Activated Epidermal Growth Factor Receptor Induces Integrin α2 Internalization via Caveolae/Raft-dependent Endocytic Pathway

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Elevated expression or activity of the epidermal growth factor (EGF) receptor is common in ovarian cancer and is associated with poor patient prognosis. Our previous studies demonstrated that expression of the constitutively active mutant form of the EGF receptor (EGFRvIII) in ovarian cancer cells led to reduction in integrin α2 surface expression, defects in cell spreading, and disruption of focal adhesions. Inhibition of EGFRvIII catalytic activity reversed the response, suggesting that EGF receptor activation regulates integrin α2. In this study we found that EGF treatment resulted in a transient loss of integrin α2 from the cell surface. Before EGF stimulation, integrin α2 and EGF receptors were associated based on biochemical and immuno-colocalization approaches. After EGF treatment, EGF receptor and integrin α2 were internalized and segregated into different compartments. Integrin α2, but not EGF receptor, was associated with caveolin-1 and GM1 (Gal_1,3GalNAc_1,4(Neu5Ac-_2,3)Gal_1,4Glc_1,1-ceramide) gangliosides, suggesting caveola-mediated endocytosis. Moreover, integrin α2 was subsequently targeted to the Golgi apparatus and the endoplasmic reticulum. Together, these findings demonstrate that activated EGF receptor transiently modulates integrin α2 cell surface expression and stimulates integrin α2 trafficking via caveolae/raft-mediated endocytosis, representing a novel mechanism by which the EGF receptor may regulate integrin-mediated cell behavior.

Epithelial ovarian carcinoma accounts for 80–90% of ovarian tumors and is the leading cause of death from gynecologic malignancy, resulting in 16,210 deaths in 2005 (1). Because of the current inability to detect disease confined to the ovary (stage I), ~75% of women are initially diagnosed with disseminated intra-abdominal disease (stage III–IV) and have a 5-year survival of <20%, whereas patients diagnosed with cancer localized to the ovary have a >90% 5-year survival. Clinically, tumors often involve the ovary and omentum, with diffuse intraperitoneal metastases and malignant ascites. Ovarian cancer metastasis results from numerous intraperitoneal adhesive events, suggesting that carcinoma cell integrins regulate subsequent invasive or metastatic behavior (2–4).

Integrins are the major family of cell surface receptors that mediate attachment to the extracellular matrix, and these integrin-mediated adhesive interactions are intimately involved in the regulation of multiple cellular functions, including tumor cell growth, apoptosis, and metastasis (5–7). After disseminated primary ovarian tumor cells attach to the peritoneal mesothelial monolayer via CD44 (8–10), integrin-mediated cell-matrix interaction potentiates intraperitoneal invasion. Ovarian carcinoma cells extend cytoplasmic processes through the junctional margins of neighboring mesothelial cells, inducing cellular retraction and exposure of the submesothelial extracellular matrix, followed by integrin-mediated adhesion to the newly exposed matrix (11, 12). Analysis of adhesive preferences and integrin expression profiles of established and primary cultures of ovarian carcinoma cells demonstrates high level expression of α2, α3, and β1 subunits and preferential adhesion to interstitial collagen types I and III (13–16) as well as laminins (17) mediated by the α2β1 and α3β1 integrins.

Numerous studies have demonstrated cooperation between integrin and epidermal growth factor (EGF)-mediated signaling pathways in the control of mitogenic, motogenic, and cell survival pathways (17–20). The EGF/ErBb family of receptor tyrosine kinases has been shown to play a key role in normal ovarian follicle development and cell growth regulation of the ovarian surface epithelium (21). Moreover, EGF receptor activation can modulate integrin function by regulating the expression and/or activity of numerous integrins, leading to altered adhesion, motility, and invasive capacity (22–24). Integrin α2 expression is selectively modulated by EGF receptor activation but not β1 integrin in several cell types (22, 25, 26), and the α2 integrin cytoplasmic domain is required for EGF-stimulated migration in NMuMG-3 cells (27, 28). Furthermore, co-localization and direct interaction between integrin α2β1 and the EGF receptor at sites of cell-cell contact has been reported in human epithelial A431 cells (18). Collectively, these observations suggest that aberrant regulation of EGF receptor activity may alter integrin α2 expression and/or function.

The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3.

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2 The abbreviations used are: EGF, epidermal growth factor; EGFR, EGF receptor; wt, wild type; ER, endoplasmic reticulum; PBS, phosphate-buffered saline; TfR, transferrin receptor; DPBS, Dulbecco’s phosphate-buffered saline; PE, phycoerythrin; GM1, Gal_1,3GalNAc_1,4(Neu5Ac-_2,3)Gal_1,4Glc_1,1-ceramide.
Our previous studies demonstrated that expression of a constitutively active mutant form of the EGFR (EGFRvIII) in ovarian cancer cells led to reduction in integrin α2 surface expression, defects in cell spreading, and disruption of focal adhesions. These responses were reversed upon inhibition of EGFRVIII catalytic activity (29). In the course of those studies we observed that integrin α2 and the wild type (wt) EGFR receptor were co-localized when EGFRVIII activity was inhibited; therefore we decided to investigate potential regulation of integrin α2 by ligand-activated EGFR receptor in ovarian tumor cells. Here we report that EGF induces transient internalization of integrin α2, but not integrin β1. In the absence of ligand, integrin α2 interacts with the EGFR receptor based on co-localization, co-immunoprecipitation, and chemical cross-linking studies. Following EGF stimulation, integrin α2 and EGFR receptor interaction was disrupted and they were internalized by distinct pathways. EGFR activation promoted integrin α2 internalization via a caveolea/lipid raft-mediated, rather than the clathrin-dependent, endocytic pathway. Furthermore, internalized integrin α2 was localized to Golgi and endoplasmic reticulum (ER). Based on these findings, we propose that activated EGFR down-regulates surface integrin α2 by a caveolea/raft-mediated endocytic pathway and presents a novel mechanism for EGFR-dependent regulation of integrins.

MATERIALS AND METHODS

Cell Culture and Treatment—Ovarian carcinoma cell line OVCA 433 was generously provided by Dr. Robert Bast Jr., MD Anderson Cancer Center, Houston, TX, and grown as described previously (29). For experiments involving EGF (Biomedical Technologies, Stoughton, MA), OVCA 433 cell lines were placed into minimal essential medium containing 0.1% bovine serum albumin for 24 h prior to growth factor stimulation was then purified and concentrated by ultrafiltration in the sodium meta-periodate. 10 mM Alexa Fluor 488 hydrazide was added to the oxidized GM1 and allowed to react with antigen/Neomarkers (Fremont, CA), and sheep anti-wtEGFR (number 55566), and mouse anti-transferrin receptor (TfR) (number 555534) were purchased from BD Biosciences. The mouse anti-EGFR (number MS311) was obtained from Lab vision/Neomarkers (Fremont, CA), and sheep anti-wtEGFR (number 06–129) was obtained from Upstate USA, Inc. (Charlottesville, VA). Goat anti-clathrin (number C8034) was purchased from Sigma. The rabbit polyclonal anti-caveolin-1 (sc-894), anti-wtEGFR (sc-120), and β-tubulin (sc-9104) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The rabbit anti-58K (ab5820) and anti-GRP94 (ab3674) were obtained from Abcam Inc. (Cambridge, MA). Chicken anti-Rab 7 was generously provided by Dr. Angela Wandinger-Ness, Dept. of Pathology, UNM School of Medicine. The rabbit polyclonal against the intracellular domain of integrin α2 (AB1936), mouse anti-integrin β1 (CBL 481), fluorescein isothiocyanate (AP265F) and Cy3 (AP192C)-conjugated anti-mouse IgG, Cy3 (AP184C)-conjugated anti-sheep IgG and rhodamine (AP182R) and Cy5 (AP182S)-conjugated anti-rabbit and Cy5-conjugated anti-goat (AP180S) antibodies were purchased from Chemicon (Temecula, CA).

Immunofluorescence and Confocal Microscopy—OVCA 433 cells were treated with 25 nM EGF and fixed with freshly prepared 3.7% (w/v) formaldehyde in PBS (137 mmol/liter NaCl, 2.7 mmol/liter KCl, 8.1 mmol/liter Na2HPO4, and 1.5 mmol/liter KH2PO4, pH 7.4) containing 0.8 mmol/liter MgCl2 and 0.18 mmol/liter CaCl2 for 10 min at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 min at room temperature, and blocked with 3% bovine serum albumin/PBS for 1 h at 37 °C. For dual staining, fixed cells were incubated with mouse anti-integrin α2, or rabbit anti-integrin α2 and sheep anti-EGFR, or mouse anti-EGFR and chicken anti-Rab 7, or mouse anti-TfR overnight at 4 °C. After washing three times with PBS, samples were incubated with fluorescein isothiocyanate-conjugated anti-mouse IgG, anti-sheep IgG or anti-rabbit IgG, and the Cy3-conjugated anti-mouse IgG, anti-sheep IgG or anti-chicken IgG. For triple staining, fixed cells were incubated with mouse anti-integrin α2, sheep anti-wt-EGFR, and rabbit anti-caveolin-1 or goat anti-clathrin and then incubated with fluorescein isothiocyanate or Cy3-conjugated anti-mouse IgG or fluorescein isothiocyanate or Cy3-conjugated anti-sheep IgG and Cy5-conjugated anti-rabbit or goat IgG antibodies. For GM1 staining, fixed cells were incubated with mouse anti-integrin α2 or mouse anti-EGFR, and then samples were incubated with Cy3-conjugated anti-mouse IgG. After washing three times with PBS, 150 μM Alexa 488-GM1 was added for 30 min at 37 °C. For Golgi and ER marker staining, OVCA 433 cells were fixed with 70% methanol/30% acetone (−20 °C) for 10 min at room temperature and incubated with rabbit anti-58K or anti-GRP94 and mouse anti-integrin α2 overnight at 4 °C; fluorescein isothiocyanate-conjugated anti-mouse and Cy3-conjugated anti-rabbit IgG were added to sample. Confocal images were acquired at room temperature using a Zeiss LSM510 system equipped with argon and HeNe lasers for excitation at 488 nm (green), 543 nm (red), and 633 nm (blue). Samples were viewed with the 63 × 1.4 oil immersion objective lens.

Cross-linking Assay—Cells were treated as described in the figure legends, washed three times with PBS, and incubated with 2 mM 3,3′ diethiobis (sulfosuccinimidylpropionate) (Pierce) in PBS at 23 °C for 30 min. The cross-linking reaction was quenched with buffer containing 10 μM Tris-HCl, pH 7.5, 0.9%
EGF-stimulated Endocytosis of Integrin α2

A. +EGF (hr) 0 0.25 0.5 2 4 24
Integrin α2

B. +EGF (hr) 0 0.25 0.5 2 4 24
Integrin α2

C. +EGF (hr) 0 0.25 0.5 2 4 24
Integrin α2

FIGURE 1. EGF receptor activation transiently modulates integrin α2 levels. A, integrin α2 expression detection by immunofluorescence microscopy. OVCA 433 cells were treated with EGF (25 nm) for the indicated times in serum-free medium and then fixed in 3.7% formaldehyde and labeled with mouse anti-integrin α2 antibody as described under "Materials and Methods." The surface expression of integrins α2, β1, and EGF receptor were compared in OVCA 433 cells using flow cytometry as described under "Materials and Methods." Data shown are expressed in arbitrary units (a.u.) and represent the mean fluorescence intensity for three independent experiments (mean ± S.D.). #, p < 0.05; *, indicates p < 0.005 compared with respective untreated control. C, OVCA 433 cells were treated with EGF (25 nm) as in panel A. Cell lysates were collected at the indicated times. Total cell protein was fractionated by polyacrylamide gel electrophoresis, and immunoblot analysis was performed to detect integrin α2 (upper panel) or β-tubulin as a loading control (lower panel).

RESULTS

Activated EGF Receptor Initiates Transient Internalization of Integrin α2—We reported previously that integrin α2 was dynamically modulated by activity of the constitutively active EGF receptor mutant EGFRVIII in an ovarian tumor cell line, OVCA 433 (29). To establish whether ligand-stimulated EGF receptor also modulates integrin α2, we examined integrin α2 localization and protein expression after EGF treatment. As shown in Fig. 1A, integrin α2 displayed cell surface localization before EGF treatment, and after addition of ligand, integrin α2 surface staining intensity was decreased and recovered at 4 h of EGF treatment (Fig. 1A). This result is supported by flow cytometry to detect cell surface integrin α2 and integrin β1 levels (Fig. 1B) and immunoblot analysis of whole cell lysates (Fig. 1C). Cell surface integrin α2 expression was decreased by 32% within 30 min and returned to control levels by 24 h (Fig. 1B). This response differed from that of the EGF receptor, which was persistently down-regulated in the presence of ligand (Fig. 1B). A similar decrease and recovery of total integrin α2 protein levels was detected by immunoblot
and chemical cross-linking approaches were used to determine whether the apparent co-localization represented biochemical interaction between the EGF receptor and integrin α2. In OVCA 433 cell lysates, anti-integrin α2 antibodies immunoprecipitated the EGF receptor, and conversely anti-EGF receptor antibodies immunoprecipitated integrin α2 (Fig. 2B) before EGF treatment. This interaction was greatly diminished within 10 min of EGF receptor activation (Fig. 2B), which was consistent with results obtained by confocal microscopy (Fig. 2A). These findings support the conclusion that the EGF receptor and integrin α2 were associated before EGF treatment.

**EGF-stimulated Internalization of EGF Receptor and Integrin α2 through Distinct Pathways**—Several mechanisms of integrin endocytosis have been reported (32–34). The trafficking of integrin αvβ6 and β1 has been reported to take place through an endosomal-mediated endocytic recycling pathway, but integrin αLβ2 was internalized and rapidly recycled upon chemoattractant stimulation via a clathrin-independent, cholesterol-sensitive pathway. Because integrin α2 is initially associated with the EGF receptor (Fig. 2), which is endocytosed primarily via clathrin-dependent mechanisms (35), and αvβ6, β1 integrins have been reported to be internalized via clathrin-dependent mechanisms (33, 34), we investigated whether integrin α2 is internalized by the clathrin endocytic pathway. Several endocytic markers (clathrin, Rab7, Tfr) of clathrin-dependent internalization were used to examine integrin α2 trafficking in response to EGF. Untreated and treated OVCA 433 cells were triple-labeled using antibodies recognizing the EGF receptor, integrin α2, and clathrin. As shown in Fig. 3A, EGF receptor co-localized with clathrin; however, was no detectable co-localization between clathrin and integrin α2. Rab7 protein belongs to a superfamily of small molecular weight GTPases associated with late endosomes. Rab7 regulates the later stages of the endocytic pathway for a number of proteins, including the Tfr, a marker of recycling endosomes that undergoes multiple rounds of clathrin-mediated endocytosis and reemergence at the cell surface (36, 37). Dual staining confocal microscopy was used to detect localization of EGF receptor or integrin α2 with Rab7 (Fig. 3B) or Tfr (Fig. 3C). As expected, there was punctate staining of EGF receptor with Rab7 (Fig. 3B, arrows) or Tfr, but no co-localization of Rab7 (Fig. 3B) or Tfr (Fig. 3C) with integrin α2 was detected. These findings suggest that EGF-stimulated internalization of integrin α2 does not occur through a clathrin-dependent pathway and prompted investigations of alternative mechanisms.

Recent studies have reported that protein kinase C-dependent integrin α2 internalization and C8-LacCer-stimulated β1 integrin internalization occur via caveolar endocytosis (38, 39). Therefore, we investigated the possibility of a caveolar-dependent mechanism for EGF-mediated integrin α2 internalization. Caveolin-1 is a hallmark protein for caveolae and caveosomes, so interactions between caveolin-1 and integrin α2 were investigated using confocal microscopy and immunoprecipitation approaches. In the absence of EGF, caveolin-1 co-localized with integrin α2 and EGF receptor (Fig. 4A, upper panels). Thirty minutes after EGF addition, caveolin-1 co-localized with integrin α2, but not the EGF receptor (Fig. 4B, lower panels). We confirmed

**FIGURE 2. Interaction between integrin α2 and EGF receptor.** A, co-localization of integrin α2 with EGF receptor. OVCA 433 cells were treated with EGF (25 nm) for 30 min or 2 h, fixed in 3.7% formaldehyde for 10 min, permeabilized with 0.1% Triton X-100 for 5 min, labeled with sheep anti-EGF receptor or mouse anti-integrin α2 antibody, and imaged by confocal immunofluorescence microscopy. The merged images present EGF receptor in green and integrin α2 in red. Co-localization of EGF receptor and integrin α2 is shown by yellow before EGF treatment, and after EGF exposure integrin α2 and EGF receptor stain separately. Scale bars, 5 μm. B, co-immunoprecipitation of integrin α2 with EGF receptor. OVCA 433 cells were treated with EGF (25 nm) for 10 or 30 min as indicated in the Fig. 1. After treatment, cells were lysed with Nonidet P-40 buffer and the lysate was immunoprecipitated with anti-EGF receptor (EGFR) or anti-integrin α2. Immunoprecipitates were run on 7.5% SDS-PAGE and immunoblotted with EGF receptor polyclonal antibody (top, left panel) or anti-integrin α2 polyclonal antibody (bottom, left panel). After treatment, OVCA 433 cells were incubated with 3’3’ dithiobis (sulfosuccinimidylpropionate) (DTSSP) for 30 min at room temperature and quenched with buffer containing glycine. Cells were lysed and immunoprecipitations conducted as described above (right panel).
the apparent co-localization of integrin α2 with caveolin-1 through co-immunoprecipitation and chemical cross-linking techniques. Before EGF treatment, caveolin-1 was associated with the EGF receptor and integrin α2 (Fig. 4B). After 10 min of EGF stimulation, caveolin-1 and EGF receptor were no longer associated but interactions between caveo-

**DISCUSSION**

Functional interactions between integrins and receptor tyrosine kinases through reciprocal activation and cooperation in signal transduction have been described (45). Often these interactions occur in an integrin-, receptor-, and cell type-dependent
α2 protein, and the loss of integrin α2 was accompanied by aberrant spreading and focal adhesion formation on type I collagen (29). Inhibition of EGFRvIII catalytic activity restored integrin α2 expression, cell spreading, and assembly of focal adhesions, suggesting that EGF receptor kinase activity regulated integrin α2 functions. In this study we find that ligand-activated EGF receptor causes transient internalization of integrin α2 through a caveolae/raft-mediated mechanism.

There are many examples of integrin trafficking from the cell membrane with evidence for selective recycling of specific integrins and internalization by different mechanisms (46). Clathrin-dependent mechanisms have been described and integrins are involved in viral entry through clathrin-mediated pathways (33, 46, 47). αvβ5 is reportedly recruited to clathrin-coated pits, and integrin β1 and L1 adhesion molecule complexes are internalized by clathrin-dependent endocytosis (46, 48). Despite the observed association between integrin α2 and the EGF receptor before ligand addition, integrin α2 did not gain entry through the clathrin-dependent pathway with the EGF receptor in ovarian tumor cells (Fig. 2).

Clathrin-independent and caveolae-mediated internalization of integrins has been described for certain integrins, including integrin α2β1 (38, 39). Caveolae are cholesterol- and sphingolipid-rich smooth invaginations of the plasma membrane that partition into raft fractions and whose expression is associated with caveolin-1 (49–52). A number of integrins, including αvβ3 and α5β1, associate with caveolin-1, and α2β1 redistribute to caveolae after integrin clustering (38, 46). In studies using human osteosarcoma cells transfected with α2 integrin, integrin α2β1 was internalized into caveosome-like structures but direct interaction with caveolin-1 was not determined (38). We found co-localization and biochemical interaction between caveolin-1 and integrin α2 in resting and EGF-stimulated cells (Fig. 4) that persisted during integrin α2 internalization. Furthermore, integrin α2 internalized with GM1, which has been extensively used as a marker for glyco-

manner. Based on co-immunoprecipitation studies, the EGF receptor forms complexes with integrin α6β4 (24) and integrin α2 (18), suggesting a close linkage between EGF receptor and the functions of these integrins. The integrin α2 cytoplasmic domain is required for EGF-stimulated migration (27, 28), and integrin α2 is reportedly required for serum-independent activation of the EGF receptor at sites of cell:cell contact in A431 cells (18). We reported previously that a constitutively active form of the EGF receptor EGFRvIII down-regulated integrin

FIGURE 4. Integrin α2 interacts with caveolin-1 in OVCA 433 cells. A, OVCA 433 cells, untreated (upper panel) and treated (lower panel) with EGF (25 nM) for 30 min, were fixed in 3.7% formaldehyde, permeabilized with 0.1% Triton X-100, labeled with sheep anti-EGFR, mouse anti-integrin α2, and rabbit anti-caveolin-1 antibody, and imaged by confocal immunofluorescence microscopy. The merged images represent EGF receptor in green, integrin α2 in red, and caveolin-1 in blue. Co-localization of EGF receptor, integrin α2, and caveolin-1 are shown in white, and co-localization between integrin α2 and caveolin is shown in purple. Scale bars, 5 μm. B, EGF receptor, integrin α2, or caveolin-1 were immunoprecipitated from OVCA 433 cell lysates treated without or with EGF for 10 min. Aliquots of the immunoprecipitates were immunoblotted with anti-caveolin-1 (left panels) or anti-EGF receptor (top right panel) or anti-integrin α2 (lower right panel) antibody. Immunoprecipitates were run on 15 or 7.5% SDS-PAGE and immunoblotted with caveolin-1 or EGF receptor, anti-integrin α2 polyclonal antibodies. Nonspecific rabbit IgG was used as a control. C, OVCA 433 cells were incubated with 3,3'-dithiobis (sulfosuccinimidylpropionate) (DTSSP) and quenched with glycine. Then EGF receptor, integrin α2 (top panel), or caveolin-1 (lower panel) were immunoprecipitated from OVCA 433 cell lysates treated without or with EGF for 10 min as described in panel B.
lipid raft domains (40, 41) and is associated with caveolae (53, 54). These findings suggest that integrin α2 is concentrated in caveolar rafts and internalized by a caveolae-mediated endocytic pathway in response to EGF receptor activation.

Caveolae do not appear to be involved in constitutive integrin endocytosis, but this internalization pathway can be activated by a variety of mechanisms (46). Trafficking by caveolae is less well understood than other endocytic pathways, but it is stimulated by a number of viruses binding to certain integrins, including α2β1 or glycosphingolipids (42). Other mechanisms promote caveolar endocytosis, such as C8-LacCer or cholesterol (39), leading to rapid internalization of β1 integrins and protein kinase C-dependent internalization of α2β1 (38). Our studies indicate that EGF receptor activation is an additional mechanism to stimulate caveolar trafficking pathways.

Recent studies (55, 56) have shown that a variety of cell surface receptors, including the EGF receptor, are present in caveolae and lipid rafts. A caveolin binding motif within the kinase domain of the EGF receptor mediates the interaction of EGF receptor with caveolin-1 (56, 57). In human glioblastoma cell lines, EGF receptor rapidly moves out of caveolae domains in response to EGF and then internalizes and degrades via a clathrin-dependent endocytic pathway (56, 58, 59). In our studies we detected co-localization and biochemical association between the EGF receptor, integrin α2, and caveolin-1 without EGF treatment. After EGF binding, interaction between the EGF receptor and integrin α2 or caveolin-1 was no longer detected and EGF receptor was located in clathrin-associated vesicles (Figs. 3 and 4). Although the EGF receptor was degraded (Fig. 2), integrin α2 was trafficked to the Golgi apparatus and ER and surface levels of integrin α2 were restored to near control levels within 4–6 h after EGF treatment. This targeting of integrin α2 is consistent with findings that cholera toxin and SV40 are delivered to the Golgi and endoplasmic reticulum, respectively, following caveolae-mediated internalization (42, 49, 60).

Trafficking is a well recognized mechanism to modulate signaling of receptors such as the EGF receptor (61); similarly, integrin endocytosis is understood to regulate cell function (42, 46). Inhibition of integrin recycling interferes with cell spreading and migration, and integrin internaliza-

![Figure 5](image-url) Localization of EGF receptor and integrin α2 in caveolae-like rafts (GM1-rich domains). OVCA 433 cells, untreated (−EGF, A and B, top panels) and treated (+EGF, A and B, bottom panels) with EGF (25 nM) for 30 min, were fixed in 3.7% formaldehyde for 10 min at room temperature, labeled with (A) mouse anti-EGFR and (B) anti-integrin α2 and Cy3-conjugated anti-mouse antibody, incubated with 150 nM Alexa 488-GM1 for 30 min at 37 °C, and then imaged by confocal immunofluorescence microscopy. The merged images present integrin α2 in red and GM1 in green with co-localization between integrin α2 and GM1 in yellow. Scale bars, 5 μm.

![Figure 6](image-url) Localization of internalized integrin α2 to Golgi and ER. OVCA 433 cells were treated with EGF (25 nM) for 30 min at 37 °C as described in Fig. 5. After fixation with dry cold 70% methanol/30% acetone, the Golgi apparatus was detected using rabbit anti-58K and ER using rabbit anti-GPR94. The merged confocal images represent integrin α2 in green and 58K or GPR94 in red with co-localization between integrin α2 and 58K or GPR94 in yellow. Scale bars, 5 μm.
tion is proposed to play a role in speed and directionality of migrating cells (46). Our findings that ligand-activated EGF receptor results in transient integrin α2 internalization and that constitutively activated EGFRII leads to persistent integrin α2 down-regulation (29) suggest a novel mechanism by which EGF receptor activation may regulate cell behavior and ovarian cancer metastasis.

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