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MUC5B Production Is Unaffected by Akt Inhibition in Human Lung Epithelial NCI-H292 Cells

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Abstract

In the human airway, the gel-forming mucin subtypes MUC5B and MUC5AC play important roles in biophylaxis. However, the regulation of MUC5B production is less clear than that of MUC5AC. Therefore, the regulation of MUC5B production by cell attachment and Akt was investigated in human lung epithelial NCI-H292 cells. We found that low cell attachment to culture plates induced the upregulated production of both MUC5B and MUC5AC. Cell attachment induces the activation of Akt, a serine/threonine kinase. Cell treatment with Akt inhibitor I decreased Akt phosphorylation and activation. However, MUC5B production was unaffected by Akt inhibition, whereas MUC5AC production was upregulated. MUC5B production was also unaffected by Akt inhibition in cells cultured on type IV collagen or fibronectin. These results suggest that the production of both MUC5B and MUC5AC is regulated by cell attachment. However, the regulation of MUC5B is unaffected by Akt inhibition, in contrast to that of MUC5AC.

Keywords

MUC5B, MUC5AC, Mucin, Akt, Asthma, Chronic Obstructive Pulmonary Disease, Low Cell Attachment

1. Introduction

The mucus layer in the human airway is produced by secretory epithelial cells and is an important component of primary host defense. It traps and transports inhaled foreign particulates out of the airway through mucociliary clearance (MCC) by mucus flow and cough [1] [2]. Mucus is mainly composed of water and mucin, a highly

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glycosylated protein. To date, 17 mucin genes have been identified in the human genome [3]. In airway mucus, the principal mucin subtypes are MUC5AC and MUC5B. In the airway of patients with asthma or chronic obstructive pulmonary disease (COPD), MUC5AC is overproduced and induces the narrowing of the airway [3]-[5]. MUC5B is a major component of the biodefence activities of mucus in the healthy airway. The lack of MUC5B causes inefficient MCC in mice [6]-[8].

The association between MUC5AC production and the mediation of asthma and COPD has been well investigated. Studies have shown that MUC5AC production is regulated by various factors and mechanisms, including the activation of inflammatory cytokines, cell-cell interactions, cellular adhesion to the extracellular matrix (ECM), and Akt activation [9]-[11]. Akt, also known as protein kinase B, is a serine/threonine kinase that plays key roles in the integrin pathway and regulates both cell growth and proliferation. MUC5AC is downregulated by Akt and is upregulated by low cell attachment. However, little is known about the regulation of MUC5B production.

Some studies that investigated the regulation of MUC5B production reported that NRG1β1, a member of NRG growth factor family, induces MUC5AC and MUC5B mucins through the activation of p38MAPK, extracellular signal-regulated kinase (ERK), and Akt in primary human bronchial epithelial cell (HBEC) cultures [12]. According to other reports, retinoic acid, the cytokines IL-6 and IL-17, and reactive oxygen increased MUC5B production in HBEC cells [13]-[17].

We previously reported that several components of ECM are involved in the regulation of MUC5AC and MUC5B production [18] [19]. ECM components relay signals to cells via integrins, which are major adhesion molecules that connect cells to ECM or culture plates [20] [21]. In the ECM-mediated integrin signaling pathway, integrin signaling activates numerous kinases, such as ERK and Akt, and thus participates in the regulation of gene expression [22] [23].

We previously reported that Akt and low cell attachment are involved in the regulation of MUC5AC production [11] [23]. Low cell attachment induces a 10-fold upregulation and Akt inhibition induces a 2-fold upregulation of MUC5AC production. However, the regulation of other mucin subtypes, such as MUC5B production by low cell attachment and Akt, has not yet been reported. Therefore, in the current study, we investigated the influence of low cell attachment and Akt on the regulation of MUC5B production.

2. Materials and Methods

2.1. Cell Culture

Human airway cancer cell line NCI-H292 was purchased from the American Type Culture Collection (Manassas, VA, USA). NCI-H292 cells were cultured in RPMI-1640 (Sigma-Aldrich, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS, Cansera International, Etobicoke, Ontario, Canada), 100 units/mL of penicillin (Gibco Oriental, Tokyo, Japan), and 100 μg/mL streptomycin (Gibco Oriental) in a 5% CO_2 incubator at 37˚C. Adherent cells were subcultured every 3 - 4 d by treatment with a trypsin–EDTA solution (Gibco Oriental).

2.2. Plate Coating with ECM Proteins

A 96-well plate polystyrene (MS8096F, Sumilon, Tokyo, Japan) was coated with 100 μL of type IV collagen (33 μg/mL, Sigma, Tokyo, Japan) or with 100 μL of fibronectin (500 μg/mL, Asahi Techno Glass, Tokyo, Japan) in phosphate-buffered saline (PBS; 0.01 mM phosphate buffer, 0.138 mM NaCl, 0.0027 mM KCl, pH 7.4) over 10 h at 4˚C. As control, the plate was pretreated with PBS. For low cell attachment, cells were seeded on a 96-well plate to a low-adhesive plate (MS8096R, Sumilon).

2.3. Reagents

Akt inhibitor I (Santa Cruz, CA, USA) was added to the cell culture medium to a final concentration of 25 μM. All reagents were dissolved to the appropriate concentration in dimethylsulfoxide (DMSO). The same concentration of DMSO was added to the controls.

2.4. MUC5B and MUC5AC Protein Assay

NCI-H292 cells were washed once with culture medium and suspended in the medium by means of syringe with a 26 G needle to create a single-cell suspension. Diluted cells (2×10^4 cells per 100 μL) were added to the wells

of coated 96-well plate and incubated at 37˚C. After removal and storage of the culture media (100 μL), the cells were harvested by lysis in 100 μL of Tris-buffered saline (TBS; 100 mM NaCl and 10 mM Tris pH 7.5) containing 0.1% SDS at the indicated time points. A total of 100 μL (2×10^4 cells) of the sample was blotted onto an Immobilon membrane (Millipore, Bedford, MA, USA) by Dot Blot Hybridization Manifold (48 wells; SCIEPLAS, Cambridge, UK). The membrane was treated with 4% skim milk (Gibco Oriental) in 0.1% Tween 20-TBS (TBS-T) for 12 h at 4˚C, and then incubated with mouse anti-human MUC5B antibody (1:2000 in 4% skim milk, ab77995, abcam, Tokyo, Japan) or with mouse anti-human MUC5AC antibody (1:2000 in 4% skim milk, MS145-P1, Thermo Scientific, Kanagawa, Japan) for 1 h. The membrane was washed five times for 5 min each with TBS-T and then incubated with rabbit anti-mouse IgG (H + L) (1:2000 in 4% skim milk, NA931V, GE Healthcare, Buckinghamshire, UK) for 1 h. After washing the membrane for five times, enzyme reactions were detected with a Luminata Forte western HRP substrate (WBLUF0500, Millipore) and a Chemidoc image analyzer (Biorad, Tokyo, Japan).

2.5. Western Blot Analysis

The cells cultured on the plates were lysed in a conventional SDS sample buffer (62.5 mMTris, 10% glycerol, 2% SDS, 0.01% bromophenol blue, pH 6.8). The samples were electrophoresed on 10% of acrylamide gels with a CM-1005 gel apparatus (Cima Biotech, Tokyo, Japan), and then blotted onto a Hybond ECL nitrocellulose membrane (GE Healthcare) with a Trans blot SD cell (Biorad). The membrane was treated with 4% skim milk (Gibco Oriental) in TBS-T (0.1% tween-20, 150 mMNaCl and 10 mMTris pH 7.5) for 12 h at 4˚C and then incubated with a rabbit anti phospho-Akt (Ser 473) antibody (4058, Cell Signaling Technology, MA, USA) or amouse antibody Akt (pan) (2920S, Cell Signaling Technology, MA, USA) at a dilution of 1:2000 in 4% skim milk for 12 h at 4˚C. The membrane was washed five times for 5 min each with TBS-T and then incubated with an anti-mouse IgG (H + L) conjugated with horseradish peroxidase (NA931V, GE Healthcare) at a dilution of 1:2000 in 4% skim milk for 1 h. After washing the membrane for five times, the enzyme reaction was detected with a Luminata Forte western HRP substrate (WBLUF0500, Millipore) and a Chemidoc image analyzer (Biorad, Tokyo, Japan). Cellular β-actin was detected as control using a mouse anti-β-actin antibody (A5316, Sigma) at a dilution of 1:2000 and an anti-mouse IgG (H + L) (GE Healthcare) at a dilution of 1:2000 in 4% skim milk for 1 h in the same way. After detection, a blot membrane was incubated with Restore Western blot Stripping buffer (21,059, Thermo Scientific, Rockford, IL, USA) for 15 min at room temperature with shaking. The membrane was washed five times for 5 min each with TBS-T and then treated with 4% skim milk (Gibco Oriental) in TBS-T for 12 h at 4˚C for reblocking.

2.6. Cell Proliferation Assay

Cell proliferation was assessed by a Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). NCI-H292 cells (2×10^4 cells in 0.1 mL) were cultured on a 96-well plate (Sumilon, Tokyo, Japan) at 37˚C. The reagent of the kit (0.01 mL) was added to each well, and then the plate was incubated for 2 h at 37˚C. Cell growth was assessed by measuring the absorbance at 450 nm with a microplate spectrophotometer Benchmark plus (BioRad).

2.7. Statistics

Analysis of variance (ANOVA) was used for comparisons among more than two groups. For other statistics, Student's t-test was performed. $^*p < 0.05$ was considered significant.

3. Results

3.1. Both MUC5B and MUC5AC Production Are Upregulated by Low Cell Attachment

We previously reported that MUC5AC production was increased 10-fold by low cell attachment [11]. Therefore, we investigated how MUC5B is regulated by cell attachment. In brief, human lung epithelial NCI-H292 cells, which produce both MUC5AC and MUC5B, were cultured for 30 h in adhesive or low adhesive plates. Then, the cells and culture medium were separately sampled. The majority of MUC5AC protein was in cells; however, the ratio of MUC5B protein in cells to that in culture medium varied according to the cell culturing conditions. Therefore, we measured the amount of MUC5B and MUC5AC in both cells and culture medium by Immuno

blot analysis using specific antibodies. The results showed that the production of both MUC5B and MUC5AC was upregulated by low cell attachment (**Figure 1**).

3.2. Regulation of MUC5B Production Is Not Affected by Akt Inhibition

Next, the influence of the Akt pathway on MUC5B regulation was investigated. In brief, NCI-H292 cells were cultured for 30 h with or without Akt inhibitor I, which inhibits Akt phosphorylation and activation (**Figure 2**). Immuno blot analysis was performed to quantify the amounts of MUC5AC and MUC5B using specific antibodies. The production of MUC5AC was upregulated in both cells and culture medium, whereas that of MUC5B was unaffected by the inhibition of Akt, in accordance with the findings of our previous study (**Figure 3** and **Figure 4**).

Type IV collagen, an ECM component, downregulates MUC5AC production via the integrin/Akt pathway [18]. MUC5B production in cells cultured on type IV collagen was also unaffected by Akt inhibition, whereas that of MUC5AC was upregulated in cells (**Figure 3** and **Figure 4**). Fibronectin, a component of ECM, upregulates MUC5B production in medium, but Akt inhibition did not affect MUC5B production in medium or in cells cultured on fibronectin (**Figure 5**). Taken together, these results suggest that the regulation of MUC5B production is unaffected by Akt inhibition.

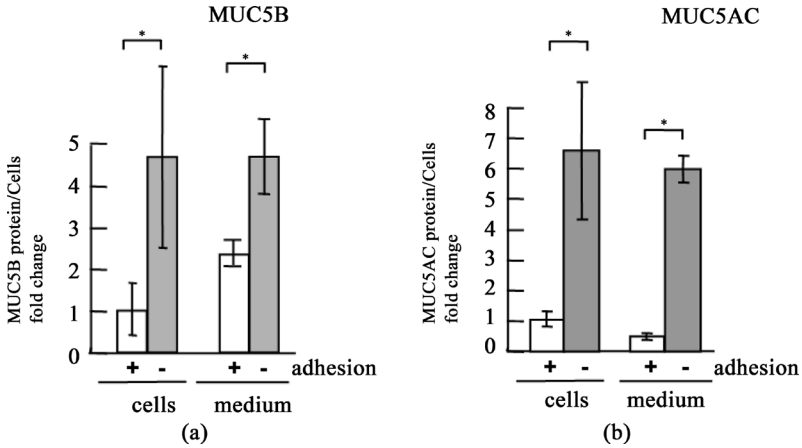

Figure 1. Evaluation of MUC5B and MUC5AC protein levels in the cells and in the medium in low adhesion plates. NCI-H292 cells (2×10^4 cells/well) were cultured in adhesive (+) or low adhesive (−) 96-well plates. (a) The cells were cultured for 30 h and sampled. Cells (cells) and culture media (medium) were sampled. The samples were analyzed using the mucin protein assay to detect the levels of MUC5B protein. Fold changes were based on MUC5B levels in the cells (mean ± SD, n = 5, one-way ANOVA); (b) the cells were cultured for 30 h and sampled. Cells (cells) and culture media (medium) were sampled. The samples were analyzed using the mucin protein assay to detect the levels of MUC5AC protein. Fold changes were based on MUC5AC levels in the cells (mean ± SD, n = 5, one-way ANOVA). Fold changes were normalized to cell numbers. Asterisks indicate statistical probability, $^*p < 0.05$ (ANOVA). The representative results of 3 independent experiments are shown.

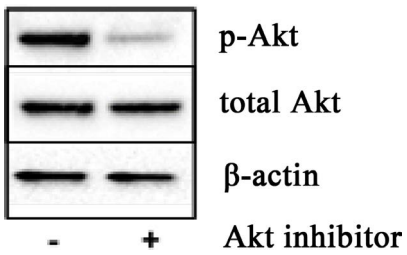

Figure 2. Evaluation of the Akt activity in the cells. NCI-H292 cells (2×10^4 cells/well) were cultured in 96-well plates pretreated with PBS. The cells were cultured with an Akt inhibitor (25 μM: Akt inhibitor: +) or with the same concentration of DMSO (−) in adhesion 96-well plates for 30 h and sampled. The samples were analyzed using western blot analysis to detect the levels of phosphorylated and activated form of p-Akt (p-Akt), total Akt, and *β*-actin. The representative results of 3 independent experiments are shown.

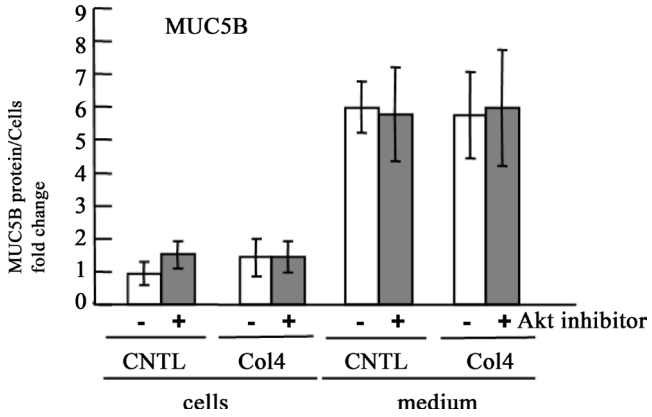

Figure 3. Evaluation of MUC5B protein in NCI-H292 cells on type IV collagen with Akt inhibitor. NCI-H292 cells (2×10^4 cells/well) were cultured in 96-well plates precoated with PBS (CNTL), or with 33 μg/ml of type IV collagen (Col4). The cells were cultured with an Akt inhibitor (25 μM: Akt inhibitor: +) or with the same concentration of DMSO (−) for 30 h and cells (cells) and culture media (medium) were sampled. The samples were analyzed using the mucin protein assay to detect the levels of MUC5B protein. Fold changes were based on CNTL level of MUC5B in cells (mean ± SD, n = 5, one-way ANOVA). The representative results of 3 independent experiments are shown.

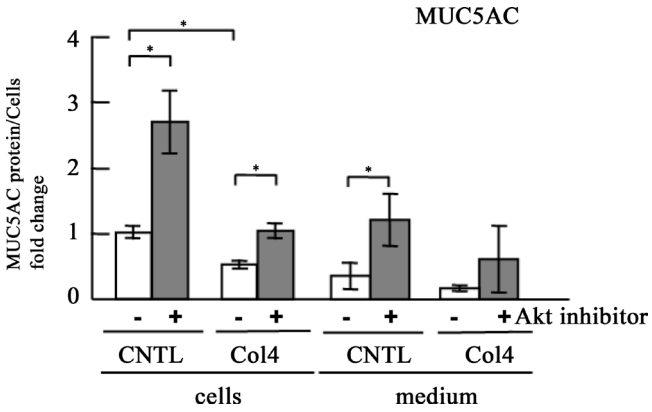

Figure 4. Evaluation of MUC5AC protein in NCI-H292 cells on type IV collagen with Akt inhibitor. NCI-H292 cells (2×10^4 cells/well) were cultured in 96-well plates precoated with PBS (CNTL), or with 33 μg/ml of type IV collagen (Col4). The cells were cultured with an Akt inhibitor (25 μM: Akt inhibitor: +) or with the same concentration of DMSO (−) for 30 h and cells (cells) and culture media (medium) were sampled. The samples were analyzed using the mucin protein assay to detect the levels of MUC5AC protein. Fold changes were based on CNTL level of MUC5AC in cells (mean ± SD, n = 5, one-way ANOVA). The representative results of 3 independent experiments are shown.

4. Discussion

The hypersecretion of airway mucus is a primary feature of certain common respiratory diseases, such as asthma and COPD. Hence, the regulation of mucus secretion is important for the treatment of respiratory diseases. MUC5AC is the major inducer of mucin, which is known to narrow the airway of patients with asthma or COPD. MUC5B is a minor mucin subtype in the asthmatic airway but a major subtype in the healthy airway and indispensable for biodefense [8]. However, MUC5B overproduction induces the narrowing of the airway. Therefore, an adequate level of MUC5B is required to maintain normal function of the airway.

Akt is a key regulator of the integrin pathway and is known to downregulate MUC5AC production. The current study is the first to recognize that the regulation of MUC5B production, in contrast to that of MUC5AC, is unaffected by Akt inhibition. The same results were obtained in cells cultured on type IV collagen or fibronectin (**Figures 2-5**). MUC5B and MUC5AC are both produced in the airway, and the respective coding genes are both located on chromosome 11p15.5, but they have different regulation mechanisms.

The result of the current study showed that low cell attachment induces the upregulation of both MUC5B and MUC5AC (**Figure 1**). Our previous study showed that integrin inhibition induced MUC5AC and MUC5B

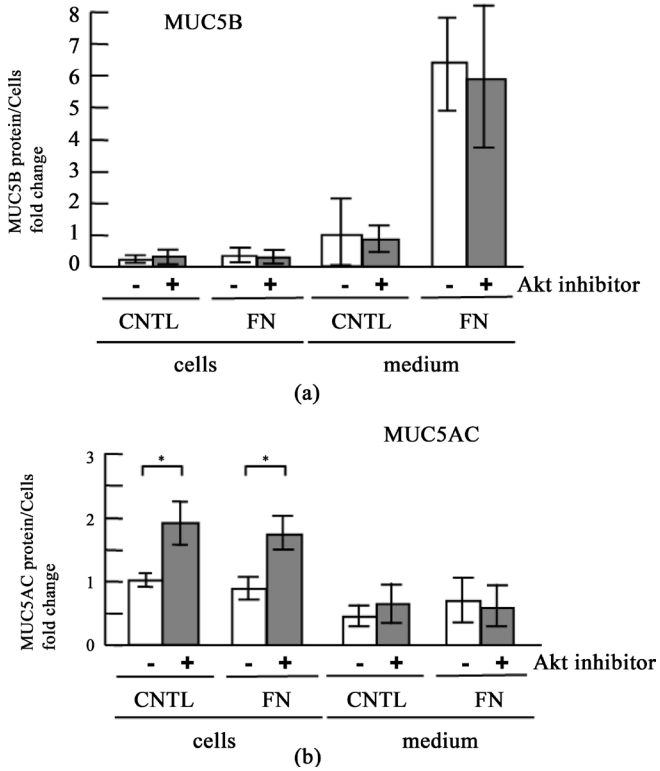

Figure 5. Evaluation of MUC5B and MUCAC protein in NCI-H292 cells on fibronectin with Akt inhibitor. NCI-H292 cells (2×10^4 cells/well) were cultured in 96-well plates precoated with PBS (CNTL), or with 500 μg/ml of fibronectin (FN). The cells were cultured with an Akt inhibitor (25 μM: Akt inhibitor: +) or with the same concentration of DMSO (−) for 30 h and cells (cells) and culture media (medium) were sampled. The samples were analyzed using the mucin protein assay to detect the levels of MUC5B (a) and MUC5AC (b) protein. Fold changes were based on CNTL level of MUC5B in medium and MUC5AC in cells (mean ± SD, n = 5, one-way ANOVA). The representative results of 3 independent experiments are shown.

upregulation [18] [19]. Although low cell attachment was found to induce the downregulation of the integrin pathway, Akt inhibition did not affect MUC5B regulation. These results suggest that MUC5B production is regulated by cell attachment molecules, such as integrins, but not by the Akt pathway. These results also demonstrate that the regulation of MUC5B production is initiated from a different point from that of MUC5AC, suggesting separate regulatory pathways. These results provide important data for the development of agents to control Akt activation to maintain MUC5B levels, along with decreasing MUC5AC levels to avoid the narrowing of the airway in patients with asthma or COPD.

5. Conclusion

The present study revealed a regulation mechanism of MUC5B production in human lung epithelial NCI-H292 cells. The production of both MUC5B and MUC5AC was upregulated by low cell attachment. However, the regulation of MUC5B production was unaffected by Akt inhibition, whereas MUC5AC production was downregulated by Akt. These results may be useful to maintain MUC5B levels, along with decreasing MUC5AC levels, in the treatment of asthma or COPD.

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Conflict of Interest

Authors have no conflict of interest.

References

[1] Fahy, J.V. and Dickey, B.F. (2010) Airway Mucus Function and Dysfunction. *The New England Journal of Medicine*, **363**, 2233-2247. http://dx.doi.org/10.1056/NEJMc1014719

[2] Button, B., Cai, L.H., Ehre, C., Kesimer, M., Hill, D.B., Sheehan, J.K., Boucher, R.C. and Rubinstein, M. (2012) A Periciliary Brush Promotes the Lung Health by Separating the Mucus Layer from Airway Epithelia. *Science* (*New York, N.Y.*), **337**, 937-941. http://dx.doi.org/10.1126/science.1223012

[3] Rose, M.C. and Voynow, J.A. (2006) Respiratory Tract Mucin Genes and Mucin Glycoproteins in Health and Disease. *Physiological Reviews*, **86**, 245-278. http://dx.doi.org/10.1152/physrev.00010.2005

[4] Fahy, J.V. (2002) Goblet Cell and Mucin Gene Abnormalities in Asthma. *Chest*, **122**, 320S-326S. http://dx.doi.org/10.1378/chest.122.6_suppl.320S

[5] Lai, H.Y. and Rogers, D.F. (2010) Mucus Hypersecretion in Asthma: Intracellular Signalling Pathways as Targets for Pharmacotherapy. *Current Opinion in Allergy and Clinical Immunology*, **10**, 67-76. http://dx.doi.org/10.1097/ACI.0b013e328334643a

[6] Davies, J.R., Svitacheva, N., Lannefors, L., Kornfalt, R. and Carlstedt, I. (1999) Identification of MUC5B, MUC5AC and Small Amounts of MUC2 Mucins in Cystic Fibrosis Airway Secretions. *The Biochemical Journal*, **344**, 321-330. http://dx.doi.org/10.1042/bj3440321

[7] Chen, Y., Zhao, Y.H., Di, Y.P. and Wu, R. (2001) Characterization of Human Mucin 5B Gene Expression in Airway Epithelium and the Genomic Clone of the Amino-Terminal and 5'-Flanking Region. *American Journal of Respiratory cell and Molecular Biology*, **25**, 542-553. http://dx.doi.org/10.1165/ajrcmb.25.5.4298

[8] Roy, M.G., Livraghi-Butrico, A., Fletcher, A.A., McElwee, M.M., Evans, S.E., Boerner, R.M., Alexander, S.N., Bellinghausen, L.K., Song, A.S., Petrova, Y.M., Tuvim, M.J., Adachi, R., Romo, I., Bordt, A.S., Bowden, M.G., Sisson, J.H., Woodruff, P.G., Thornton, D.J., Rousseau, K., De la Garza, M.M., Moghaddam, S.J., Karmouty-Quintana, H., Blackburn, M.R., Drouin, S.M., Davis, C.W., Terrell, K.A., Grubb, B.R., O'Neal, W.K., Flores, S.C., Cota-Gomez, A., Lozupone, C.A., Donnelly, J.M., Watson, A.M., Hennessy, C.E., Keith, R.C., Yang, I.V., Barthel, L., Henson, P.M., Janssen, W.J., Schwartz, D.A., Boucher, R.C., Dickey, B.F. and Evans, C.M. (2014) Muc5b Is Required for Airway Defence. *Nature*, **505**, 412-416. http://dx.doi.org/10.1038/nature12807

[9] Song, K.S., Lee, W.J., Chung, K.C., Koo, J.S., Yang, E.J., Choi, J.Y. and Yoon, J.H. (2003) Interleukin-1 Beta and Tumor Necrosis Factor-Alpha Induce MUC5AC Overexpression through a Mechanism Involving ERK/p38 Mitogen-Activated Protein Kinases-MSK1-CREB Activation in Human Airway Epithelial Cells. *The Journal of Biological Chemistry*, **278**, 23243-23250. http://dx.doi.org/10.1074/jbc.M300096200

[10] Iwashita, J., Ose, K., Ito, H., Murata, J. and Abe, T. (2011) Inhibition of E-Cadherin Dependent Cell-Cell Contact Promotes MUC5AC Mucin Production through the Activation of Epidermal Growth Factor Receptors. *Bioscience, Biotechnology, and Biochemistry*, **75**, 688-693. http://dx.doi.org/10.1271/bbb.100830

[11] Iwashita, J., Hongo, K., Ito, Y., Abe, T. and Murata, J. (2013) Regulation of MUC5AC Mucin Production by the Cell Attachment Dependent Pathway Involving Integrin b1 in NCI-H292 Human Lung Epithelial Cells. *Advances in Biological Chemistry*, **3**, 1-10.

[12] Kettle, R., Simmons, J., Schindler, F., Jones, P., Dicker, T., Dubois, G., Giddings, J., Van Heeke, G., and Jones, C.E. (2010) Regulation of Neuregulin 1beta1-Induced MUC5AC and MUC5B Expression in Human Airway Epithelium. *American Journal of Respiratory Cell and Molecular Biology*, **42**, 472-481. http://dx.doi.org/10.1165/rcmb.2009-0018OC

[13] Bernacki, S.H., Nelson, A.L., Abdullah, L., Sheehan, J.K., Harris, A., Davis, C.W. and Randell, S.H. (1999) Mucin Gene Expression during Differentiation of Human Airway Epithelia *in Vitro*. Muc4 and muc5b Are Strongly Induced. *American Journal of Respiratory Cell and Molecular Biology*, **20**, 595-604. http://dx.doi.org/10.1165/ajrcmb.20.4.3442

[14] Koo, J.S., Yoon, J.H., Gray, T., Norford, D., Jetten, A.M. and Nettesheim, P. (1999) Restoration of the Mucous Phenotype by Retinoic Acid in Retinoid-Deficient Human Bronchial Cell Cultures: Changes in Mucin Gene Expression. *American Journal of Respiratory Cell and Molecular Biology*, **20**, 43-52. http://dx.doi.org/10.1165/ajrcmb.20.1.3310

[15] Gray, T., Koo, J.S. and Nettesheim, P. (2001) Regulation of Mucous Differentiation and Mucin Gene Expression in the Tracheobronchial Epithelium. *Toxicology*, **160**, 35-46. http://dx.doi.org/10.1016/S0300-483X(00)00455-8

[16] Chen, Y., Thai, P., Zhao, Y.H., Ho, Y.S., DeSouza, M.M. and Wu, R. (2003) Stimulation of Airway Mucin Gene Expression by Interleukin (IL)-17 through IL-6 Paracrine/Autocrine Loop. *The Journal of Biological Chemistry*, **278**, 17036-17043. http://dx.doi.org/10.1074/jbc.M210429200

[17] Casalino-Matsuda, S.M., Monzon, M.E., Day, A.J. and Forteza, R.M. (2009) Hyaluronan Fragments/CD44 Mediate Oxidative Stress-Induced MUC5B Up-Regulation in Airway Epithelium. *American Journal of Respiratory Cell and Molecular Biology*, **40**, 277-285.

[18] Iwashita, J., Yamamoto, T., Sasaki, Y. and Abe, T. (2010) MUC5AC Production Is Downregulated in NCI-H292 Lung

Cancer Cells Cultured on Type-IV Collagen. *Molecular and Cellular Biochemistry*, **337**, 65-75. http://dx.doi.org/10.1007/s11010-009-0286-z

[19] Ito, Y., Iwashita, J., Kudoh, A., Kuramata, C. and Murata, J. (2015) MUC5B Mucin Production Is Upregulated by Fibronectin and Laminin in Human Lung Epithelial Cells via the Integrin and ERK Dependent Pathway. *Bioscience, Biotechnology, and Biochemistry*, **79**, 1794-1801. http://dx.doi.org/10.1080/09168451.2015.1052769

[20] Heino, J. and Kapyla, J. (2009) Cellular Receptors of Extracellular Matrix Molecules. *Current Pharmaceutical Design*, **15**, 1309-1317. http://dx.doi.org/10.2174/138161209787846720

[21] De Arcangelis, A. and Georges-Labouesse, E. (2000) Integrin and ECM Functions: Roles in Vertebrate Development. *Trends in Genetics*: *TIG*, **16**, 389-395. http://dx.doi.org/10.1016/S0168-9525(00)02074-6

[22] Roskelley, C.D., Srebrow, A. and Bissell, M.J. (1995) A Hierarchy of ECM-Mediated Signalling Regulates Tissue-Specific Gene Expression. *Current Opinion in Cell Biology*, **7**, 736-747. http://dx.doi.org/10.1016/0955-0674(95)80117-0

[23] Iwashita, J., Ito, Y., Yokoo, M., Takahashi, S. and Murata, J. (2014) Akt Induces Down Regulation of MUC5AC Production in NCI-H292 Human Airway Epithelial Cells Cultured on Extracellular Matrix. *Bioscience, Biotechnology, and Biochemistry*, **78**, 212-221. http://dx.doi.org/10.1080/09168451.2014.877829