



Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Integrin α_5 interacts with EGFR, is necessary for Fc ϵ RI signaling and is necessary for allergic inflammation in relation with angiogenesis

Youngmi Kim^{a,1}, Kyungjong Kim^{a,1}, Deokbum Park^a, Sangkyung Eom^a, Hyunmi Park^a, Hansoo Lee^b, Yun-Sil Lee^c, Jongseon Choe^d, Jang-Hee Hahn^d, Young-Myeong Kim^d, Jai Youl Ro^e, Dooil Jeoung^{a,*}

^a Department of Biochemistry, College of Natural Sciences, Kangwon National University, Chunchon 200-701, Republic of Korea

^b Department of Biological sciences, College of Natural Sciences, Kangwon National University, Republic of Korea

^c College of Pharmacy, Ewha Womans University, Seoul 120-750, Republic of Korea

^d College of Medicine, Kangwon National University, Chunchon 200-701, Republic of Korea

^e College of Medicine, Sungkyunkwan University, Suwon 440-746, Republic of Korea

ARTICLE INFO

Article history:

Received 21 September 2010

Received in revised form 24 January 2011

Accepted 25 January 2011

Available online xxx

Keywords:

Allergic inflammation

Angiogenesis

EGFR

Fc ϵ RI

Integrin

ABSTRACT

Recent reports have suggested role for epidermal growth factor receptor (EGFR) in asthma and skin inflammation. Integrin(s) are known to be necessary for the transactivation of EGFR. The roles of EGFR and integrin(s) in allergic inflammation were investigated. Antigen stimulation induced activation of EGFR and interaction between EGFR and integrin α_5 in Rat Basophilic Leukemia (RBL2H3) cells and bone marrow-derived mouse mast cells (BMMCs). Flow cytometry revealed increased phosphorylation of EGFR on cell surfaces. Antigen stimulation induced interaction between EGFR and Fc ϵ RI in both RBL2H3 cells and BMMCs. Blocking of EGFR or integrin α exerted negative effects on rac1 activity and secretion of β -hexosaminidase in both RBL2H3 cells and BMMCs. EGFR and integrin α_5 were found to be necessary for IgE-dependent cutaneous anaphylaxis. FAK (focal adhesion kinase), interacted with EGFR and with Fc ϵ RI upon antigen stimulation, and it was necessary for the increased secretion of β -hexosaminidase in both RBL2H3 cells and BMMCs. EGFR and integrin α_5 were necessary for interactions between activated RBL2H3 cells, BMMCs and rat aortic endothelial cells (RAECs). Conditioned medium of antigen-stimulated RBL2H3 cells promoted RAECs tube formation, rat aortic ring formation and blood vessel formation. Conditioned medium of antigen-stimulated BMMCs also had the same effects on RAECs. This enhanced angiogenic potential of RAECs was dependent on EGFR and integrin α_5 . In conclusion, EGFR, via interaction with Fc ϵ RI and integrin α_5 , is necessary for allergic inflammation associated with cellular interaction.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Various intracellular signaling molecules are activated by integrin-mediated cell adhesion (Gilcrease, 2007; Parekh et al., 2000). Chemical inhibition of integrin $\alpha_5\beta_1$ exerts a negative effect on airway inflammation (Okazaki et al., 2009). Integrin α_5 is a key receptor for adhesion of mast cells to the extracellular matrix-component fibronectin (Yasuda et al., 1995). Blocking of integrin α_5 strongly suppresses passive cutaneous anaphylaxis (Ra et al., 1994). Src kinase activity is required for integrin-mediated transactivation of EGFR during mitogenesis (Moro et al., 2002). Integrin $\alpha_3\beta_1$ and integrin $\alpha_6\beta_4$ crosstalk with EGFR (Edick et al., 2007). Integrin α_5 -Fc ϵ Ry clustering triggers the Fc ϵ RI signaling cascade

(Houtman et al., 2001). The above reports suggest possible roles for EGFR and integrin(s) in allergic inflammation. However, the functional roles of integrin(s) in *in vitro* and *in vivo* allergic inflammation have not been studied in detail.

Epidermal growth factor receptor (EGFR) responds to a diverse array of ligands, and plays an important role in various cellular processes, such as proliferation and invasion (Kim et al., 2008a,b). EGFR signaling is necessary for sustaining asthma (McNamara et al., 2004; Hamilton et al., 2003). Adenosine induces mucin expression in asthma through the activation of EGFR (McNamara et al., 2004). EGFR is required for sustaining neutrophil inflammation in asthma (Hamilton et al., 2003). EGFR signaling induces expression of MMP-2 (Gong et al., 2008). Inhibition of MMPs prevents allergen-induced asthma (Kumagai et al., 1999). The above reports suggest potential role for EGFR in *in vitro* and *in vivo* allergic inflammation.

In this study, we found that allergic inflammation involved activation of EGFR as well as interactions between EGFR and Fc ϵ RI and between EGFR and integrin α_5 . EGFR and integrin α_5 were

* Corresponding author. Tel.: +82 33 250 8518; fax: +82 33 242 0459.

E-mail address: jeoungd@kangwon.ac.kr (D. Jeoung).

¹ These authors contributed equally.

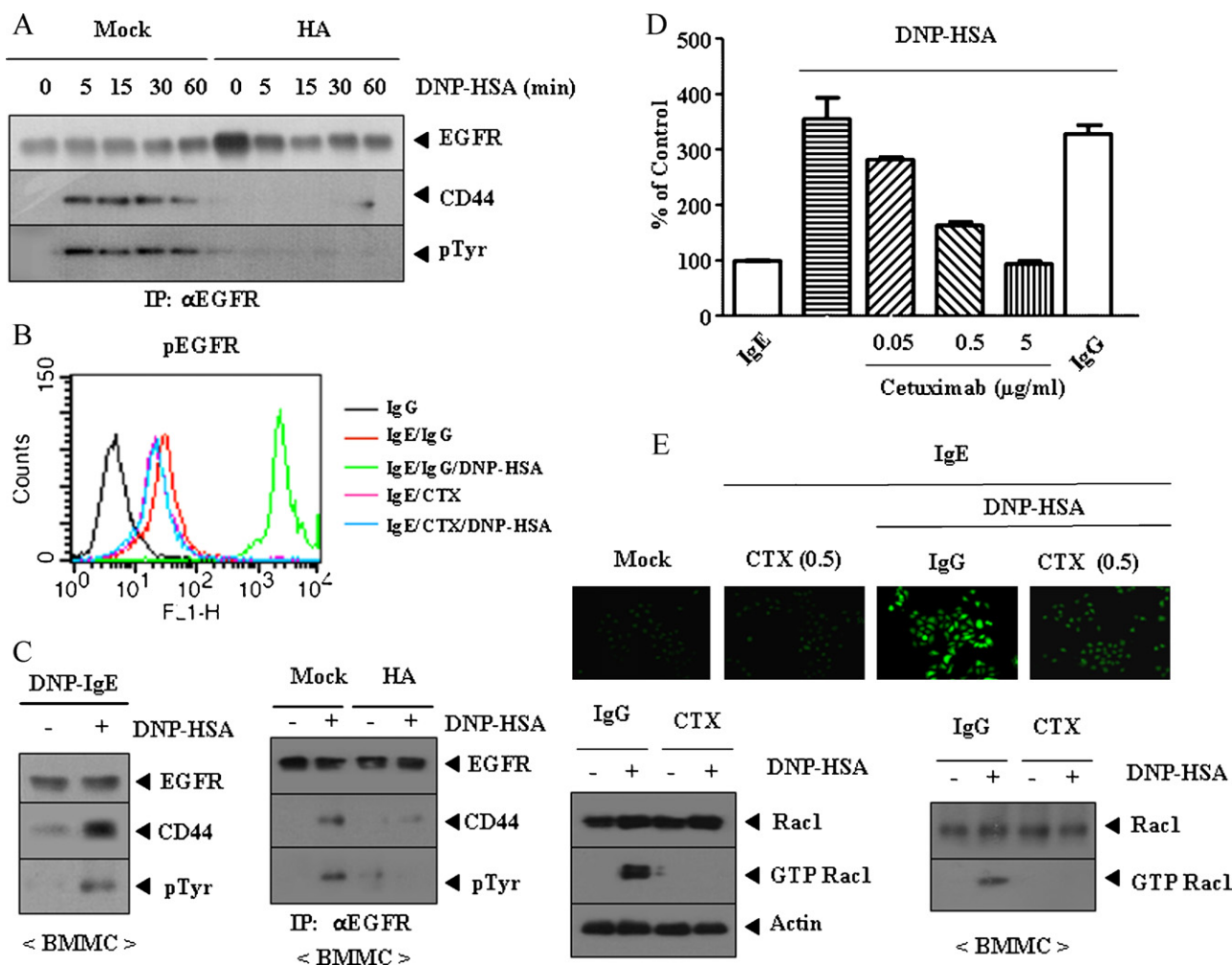


Fig. 1. EGFR is necessary for hallmarks of allergic inflammation. (A) IgE-sensitized RBL2H3 cells were pretreated with or without HA (200 μg/ml) for 30 min, followed by antigen (DNP-HSA) stimulation (100 ng/ml) for various time intervals. Cell lysates prepared at each time point were immunoprecipitated with anti-EGFR antibody (2 μg/ml), followed by Western blot analysis. (B) Flow cytometry for detecting the cell surface expression of p-EGFR was performed as described. CTX (0.5 μg/ml), an EGFR blocking antibody, or isotype-matched control IgG (2 μg/ml) was added to IgE-sensitized RBL2H3, prior to antigen stimulation. (C) IgE-sensitized bone marrow-derived mouse mast cells (BMMCs) were stimulated with DNP-HSA for 30 min. Cell lysates were prepared and subjected to Western blot analysis (left panel). Cell lysates prepared from antigen-stimulated BMMCs pretreated with or without HA for 30 min were immunoprecipitated with anti-EGFR antibody (2 μg/ml), followed by Western blot analysis (right panel). (D) IgE-sensitized RBL2H3 cells were pretreated with or without IgG (2 μg/ml) or various concentrations of CTX for 30 min, followed by stimulation with DNP-HSA for 30 min. Enzyme assays were carried out as described in Section 2. **p* < 0.05. (E) IgE-sensitized RBL2H3 cells were pretreated with or without IgG (2 μg/ml) or CTX (0.5 μg/ml) for 30 min, followed by stimulation with DNP-HSA. Reactive oxygen species level (upper panel) or rac1 activity (lower panel) in RBL2H3 cells was determined as described. The effect of EGFR blocking on rac1 activity in antigen-stimulated BMMCs was also determined as described (lower panel).

necessary for IgE-dependent anaphylaxis and also necessary for interactions between activated basophils, activated mast cells and endothelial cells. Our data reveal the potential value of EGFR as a target for the development of anti-anaphylaxis and provide a novel mechanism of allergic inflammation based on the interaction of EGFR with FcεRI.

2. Materials and methods

2.1. Cell lines and cell culture

RBL2H3 cells were grown in Dulbecco's modified Eagle's medium containing heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (Invitrogen, San Diego, CA). Cultures were maintained in 5% CO₂ at 37 °C. Rat aortic endothelial cells (RAECs) were isolated and maintained as previously reported. Bone marrow-derived mouse mast cells (BMMCs) were isolated according to the standard procedures (Kim et al., 2010a,b).

2.2. Mice

Five week-old female BALB/c mice were purchased from SLC Japan (Shizuoka, Japan) and maintained in SPF condition. All animal experiments were approved by Institutional Review Board for animal studies of Kangwon National University.

2.3. Chemicals and reagents

DNP-HSA and DNP-specific IgE antibody were purchased from Sigma Chemical Company. Chemicals used in this study were purchased from Sigma Chemical Company. Anti mouse and anti rabbit IgG-horse radish peroxidase conjugate antibody were purchased from Pierce Company (Rockford, IL). All other antibodies used in this study were purchased from Santa Cruz Company. Lipofectamin and PlusTM reagent for transfection were purchased from Invitrogen (San Diego, CA). Cytokine ELISA kit was purchased from Koma Biotech (Korea).

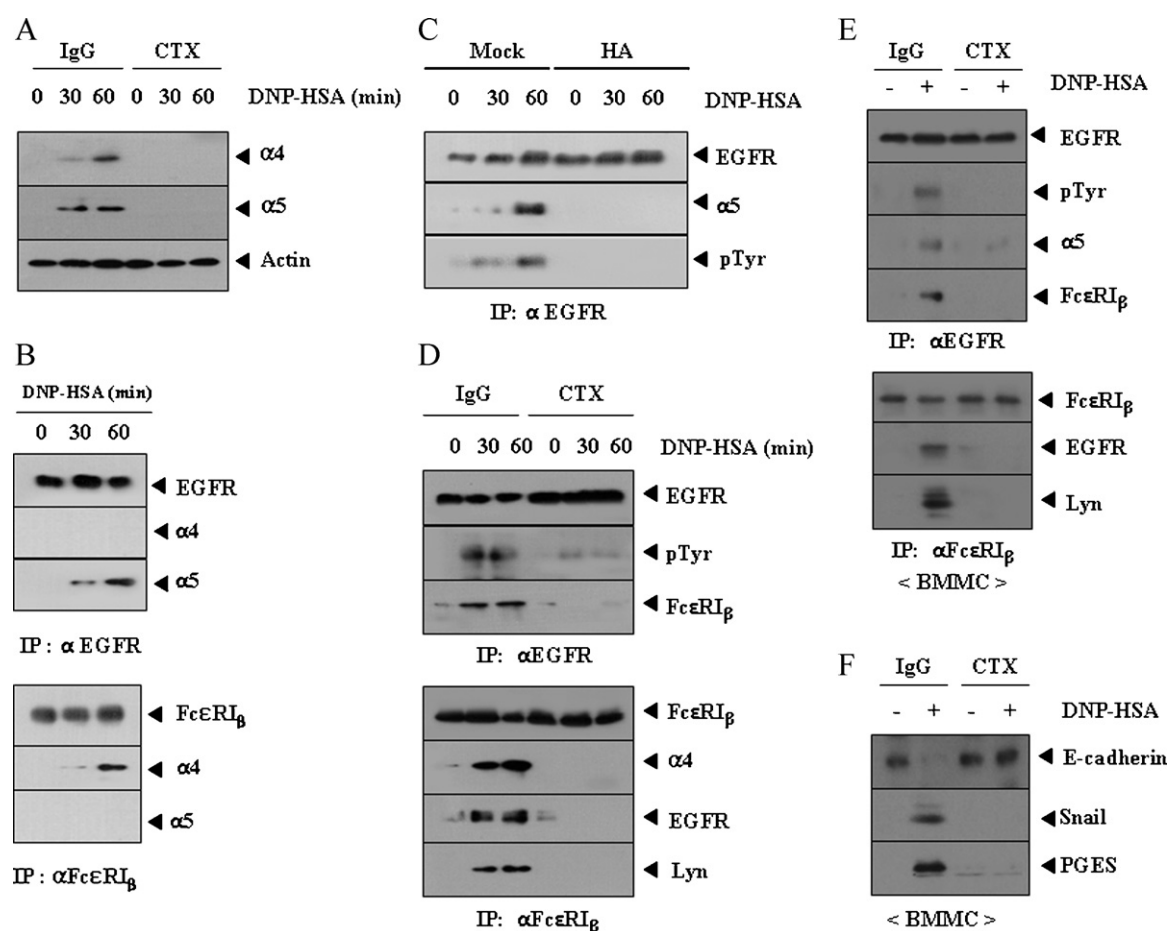


Fig. 2. Antigen stimulation induces interaction between EGFR and integrin α_5 and between Fc ϵ RI β and integrin α_4 . (A) IgE-sensitized RBL2H3 cells were pretreated with IgG (2 μ g/ml) or CTX (0.5 μ g/ml) for 30 min, followed by stimulation with DNP-HSA for various time intervals. Cell lysates prepared at each time point were subjected to Western blot analysis. (B) IgE-sensitized RBL2H3 cells were stimulated with DNP-HSA for various time intervals. Cell lysates prepared at each time point were immunoprecipitated with indicated antibody, followed by Western blot analysis. (C) IgE-sensitized RBL2H3 cells were pretreated with or without HA for 30 min, followed by stimulation with DNP-HSA for various time intervals. Immunoprecipitation was performed with the indicated antibody and was followed by Western blot analysis. (D) IgE-sensitized RBL2H3 cells were pretreated with the indicated antibody, followed by stimulation with DNP-HSA for various time intervals. Cell lysates were immunoprecipitated with the indicated antibody, followed by Western blot analysis. (E) IgE-sensitized BMMCs were pretreated with the indicated antibody (0.5 μ g/ml), followed by stimulation with DNP-HSA for 30 min. Cell lysates were immunoprecipitated with the indicated antibody, followed by Western blot analysis. (F) is the same as (E), except that Western blot analysis was performed.

2.4. β -Hexosaminidase secretion assay

Degranulation was determined by measuring the release of β -hexosaminidase. The assays were carried out according to the standard procedures (Kim et al., 2010a,b). To determine effect of EGFR or integrin α_5 on β -hexosaminidase secretion, IgE-sensitized RBL2H3 cells were pretreated with IgG (2 μ g/ml), 5H10-27 (2 μ g/ml) or CTX (0.5 μ g/ml), an EGFR-blocking antibody, for 15 min. Cells were then stimulated with DNP-HSA (100 ng/ml) for 1 h.

2.5. Flow cytometry

To detect phospho-EGFR on cell surface, the trypsinized RBL2H3 (1×10^6) cells were harvested, washed with PBS containing 5% BSA and then fixed in PBS containing 3% formaldehyde. After removal of fixing solution, cells were incubated with anti-pEGFR antibody (1:100, Santa Cruz) for 1 h. The cells were washed with PBS and incubated with Alexa Fluor 488-conjugated secondary antibody (1:1000, Santa Cruz) for 30 min. After incubation, cells were washed and resuspended in PBS, and then analyzed by flow cytometry (FACS Scan, BD Biosciences).

2.6. Immunohistochemistry

Paraffin-embedded ear tissue sections were immunostained using the Vectastain ABC Elite Kit (Vector Laboratories). Ear tissue sections were deparaffinized with xylene and washed in ethanol. Endogenous peroxidase activity is blocked with 3% hydrogen peroxide in dH₂O for 10 min. Slides were then blocked with 5% normal goat serum in TBS containing 0.1% Tween-20 (TBS-T) for 1 h. For immunohistochemistry, a primary antibody to pEGFR (1:100, Santa Cruz), or integrin α_5 (1:100, Santa Cruz) was added and incubation continued at 4 °C for 24 h. After washing with TBS-T, slides were treated with biotinylated secondary antibody for 30 min. After washing, slides were incubated in the ABC complex for 30 min, and then stained with diaminobenzidine (DAB, Sigma). Sections were counterstained with hematoxylin and finally mounted using Fixogum rubber cement (Mercateo, München, Germany).

2.7. Western blot analysis

Western immunoblot analyses were performed according to standard procedures (Kim et al., 2008a,b). For immunoprecipitation, cell lysates were immunoprecipitated with respective antibody (each at 2 μ g) on ice for 1 h. Protein G-sepharose was then

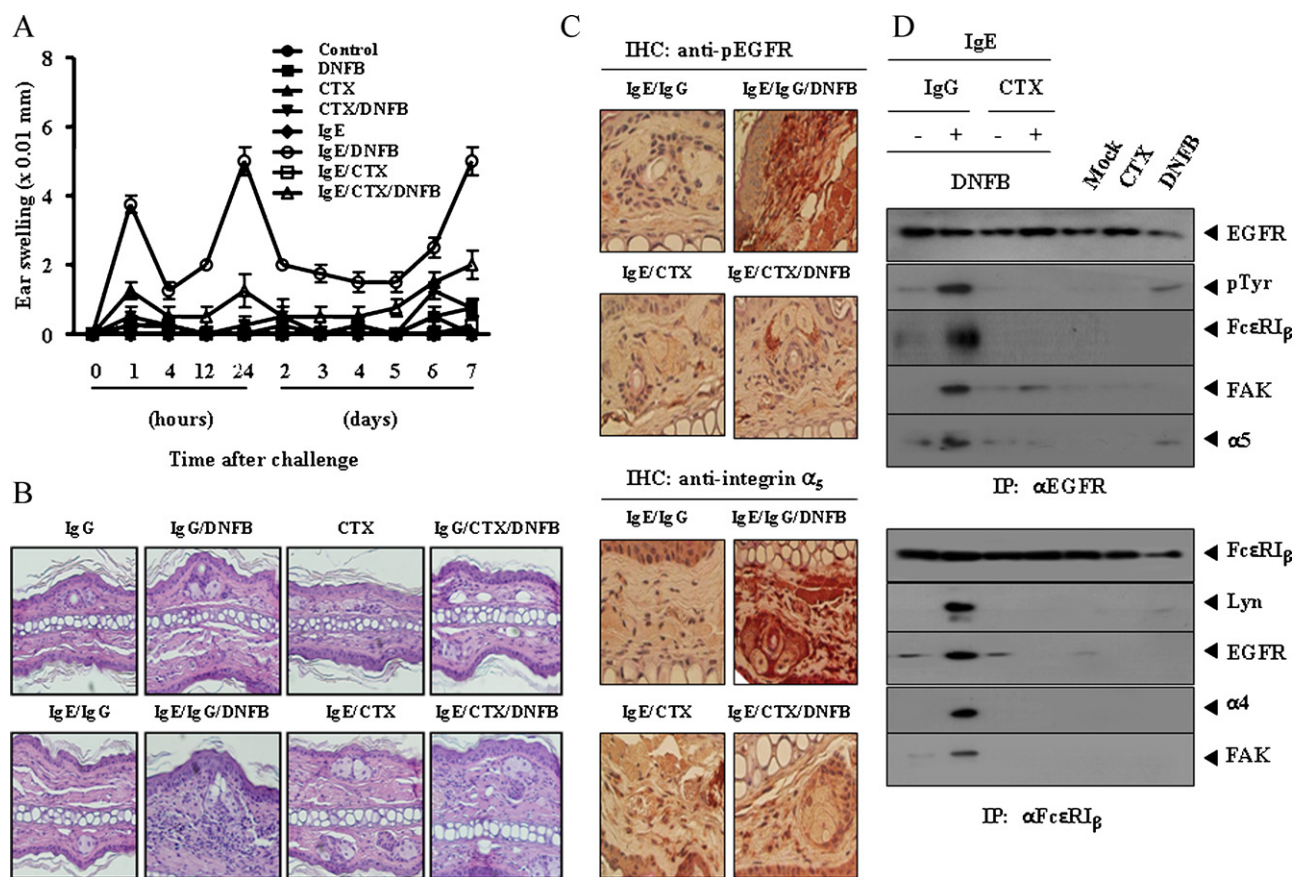


Fig. 3. EGFR is necessary for IgE-dependent cutaneous anaphylaxis (CA) in BALB/c mice. (A) Ears of BALB/c mice were injected with DNP-specific IgE antibody (10 μ g). The next day, ears of mice were painted with dinitrofluorobenzene (DNFB). Ear thickness was measured for 7 days as described. CTX (1 mg/kg) or isotype-matched IgG antibody (1 mg/kg) was added along with DNFB. Each value represents average values obtained from five BALB/c mice. (B) Seven days after DNFB challenge, ear tissues of BALB/c mouse were subjected to H&E staining. (C) is the same as (B) except that immunohistochemistry employing anti-pEGFR or anti-integrin α_5 antibody was performed. (D) BALB/c mouse ear tissue lysates isolated 7 days after DNFB challenge were subjected to immunoprecipitation, followed by Western blot analysis.

added and the reaction was performed at 4°C for 2 h on a rotary shaker. Immune complexes were washed three times with lysis buffer, and 2 $\times$ sample buffer was added to the beads. Boiled samples were then loaded on gels. Western immunoblot analyses were followed according to standard procedures (Kim et al., 2010a,b). The optimal dilution for each antibody was determined empirically.

2.8. Cytokine ELISA

Assays were performed according to manufacturer's instruction (Koma Biotech, Korea).

2.9. Rac1 activity assay

Rac1 activity assays and measurement of reactive oxygen species production were performed according to the well-established procedures (Kim et al., 2008a,b).

2.10. Reactive oxygen species measurements

The IgE-sensitized RBL2H3 cells were pretreated with or without IgG (2 μ g/ml), 5H10-27 (2 μ g/ml) or CTX (0.5 μ g/ml) before antigen stimulation. After 30 min incubation, intracellular ROS level was measured using the 2',7'-dichlorofluorescein-diacetate (DCFH-DA) by confocal microscopy.

2.11. Histological analyses

Ear samples were fixed in 10% (v/v) buffered formalin, embedded in paraffin, sectioned at 4 μ m, and then stained with hematoxylin and eosin to examine lymphocyte infiltration.

2.12. IgE-dependent triphasic cutaneous reaction employing DNFB

IgE-dependent triphasic cutaneous reaction in the ear of female BALB/c mice was induced as reported previously (Nakamura et al., 2003). In brief, mice were passively sensitized with intravenous injection of 10 μ g of mouse anti-DNP monoclonal IgE antibody dissolved in 0.5 ml of PBS. Twenty-four hours later, cutaneous reaction was evoked by painting with 25 μ l of 0.15% DNFB acetone–olive oil (3:1) solution onto each surface of both ear lobes. Both ears of mice were pretreated topically with or without IgG (1 mg/kg) or CTX (1 mg/kg) before DNFB challenge. Mice that received no IgE served as control. Ear thickness was measured by using a digital gauge immediately before and after the DNFB challenge in a total of seven days. The experimental result was expressed as the average increase of in ear thickness. Each group consists of five mice.

2.13. IgE-dependent cutaneous anaphylaxis induced by DNP–HSA

To examine whether allergic inflammation would involve vascular leakage, anti DNP-specific IgE (0.5 μ g) was injected into a mouse ear. The next day, IgG (1 mg/kg), 5H10-27 (0.12 mg/kg)

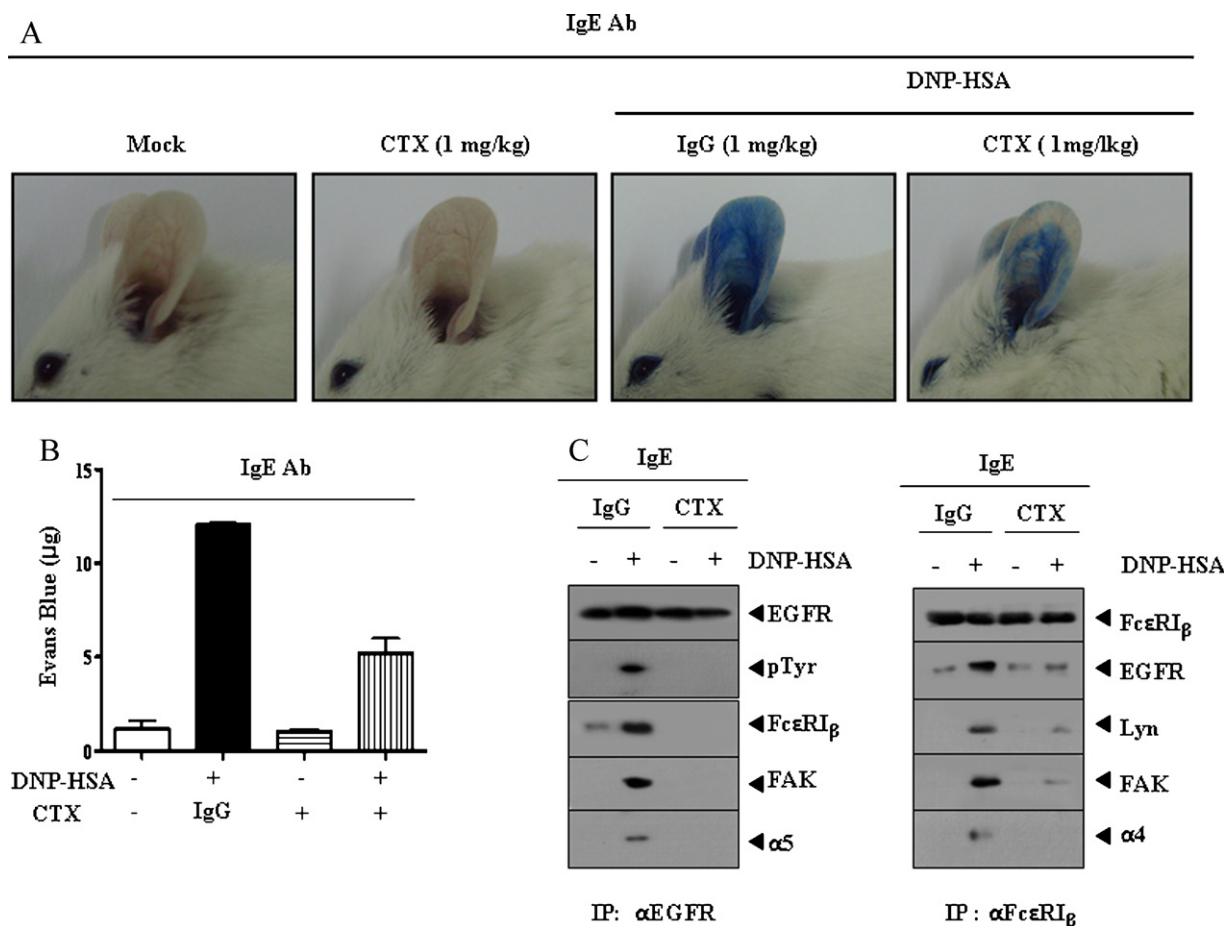


Fig. 4. EGFR is responsible for enhanced vascular leakage in BALB/c mouse model of cutaneous anaphylaxis. Mice were sensitized by injecting 0.5 μg of anti DNP-specific IgE into the ear lobe. The next day, IgG (1 mg/kg) or CTX (1 mg/kg) was intravenously injected via tail vein before antigen stimulation. One hour later, the mice were challenged by intravenously injecting 250 μl of 250 μg DNP-HSA containing 2% Evansblue solution. Blocking of EGFR (A) exerts a negative effect on enhanced vascular leakage in BALB/c mouse model of cutaneous anaphylaxis. The extent of vascular leakage was determined by measuring the amount of eluted Evansblue as described (B). (C) Twenty-four hours after DNP-HSA challenge, mouse ear tissue lysates were prepared and immunoprecipitated with the indicated antibody (2 μg/ml), followed by Western blot analysis.

or CTX (1 mg/kg) was injected via tail vein. One hour later, the mice were challenged with intravenous injection of a 250 μg of DNP-HSA in 250 μl PBS containing 4% Evansblue solution. The mouse were killed an hour after antigen challenge, followed by the removal of an ear for measurement of the amount of dye extravasated by DNP-HSA. The dye was eluted from the ear in 700 μl of formamide at 63 °C. The absorbance was measured at 620 nm.

2.14. Endothelial cell tube formation assay

Tube formation assays were performed to examine whether angiogenic potential of endothelial cells would be regulated by allergic inflammation. For this, growth factor-reduced matrigel was added to 24-well plates (200 μl matrigel per well) and polymerized for 30 min at 37 °C. Rat aortic endothelial cells were first incubated in M199 containing 1% FBS for 1 h, followed by the addition of conditioned medium of RBL2H3 or BMMCs pretreated with or without IgG (2 μg/ml), 5H10-27 (2 μg/ml) or CTX (0.5 μg/ml) before antigen stimulation. After 6–8 h of incubation at 37 °C, the endothelial cells were photographed using an inverted microscope (magnification, 100×; Olympus).

2.15. Chemoinvasion assay

The invasive potential of RBL2H3 cells toward HUVEC cells was determined by using a transwell chamber system with

8 μm pore polycarbonate filter inserts (Costar, Acton, MA). The lower and upper sides of the filter were coated with gelatin and matrigel, respectively. The IgE-sensitized RBL2H3 cells were pre-treated with or without AG1478 (10 μM), IgG (2 μg/ml) or 5H10-27 (1 μg/ml) for 1 h, followed by stimulation with DNP-HSA (100 ng/ml) for 4 h. Cells were harvested by trypsinization and counted. The trypsinized RBL2H3 cells (2×10^4) in the serum-free DMEM medium containing 0.1% bovine serum albumin were then added to each upper chamber of the transwell. Rat aortic endothelial cells were seeded in M199 containing 20% FBS in the lower chamber, and cells were incubated at 37 °C for 14 h. The cells were fixed with methanol, and invaded cells were stained and counted.

2.16. Aortic ring formation assays

Rat aortic ring assay was performed according to the standard procedures (Chung et al., 2010). In brief, 96-well plates were coated with 30 μl of matrigel per well and polymerized in an incubator. Aortas isolated from 6-week-old male Sprague–Dawley rats were cleared of periadventitial fat and connective tissues in cold PBS and cut into rings of 1–1.5 mm in circumference. The aortic rings were randomized into wells and sealed with a 30 μl overlay of matrigel. Each well contained one aortic ring. Conditioned medium of antigen-stimulated RBL2H3 cells pretreated with or without AG1478 (1 μg/ml) or 5H10-27 (2 μg/ml) before antigen stimulation was added. After six days, extent of microvessel sprouting was

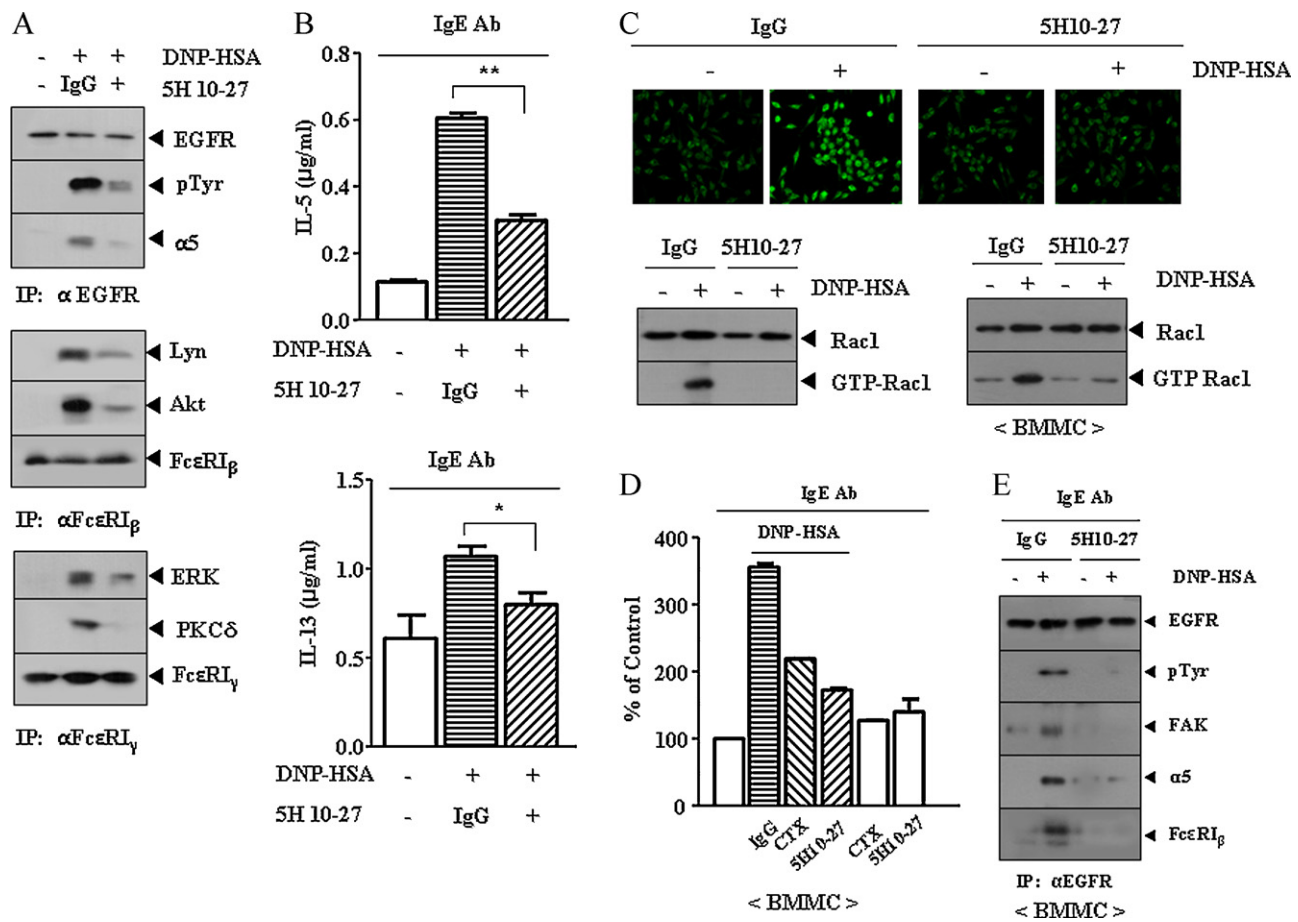


Fig. 5. Integrin α_5 is necessary for the hallmarks of allergic inflammation and is necessary for interaction between EGFR and Fc ϵ RI. (A) IgE-sensitized RBL2H3 cells were pretreated with or without 5H10-27, an integrin α_5 blocking antibody (0.5 μ g/ml) or IgG (2 μ g/ml) for 1 h, followed by antigen stimulation. Cell lysates prepared were immunoprecipitated with the indicated antibody, followed by Western blot analysis. (B) IgE-sensitized RBL2H3 cells were pretreated with 5H10-27 (0.5 μ g/ml) or IgG (2 μ g/ml) for 1 h, followed by antigen stimulation. Supernatants were used for measurement of IL-5 and IL-13. (C) IgE-sensitized RBL2H3 cells were pretreated with or without 5H10-27 for 1 h. Reactive oxygen species level and rac1 activity were measured as described. (D) IgE-sensitized BMMCs were pretreated with the indicated antibody for 1 h, followed by antigen stimulation. BMMCs treated with IgG alone serve as a negative control. Secretion of β -hexosaminidase was determined as described. (E) IgE-sensitized BMMCs were pretreated with 5H10-27 or IgG for 1 h, followed by antigen stimulation. Cell lysates were immunoprecipitated with anti-EGFR antibody (2 μ g/ml), followed by Western blot analysis.

determined by using an inverted microscope (magnification, 100 $\times$; Olympus).

2.17. *In vivo intravital microscope angiogenesis assays*

Male BALB/c mice (6–8 week old) were obtained from Dae-han Biolink (Seoul, Korea). Mice were maintained at the specific pathogen-free housing facility at the School of Medicine, Kangwon National University. *In vivo* angiogenesis was assessed as follows. The mice were anesthetized by 1.5% isofurane and O₂–N₂O using a vaporizer (Surgivet, Waukesha, WI, USA), and abdominal wall windows were implanted. A titanium circular mount with eight holes on the edge was inserted between the skin and abdominal wall. Growth factor-reduced matrigel containing RBL2H3 cells (10⁶ cells) pretreated with or without AG1478 (1 μ g/ml) or 5H10-27 (2 μ g/ml) before antigen stimulation was applied to the space between the windows, and a circular glass cover slip was placed on top and fixed by a snap ring. After four days, the animals were anesthetized and were injected intravenously with 50 μ l of 25 ng/ml fluorescein isothiocyanate-labeled dextran (molecular weight, Mr ~ 2,000,000) via the tail vein. The mice were then placed on a Zeiss Axiovert 200 M microscope. The epi-illumination microscopy setup included a 100-W mercury lamp and filter set for blue light. Fluorescence images were recorded in random locations

of each window by electron-multiplying charge-coupled device camera (Photo Max 512, Princeton Instruments, Trenton, NJ, USA) and digitalized for subsequent analysis using the Metamorph program (Universal Imaging, Downingtown, PA, USA).

3. Results

3.1. Antigen stimulation induces EGFR activation in RBL2H3 cells and bone marrow-derived mast cells (BMMCs)

Antigen stimulation induced interaction between EGFR and CD44 and increased phosphorylation of EGFR in RBL2H3 cells (Fig. 1A). HA, an anti-allergic agent, disrupted interaction between EGFR and CD44 and exerted a negative effect on the phosphorylation of EGFR in RBL2H3 cells (Fig. 1A). Flow cytometry showed increased phosphorylation of EGFR in both RBL2H3 cells (Fig. 1B). CTX (CTX), an EGFR-blocking antibody prevented antigen from increasing EGFR phosphorylation based on flow cytometry analysis (Fig. 1B). Increased phosphorylation of EGFR was also observed in BMMCs in response to antigen stimulation (Fig. 1C, left panel). HA exerted negative effects on EGFR phosphorylation and the interaction between EGFR and CD44 by antigen stimulation in BMMCs (Fig. 1C, right panel). Blocking of EGFR by CTX exerted a negative effect on β -hexosaminidase secretion in RBL2H3 cells (Fig. 1D). CTX

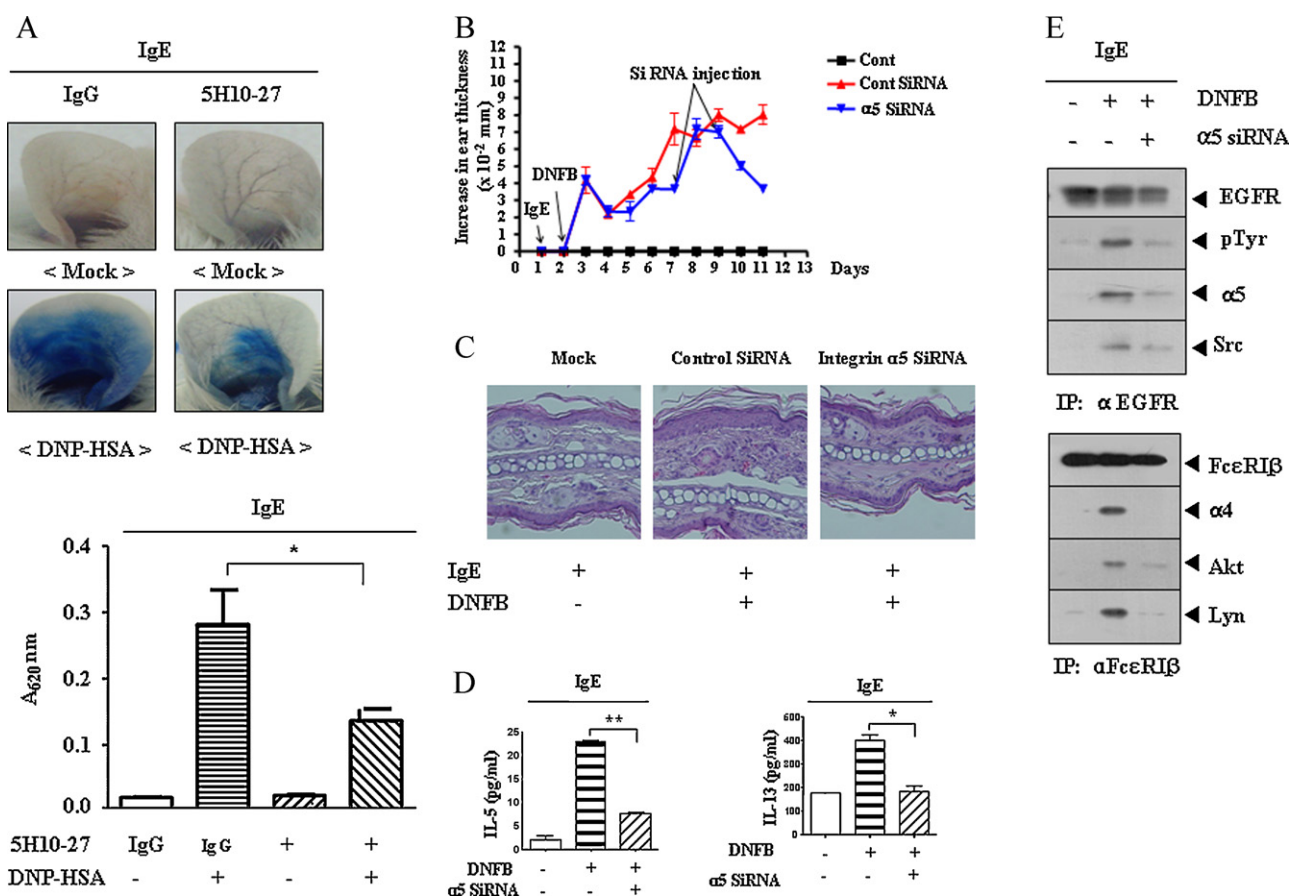


Fig. 6. Integrin α_5 is necessary for IgE-dependent cutaneous anaphylaxis. (A) Mice were sensitized by injecting 0.5 μ g of anti DNP-specific IgE into the ear lobe. The next day, IgG (1 mg/kg) or 5H10-27 (1 mg/kg) was intravenously injected via tail vein before antigen stimulation. One hour later, the mice were challenged by intravenously injecting 250 μ l of 250 μ g DNP-HSA containing 2% Evansblue solution. The extent of vascular leakage was determined by measuring the amount of eluted Evansblue as described. (B) DNP-specific IgE antibody was injected via the tail vein before antigen stimulation. Control siRNA or (100 nM) or integrin α_5 siRNA (100 nM) was injected via the tail vein of a BALB/c mouse twice in a total of 11 days. Ear thickness was measured as described. (C) BALB/c mouse ear tissues obtained 11 days after IgE sensitization were subjected to H&E staining. (D) *In vivo* downregulation of integrin α_5 decreases serum level of IL-5 and IL-13 in animal model of cutaneous anaphylaxis. (–) denotes either control siRNA or DMSO (D).

also prevented antigen from increasing reactive oxygen species production in RBL2H3 cells (Fig. 1F, upper panel) and prevented antigen from increasing rac1 activity in RBL2H3 cells (Fig. 1F, lower panel). CTX also prevented antigen from increasing rac1 activity in BMMCs (Fig. 1F, lower panel). These results suggest that EGFR may be necessary for allergic inflammation.

3.2. Antigen stimulation induces EGFR–integrin α_5 and EGFR–FcεRI interaction

Loss of E-cadherin promotes cancer metastasis via integrin α_5 (Sawada et al., 2008). Antigen stimulation decreases expression of E-cadherin in RBL2H3 cells (Kim et al., 2008a,b). Therefore, we investigated the role of integrin(s) in allergic inflammation. Blocking of EGFR by CTX prevented antigen from increasing the expression of integrin α_4 and integrin α_5 in RBL2H3 cells (Fig. 2A). Antigen stimulation induced interaction between EGFR and integrin α_5 as well as between FcεRIβ and integrin α_4 in RBL2H3 cells (Fig. 2B). HA disrupted interaction between EGFR and integrin α_5 in RBL2H3 cells (Fig. 2C). Immunoprecipitation showed interaction between EGFR and FcεRIβ upon antigen stimulation in RBL2H3 cells, whereas CTX disrupted this interaction (Fig. 2D). The interaction between EGFR and FcεRIβ was also observed in antigen-stimulated BMMCs (Fig. 2E). CTX negatively regulated the expression of hallmarks of allergic inflammation, such as E-cadherin, Snail, and prostaglandin E synthase (PGES), in BMMCs

(Fig. 2F). These results suggest that EGFR, in association with FcεRI, may mediate allergic inflammation.

3.3. EGFR is necessary for *in vivo* IgE-dependent cutaneous anaphylaxis

We examined the role of EGFR in IgE-dependent cutaneous anaphylaxis (CA) employing BALB/c mice. Addition of 2,4-dinitrofluorobenzene (DNFB) increased ear thickness, as expected (Fig. 3A). Mice that received no IgE did not experience changes in ear thickness (Fig. 3A). Blocking of EGFR by CTX prevented DNFB from increasing ear thickness (Fig. 3A). CTX exerted a negative effect on lymphocyte infiltration in a CA model (Fig. 3B). Chemical inhibition of EGFR by AG1478 exerted a negative effect on increased ear thickness by DNFB (Supplemental Fig. 1A). Immunohistochemistry of BALB/c mouse ear tissue showed increased phosphorylation of EGFR as well as increased expression of integrin α_5 by antigen stimulation (Fig. 3C). Western blotting of BALB/c mouse ear tissue showed that blocking of EGFR by CTX exerted a negative effect on EGFR phosphorylation, interaction between EGFR and FAK and interaction between EGFR and FcεRIβ (Fig. 3D). AG1478 exerted a negative effect on the increased serum level of IL-5 and IL-13 (Supplemental Fig. 1B) as well as on lymphocyte infiltration induced by DNFB in BALB/c mice (Supplemental Fig. 1C). AG1478 exerted a negative effect on the interaction of FcεRIβ or FcεRIγ with various cellular signaling molecules in BALB/c mice

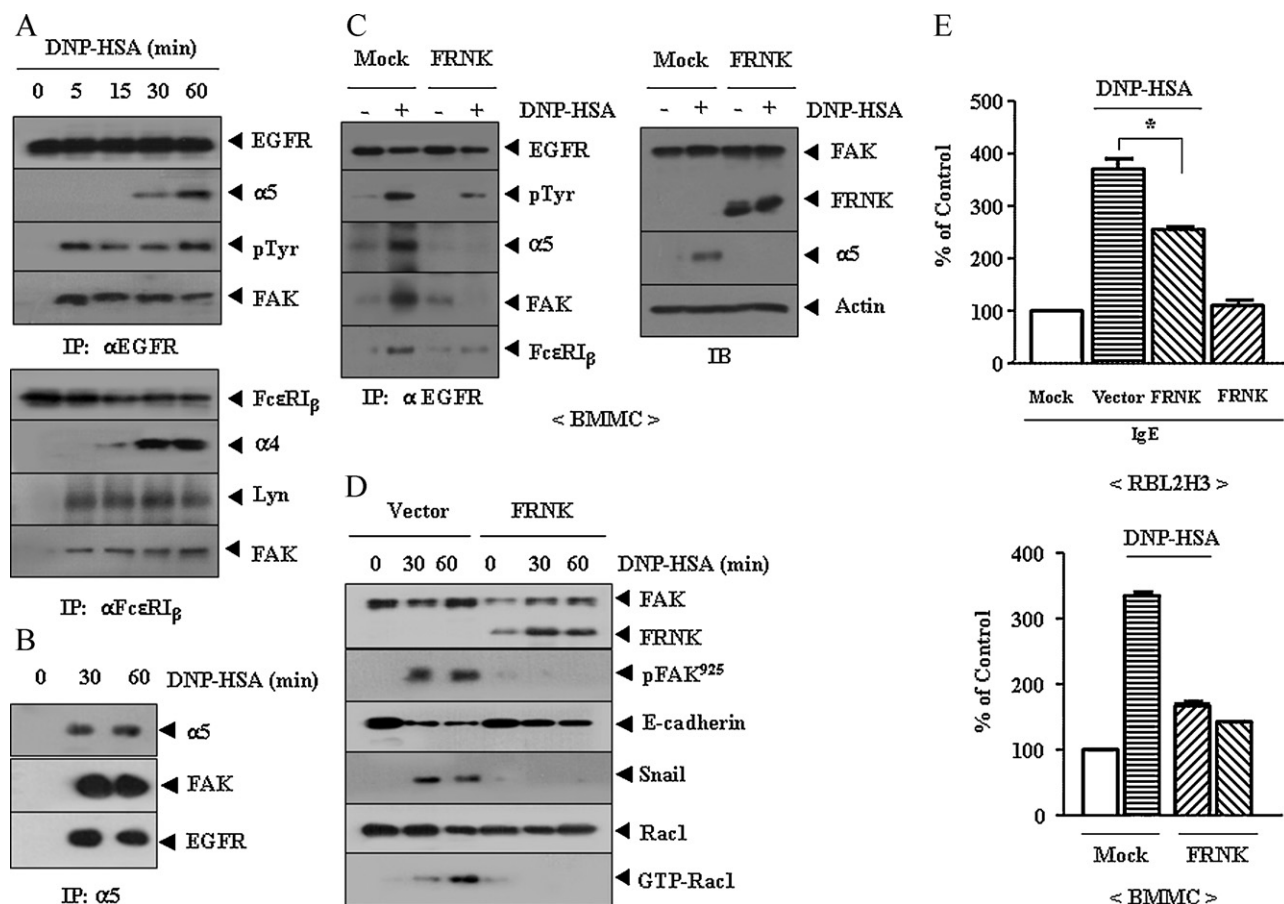


Fig. 7. FAK interacts with integrin α_5 and is necessary for hallmarks of allergic inflammation. (A) IgE-sensitized RBL2H3 cells were stimulated with DNP-HSA for various time intervals. Cell lysates prepared were immunoprecipitated with the indicated antibody, followed by Western blot analysis. (B) Immunoprecipitation shows an interaction between integrin α_5 and FAK. (C) BMMCs were transiently transfected with control vector (1 μ g) or FRNK (1 μ g), an endogenous inhibitor of FAK, prior to sensitization with DNP-specific IgE. Cell lysates were immunoprecipitated with anti-EGFR antibody (2 μ g/ml), followed by Western blot analysis. (D) RBL2H3 cells were transiently transfected with control vector (1 μ g) or FRNK (1 μ g), an endogenous inhibitor of FAK, prior to sensitization with DNP-specific IgE. Western blot analysis was performed. (E) RBL2H3 (upper panel) or BMMCs (lower panel) were transiently transfected with the indicated constructs, prior to sensitization with DNP-specific IgE. After antigen stimulation, β -hexosaminidase secretion assays were performed as described.

(Supplemental Fig. 1D). Further, AG1478 exerted a negative effect on induction of integrin α_5 and integrin α_4 by DNFB in BALB/c mice (Supplemental Fig. 1D). Activation of EGFR was necessary for the induction of downstream signaling molecule during allergic inflammation, such as Snail in BALB/c mice (Supplemental Fig. 1D). These results suggest that activation of EGFR and interaction between EGFR and Fc ϵ RI are necessary for *in vivo* allergic inflammation.

3.4. EGFR is necessary for cutaneous anaphylaxis accompanied by enhanced vascular leakage

DNP-specific IgE injection followed by antigen (DNP-HSA) stimulation induced vascular leakage in BALB/c mice (Fig. 4A). CTX exerted a negative effect on induced vascular leakage by DNP-HSA stimulation (Fig. 4A and B). Immunoprecipitation of BALB/c ear tissue lysates revealed that CTX prevented antigen from increasing EGFR phosphorylation and from inducing interaction between EGFR and Fc ϵ RI β and between FAK and integrin α_5 (Fig. 4C, left panel). CTX also exerted a negative effect on interaction between Fc ϵ RI β and EGFR, FAK, lyn and integrin α_4 (Fig. 4C, right panel).

3.5. Integrin α_5 is necessary for activation of Fc ϵ RI in antigen-stimulated RBL2H3 cells

Blocking of integrin α_5 by 5H10-27 showed that integrin α_5 was necessary for activation of EGFR and Fc ϵ RI (Fig. 5A, upper panel).

5H10-27 exerted negative effects on the interactions between Fc ϵ RI β and lyn and between Fc ϵ RI β and Akt (Fig. 5A, middle panel). 5H10-27 also exerted a negative effect on the interaction between Fc ϵ RI γ and ERK and between Fc ϵ RI γ and PKC δ (Fig. 5A, lower panel). 5H10-27 exerted a negative effect on secretion of Th2 cytokines such as IL-5 and IL-13 in antigen-stimulated RBL2H3 cells (Fig. 5B). 5H10-27 prevented antigen from increasing reactive oxygen species production and rac1 activity in antigen-stimulated cells (Fig. 5C). Integrin α_5 was also necessary for increased rac1 activity by antigen in BMMCs (Fig. 5C). EGFR and integrin α_5 were both necessary for the secretion of β -hexosaminidase by antigen in BMMCs (Fig. 5D). Further, 5H10-27 prevented antigen from inducing interactions between EGFR and Fc ϵ RI β and between EGFR and FAK (Fig. 5E). Lastly, 5H10-27 prevented antigen from increasing phosphorylation of EGFR in BMMCs (Fig. 5E). These results suggest that integrin α_5 is necessary for activation of EGFR and Fc ϵ RI signaling and mediates allergic inflammation.

3.6. Integrin α_5 is necessary for IgE-dependent cutaneous anaphylaxis

We examined the role of integrin α_5 in IgE-dependent cutaneous anaphylaxis. Blocking of integrin α_5 by 5H10-27 prevented vascular leakage induced by antigen (DNP-HSA) stimulation (Fig. 6A). To further confirm the role of integrin α_5 in cutaneous anaphylaxis,

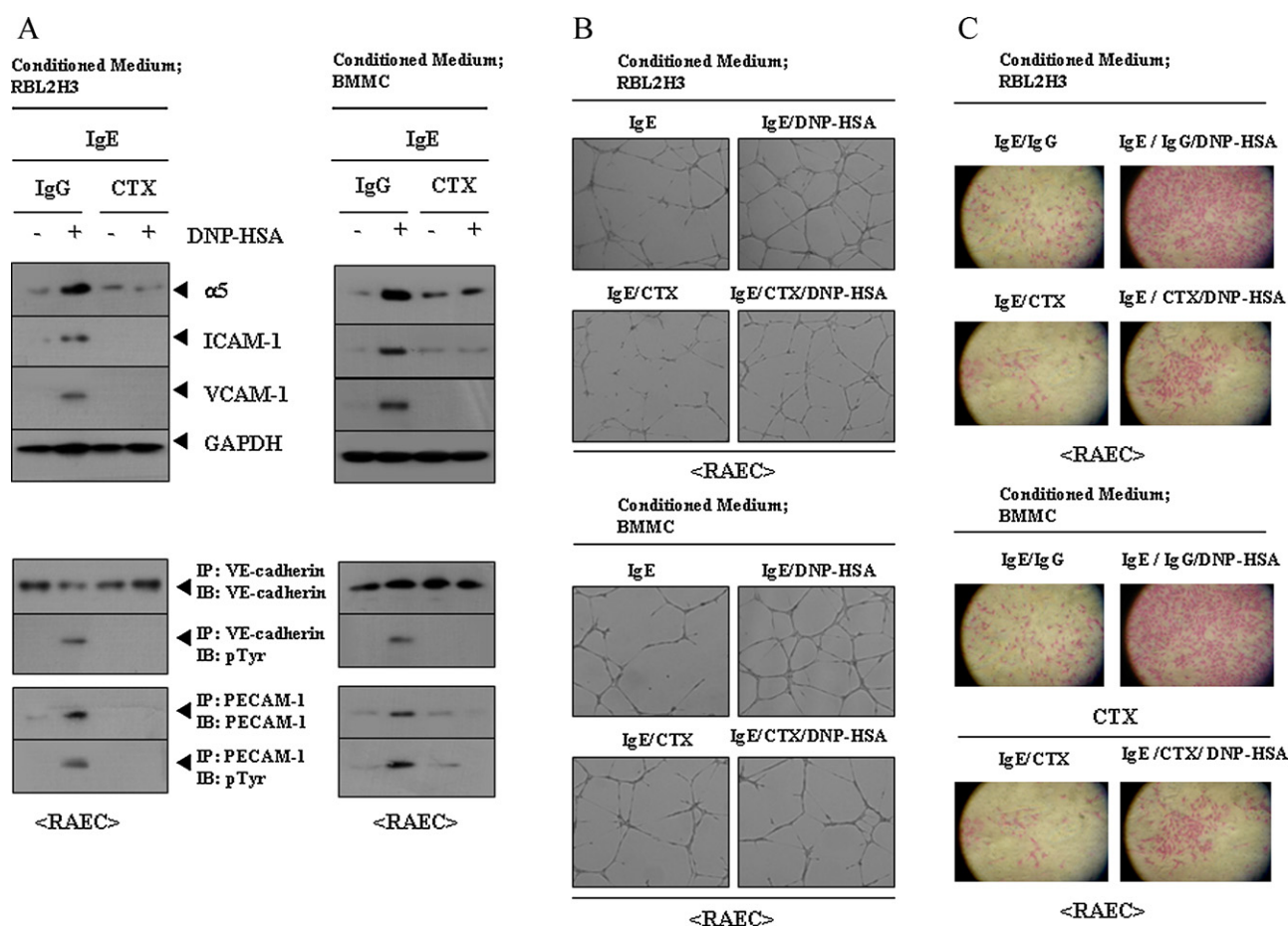


Fig. 8. EGFR and integrin α_5 are necessary for interaction between activated basophils and endothelial cells. (A) IgE sensitized RBL2H3 cells or BMMCs were pretreated with IgG (2 μ g/ml) or CTX (0.5 μ g/ml) for 1 h before antigen stimulation. Conditioned medium of antigen-stimulated RBL2H3 cells or BMMCs was transferred to RAECs. Cell lysates prepared from RAECs after addition of conditioned medium were subjected to Western blot analysis (upper panel) or immunoprecipitated with the indicated antibody, followed by Western blot analysis (lower panel). (B) IgE-sensitized RBL2H3 cells or BMMCs were pretreated with or without IgG (2 μ g/ml) or CTX (0.5 μ g/ml) for 1 h before antigen stimulation. Supernatants were added to each well coated with matrigel, and endothelial cell tube formation assays were performed. (C) is the same as (B) except that cellular invasion assays were performed.

control or integrin α_5 siRNA was injected into the tail vein of BALB/c mice 5 and 7 days after DNFB stimulation. Injection was carried out twice during a time period of 10 days after IgE sensitization. Downregulation of integrin α_5 exerted a negative effect on ear thickness (Fig. 6B) and decreased lymphocyte infiltration (Fig. 6C). Downregulation of integrin α_5 also decreased the serum level of IL-5 and IL-13 (Fig. 6D). Immunoprecipitation found that downregulation of integrin α_5 prevented activation of EGFR (Fig. 6E). Downregulation of integrin α_5 also inhibited the interaction between Fc ϵ RI β and integrin α_4 (Fig. 6E). These results suggest a role for integrin α_5 in cutaneous anaphylaxis.

3.7. FAK is necessary for EGFR–Fc ϵ RI interaction and mediates allergic inflammation

FAK is involved in EGFR signaling (Kim et al., 2008a,b). *In vivo* anaphylaxis was accompanied by interactions between EGFR and FAK and between Fc ϵ RI β and FAK (Fig. 3C). Therefore, we hypothesized that FAK would be necessary for allergic inflammation. Antigen stimulation induced interaction between EGFR and FAK and between FAK and Fc ϵ RI in RBL2H3 cells (Fig. 7A). FAK interacted with integrin α_5 in antigen-stimulated RBL2H3 cells (Fig. 7B). FRNK, an endogenous inhibitor of FAK, exerted negative effects on increased phosphorylation of EGFR and on the interaction between EGFR and Fc ϵ RI β in antigen-stimulated BMMCs (Fig. 7C). FRNK prevented antigen from regulating expression of EMT-related proteins,

such as E-cadherin and Snail and also prevented antigen from activating rac1 in RBL2H3 cells (Fig. 7D). Inactivation of FAK exerted an inhibitory effect on the secretion of β -hexosaminidase in RBL2H3 cells (Fig. 7E, upper panel) and BMMCs (Fig. 7E, lower panel). These results suggest that FAK, via interaction with EGFR and Fc ϵ RI β , mediates allergic inflammation.

3.8. EGFR is necessary for cellular interaction and enhanced angiogenic potential in relation with allergic inflammation

Conditioned medium of antigen-stimulated RBL2H3 cells induced expression of integrin α_5 , ICAM-1 and VCAM-1, which are necessary for the adhesion of leukocytes to endothelial cells and for angiogenesis in rat aortic endothelial cells (RAECs) (Fig. 8A, left panel). This induction was dependent on EGFR (Fig. 8A, left panel). Conditioned medium of antigen-stimulated RBL2H3 cells induced phosphorylation of VE-cadherin and PECAM-1 in RAECs (Fig. 8A, left panel). Conditioned medium of antigen-stimulated BMMCs showed the same effects on RAECs (Fig. 8A, right panel). Conditioned medium of activated RBL2H3 cells and BMMCs enhanced endothelial cell tube formation (Fig. 8B) as well as invasion potential of endothelial cells (Fig. 8C). These effects were dependent on EGFR (Fig. 8D). Taken together, these results suggest that activated basophils and BMMCs may activate signaling in endothelial cells to enhance the angiogenic potential of endothelial cells.

4. Discussion

Inhibition of EGFR exerts a negative effect on skin inflammation (Lee et al., 2010; Li and Luan, 2010; Liu et al., 2008a,b). PKC α is necessary for the coupling of Fc ϵ RI to mast cell activation (Melendez et al., 2007). Since PKC is an activator of EGFR (Parekh et al., 2000) and is involved in allergic inflammation in RBL2H3 cells (Kim et al., 2008a,b), it is reasonable that EGFR is necessary for allergic inflammation in RBL2H3 cells. CD44-PKC α interaction is necessary for allergic inflammation in RBL2H3 cells (Kim et al., 2008a,b). Hyaluronic acid (HA), an anti-allergic agent, disrupts the interaction between CD44-PKC α and exerts a negative effect on *in vitro* and *in vivo* allergic inflammation (Kim et al., 2008a,b). The fact that hyaluronic acid (HA) promotes cancer cell motility by induction of CD44-EGFR (Kim et al., 2008a,b) suggests that there may be an interaction between CD44 and EGFR in RBL2H3 cells. Antigen stimulation induces activation of EGFR in both RBL2H3 cells (Fig. 1A) and BMMCs (Fig. 1C).

Integrin activation is necessary for eosinophilic inflammation of asthma (Barthel et al., 2008). Integrin IIb beta-3 is necessary for interaction of mast cells with fibrinogen and exacerbates inflammation (Oki et al., 2009). Beta3 integrin mediates activation of EGFR to inhibit neurite outgrowth (Schachtrup et al., 2007). Integrin $\alpha_6\beta_4$ is necessary for the transactivation of EGFR in breast cancer cells (Yoon et al., 2006). EGFR cross talks with integrin $\alpha\nu\beta_5$ and promotes cancer metastasis (Ricono et al., 2009). The fact that integrin α_5 interacts with EGFR and integrin α_4 interacts with Fc ϵ RI β in response to antigen stimulation (Fig. 2B) suggests that EGFR interacted with Fc ϵ RI to mediate allergic inflammation. Our data show an interaction between EGFR and Fc ϵ RI β in RBL2H3 cells (Fig. 2D) and BMMCs (Fig. 2E). This further confirms role of EGFR in allergic inflammation. Our data clearly show a role for EGFR in *in vivo* anaphylaxis (Fig. 3). It is therefore reasonable that EGFR can be employed as a target for the development of allergy therapeutics. However, the interaction between EGFR and Fc ϵ RI β has not been previously reported.

FAK mediates activation of ERK and is involved in infection-induced allergic inflammation (Cheung et al., 2008). Integrin $\alpha_3\beta_1$ activates FAK/Src to regulate rac1-dependent polarization of keratinocytes on laminin-5 (Choma et al., 2007). Urokinase-type plasminogen activator receptor transactivates EGFR via Src (Das et al., 2004). Downregulation of integrin α_2 by mutant EGFR leads to aberrant focal adhesion formation (Ning et al., 2005). In our data, FAK interacts with integrin α_5 (Fig. 7B) and is necessary for interaction between EGFR and Fc ϵ RI β (Fig. 7C). FAK is necessary for increased secretion of β -hexosaminidase (Fig. 7E).

VEGFR1 and VEGFR2 are expressed by human mast cells (Detoraki et al., 2009) and can be induced by PGE(2) (Detoraki et al., 2009). PGE(2)-induced human mast cells induce angiogenesis in CAM assays (Liu et al., 2009). Previously we reported that antigen stimulation increases secretion of PGE(2) in RBL2H3 cells (Kim et al., 2010a,b). Leptin enhances angiogenesis via integrin $\alpha\nu\beta_5$ (Heida et al., 2010). Chemical inhibition of integrin $\alpha\nu\beta_3$ suppresses hepatic angiogenesis (Patsenker et al., 2009). Integrin $\alpha\nu\beta_3$ enhances angiogenesis and tumor growth in the brain (Lorger et al., 2009). Integrin $\alpha_5\beta_1$ is necessary for angiogenesis in placental multipotent mesenchymal stromal cells (Lee et al., 2009). Therefore, we hypothesized that activated RBL2H3 cells might enhance the angiogenic potential of endothelial cells via integrin(s). Conditioned medium of antigen-stimulated RBL2H3 cells promoted rat aortic ring formation, which was dependent on EGFR and integrin α_5 (Supplemental Fig. 2A). As seen in intravital microscopy (Supplemental Fig. 2B), chemical inhibition of EGFR or blocking of integrin α_5 exerted a negative effect on angiogenic potential of antigen-stimulated RBL2H3 cells. Conditioned medium of antigen-stimulated RBL2H3 cells enhanced RAEC tube formation, which

was dependent on EGFR and integrin α_5 (Supplemental Fig. 2C). Antigen-stimulated RBL2H3 cells showed high invasion potential toward EAECs, which was dependent on EGFR and integrin α_5 (Supplemental Fig. 2D). These results suggest that antigen-stimulated basophilic leukemia cells may interact with endothelial cells to promote angiogenesis.

In this study, we found that the EGFR–integrin α_5 interaction was necessary for both *in vitro* and *in vivo* allergic inflammation. We also found an interaction between EGFR and Fc ϵ RI β . Allergic inflammation involved interactions between basophil, mast cells and endothelial cells, and was accompanied by enhanced angiogenic potential of endothelial cells. Overall, this study shows that EGFR can be employed as a target for the development of allergy therapeutics.

Acknowledgments

This work was supported by a grant from the Korea Research Foundation (C1001478-01-01, C1006272-01-01, C1006625-01-02 and C1007565-01-01). This work was supported by the Regional Innovation Center Program of the Ministry of Education, Science and Technology. This work was also supported by a grant from Korea Research Foundation Grant funded by the Korean Government (MEST) (The Regional Research Universities Program/Medical & Bio-Materials Research Center).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molimm.2011.01.013.

References

- Barthel, S.R., Johansson, M.W., McNamee, D.M., Mosher, D.F., 2008. Roles of integrin activation in eosinophil function and the eosinophilic inflammation of asthma. *J. Leukoc. Biol.* 83, 1–12.
- Cheung, P.F., Wong, C.K., Ip, W.K., Lam, C.W., 2008. FAK-mediated activation of ERK for eosinophil migration: a novel mechanism for infection-induced allergic inflammation. *Int. Immunol.* 20, 353–363.
- Choma, D.P., Milano, V., Pumiglia, K.M., DiPersio, C.M., 2007. Integrin $\alpha_3\beta_1$ -dependent activation of FAK/Src regulates Rac1-mediated keratinocyte polarization on laminin-5. *J. Invest. Dermatol.* 127, 31–40.
- Chung, B.H., Lee, J.J., Kim, J.D., Jeoung, D., Lee, H., Choe, J., Ha, K.S., Kwon, Y.G., Kim, Y.M., 2010. Angiogenic activity of sesamin through the activation of multiple signal pathways. *Biochem. Biophys. Res. Commun.* 391, 254–260.
- Das, R., Mahabeshwar, G.H., Kundu, G.C., 2004. Osteopontin induces AP-1-mediated secretion of urokinase-type plasminogen activator through c-Src-dependent epidermal growth factor receptor transactivation in breast cancer cells. *J. Biol. Chem.* 279, 11051–11064.
- Detoraki, A., Staiano, R.L., Granata, F., Giannattasio, G., Prevete, N., de Paulis, A., Ribatti, D., Genovese, A., Triggiani, M., Marone, G., 2009. Vascular endothelial growth factors synthesized by human lung mast cells exert angiogenic effects. *J. Allergy Clin. Immunol.* 123, 1142–1149.
- Edick, M.J., Tesfay, L., Lamb, L.E., Knudsen, B.D., Miranti, C.K., 2007. Inhibition of integrin-mediated crosstalk with epidermal growth factor receptor/Erk or Src signaling pathways in autophagic prostate epithelial cells induces caspase-independent death. *Mol. Biol. Cell* 18, 2481–2490.
- Gilcrease, M.Z., 2007. Integrin signaling in epithelial cells. *Cancer Lett.* 247, 1–25.
- Gong, M., Meng, L., Jiang, B., Zhang, J., Yang, H., Wu, J., Shou, C., 2008. p37 from *Mycoplasma hyorhinis* promotes cancer cell invasiveness and metastasis through activation of MMP-2 and followed by phosphorylation of EGFR. *Mol. Cancer Ther.* 7, 530–537.
- Hamilton, L.M., Torres-Lozano, C., Puddicombe, S.M., Richter, A., Kimber, I., Dearman, R.J., Vrugt, B., Aalbers, R., Holgate, S.T., Djukanović, R., Wilson, S.J., Davies, D.E., 2003. The role of the epidermal growth factor receptor in sustaining neutrophil inflammation in severe asthma. *Clin. Exp. Allergy* 33, 233–240.
- Heida, N.M., Leifheit-Nestler, M., Schroeter, M.R., Müller, J.P., Cheng, I.F., Henkel, S., Limbourg, A., Limbourg, F.P., Alves, F., Quigley, J.P., et al., 2010. Leptin enhances the potency of circulating angiogenic cells via src kinase and integrin (α) ν beta5: implications for angiogenesis in human obesity. *Arterioscler. Thromb. Vasc. Biol.* 30, 200–206.
- Houtman, R., Koster, A.S., Nijkamp, F.P., 2001. Integrin VLA-5: modulator and activator of mast cells. *Clin. Exp. Allergy* 31, 817–822.
- Kim, D.Y., Jeoung, D., Ro, J.Y., 2010a. Signaling pathways in the activation of mast cells cocultured with astrocytes and colocalization of both cells in experimental allergic encephalomyelitis. *J. Immunol.* 185, 273–283.

- Kim, Y., Eom, S., Kim, K., Lee, Y.S., Choe, J., Hahn, J.H., Lee, H., Kim, Y.M., Ha, K.S., Ro, J.Y., Jeoung, D., 2010b. Transglutaminase II interacts with rac1, regulates production of reactive oxygen species, expression of snail, secretion of Th2 cytokines and mediates *in vitro* and *in vivo* allergic inflammation. *Mol. Immunol.* 47, 1010–1022.
- Kim, Y., Lee, Y.S., Choe, J., Lee, H., Kim, Y.M., Jeoung, D., 2008a. CD44 epidermal growth factor receptor interaction mediates hyaluronic acid-promoted cell motility by activating protein kinase C signaling involving Akt, Rac1 Phox, reactive oxygen species, focal adhesion kinase, and MMP-2. *J. Biol. Chem.* 283, 22513–22528.
- Kim, Y., Lee, Y.S., Hahn, J.H., Choe, J., Kwon, H.J., Ro, J.Y., Jeoung, D., 2008b. Hyaluronic acid targets CD44 and inhibits FcεpsilonRI signaling involving PKCdelta, Rac1 ROS, and MAPK to exert anti-allergic effect. *Mol. Immunol.* 45, 2537–2547.
- Kumagai, K., Ohno, I., Okada, S., Ohkawara, Y., Suzuki, K., Shinya, T., Nagase, H., Iwata, K., Shirato, K., 1999. Inhibition of matrix metalloproteinases prevents allergen-induced airway inflammation in a murine model of asthma. *J. Immunol.* 162, 4212–4219.
- Lee, E.J., Jeon, M.S., Kim, B.D., Kim, J.H., Kwon, Y.G., Lee, H., Lee, Y.S., Yang, J.H., Kim, T.Y., 2010. Capsiate inhibits ultraviolet B-induced skin inflammation by inhibiting Src family kinases and epidermal growth factor receptor signaling. *Free Radic. Biol. Med.* 48, 1133–1143.
- Lee, M.Y., Huang, J.P., Chen, Y.Y., Aplin, J.D., Wu, Y.H., Chen, C.Y., Chen, P.C., Chen, C.P., 2009. Angiogenesis in differentiated placental multipotent mesenchymal stromal cells is dependent on integrin alpha5beta1. *PLoS One* 4, e6913.
- Li, X.H., Luan, B., 2010. Effect of epidermal growth factor receptor on airway remodeling in asthmatic mice and its mechanism. *Zhongguo Dang Dai Er Ke Za Zhi* 12, 137–140.
- Liu, K., Anderson, G.P., Bozinovski, S., 2008a. DNA vector augments inflammation in epithelial cells via EGFR-dependent regulation of TLR4 and TLR2. *Am. J. Respir. Cell. Mol. Biol.* 39, 305–311.
- Liu, K., Gualano, R.C., Hibbs, M.L., Anderson, G.P., Bozinovski, S., 2008b. Epidermal growth factor receptor signaling to Erk1/2 and STATs control the intensity of the epithelial inflammatory responses to rhinovirus infection. *J. Biol. Chem.* 283, 9977–9985.
- Liu, Z., Kobayashi, K., van Dinther, M., van Heiningen, S.H., Valdimarsdottir, G., van Laar, T., Scharpfenecker, M., Löwik, C.W., Goumans, M.J., Ten Dijke, P., et al., 2009. VEGF and inhibitors of TGFbeta type-I receptor kinase synergistically promote blood-vessel formation by inducing alpha5-integrin expression. *J. Cell Sci.* 22, 3294–3302.
- Lorger, M., Krueger, J.S., O'Neal, M., Staflin, K., Felding-Habermann, B., 2009. Activation of tumor cell integrin alphavbeta3 controls angiogenesis and metastatic growth in the brain. *Proc. Natl. Acad. Sci. U.S.A.* 106, 10666–10671.
- McNamara, N., Gallup, M., Khong, A., Sucher, A., Maltseva, I., Fahy, J., Basbaum, C., 2004. Adenosine up-regulation of the mucin gene MUC2, in asthma. *FASEB J.* 18, 1770–1772.
- Melendez, A.J., Harnett, M.M., Pushparaj, P.N., Wong, W.S., Tay, H.K., McSharry, C.P., Harnett, W., 2007. Inhibition of Fc epsilon RI-mediated mast cell responses by ES-62, a product of parasitic filarial nematodes. *Nat. Med.* 13, 1375–1381.
- Moro, L., Dolce, L., Cabodi, S., Bergatto, E., Boeri Erba, E., Smeriglio, M., Turco, E., Retta, S.F., Giuffrida, M.G., Venturino, M., et al., 2002. Integrin-induced epidermal growth factor (EGF) receptor activation requires c-Src and p130Cas and leads to phosphorylation of specific EGF receptor tyrosines. *J. Biol. Chem.* 277, 9405–9414.
- Nakamura, N., Ochi, T., Sawada, M., Tanaka, H., Inagaki, N., Saiki, I., et al., 2003. Role of T cells in IgE-dependent triphasic cutaneous reaction caused by dinitrofluorobenzene in the mouse ear: participation of CD8⁺ T cells. *Allergol. Int.* 52, 31–36.
- Ning, Y., Zeineldin, R., Liu, Y., Rosenberg, M., Stack, M.S., Hudson, L.G., 2005. Down-regulation of integrin alpha2 surface expression by mutant epidermal growth factor receptor (EGFRvIII) induces aberrant cell spreading and focal adhesion formation. *Cancer Res.* 65, 9280–9286.
- Okazaki, T., Ni, A., Ayeni, O.A., Baluk, P., Yao, L.C., Vossmeier, D., Zischinsky, G., Zahn, G., Knolle, J., Christner, C., et al., 2009. alpha5beta1 Integrin blockade inhibits lymphangiogenesis in airway inflammation. *Am. J. Pathol.* 174, 2378–2387.
- Oki, T., Eto, K., Izawa, K., Yamanishi, Y., Inagaki, N., Frampton, J., Kitamura, T., Kitaura, J., 2009. Evidence that integrin alpha IIb beta 3-dependent interaction of mast cells with fibrinogen exacerbates chronic inflammation. *J. Biol. Chem.* 284, 31463–31472.
- Parekh, D.B., Katso, R.M., Leslie, N.R., Downes, C.P., Procyk, K.J., Waterfield, M.D., Parker, P.J., 2000. Beta1-integrin and PTEN control the phosphorylation of protein kinase C. *Biochem. J.* 352, 425–433.
- Patsenker, E., Popov, Y., Stickel, F., Schneider, V., Ledermann, M., Sägeser, H., Niedobitek, G., Goodman, S.L., Schuppan, D., 2009. Pharmacological inhibition of integrin alphavbeta3 aggravates experimental liver fibrosis and suppresses hepatic angiogenesis. *Hepatology* 50, 1501–1511.
- Ra, C., Yasuda, M., Yagita, H., Okumura, K., 1994. Fibronectin receptor integrins are involved in mast cell activation. *J. Allergy Clin. Immunol.* 94, 625–628.
- Ricono, J.M., Huang, M., Barnes, L.A., Lau, S.K., Weis, S.M., Schlaepfer, D.D., Hanks, S.K., Cheresch, D.A., 2009. Specific cross-talk between epidermal growth factor receptor and integrin alphavbeta5 promotes carcinoma cell invasion and metastasis. *Cancer Res.* 69, 1383–1391.
- Sawada, K., Mitra, A.K., Radjabi, A.R., Bhaskar, V., Kistner, E.O., Tretiakova, M., Jagadeeswaran, S., Montag, A., Becker, A., Kenny, H.A., et al., 2008. Loss of E-cadherin promotes ovarian cancer metastasis via alpha 5-integrin, which is a therapeutic target. *Cancer Res.* 68, 2329–2339.
- Schachtrup, C., Lu, P., Jones, L.L., Lee, J.K., Lu, J., Sachs, B.D., Zheng, B., Akassoglou, K., 2007. Fibrinogen inhibits neurite outgrowth via beta 3 integrin-mediated phosphorylation of the EGF receptor. *Proc. Natl. Acad. Sci. U.S.A.* 104, 11814–11819.
- Yasuda, M., Hasunuma, Y., Adachi, H., Sekine, C., Sakanishi, T., Hashimoto, H., Ra, C., Yagita, H., Okumura, K., 1995. Expression and function of fibronectin binding integrins on rat mast cells. *Int. Immunol.* 7, 251–258.
- Yoon, S.O., Shin, S., Lipscomb, E.A., 2006. A novel mechanism for integrin-mediated ras activation in breast carcinoma cells: the alpha6beta4 integrin regulates ErbB2 translation and transactivates epidermal growth factor receptor/ErbB2 signaling. *Cancer Res.* 66, 2732–27399.