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Wound Repair and Proliferation of Bronchial Epithelial Cells Regulated by CTNNAL1

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Abstract Adhesion molecules play vital roles in airway hyperresponsiveness (AHR) or airway inflammation. Our previous study indicated that adhesion molecule catenin alpha-like 1 (CTNNAL1) is relevant closely to asthma susceptibility, but its biological function or significance is still unclear. In the present study, we observed the temporal and spatial distribution of CTNNAL1 expression in mouse lung tissue with the OVA-sensitized asthma model and found that the level of CTNNAL1 mRNA showed a prominent negative correlation with pulmonary resistance (R_L). To study the function of CTNNAL1 in airway, effects of CTNNAL1 on proliferation and wound repair activity of human bronchial epithelial cells (HBEC) was investigated with antisense oligonucleotide (ASO) technique. The results showed that: (1) CTNNAL1 ASO could decelerate the repairing velocity and proliferation of HBEC; (2) CTNNAL1 expression was increased on the edge cells of mechanic wounded area in culture; (3) extracellular matrix component fibronectin (Fn) obviously promoted wound repair activity and proliferation of HBEC, which could be blocked by CTNNAL1 ASO; (4) Western blot showed that Fn could promote FAK phosphorylation, which also be inhibited by CTNNAL1 ASO. In conclusion, the level of CTNNAL1 mRNA expression is highly correlated to airway resistance; CTNNAL1 may contribute to the wound repair and proliferation of HBEC. Furthermore, it may serve to Fn mediated cell-extracellular adhesion and its signal transduction. J. Cell. Biochem. 103: 920–930, 2008.

Key words: CTNNAL1; bronchial epithelial cells (BEC); asthma

In airway hyperresponsive diseases such as asthma, the bronchial epithelium is frequently desquamated, leaving areas of denuded basement membrane, under which submucosal terminal sensory nerve are directly exposed to inhaled air and the sensitivity of sensory nerve increase aberrantly [Montefort et al., 1992]. The epithelium both provides a physical barrier to the external environment and regulates several metabolic functions of airways, including fluid and ion transport to the airway lumen, mucociliary clearance, and airway diameter. Reconstitution of a functional epithelia barrier requires an ordered sequence of events, including cell spreading and migration, adhesion, production of provisional matrix, proliferation, and differentiation into needed epithelial cell subtypes. Each of these processes may depend on adequate expression and distribution of adhesive molecules on epithelial cells. HBEC express four kind of adhesive molecules, including cadherin, integrin, ICAM-1, and selectin. In normal state, HBEC express intergrins and cadherins, which mediate and maintain intercellular homologous and heterogeneous adhesions. In injury state, HBEC can arrest leukocytes, evoke an inflammation reaction in airway by expressing selectins and intercellular adhesive molecule-1 (ICAM-1) [Campbell et al., 1994].

Yang Xiang and Yu-Rong Tan contributed equally to this work.

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Since the adhesion molecule family has many types with diverse functions, the issue of what homeostasis is maintained with regard to cells' constitutive adhesion and the inflammation adhesion mechanism is far from being clear. Therefore, we hypothesized that the defect in structures and functions of airway epithelium may be the initiator of AHR. The imbalance of the adhesion molecules expression may contribute to the structural defect or functional disorder on airway epithelium and therefore be associated with asthma pathogenicity. In order to confirm the hypothesis, this present study used a cDNA microarray to screen the differential expression of adhesion molecules in human peripheral blood leukocytes, and found that an alpha-catenin-related protein, catenin alpha-like 1 (CTNNAL1) was downregulated in asthma patients, which was further confirmed by Real time PCR (These results will be published in an other article). However, the expression of CTNNAL1 in airway and the function associated with airway epithelium are remained unknown.

CTNNAL1 was first characterized as a 2.45-kb transcript that was down-regulated in human pancreatic cancer cells [Zhang et al., 1998]. CTNNAL1 shows extensive homology to α-catenin, a vinculin family member that plays a crucial role in cell differentiation and cancer invasion. The human CTNNAL1 gene maps to chromosome 9q31–q32, a region where frequent alterations were observed in several types of tumors including bladder, ovarian, and esophageal cancer. CTNNAL1 mRNA is expressed ubiquitously, although the levels are lower in neural tissues [Janssens et al., 1999]. Even though a functional role has not been assigned to CTNNAL1, its extensive homology to α-catenin, and its chromosomal localization make it an intriguing candidate for further study relative to cell differentiation, growth and motility.

As of now, there have no papers published about the expression of CTNNAL1 in adult lungs and whether it is involved in wound repair or cell proliferation during airway inflammation or airway hyperresponsiveness (AHR). The aim of this study is to investigate the temporal and spatial distribution of CTNNAL1 mRNA in a well-characterized Ovalbumin (OVA)-sensitized Balb/c mice asthma model. CTNNAL1 antisense oligonucleotide (ASO) was used to observe the effect of CTNNAL1 on the repair and proliferation of HBEC.

CTNNAL1 is a member of the vinculin superfamily of proteins, which includes both α-catenin and vinculin. Vinculin is present both in cadherin–catenin adhesion complexes and in focal adhesion complexes that mediate cell-extracellular matrix adhesion. As vinculin proteins are linked via talin to integrins, which are transmembrane receptors for the extracellular matrix such as fibronectin (Fn) [Weiss et al., 1998], and the linkage of Fn receptor proteins to the actin cytoskeleton are required for proper tissue differentiation and homeostasis, we also detect the effect of CTNNAL1, a member of vinculin family, on Fn mediated wound repair, cell proliferation, and focal adhesion kinase phosphorylation.

MATERIALS AND METHODS

Animal Model of Asthma

Balb/c mice were obtained from the experimental animal center, XiangYa Medical College, Central South University. All mice were housed under specific pathogen-free conditions and had ad libitum access to food and water.

Asthma models were induced by intraperitoneal injection with 50 μg of OVA (grade V; Sigma, St. Louis, MO) and 1 mg aluminum hydroxide (EM Industries, Hawthorne, NY) on day 0, and then boost with a second injection 7–10 days later. On day 21, we challenge the animals via the intratracheal route with 50 μg of antigen in 30 μl aliquots. We repeat the challenge on days 22 and 23. This schedule and dose pattern allows optimal responses in both inflammatory infiltrate and AHR [Isenberg-Feig et al., 2003; Nikolaidis et al., 2003]. Thirty mice were randomly divided into five experiment groups and one control group. Control mice were sensitized or challenged with saline. Airway parameters measurement and lung tissues collection were performed on days 24, 26, 30, 32 and 34, corresponding to the 1, 2, 4, 6, and 8 days after the last challenge, respectively.

Measurement of Airway Responsiveness In Vivo

Airway responsiveness was measured using barometric whole-body plethysmography by recording airflow and respiratory pressure curves (Buxco; EMKA Technologies, Paris, France) in response to inhaled histamine (Sigma).

Airway responsiveness was expressed in pulmonary resistance (R_L), which was determined by multiple linear regressions from transpulmonary pressure and airflow.

Briefly, mice were placed in a whole-body chamber, and basal readings were obtained and averaged for 3 min. Aerosolized saline, followed by histamine (1.28 mg/ml), were nebulized for 3 min, and readings were taken and averaged for 3 min after each nebulization.

Cells Culture

The 16HBE14o$^-$cells, a generous gift of Dr. Dieter Gruenert, University of California San Francisco, were SV_{40}-transsformed human central airway epithelial cells. Cells were cultured in a mixture medium of DMEM: F12 (1:1) (Sigma) containing 100 U/ml penicillin, 100 U/ml streptomycin, and 10% fetal bovine serum (Hangzhou Sijiqing Biotech., Hangzhou, China) and incubated at 37°C in 5% CO_2.

Real Time PCR Measurement for Expression of CTNNAL1 mRNA in Lung Tissues and Cultured HBEC

RNA was extracted from mouse lung tissues and cultured HBEC and reverse transcription was performed by *AMV* reverse transcriptase (QIAGEN, Germany). PCR was then carried out using primers and taqman probes (TaKaRa, China). Taqman probes were labeled at their 5′-end with the reporter dye 5-carboxyfluorescein (FAM) and at their 3′-end with the quencher 6-carboxylelra-methyl rhodamine (TAMRA). The primers and taqman probes were synthesized as Table I. Briefly, 1 μl of the reverse-transcripts was added to a 30 μl PCR mixture for 40 cycles. Each cycle included 93°C for 30 s, 60°C for 60 s by using Taq polymerase. Negative controls consisted of an equal volume of water substituted for the volume of RNA in the RT reaction. Normalization of mRNA expression data for sample-to-sample variability in RNA input, RNA quality, and reverse transcription efficiency was achieved by comparing the copy numbers of target mRNAs with that of mouse or human GAPDH for each run.

TABLE I. Oligonucleotide Primers Used for Real-Time PCR Analysis

Sequence of primers
CTNNAL1-human
Forward 5′-TCTCGCAACTATGGAAAG-3′
Reverse 5′-GTAGGCAGAATCAGTAAAG-3′
FAM+AACTGCGAATCAGCCCAT+TAMRA
GAPDH-human
Forward 5′-CCACTCCTC CACCTTTGAC-3′
Reverse 5′-ACCCTGTTGCTGTAGCCA-3′
FAM+TTGCCCTCAACGACCACTTTGTC+TAMRA
CTNNAL1-mouse
Forward 5′-TCTTCGGGAGAATGTTTGCTT-3′
Reverse 5′-TGTGCTCGTGGCTGGTGTAG-3′
FAM+CCGCATTGGAGGCCGTCTTG+TAMRA
GAPDH-mouse
Forward 5′-TGTGTCCGTCGTGGATCTGA-3′
Reverse 5′-CCTGCTTCACCACCTTCTTGA-3′
FAM+ CCGCCTGGAGAAACCTGCCA
AGTATG+ TAMRA

In Situ Hybridization (ISH) Detection for CTNNAL1 mRNA in Cultured HBEC and Lung Tissue

The following probes labeled 5-end with digoxin (purchased from TaKaRa Biotechnology Company) were used for ISH to localize mouse CTNNAL1 mRNA: 5′-ATCACCACACTTATAAACCATAAAGATAATACCAA-3′, 5′-GCATACATGCAGAGGAGACATTTCAGGTGACTGGC-3′, 5′-ATGTCCAGGATGGCCTATTCTCTGTATTTATTTAC-3′, or human CTNNAL1 mRNA: 5′-ACCTTCCGGAGAAGCCCCAGGAAGACATTA-3′, 5′-ATGGAAGGGGTCTGAGAAGAGATAAGGGCG-3′, 5′-GATCAAAACTCGCTCGGTGGAGCAGACGCT-3′. Briefly, the lung tissues were fixed in 4% paraformaldehyde, embeded in paraffin, cut into 6 mm paraffin sections and placed on slides, the coverglasses with HBEC on them were fixed in 4% paraformaldehyde. They were incubated with 30%H_2O_2 and 5% pepsin in turn and then hybridized at 42°C overnight with labeled probes (2.0 g/ml). After washing, the slides and coverglasses were sequentially incubated with blocking buffer, biotin labeled rabbit-anti-digoxin antibody, streptavidin biotin peroxidase complex (sABC) and biotin labeled peroxidase, followed by rinsing with PBS after every step. The peroxidant activity was visualized by the 3, 3-diaminobenzidine tetrahydrochloride (DAB). The coverglasses were incubated with normal mouse serum instead of the mouse-anti-digoxin antibody in absence of the labeled probe for the negative control.

Antisense Oligonucleotide (ASO) of CTNNAL1

CTNNAL1 ASO was designed according to the human CTNNAL1 mRNA sequence (nucleotides 1–20 from ATG, AUGGCCGCCUCUCCCGGACC). The ASO was synthesized at TaKaRa Biotechnology Company as a 20-base phosphorothioate chemic oligonucleotides, where bases

1–5 and 16–20 were modified with 2′-O-(2-methoxy)-ethyl. CTNNAL1 ASO was transfected into cells with lipofectin (Sigma) for 4 h in serum-free DMEM. Then the ASO reaction mixture was replaced with normal growth media (with 10% FBS) and the cells were incubated under normal conditions for an additional 16–20 h. The efficacy of the ASO in inhibiting the activity of CTNNAL1 was verified by real time PCR. A nonsense oligonucleotide (5′-TCTGGACTGTC-CCCCATTTC-3′) was used as control.

Monolayer Wound Repair Assay

This assay was used to demonstrate the effect of CTNNAL1 ASO on epithelial cell migration and wound repair. We have previously published details of this method [Guan et al., 2006; Tan et al., 2006]. Briefly, 16HBE14o$^-$ cells were grown until confluent in 12-well plates with DMEM: F12 (1:1), and a small wound was made in the confluent monolayer with a rubber stylet. The edge of wound was recognized and the remaining wound area was measured serially per 4 h in 24 h by video microscopy (Olympus Company, Japan). A linear regression equation of the remaining wound area to time was obtained. Repair index (RI), equal to the absolute value of slope, was used to judge the repair speed of HBEC. HBEC were pretreated with CTNNAL1 ASO (0.4, 0.8, 1.0 μM) or Fn (10 μg/ml, Sigma) or both. A positive control, 10 μg/ml EGF, was done at the same time to ensure valid experiments.

Proliferation Assay

The cells were trypsinized in a 0.25% trypsin solution and seeded in a 96-well plate at a density of 10^4 cells (0.1 ml/well). After the cells grown for 24 h to approximately 80% subconfluent state, 0.1 ml serum-free medium was added to each well to synchronize the growth of cells for 24 h. Then EGF (10 μg/ml), Fn (10 μg/ml), CTNNAL1 ASO (0.4, 0.8, 1.0 μM) or both of Fn and CTNNAL1 ASO were added to each well and incubated for another 24 h. Each treatment was tested in at least six wells. Next, 15 μl of 0.5% MTT solution was added to each well and incubated for 4 h. Then, the medium and MTT were removed and 150 μl of DMSO was added to each well. The mixture was shaken for 10 min to dissolve the crystal. The OD of each well solution was determined at 570 nm by using an ELISA reader.

Western Blot Analysis for FAK Phosphorylation

HBE cells(5×10^6) were lysed in ice-cold cell lysis buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris-HCl (pH 7.4), 2 mM sodium orthovanadate, 10 μg/ml leupeptin, 50 mM NaF, 5 mM EDTA, 1 mM EGTA, and 1 mM PMSF) by stirring for 1 h at 4°C. The lysate was obtained after a centrifugation at 13,000*g* for 15 min. An equal volume of Laemmli sample buffer was added to each cell lysate. Samples were boiled for 10 min, and then equal amounts of protein were separated by 7.5% SDS–PAGE before being transferred to nitrocellulose membrane. The membrane were blocked with 3% BSA in PBS for 2 h and then incubated with the anti-FAK mAb or anti-phospho-FAK mAb. Following incubation with the appropriate horseradish peroxidase-conjugated secondary antibody, detection was made using the enhanced chemiluminescene system.

Statistical Analyses

The data were analyzed using unpaired Student's *t*-test. Values were expressed by mean ± SE. $P < 0.05$ was considered as statistically significant.

RESULTS

The Expression of CTNNAL1 mRNA in Mouse Lung Tissue

To fully understand the dynamic changes of CTNNAL1 expression during the development of asthma, we observed the temporal and spatial distribution of CTNNAL1 expression in mouse lung tissue with the OVA-sensitized asthma model.

In the normal group, the structure of bronchi walls and pulmonary alveoli were intact and regular, which showed no sign of inflammationary infiltration, mucosal exudation, and cavitary stricture. However, inflammationary infiltration, mucosal exudation, cavitary stricture, and bronchial epithelial denudation were observed in asthma groups.

In situ hybridization showed that CTNNAL1 mRNA was predominantly localized in the ciliated columnar epithelium of bronchioles (Fig. 1A), secretory cells of terminal bronchioles (Fig. 1B), vascular endothelial cells (Fig. 1C) and alveolar cells (Fig. 1D).

Real time PCR showed that the expression of CTNNAL1 mRNA was significantly down

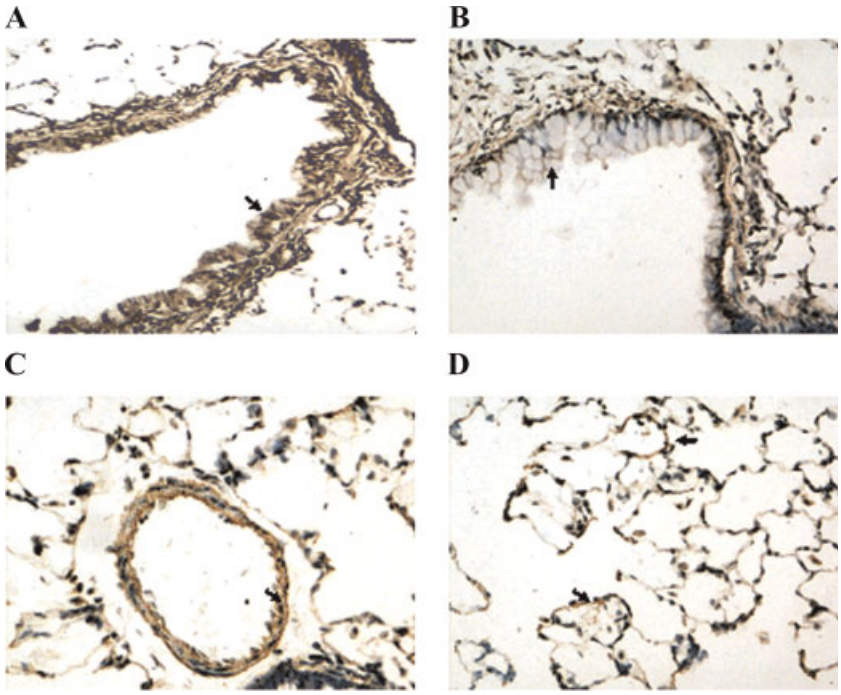

Fig. 1. Distribution of CTNNAL1 by in situ hybridization for mRNA expression. CTNNAL1 mRNA was predominantly localized in the ciliated columnar epithelium of bronchioles (**A**), secretory cells of terminal bronchioles (**B**), vascular endothelial cells (**C**) and alveolar cells (**D**). Arrows indicate positive staining in cell types referred to in the four panels (×100). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

regulated in the asthma model($P < 0.01$), and then began to increase to the baseline in the sixth day after the last OVA challenge (Fig. 2A).

Airway Responses

To verify that hyperresponsiveness was induced in OVA-sensitized mice, airway responsiveness to aerosolized histamine was measured in each group. Figure 2B shows the time course for changes in airway resistance after histamine challenge in control and OVA-sensitized mice. The value of pulmonary resistance (R_L) for the OVA-sensitized mice was higher than the control mice. Thus, OVA-challenged mice show airway hyperresponsiveness in vivo. Among the OVA-sensitized groups, R_L peaked in the second day after the last OVA challenge, and then began to decrease in the sixth day after the last OVA challenge obviously (Fig. 2B). The level of CTNNAL1 mRNA showed a prominent negative correlation with R_L ($r = -0.756$, $P = 0.004$; Fig. 2C).

The Expression of CTNNAL1 mRNA in Cultured HBEC

The CTNNAL1 mRNA expression was determined by Real time PCR. It was shown that CTNNAL1 mRNA was detectable in unstressed HBEC and CTNNAL1 ASO (0.4, 0.8, and 1.0 μM, respectively) could decrease the expression of CTNNAL1 in a concentration-dependent manner, while nonsense oligonucleotide (1.0 μM) had no effect (Fig. 3).

Effects of CTNNAL1 ASO on Wound Repair and Proliferation of HBEC

A small wound was mechanically made in each well with an area of approximate 2.713 ± 0.032 mm^2. The remaining wound area in the control group was decreased to $45.43 \pm 7.67\%$ of the initial area 24 h later (Fig. 4A). Satisfactory correlation was detectable between the time and the remaining wound area ($r = -0.967$, $P < 0.01$), with the repair index (RI) as 1.34. The repair of HBEC was accelerated by EGF (RI, 1.77) and slowed by CTNNAL1 ASO (1.0 μM, RI, 1.12, $P < 0.05$; Fig. 4A). Proliferation of HBEC measured by MTT showed that pretreatment with EGF could promote the proliferation of HBEC. CTNNAL1 ASO could obviously inhibit the proliferation of HBEC (Fig. 4B). Inhibition of wound closure and proliferation was concentration-dependent but was not complete.

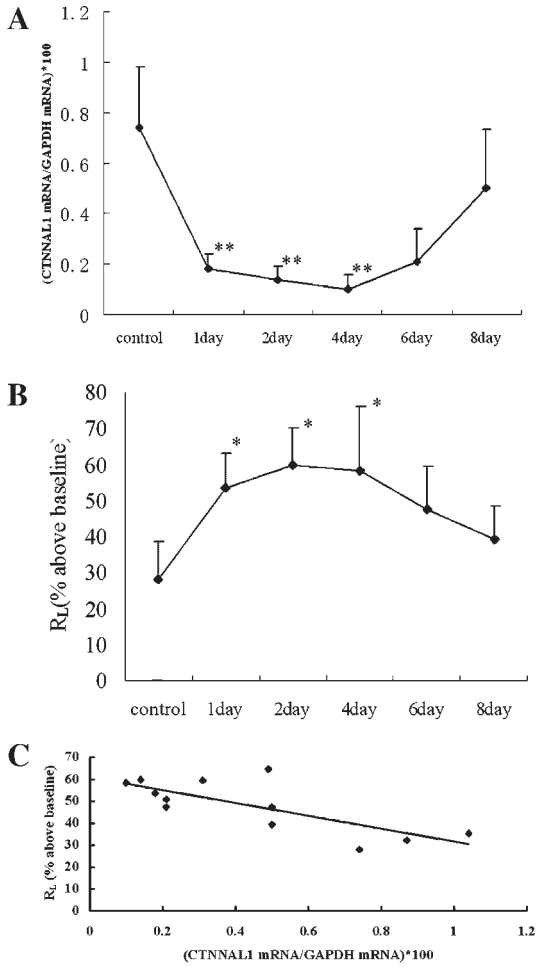

Fig. 2. The relationship of CTNNAL1 expression and airway response. **A**: Real time PCR showed the time course of CTNNAL1expression in mice lung tissue. Group 1 day, 2 days, 4 days, 6 days, and 8 days corresponding to the 1, 2, 4, 6, and 8 days after the last challenge respectively. The result showed that the expression of CTNNAL1 mRNA was significantly down regulated in the asthma model($P < 0.01$), and then began to increase to the baseline in the sixth day after the last OVA challenge (n = 5, $^{**}P < 0.01$). **B**: The time course for changes in pulmonary resistance after histamine challenge in control and OVA-sensitized mice. The value of R_L for the OVA-sensitized mice was higher than the control mice. Thus, OVA-challenged mice show airway hyperresponsiveness in vivo. Among the OVA-sensitized groups, R_L peaked in the second day after the last OVA challenge, and then began to decrease in the sixth day after the last OVA challenge obviously. (n = 5,$^{*}P < 0.05$). **C**: The correlation of CTNNAL1 mRNA level and pulmonary resistance (R_L).There is a negative correlation between them ($r = -0.756$, $P = 0.004$).

CTNNAL1 Expression on the Edge of Cells in Wounded Cell Culture

To clarify the biological role of CTNNAL1 in repair and proliferation, examination of wound edges creation in monolayer for CTNNAL1 expression by in situ hybridization was performed. The results showed that few expression of CTNNAL1 mRNA was detectable in the initial wound area, but its expression explicitly increased on the cells in wound edges 18 h after creation (Fig. 5).

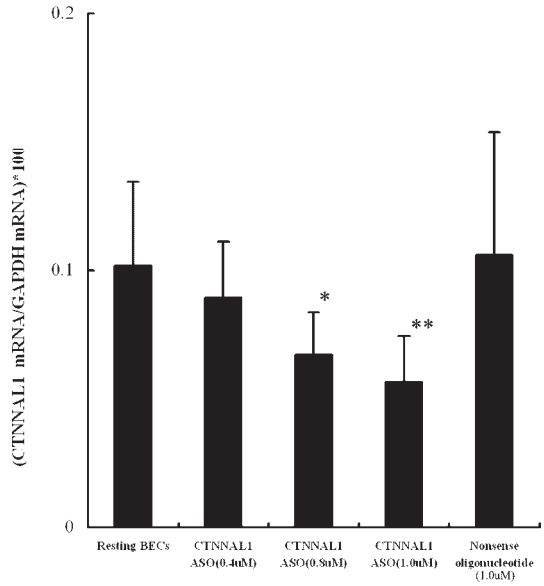

Fig. 3. The expression of CTNNAL1 on HBEC assayed by real time PCR. Real time PCR showed the expression of CTNNAL1 on a human epithelial cell line, 16HBE14o-. CTNNAL1 ASO (0.4, 0.8, 1.0 μM, respectively) could inhibit CTNNAL1 expression in a concentration-dependent manner, while nonsense oligonucleotide(1.0 μM) had no effect (n = 3, $^{*}P < 0.05$ vs. control; $^{**}P < 0.01$ vs. Control).

CTNNAL1 ASO Inhibited Fn Mediated Wound Repair and Proliferation of HBEC

The results showed that Fn obviously promoted wound repair and proliferation of HBEC. HBEC incubated with 1.0 μM of CTNNAL1 ASO for 30 min blocked the acceleration of both wound repair and proliferation induced by Fn (Fig. 6A,B).

Effects of CTNNAL1 ASO on FAK Phosphorylation

Western blot showed that Fn could promote FAK phosphorylation, which reached the peak in 30 min, then weakened and disappeared 90 min later (Fig. 7A). CTNNAL1 ASO alone had no effects on FAK phosphorylation. However, it could inhibit the effects induced by Fn (Fig. 7B).

DISCUSSION

Destruction in airway epithelium structure and integrity, which is often induced by flaws of constitutive adhesion, is the essential link

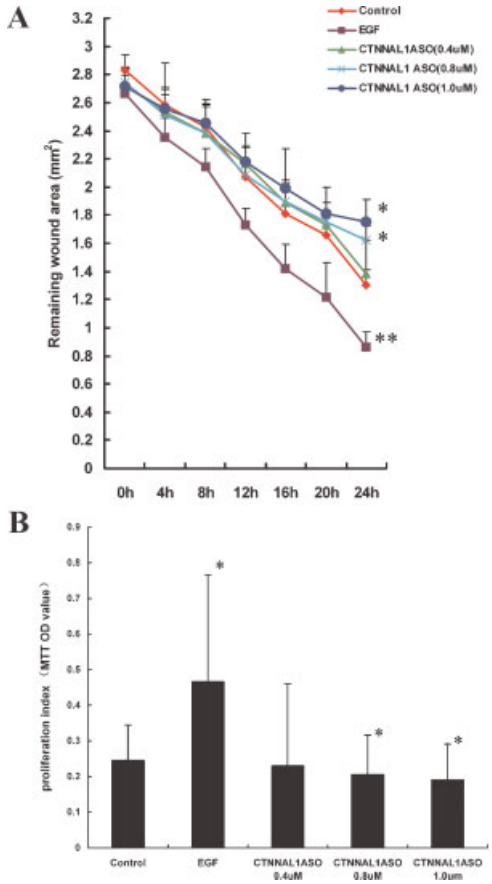

Fig. 4. The effects of CTNNAL1 ASO treatments on HBEC wound repair (**A**) and proliferation (**B**). EGF could promote HBEC wound repair and proliferation. CTNNAL1 ASO (0.4, 0.8, 1.0 μM, respectively) could partly inhibit the HBEC wound repair and proliferation ($n = 18$, $^{**}P < 0.01$ vs. control, $^{*}P < 0.05$ vs. Control). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

of asthma pathogenicity. Therefore it can be reasonably speculated that abnormal expression of adhesive molecules in airway epithelial cells, which might be possibly due to either correspond coding gene alteration or imbalance expression among various kind of adhesion molecules, contribute to aberrant susceptibility to asthma. Based on the result of asthma-associated adhesion molecules expression spectrum, CTNNAL1, whose expression was down regulated in asthma patients, was selected as a candidate in this present study for a further functional investigation. Using a well-characterized OVA-sensitized Balb/c mice asthma model, we carried out a time course study of the expression of CTNNAL1 in lung following the AHR progression. Our data provide a dynamic picture of CTNNAL1 expression and airway responses. For the first time we demonstrated that, CTNNAL1 may participate in the wound repair and proliferation of HBEC in vitro. Our data indicates that the down regulation of CTNNAL1 expression might contribute to the asthma development.

CTNNAL1 is firstly identified and chromosomal localized by Zhang et al. [1998]. It is a novel protein homologous to α-catenin and its function remains unclear.

α-catenin is essential for cadherin adhesive activity which forms functional cell–cell adhesion and links cadherin complexes to the actin cytoskeleton, so that is able to provide flexible dynamic adhesion during wound repair of adult tissues and embryonic development [Tepass, 2002; Perez-Moreno et al., 2003]. In bronchial epithelium, expression of α-catenin was noted at cell–cell borders in both bronchial pseudostratified columnar and basal cells at relatively high levels. Reduced expression of α-catenin was closely correlated with an atypical grade of dysplasia in the basal layer and was observed in squamous cell carcinoma [Pirinen et al., 2001; Kato et al., 2005].

With 734 amino acids, the predicted CTNNAL1 polypeptide is smaller than α-catenin. It is not yet known whether CTNNAL1 localizes to cell–cell borders or is a component of adherens junctions. However, the amino-terminal amphipathic helices of α-catenin that are thought to ensure an interaction with β-catenin are also present in CTNNAL1. Furthermore, amphipathic helices in the carboxy-terminal part of vinculin and α-catenin, which allow these proteins to bind to the actin cytoskeleton, are also present in CTNNAL1 [Park, 2002]. Therefore, sequence homology between CTNNAL1 and α-catenin or vinculin implies that CTNNAL1, whose function was not described previously, might have the potential to mediate cell–cell adhesion.

Here we showed that mRNA level of CTNNAL1 was decreased in our asthma animals. There was a negative correlation between the pulmonary resistance (R_L) in asthma mice and the levels of CTNNAL1 mRNA in the 8 days' time course after the last OVA challenge. It is conceivable that CTNNAL1 contribute to BEC constitutive adhesion. The down regulation of it might hinder the maintenance of epithelium integrity in stressed condition. It was convinced that the integrity of airway epithelium in asthmatic patients or normal people is significantly relevant with airway responsiveness

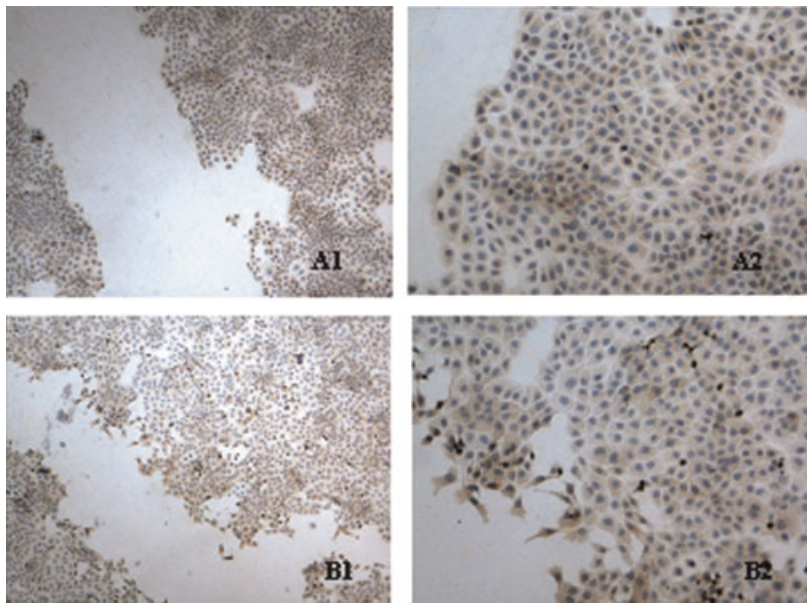

Fig. 5. CTNNAL1 expression on the edge of cells in wounded cell culture. Few expression of CTNNAL1 mRNA was detectable in the initial wound area (**A1**, 50×; **A2**, 200×), but its expression was explicitly increased on the cells in wound edges 18 h after creation (**B1**, 50×; **B2**, 200×). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

[Takizawa, 2005]. The reduction of CTNNAL1 could account for the observed augmentation of the R_L.

In order to verify the role of CTNNAL1 in BEC constitutive adhesion, we performed in vitro experiments to get further understanding of associated functions of CTNNAL1 in airway.

Here we report the expression of CTNNAL1 on a human epithelial cell line, 16HBE14o-, by in situ hybridization and real time PCR, and the expression was obviously decreased after the treatment of CTNNAL1 ASO.

Repair of a damaged epithelium may be a necessary process in restoring airway function to its normal state. Repair generally involves several steps, including migration and spreading of epithelial cells at the margin of the injury into the damaged region and proliferation of new epithelial cells [Keenan et al., 1982a,b,c]. The migration and dissemination to the wound occur within 16–20 h after damage and the proliferation occurs around 24 h after the damage [Kim et al., 1998]. It is reported that α-catenin links to growth signaling pathways [Barth et al., 1997] and regulates skin cell proliferation through a mitogen-activated protein kinase dependent pathway [Vasioukhin et al., 2001]. Interestingly, CTNNAL1 mRNA appears to be differentially regulated following growth arrest of cells, suggesting that it may play a role in growth regulation [Zhang et al., 1998], although not any function of it was described previously. In this study, we found that the repair and proliferation of HBEC were slowed down after treated with CTNNAL1 ASO, and CTNNAL1 expression was explicitly increased on the cells in wound edges, which indicating that CTNNAL1 might involved in growth regulation and may be beneficial for the recovery of epithelium damage. Our findings showed some functional similarities between CTNNAL1 and α-catenin.

Yeast two-hybrid analyses have uncovered the lymphoid blast crisis oncogene (LBC) Rho guanine nucleotide-exchange factor as a partner for CTNNAL1 but not for α-catenin, which indicates that CTNNAL1 might have a new role in modulating signaling by the Rho pathway [Park, 2002]. α-catenin and CTNNAL1 have distinct activities that down regulate, respectively, β-catenin and Ras signals converging on the cyclin D1 promoter [Merdek et al., 2004]. It will be interesting to see how the functions and associations of CTNNAL1 differ from those of the 'classic' α-catenins.

The binding of fibronectin (Fn), a extracellular matrix protein, to integrins has been showed to alter expression of several genes, and to regulate adhesion, migration, proliferation and apoptosis of epithelial cells [Harkonen et al.,

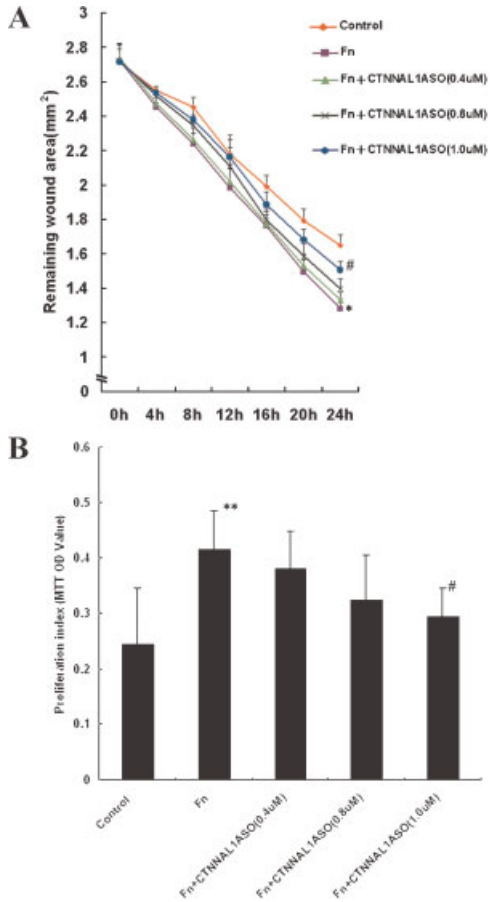

Fig. 6. The effects of CTNNAL1binding with Fn on wound closure (**A**) and proliferation (**B**) in HBEC monolayers. Fn could accelerate the repair and proliferation of HBEC. CTNNAL1 ASO (0.4, 0.8, 1.0 μM, respectively) could partly inhibit the HBEC wound repair and proliferation induced by Fn. (n = 18, $^{**}P < 0.01$ vs. control, $^{*}P < 0.05$ vs. control, $^{\#}P < 0.05$ vs. Fn). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

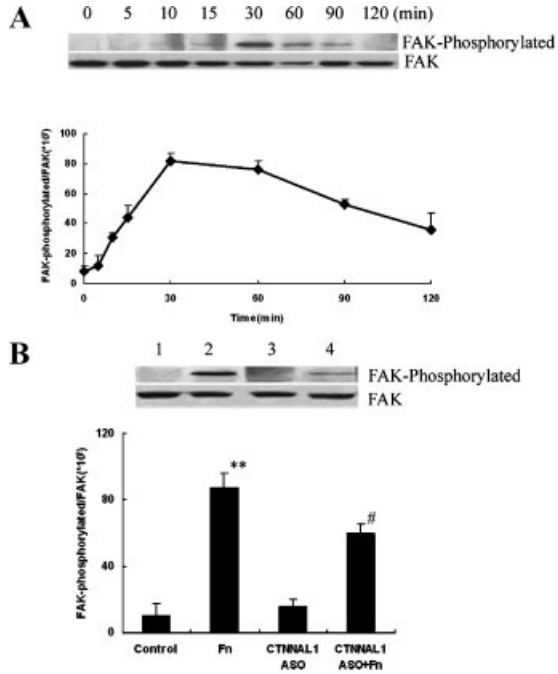

Fig. 7. Western blot showed that the effects of various treatments on FAK phosphorylation. **A**: The time course of FAK phosphorylation caused by Fn. The result showed that FAK phosphorylation became strong from 10 to 30 min, and then declined gradually. **B**: Western blot showed that Fn could promote the phosphorylation of FAK.CTNNAL1 ASO alone did not affect FAK phosphorylation. However, CTNNAL1 ASO could inhibit FAK phosphorylation induced by Fn (n = 3, $^{**}P < 0.01$ vs. control, $^{\#}P < 0.05$ vs. Fn).

1995; Matter and Ruoslahti, 2001; Ohke et al., 2001; Giuffrida et al., 2004; Jarvis and Bryers, 2005; Yamazaki et al., 2005]. Signal transduction from integrins after binding of matrix protein proceeds via several intermediate pathways, such as alteration in calcium influx [Canti et al., 2005], changes in inositol phosphate metabolism [Jones et al., 2005; Ruzzi et al., 2005], and activation of multiple protein tyrosine kinases, including focal adhesion kinase (FAK) and certain MAP kinases [Wierzbicka-Patynowski and Schwarzbauer, 2002].

Vinculin is present both in cadherin-catenin adhesion complexes that mediate cell-cell adhesion and in focal adhesion complexes that mediate cell-extracellular matrix adhesion, while α-catenin is present only in cadherin-catenin adhesion complexes [Weiss et al., 1998]. As a member of the vinculin superfamily, the genomic organization of CTNNAL1 is closely related to that of the αE-catenin gene CTNNA1, but not at all to that of the vinculin gene [Janssens et al., 1999]. Here we showed Fn could obviously promoted wound repair and proliferation of HBEC, these effects can be suppressed by pretreatment of CTNNAL1 ASO. CTNNAL1 might be concerned with the migration and proliferation mediated by Fn. Western blot showed that Fn could promote FAK phosphorylation in a transient time-dependent manner. CTNNAL1 ASO alone did not cause FAK phosphorylation. However, it could inhibit FAK phosphorylation induced by Fn, indicating that CTNNAL1 might have a role in modulating migration and proliferation via FAK phosphorylation signal transduction from Fn. Our results showed a novel link between CTNNAL1 with Fn mediated cell-extracellular matrix adhesion. However, the role of CTNNAL1 in fibronectin induced FAK-phosphorylation and

its position in signaling cascade need to be studied in our next step.

Our result showed that CTNNAL1 ASO could decelerate the repairing velocity and proliferation of HBEC mediated by Fn or alone in vitro. One potential explanation is that CTNNAL1 activate processes that lead to migration and proliferation in addition to Fn mediated tyrosine kinase cascade signals. Inhibition of HBEC migration and proliferation in the present study with the use of CTNNAL1 ASO, then, may be due to blockade of not only cell-matrix but also cell–cell interactions, as either or both processes may be available during repair.

In asthma, the bronchial epithelium shows evidence of damage with loss of columnar cells from their basal cell attachments [Takizawa, 2005] and the enhanced expression of the epidermal growth factor receptor (EGFR, HER1, c-erbB1) [Puddicombe et al., 2000]. Epithelial damage in asthma might due to increased susceptibility to a damaging agent and/or an inadequate repair response. Our findings reveal that the level of CTNNAL1 mRNA is highly correlated to the degree of pulmonary resistance; CTNNAL1 may contribute to the wound repair and proliferation of HBEC. Furthermore, it may serve to Fn mediated cell–extracellular adhesion and its signal transduction via FAK phosphorylation. These results raise the possibility that the down regulation of CTNNAL1 might contribute to the asthma development because of the attenuated cell–cell and cell–matrix adhesion, which lead to the bronchial epithelium desquamated and AHR. Detailed functional analysis of CTNNAL1 may provide important clues to the understanding of formation and regulation of cell adhesion.

GLOSSARY

AHR	airway hyperresponsiveness
CTNNAL1	catenin alpha-like 1
R_L	pulmonary resistance
HBEC	human bronchial epithelium cells
ASO	antisense oligonucleotide
ISH	in situ hybridization
RI	repair index
Fn	fibronectin

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