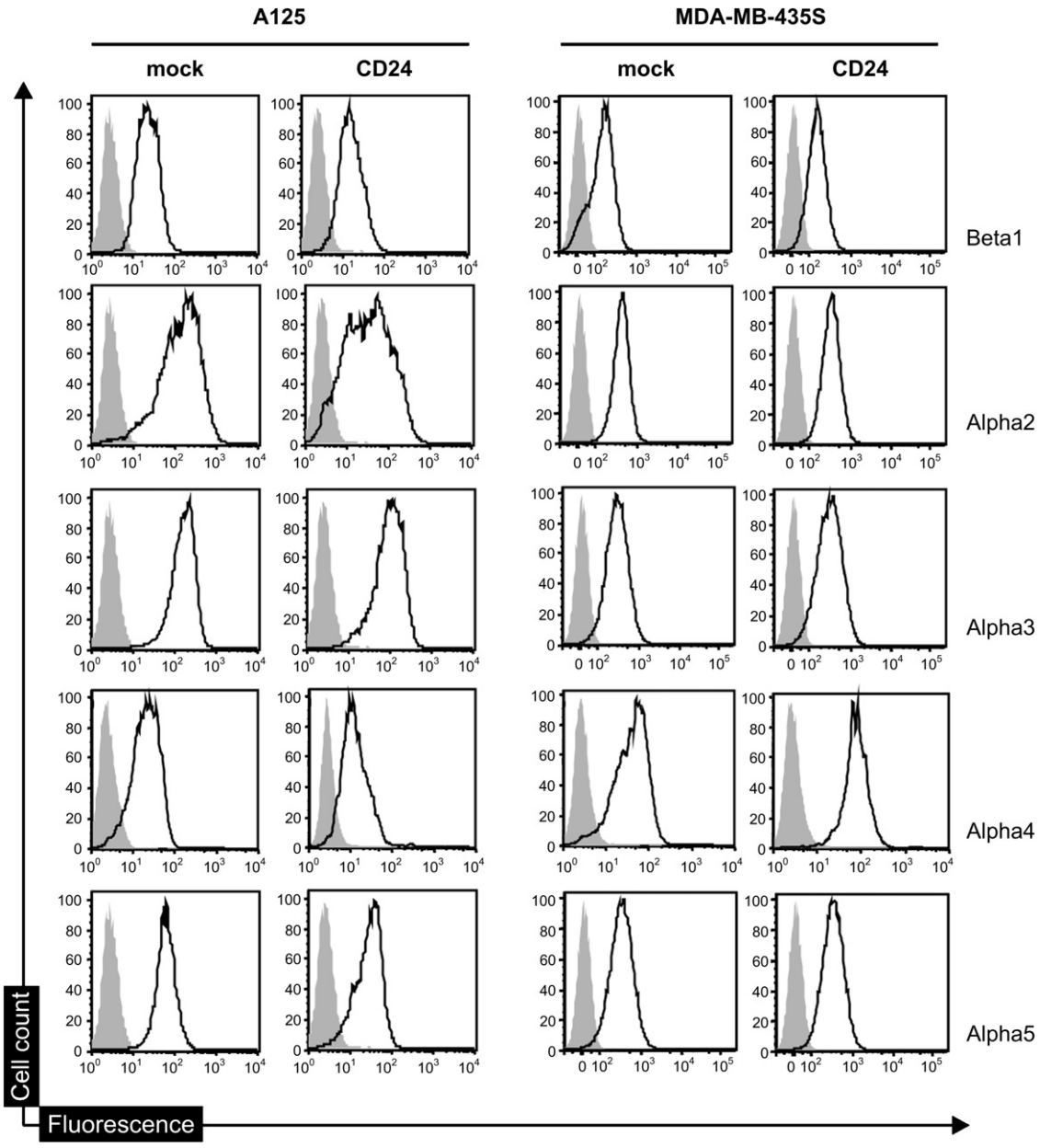

Fig. 3. CD24 has no effect on the cell surface expression of integrins. Flow cytometric analysis of integrin subunits β1, α2, α3, α4, and α5 on the cell surface of A125 and MDA-MB-435S transfectants. Cells were incubated with specific primary antibodies followed by a PE-conjugated anti-mouse IgG antibody. The grey histogram represents the secondary antibody control.

achieved by augmenting the gene expression or recycling of integrins. As confirmed by FACS staining, changes in integrin expression levels were not observed in CD24-transfected cells (Fig. 3). (ii) The binding capacity of integrins can also be elevated by increasing their affinity towards their ligands. This can be influenced by affecting the inside-out-signalling. Furthermore, the association of integrins with lipid rafts is an important factor in the regulation of integrin activation. Previous studies of our group indicated, that αLβ2 integrins localized to DRMs of murine T-cells. Cross-linking of lipid raft components as HSA or the ganglioside GM-1 induced the activation of αLβ2 and increased its binding to ICAM-1 [28]. Moreover, the association of αLβ2 with lipid rafts stimulated the recruitment of α4β1 integrins to these membrane domains [25]. The same study indicated, that the adhesion of thymocytes to ICAM-1 via αLβ2 or to fibronectin via α4β1 and α5β1 depended on the localization of these integrins to DRMs [25]. In a different set of experiments, the integrins α2β1 and α4β1 were transiently recruited to lipid rafts domains of Jurkat T-cells, if these cells were seeded on fibronectin- or collagen-4 [29]. Recent publications described an influence of lipid raft-associated β1 integrin on the adhesion and migration of tumor cells. Binding of MDA-MB-231 cells to fibronectin induced the translocation of β1 integrin to DRMs. Treatment of these cells with the lipid raft-

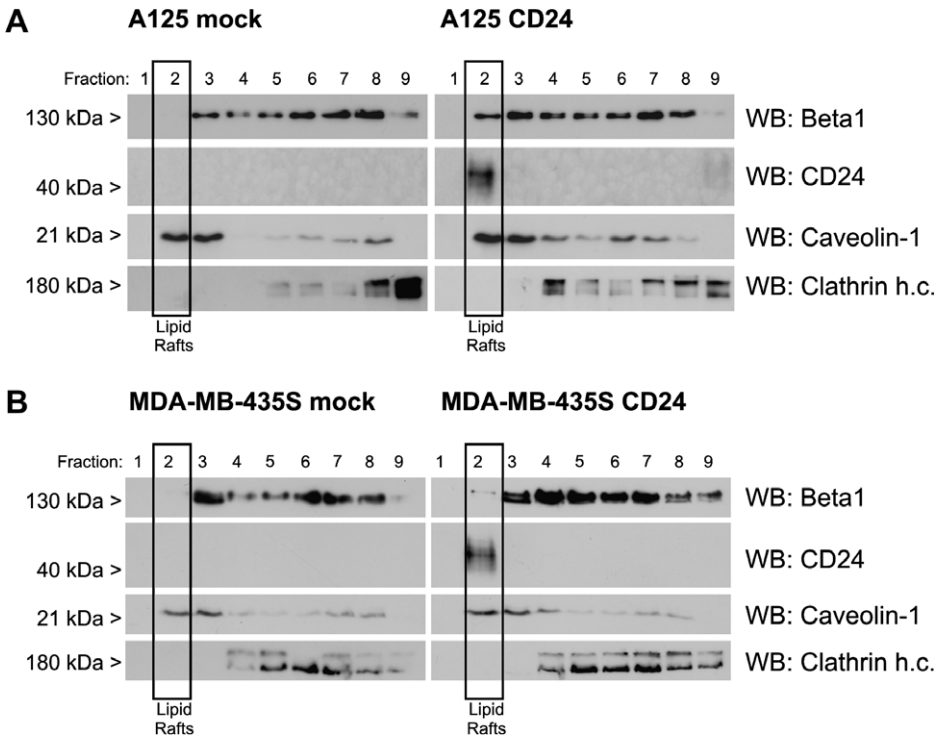

Fig. 4. β1 Integrin is recruited to lipid rafts in CD24-positive cells. To analyse the lateral distribution of β1 integrin in CD24- or control-transfected A125 (A) or MDA-MB-435S (B) cells, DRMs were isolated by sucrose density gradient centrifugation of Triton X-100 lysates. The gradient was harvested from the top. The fractions were precipitated with acetone and analysed by Western blotting with mAbs to β1 and CD24. The lipid raft fraction was identified using a pAb to Caveolin-1.

destructing agent methyl-β-D-cyclodextrin (MCD) inhibited both the translocation of β1 integrin to lipid rafts and the adhesion to fibronectin [30]. As an important regulator of the β1 localization, the bone morphogenetic protein (BMP)-2 was recently identified. Treatment of murine osteoblastic and osteosarcoma cells with recombinant BMP-2 stimulated the haptotactic migration on fibronectin, collagen-1, and laminin. Importantly, BMP-2-treated cells showed an elevated level of DRM-associated β1-integrin [31].

Similarly, the data presented here demonstrate, that, although no β1 integrin localizes to lipid raft domains in CD24-negative cells, transfection of CD24 induces the recruitment of β1 integrin into these cholesterol- and sphingolipid-enriched membrane domains (Fig. 4).

The results presented supports a previous study of our group, in which CD24 regulated the activity of the chemokine receptor CXCR4 by affecting its localization in the plasma membrane of pre-B cells and breast carcinoma cells [17]. In CD24-positive cells, CXCR4 lost its association with lipid rafts that is necessary for effective binding of its ligand SDF-1. This, in turn, resulted in a subsequent inhibition of downstream signalling, cell migration and invasion [17]. Conclusively, while CD24 restricted the lipid raft-localization of CXCR4, we here show that it recruits β1 integrins to these membrane domains. Given that CD24 is a major GPI-anchored and lipid rafts-associated protein in many cell types, it might therefore act as a general "gate-keeper" to lipid rafts.

Taken together, the results presented in this study demonstrate, that CD24 is a stimulator of transmigration and invasion. The CD24-mediated increase in cell migration depends on the β1 integrin subunit. Furthermore, β1 integrin localizes to DRMs in CD24-positive cells, although a correlation between this finding and the elevated cell migration of CD24-transfectants remains to be elucidated. Nevertheless, we suggest that CD24 exerts its functions by regulating the association of different proteins with lipid rafts that act as signalling platforms. Therefore, the effects of CD24 might depend on the function of the proteins whose lateral localization it regulates.

Acknowledgments

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References

[1] G. Kristiansen, C. Denkert, K. Schluns, E. Dahl, C. Pilarsky, S. Hauptmann, CD24 is expressed in ovarian cancer and is a new independent prognostic marker of patient survival, Am. J. Pathol. 161 (2002) 1215–1221.

[2] G. Kristiansen, K.J. Winzer, E. Mayordomo, J. Bellach, K. Schluns, C. Denkert, E. Dahl, C. Pilarsky, P. Altevogt, H. Guski, M. Dietel, CD24 expression is a new prognostic marker in breast cancer, Clin. Cancer Res. 9 (2003) 4906–4913.

[3] J. Jacob, J. Bellach, R. Grutzmann, I. Alldinger, C. Pilarsky, M. Dietel, G. Kristiansen, Expression of CD24 in adenocarcinomas of the pancreas correlates with higher tumor grades, Pancreatology 4 (2004) 454–460.
[4] G. Kristiansen, K. Schluns, Y. Yongwei, C. Denkert, M. Dietel, I. Petersen, CD24 is an independent prognostic marker of survival in nonsmall cell lung cancer patients, Br. J. Cancer 88 (2003) 231–236.
[5] W. Weichert, C. Denkert, M. Burkhardt, T. Gansukh, J. Bellach, P. Altevogt, M. Dietel, G. Kristiansen, Cytoplasmic CD24 expression in colorectal cancer independently correlates with shortened patient survival, Clin. Cancer Res. 11 (2005) 6574–6581.
[6] Y.Y. Chou, Y.M. Jeng, T.T. Lee, F.C. Hu, H.L. Kao, W.C. Lin, P.L. Lai, R.H. Hu, R.H. Yuan, Cytoplasmic CD24 expression is a novel prognostic factor in diffuse-type gastric adenocarcinoma, Ann. Surg. Oncol. 14 (2007) 2748–2758.
[7] T. Suzuki, N. Kiyokawa, T. Taguchi, T. Sekino, Y.U. Katagiri, J. Fujimoto, CD24 induces apoptosis in human B cells via the glycolipid-enriched membrane domains/rafts-mediated signaling system, J. Immunol. 166 (2001) 5567–5577.
[8] T. Taguchi, N. Kiyokawa, K. Mimori, T. Suzuki, T. Sekino, H. Nakajima, M. Saito, Y.U. Katagiri, N. Matsuo, Y. Matsuo, H. Karasuyama, J. Fujimoto, Pre-B cell antigen receptor-mediated signal inhibits CD24-induced apoptosis in human pre-B cells, J. Immunol. 170 (2003) 252–260.
[9] J.A. Zarn, S.M. Zimmermann, M.K. Pass, R. Waibel, R.A. Stahel, Association of CD24 with the kinase c-fgr in a small cell lung cancer cell line and with the kinase lyn in an erythroleukemia cell line, Biochem. Biophys. Res. Commun. 225 (1996) 384–391.
[10] M. Sammar, S. Aigner, P. Altevogt, Heat-stable antigen (mouse CD24) in the brain: dual but distinct interaction with P-selectin and L1, Biochim. Biophys. Acta 1337 (1997) 287–294.
[11] I. Stefanova, V. Horejsi, I.J. Ansotegui, W. Knapp, H. Stockinger, GPI-anchored cell-surface molecules complexed to protein tyrosine kinases, Science 254 (1991) 1016–1019.
[12] G.F. Fischer, O. Majdic, S. Gadd, W. Knapp, Signal transduction lymphocytic in myeloid cells via CD24 a new member of phosphoinositol-anchored membrane molecules, J. Immunol. 144 (1990) 638–641.
[13] S. Aigner, Z.M. Sthoeger, M. Fogel, E. Weber, J. Zarn, M. Ruppert, Y. Zeller, D. Vestweber, R. Stahel, M. Sammar, P. Altevogt, CD24, a mucin-type glycoprotein, is a ligand for P-selectin on human tumor cells, Blood 89 (1997) 3385–3395.
[14] J. Friederichs, Y. Zeller, A. Hafezi-Moghadam, H.J. Grone, K. Ley, P. Altevogt, The CD24/P-selectin binding pathway initiates lung arrest of human A125 adenocarcinoma cells, Cancer Res. 60 (2000) 6714–6722.
[15] R. Kleene, H. Yang, M. Kutsche, M. Schachner, The neural recognition molecule L1 is a sialic acid-binding lectin for CD24, which induces promotion and inhibition of neurite outgrowth, J. Biol. Chem. 276 (2001) 21656–21663.
[16] P. Baumann, N. Cremers, F. Kroese, G. Orend, R. Chiquet-Ehrismann, T. Uede, H. Yagita, J.P. Sleeman, CD24 expression causes the acquisition of multiple cellular properties associated with tumor growth and metastasis, Cancer Res. 65 (2005) 10783–10793.
[17] H. Schabath, S. Runz, S. Joumaa, P. Altevogt, CD24 affects CXCR4 function in pre-B lymphocytes and breast carcinoma cells, J. Cell Sci. 119 (2006) 314–325.
[18] D. Jackson, R. Waibel, E. Weber, J. Bell, R.A. Stahel, CD24, a signal-transducing molecule expressed on human B cells, is a major surface antigen on small cell lung carcinomas, Cancer Res. 52 (1992) 5264–5270.
[19] C.T. Mierke, D.P. Zitterbart, P. Kollmannsberger, C. Raupach, U. Schlötzer-Schrehardt, T.W. Goecke, J. Behrens, B. Fabry, Breakdown of the endothelial barrier function in tumor cell transmigration: CXCR2 mediated regulation of invasiveness, Biophys. J. (in press).
[20] C.T. Mierke, M. Ballmaier, U. Werner, M.P. Manns, K. Welte, S.C. Bischoff, Human endothelial cells regulate survival and proliferation of human mast cells, J. Exp. Med. 192 (2000) 801–811.
[21] J.D. Humphries, A. Byron, M.J. Humphries, Integrin ligands at a glance, J. Cell Sci. 119 (2006) 3901–3903.
[22] E.S. Yao, H. Zhang, Y.Y. Chen, B. Lee, K. Chew, D. Moore, C. Park, Increased beta1 integrin is associated with decreased survival in invasive breast cancer, Cancer Res. 67 (2007) 659–664.
[23] F. Oshita, H. Ito, M. Ikehara, N. Ohgane, N. Hamanaka, H. Nakayama, H. Saito, K. Yamada, K. Noda, A. Mitsuda, Y. Kameda, Prognostic impact of survivin, cyclin D1, integrin beta1, and VEGF in patients with small adenocarcinoma of stage I lung cancer, Am. J. Clin. Oncol. 27 (2004) 425–428.
[24] T.C. Bottger, H. Maschek, M. Lobo, R.G. Gottwohl, W. Brenner, T. Junginger, Prognostic value of immunohistochemical expression of beta-1 integrin in pancreatic carcinoma, Oncology 56 (1999) 308–313.
[25] B. Leitinger, N. Hogg, The involvement of lipid rafts in the regulation of integrin function, J. Cell Sci. 115 (2002) 963–972.
[26] M. Hahne, R.H. Wenger, D. Vestweber, P.J. Nielsen, The heat-stable antigen can alter very late antigen 4-mediated adhesion, J. Exp. Med. 179 (1994) 1391–1395.
[27] R.O. Hynes, Integrins: bidirectional, allosteric signaling machines, Cell 110 (2002) 673–687.
[28] K. Krauss, P. Altevogt, Integrin leukocyte function-associated antigen-1-mediated cell binding can be activated by clustering of membrane rafts, J. Biol. Chem. 274 (1999) 36921–36927.
[29] B.J. Holleran, E. Barbar, M.D. Payet, G. Dupuis, Differential recruitment of alpha2beta1 and alpha4beta1 integrins to lipid rafts in Jurkat T lymphocytes exposed to collagen type IV and fibronectin, J. Leukoc. Biol. 73 (2003) 243–252.
[30] Q. Huang, H.M. Shen, G. Shui, M.R. Wenk, C.N. Ong, Emodin inhibits tumor cell adhesion through disruption of the membrane lipid Raft-associated integrin signaling pathway, Cancer Res. 66 (2006) 5807–5815.
[31] T. Sotobori, T. Ueda, A. Myoui, K. Yoshioka, M. Nakasaki, H. Yoshikawa, K. Itoh, Bone morphogenetic protein-2 promotes the haptotactic migration of murine osteoblastic and osteosarcoma cells by enhancing incorporation of integrin beta1 into lipid rafts, Exp. Cell Res. 312 (2006) 3927–3938.