Compressive stress induces collective migration through cytoskeletal remodelling in nasal polyp epithelium*

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Abstract

Background: Nasal polyps in the nasal cavity and mucous discharge inside the maxillary sinus exhibit compressive stress on the nasal mucosal epithelium. However, there have been only a few studies on how compressive stress impacts the human nasal mucosal epithelium.

Methodology: We investigated the effect of compressive stress on collective migration, junctional proteins, transepithelial electrical resistance, epithelial permeability, and gene expression in well-differentiated normal human nasal epithelial (NHNE) cells and human nasal polyp epithelial (HNPE) cells.

Results: NHNE cells barely showed collective migration at compressive stress up to 150 mmH_20. However, HNPE cells showed much greater degree of collective migration at a lower compressive stress of 100 mmH_20. The cell migration of HNPE cells subjected to 100 mmH_2O compression was significantly decreased at day 3 and was recovered to the status prior to the compressive stress by day 7, indicating that HNPE cells are relatively more sensitive to mechanical pressure than NHNE cells. Compressive stress also increased transepithelial electrical resistance and decreased epithelial permeability, indicating that the compressive stress disturbed the structural organization rather than physical interactions between cells. In addition, we found that compressive stress induced gene expressions relevant to airway inflammation and tissue remodelling in HNPE cells.

Conclusion: Taken together, these findings demonstrate that compressive stress on nasal polyp epithelium is capable of inducing collective migration and induce increased expression of genes related to airway inflammation, innate immunity, and polyp remodelling, even in the absence of inflammatory mediators.

Key words: cell movement, mechanical force, nasal polyps, tight junction, airway remodelling

Introduction

Chronic sinusitis with nasal polyps and acute sinusitis are major diseases studied in the rhinology field. Nasal polyps in the nasal cavity and mucous discharge inside the maxillary sinus exhibit compressive stress on the nasal mucosal epithelium. However, since the nasal epithelium is frequently injured by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs)[(1)], most mechanical studies have focused on biological events and not on physical forces in the upper and lower airway.

A few studies have been published about the effect of compressive stress on cell mechanosensing focusing on cell physics[(2-4)]. Physiologically relevant oscillatory compressive stress increased ciliary beat frequency and mucociliary clearance by stimulating ATP release in human bronchial epithelial cells[(2,3)]. Extracellular ATP comes from damaged mitochondria, indicating epithelial cell death.

New impulses for cell migration have been discovered with the

technological progress in microscopy and visualisation techniques[5]. Collective cell migration is the coordinated migration of a group of cells during which cells are influenced by interactions with their neighbours. Collective movements have been observed in diverse processes including wound healing, development, and invasion of cancer cells[6]. Collective migration involves transient loss of epithelial features and a reduction in intercellular junctions[7,8].

The effect of compressive pressure on bronchial cell collective migration and intercellular permeability has been studied recently[9,10]. In primary normal human bronchial epithelial (NHBE) cells in air-liquid interface (ALI) culture, intermittent compressive stress leads to mucus hypersecretion contributing to airway obstruction by inducing goblet-cell hyperplasia[9]. In addition, the physical processes of cell jamming (a nonmotile state) and unjamming (a motile state) are induced by mechanical compression during bronchial constriction in asthma[10], leading to tissue remodelling and collective cellular migration.

The nasal polyp mucosa is shown to have reduced expression of claudin-1 and occludin tight junction proteins[11]. Exposure to representative inflammatory cytokines, IFN-γ and TNF-α, has been shown to down-regulate claudin-1 and occludin tight junction proteins in a respiratory epithelium tissue culture model. IL-4 also disrupted the expression and localisation of the tight junction proteins zonula occludens-1 and occludin, and it induced the cleavage and asymmetric distribution of E-cadherin in the human nasal epithelial cells. These events induced collective epithelial migration and cell shape changes driven by actin cytoskeleton reorganisation[11]. Eventually, these mechanical forces can lead to altered biochemical events or cell behaviours[12]. In addition, physical forces elicit biochemical signalling through an autocrine ligand-receptor circuit operating in a dynamically regulated extracellular volume, without induction of force-dependent biochemical processes within the cell or cell membrane[13]. Therefore, the compressive forces in pathological conditions may activate various signalling pathways of epithelial cells and contribute to the persistence of disease conditions and inflammation.

However, how compressive stress impacts human nasal epithelial cells has not been studied previously. We investigated the effect of compressive stress on collective movement, examined effect on epithelial permeability due to changes in the tight junction proteins, and determined the genes influenced by compressive stress in human nasal epithelial cells.

Materials and methods

Antibodies and reagents

Mouse monoclonal anti-β-actin was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit monoclonal anti-FAK, rabbit monoclonal anti-FAK (phosphor Y397 & Y925), rabbit monoclonal anti-Src, and rabbit monoclonal anti-pSrc were obtained from Abcam (Cambridge, UK). Rabbit polyclonal anti-ZO-1 and mouse monoclonal anti-occludin were purchased from Thermo Scientific (Fremont, CA, USA). Mouse monoclonal anti-E-cadherin was obtained from BD Biosciences (San Jose, CA, USA). Mouse monoclonal anti-acetylated tubulin, Fluorescein isothiocyanate (FITC)--phallloidin, and FITC-dextran (4 kDa) were purchased from Sigma-Aldrich (Saint Louis, MO). Mouse monoclonal anti-MUC5AC was obtained from Thermo Scientific (Fremont, CA).

Cell culture

The Institutional Review Board of Yonsei University College of Medicine approved the study protocols (IRB# 4-2016-1153). All participants provided their written consents to participate in this study. Primary normal human nasal epithelial (NHNE) cells were isolated by nasal brushing from the inferior turbinate of healthy subjects who have no mucosal inflammation. Human nasal polyp epithelial (HNPE) cells were isolated from nasal polyps obtained from patients with chronic rhinosinusitis who had no clinical history of allergy, asthma, aspirin sensitivity, or cystic fibrosis. The ALI culture system was used for HNE cells, as previously described [14,15]. Briefly, epithelial basal cells were expanded on plastic dish with BEGM (Lonza, Basel, Switzerland), supplemented with growth factors according to the manufacturer's instructions: hydrocortisone (0.5 μg/ml), insulin (5 μg/ml), transferrin (10 μg/ml), epinephrine (0.5 μg/ml), triiodothyronine (6.5 μg/ml), gentamicin (50 μg/ml), amphotericin-B (50 μg/ml), retinoic acid (15 ng/ml), bovine pituitary extract (50 μg/ml), bovine serum albumin (1.5 μg/ml), and epidermal growth factor (0.5 ng/ml). At passage-2, cells were seeded at a density of 2×10^5 cells/cm^2 on a 12 mm, 0.45 μm pore Transwell-clear culture insert (Costar Co, Cambridge, MA, USA) and cultured in a 1:1 mixture of BEGM and DMEM (Lonza, Basel, Switzerland). As soon as the cells reached confluence, the apical surfaces of the cells were exposed to air (ALI day 0) and the ALI medium supplemented with additional 50 nM retinoic acid was added to the basolateral chamber. The medium was changed every other day for 2 weeks.

Mechanical compression

On day 14 of ALI culture, mechanical compression was applied to the apical surface of the differentiated primary NHNE cell and HNPE cell cultures using a custom-made device that is able to produce gas pressure. A 5% CO_2 premixed gas passed through a water reservoir inside the incubator and was pressurised into the apical chamber, which was sealed with a rubber plug. The applied pressure was set at 50, 100, or 150 mmH_2O, which was similar and higher[16,17] compared to the pressure variation occurred during the normal nasal respiration. After 3 h of compression, NHNE cell and HNPE cell cultures were removed from the compression device and were left idle in the ambient pressure until cell migration was recorded. (Figure 1A)

Image acquisition

Collective migration of the cells was monitored using an inverted microscope (Axio Observer Z1, Carl Zeiss, Germany) equipped with an Incustage incubating chamber (Live Cell Instrument, South Korea), which was capable of maintaining a 37°C and 5% CO_2 level during image acquisition. Phase contrast images of the apical surface of NHNE cell and HNPE cell cultures were taken by a 40× objective at 9 min intervals for a 3 h duration. For each experiment, six different locations on an apical surface were imaged.

Analysis of cell migration

We analysed the cell migration by acquiring the velocity vector field of the migrating NHNE cells and HNPE cells at the apical surface using an open source particle image velocimetry (PIV) analysis software PIVLab[18] written in Matlab (Mathwoks, USA) as described previously[19].

Microarrays and data analysis

Total RNA was extracted using TRIzol reagent (Invitrogen, Gaithersburg, MD, USA) and microarray analysis was performed as described previously[20]. All data analysis of differentially expressed genes was conducted using R 3.3.3 (www.r-project.org).

Analysis of protein expression

Western blot analysis of whole cell lysates was performed as described previously[19].

Measurements of barrier integrity

Epithelial barrier integrity of the Transwell-grown HNE cell layers was assessed by measuring transepithelial electrical resistance (TEER) using an EVOM Voltohmmeter (World Precision Instruments). Epithelial barrier function was assessed by determining paracellular permeability with the FITC-dextran flux assay. In brief, FITC-dextran (4 kDa; 1 mg/ml) was added to the apical compartment of the Transwell-grown HNPE cell layers. At 3 or 24 h after application of compressive stress, basal media were analysed to measure the accumulation of fluorescence signal across the layers using a SPECTRAmax microplate spectrofluorometer (Molecular Devices) with an excitation of 492 nm and emission of 520 nm.

Immunofluorescence staining

Transwell-grown HNPE cell layers were processed for immunofluorescence analysis for MUC5AC, acetylated α-tubulin, ZO-1, E-cadherin, and phalloidin using previously described protocols[19]. Images were captured and analysed with confocal laser-scanning microscopy (LSM700, Carl Zeiss MicroImaging GmbH, Jena, Germany).

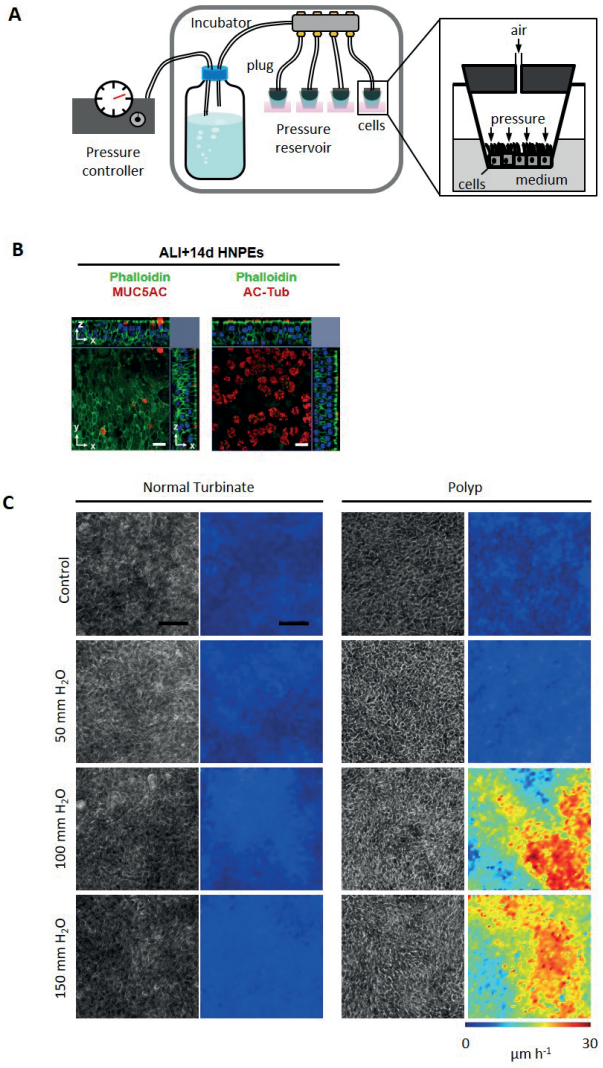


Figure 1. Compressive stress causes collective migration of NHNE cells and HNPE cells. (A) Custom compression device that supplied humidified 5% CO_2 air to apply compression on the apical surface of the differentiated NHNE cells and HNPE cells. (B) Representative confocal microscope images of HNPE cells differentiated at the ALI culture for 2 weeks. Cells were stained for the secretory cell marker MUC5AC (red) and the ciliated cell marker acetylated α-tubulin (Ac-Tub; red), and counterstained with DAPI (blue) and FITC-phalloidin for F-actin (green). Immunofluorescence images show xy and xz sections. Scale bar, 20 µm. (C) Phase contrast images (left) and corresponding speed maps (right) of the migrating NHNE cells and HNPE cells at 24 h after exposure to compressive stress of 0 (red), 50 (orange), 100 (yellow), or 150 mmH_2O (green) for 3 h. Colour scale is indicated at the bottom.

Statistical analysis

We performed all statistical analysis on GraphPad Prism 5.0 software. Results are reported as the mean ± SD. Significant differences between two groups were determined using the Mann-Whitney test. A difference was considered significant when $P < 0.05$.

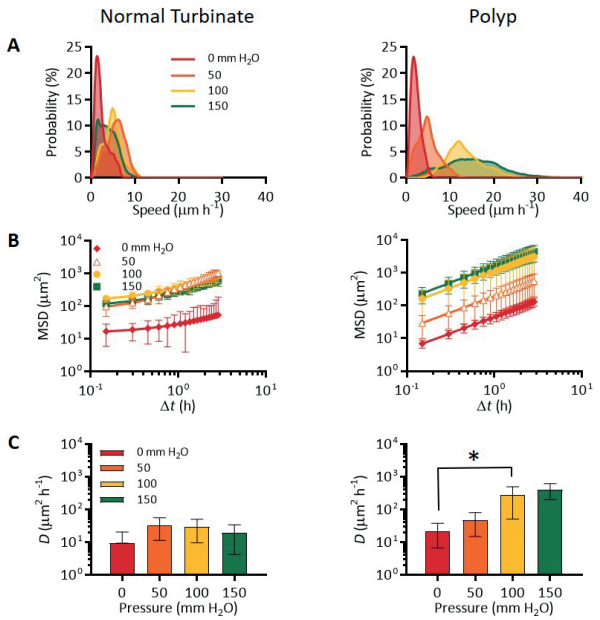


Figure 2. Analysis of cell migration. (A) Mean squared displacement (MSD) (B) speed distribution (C) Diffusion constant D of NHNE cells (left) and HNPE cells (right) after applying compressive stress of 0, 50, 100, and 150 mmH_2O. Scale bar is 50 μm. * Significant differences between two groups were determined using the Mann-Whitney test. $P < 0.05$ was considered significantly different.

Results

Compression induced collective migration in HNPE cells, but not in NHNE cells

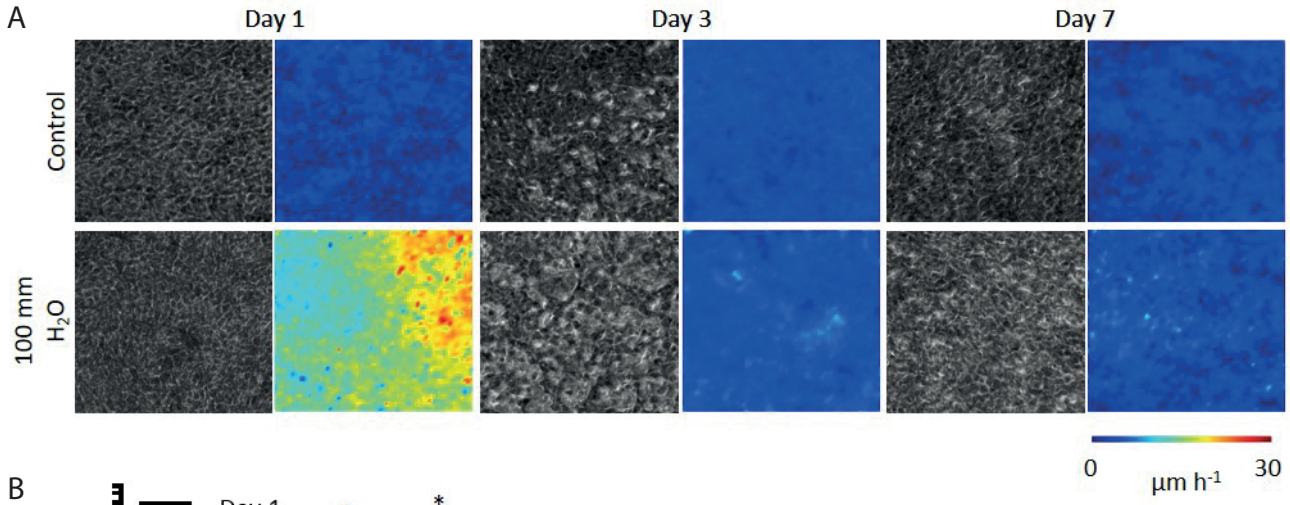
To study collective epithelial behaviours including cell rearrangement and organization in human nasal epithelium under physiological and pathological conditions, we investigated the effect of applied compression on well-differentiated NHNE and HNPE cells grown at the ALI for 2 weeks. We first examined the differentiation state of HNPE cells at day 14 of ALI culture by immunofluorescent staining. The presence of goblet and ciliated cells was identified from the staining of mucin MUC5AC and acetylated α-tubulin (Ac-Tub) on the apical surface, respectively, and cell morphology was visualized by FITC-phalloidin staining of filamentous actin (F-actin). Both NHNE cells and HNPE cells were differentiated into ciliated pseudostratified columnar epithelium with goblet cells (Figure 1B). The PIV analysis for the time-lapse images of NHNE cells subjected to gas pressure demonstrated how the cell migration behaviour depends on the applied compression. For the NHNE cells, no significant change in the migration speed was observed when the applied compression increased. In contrast, for the HNPE cells, the migration speed increased significantly at the applied compression pressure of 100 mmH_2O and higher (Figure 1C).

The average migration speeds in the HNPE cells were estimated to be 2.1, 4.5, 12.3, and 15.3 μm h-1 at 0, 50, 100, and 150 mmH_2O, respectively (Figure 2A), The collective migration of epithelial cells was further characterized by analysing the mean squared displacement (MSD). NHNE cells to the compression of 50, 100, and 150 mmH_2O exhibited the similar MSDs independent of the compression magnitude. Compared to the NHNE cells, HNPE cells exhibited an increasing degree of MSD as the applied compression. A more significant increase of MSD was observed at 100 mmH_2O (Figure 2B). MSD is described by a power law with a time interval: MSD ~ Δt^{α}. The exponent is equal to 1 when the trajectory corresponds to Brownian motion and is greater or smaller than 1 when corresponding to super-diffusive or sub-diffusive motion, respectively. Both the NHNE cells and HNPE cells exhibited the Brownian motion at all experiment conditions. α values of NHNE cells were 0.99, 1.0, 1.0, and 0.99, and those of HNPE cells were 0.98, 0.99, 1.0 and 1.0 for 0, 50, 100, 150 mmH_2O, respectively. Although the exponents of MSD were similar, the magnitude of MSD increased with the applied compression, indicative of an increased motility. For the NHNE cells, the diffusion coefficients (D) estimated from the MSD was not significantly altered by the applied compression. Compared to the NHNE cells, an increase of the diffusion coefficient proportional to the applied compression was observed for the HNPE cells (Figure 2C). The critical compression to cause the increase in motility was 100mmH_2O in HNPE cells. These results suggest that the HNPE cells are more sensitive to the applied compression and liable to be migratory compared to the NHNE cells. Because NHNE cells barely showed collective migration, we used only HNPE cells for the subsequent experiments.

Collective migration in HNPE cells showed time-dependent decay upon removal of compressive stress

To better understand the effect of mechanical stimulation on the HNPE cells, we observed how HNPE cells migratory behaviours that were influenced by compression application changed after removal of the applied mechanical stimulation. The cell migration subjected to 100 mmH_2O compression was significantly decreased at day 3 and was recovered to the status prior to the compressive stress by day 7 (Figure 3A). The diffusion coefficients recovered to the value at the condition without an applied compression at day 7 for the HNPE cells (Figure 3B). The recovery time might be attributed to a delay in time from when the phenotype of an individual cell is altered or the force integrity between cells being stabilised after disruption by compression. Our results indicate that nasal mucosa reorganisation can occur in a more complex manner in a dynamic condition when both biological conditions and characteristics of mechanical stimulation such as its frequency and magnitude vary.

Epithelial permeability was not increased, and tight junction proteins were not decreased by compressive stress in HNPE cells



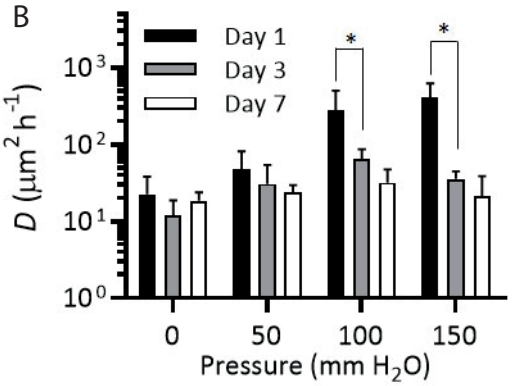


Figure 3. Migration of HNPE cells induced by compressive stress attenuates over time. (A) Phase contrast images (left) and corresponding speed maps (right) of the migrating cells at day 1, 3, and 7 after 100 mmH2O compression for 3 h. Colour scale is indicated at the bottom. (B) Histograms showing the HNPE cells stabilising 1, 3, and 7 days after compression under 0, 50, 100 and 150 mmH_2O. * Significant differences between two groups were determined using the Mann-Whitney test. P < 0.05 was considered significantly different.

We next examined whether collective migration of HNPE cells was related to the change in transepithelial electrical resistance, paracellular permeability, or junctional components. Contrary to our expectations, after 3-h compression at 0, 50, 100, and 150 mmH_2O, transepithelial electrical resistance (TEER) increased at 3 and 24 h after compression (Figure 4A). Consistently, the dextran permeability was decreased to the same level in HNPE cells after 3-h exposure to compressive stress of 50, 100, and

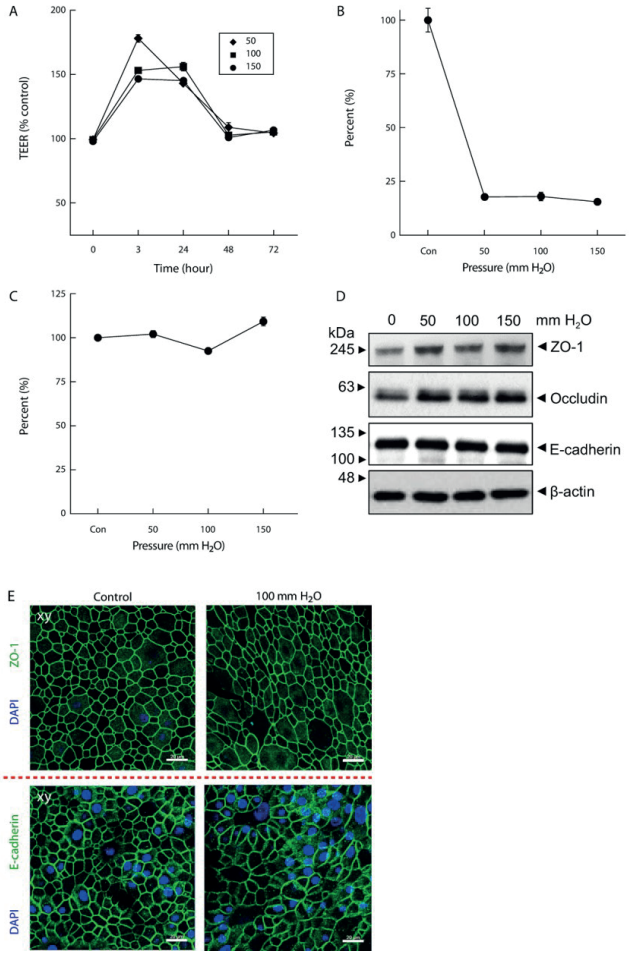


Figure 4. Analysis of tight junctions of compressed HNPE cells. (A) Transepithelial electrical resistance (TEER) results for the migrating cells after exposure to compressive stress of 50, 100, and 150 mmH_2O for 3 h. The background signal was subtracted (using TEER values from a blank Transwell), and data were normalized to uncompressed controls. (B) Dextran permeability results for the HNPE cells at one exposure to compressive stress of 0, 50, 100, or 150 mmH_2O for 3 h. (C) Dextran permeability result for the HNPE cells 24 h after of exposure to compressive stress of 0, 50, 100, or 150 mmH_2O for 3 h. (D) Western blot result for ZO-1, occludin, and E-cadherin at 24 h after of exposure to compressive stress of 0, 50, 100, and 150 mmH_2O. β-actin was used as the control. (E) Representative immunofluorescence staining for ZO-1 and E-cadherin (green) and merged images with DAPI (blue) on the HNPE cells at 24 h after exposure to compressive stress of 0 and 100 mmH_2O for 3 h. Scale bar is 20 μm.

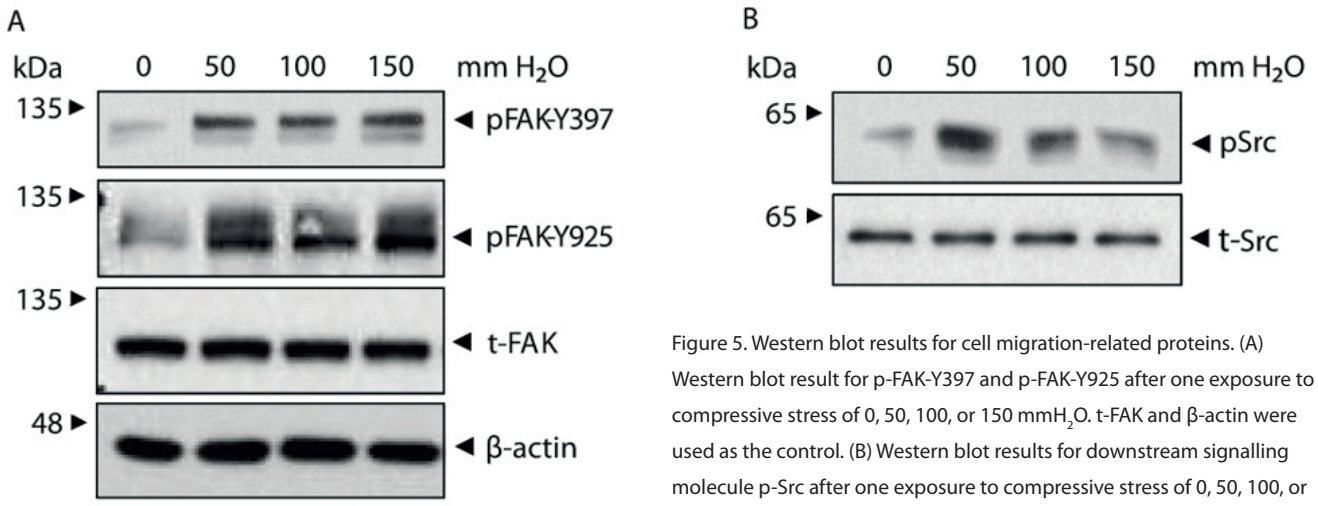


Figure 5. Western blot results for cell migration-related proteins. (A) Western blot result for p-FAK-Y397 and p-FAK-Y925 after one exposure to compressive stress of 0, 50, 100, or 150 mmH_2O. t-FAK and β-actin were used as the control. (B) Western blot results for downstream signalling molecule p-Src after one exposure to compressive stress of 0, 50, 100, or 150 mmH_2O. t-Src was used as the control.

150 mmH_2O compared with the uncompressed control cells (Figure 4B) and then returned to control levels at 24 h after 3-h compression (Figure 4C). Since the permeability of the epithelial barrier is controlled by the tight junction (TJ) proteins, we next examined the protein levels of ZO-1 and occludin at 24 h after 3-h exposure to compressive stress of 50, 100, and 150 mmH_2O. The expression levels of ZO-1 and occludin in HNPE cells showed a slight increase or no change regardless of compressive pressure application (Figure 4D). We also observed that the expression of another component of adherens junctions (AJs), E-cadherin, was not changed by compression (Figure 4D). Further, immunofluorescence analyses revealed that the compressed HNPE cells exhibited more enlarged and elongated shapes compared with the uncompressed controls, but the overall structure of TJs and AJs remained intact (Figure 4E).

Therefore, these data suggest that the decrease in permeability of the epithelial barrier by compressive stress is attributed to the change in structural arrangement of the cytoskeleton rather than alteration in expression level of junction proteins.

Compressive stress induced collective HNPE cells migration via FAK signalling pathways

Focal Adhesion Kinase (FAK) is a crucial mediator of intracellular signalling by integrins in the regulation of cytoskeleton organisation and cell migration[21]. Integrin-mediated FAK autophosphorylation at Tyr-397 (pFAK-Tyr-397) forms a complex with Src, which leads to the conformational activation of Src, promoting further phosphorylation of FAK at Tyr-925 (pFAK-Y925) for focal adhesion turnover and cell protrusion[21].

To investigate the effect of compressive stress on activation of FAK and Src in HNPE cells, we subjected HNPE cells to compressive stress at a magnitude of 0, 50, 100, or 150 mmH_2O pressure for 3 h and examined the status of FAK and Src. Western blot analysis showed that compressive stress increased the levels of pFAK-Y397, pFAK-Y925, and pSrc in HNPE cells (Figure 5A&B), suggesting that compression can induce collective migration of HNPE cells through the Src/FAK-dependent pathway.

Compressive stress increased expression of genes associated with airway inflammation and remodelling

To characterise the changes elicited by compressive stress on HNPE cells on the genome-wide scale, total RNA was isolated from HNPE cells subjected to the ambient pressure for 24 h after 0 and 3 h of a static compression at a magnitude of 100 mmH_2O and hybridised to an Affymetrix GeneChip Human ClariomS array containing 21,448 probes. Comparative microarray analysis revealed that 36 genes showed a ≥2-fold change in expression in the compressed cells in comparison to uncompressed cells ($P \leq 0.01$, FDR < 0.01); 26 up- and 10 down-regulated genes are shown in Table 1. Gene ontology analysis for the data revealed enhanced biological functions in terms of cell proliferation (ERRFI1, PRDM1, TRPS1, AKIRIN2, EGR1, IL1A, and PLAU), cell migration (PHLDB2, PLAU, GRAMD1B, DUSP5, DUSP6, ASAP1, and KRT23), cell adhesion (PHLDB2, EGR1, and PLAU), response to wounding (PLAUR, SERPINB2, PHLDB2, SEMA7A, IL1A, and PLAU), and innate immune response (CCL20, IL1A, and TSLP). Of note, most of the down-regulated genes had unknown functions.

Interestingly, the compressive stress on HNPE cells significantly stimulated the expression of genes related to the plasminogen system, including plasminogen activator, urokinase (PLAU), and plasminogen activator, urokinase receptor (PLAUR), which leads to subepithelial fibrosis[22]. In addition, the expression of early growth response-1 (EGR1), a key mediator of profibrotic TGF-β responses[23], was up-regulated in the compressed HNPE cells compared to the uncompressed cells. Furthermore, compressive stress stimulated the expression of thymic stromal lymphopoietin (TSLP) and the chemokine CCL20, which play critical roles in

Table 1. List of genes that were differentially expressed in response to compressive stress in the HNPE cells.

Probe ID	Gene_Symbol	Description	FC Comp/C
Up-regulated genes			
TC0100018403.hg.1	ERRFI1	ERBB receptor feedback inhibitor 1	3.70
TC0200010980.hg.1	CCL20	chemokine (C-C motif) ligand 20	3.63
TC0600008972.hg.1	PRDM1	PR domain containing 1, with ZNF domain	3.49
TC0700007034.hg.1	CREB5	cAMP responsive element binding protein 5	3.45
TC0600009364.hg.1	NCOA7	nuclear receptor coactivator 7	3.45
TC0500008307.hg.1	TSLP	thymic stromal lymphopoietin	3.30
TC1900010856.hg.1	PLAUR	plasminogen activator, urokinase receptor	3.27
TC0800011533.hg.1	TRPS1	Transcript Identified by AceView, Entrez Gene ID(s) 7227	2.78
TC0100015797.hg.1	POGZ	Transcript Identified by AceView, Entrez Gene ID(s) 23126	2.58
TC1100013108.hg.1	GRAMD1B	GRAM domain containing 1B	2.34
TC1000008891.hg.1	DUSP5	dual specificity phosphatase 5	2.33
TC1800009242.hg.1	SERPINB2	serpin peptidase inhibitor, clade B (ovalbumin), member 2	2.30
TC0300008324.hg.1	PHLDB2	pleckstrin homology-like domain, family B, member 2	2.27
TC0600012514.hg.1	AKIRIN2	akirin 2	2.26
TC1200011470.hg.1	DUSP6	dual specificity phosphatase 6	2.25
TC1700011794.hg.1	QRICH2	Jeck2013 ALT_DONOR, coding, INTERNAL, intronic best transcript NM_032134	2.21
TC0500011173.hg.1	POC5	POC5 centriolar protein	2.20
TC1500010018.hg.1	SEMA7A	semaphorin 7A, GPI membrane anchor (John Milton Hagen blood group)	2.20
TC0800011826.hg.1	ASAP1	ArfGAP with SH3 domain, ankyrin repeat and PH domain 1	2.11
TC0500008785.hg.1	EGR1	early growth response 1	2.10
TC0100016476.hg.1	KIAA0040	KIAA0040	2.09
TC0100011022.hg.1	RGS2	regulator of G-protein signalling 2	2.08
TC0200007421.hg.1	CAMKMT	Memczak2013 ALT_ACCEPTOR, ALT_DONOR, coding, INTERNAL, intronic best transcript NM_024766	2.06
TC0200013913.hg.1	IL1A	interleukin 1 alpha	2.04
TC1000008054.hg.1	PLAU	plasminogen activator, urokinase	2.02
TC1700010641.hg.1	KRT23	keratin 23, type I	2.00
Down-regulated genes			
TC0X00006877.hg.1	MAGEB3	Transcript Identified by AceView, Entrez Gene ID(s) 4114	-2.66
TC1000011379.hg.1	LIPA	Transcript Identified by AceView, Entrez Gene ID(s) 3988	-2.28
TC0500009984.hg.1	FLJ33360	FLJ33360 protein	-2.21
TC1300009169.hg.1	PCDH9	Memczak2013 ALT_ACCEPTOR, ALT_DONOR, coding, INTERNAL, intronic best transcript NM_203487	-2.15
TC0100018105.hg.1	OR13G1	olfactory receptor, family 13, subfamily G, member 1	-2.12
TC1500010738.hg.1	CTXN2	cortexin 2	-2.12
TC0600012562.hg.1	BACH2	Memczak2013 ALT_ACCEPTOR, ALT_DONOR, coding, INTERNAL, intronic best transcript NM_001170794	-2.11
TC0200015127.hg.1	PDE1A	Transcript Identified by AceView, Entrez Gene ID(s) 5136	-2.04
TC0300009194.hg.1	SUCNR1	succinate receptor 1	-2.04
TC1100007767.hg.1	MS4A4A	membrane-spanning 4-domains, subfamily A, member 4A	-2.02

Genes with significant changes of at least 2-fold compared with uncompressed cells were considered test ($P \leq 0.01$, FDR < 0.01). Abbreviations: Comp, compressed cells; FC, fold change; FDR, false discovery rate.

the development of allergic airway inflammation by promoting the maturation of dendritic cells (DCs) as well as the recruitment of DCs and T-cells to the airways [24]. Collectively, these results suggest that the mechanical stress applied to HNPE cells can lead to the signalling pathways related to airway inflammation and remodelling.

Discussion

In nasal airway disease, including eosinophilic/non-eosinophilic polyps, mucus stagnation from acute/chronic bacterial sinusitis, and fungal sinusitis, both biological and physical events occur simultaneously. Numerous studies reported biological events in the nasal mucosa[25], but there are few reports about the effect of physical stress on the nasal mucosa. Tissue-remodelling is induced during wound repair, and it has been linked to collective cellular migration[26]. Almost all living tissue is constructed by collective cell migration, and it has a significant role in the leader-follower organisation of cell groups during embryonic development[27]. In the lower airway, collective migrations occur with the application of mechanical compression[9]. However, there are no studies about collective migration in the upper airway.

IL-4, a key mediator in driving and exacerbating allergic inflammation, induced collective migration through integrin αvß5 and αvß6 in well-differentiated HNPE cells[19]. During normal nasal respiration, Solomon et al.[16] reported that the nasal air pressure was varied between -30 mmH_2O and 30 mmH_2O during normal nasal respiration. Moreover, Calmet et al.[17] showed that the air pressure was up to 40 mmH_2O at the mid-portion of the nasal cavity with the highest inspiratory velocity in normal conditions. However, the structural changes in the nasal mucosa due to nasal polyps and chronic infection can generate pressures by reduction of airway path or compression of epithelial cells against each other in contact areas with adjacent nasal mucosa. We also note that the type of pressure as well as its magnitude should be taken into account. While the airway pressure is varying from negative to positive values, the applied pressure in our experiment was a constant. Therefore, although it is difficult to compare directly between the pressure in the experiments and one measured in vivo, the applied pressure in our study can represent an increased pressure by reduction of airway path in pathology to a certain extent and the condition where cell-cell contact force is increased.

In this study, NHNE cells barely showed collective migration at compressive stress up to 150 mmH_2O, which is similar to the result from the normal bronchial epithelial cells[10]. These data indicate that both normal airway epithelium of human nose and bronchus show similar response to compressive stress. However, HNPE cells showed much greater degree of collective migration at a lower compressive stress of 100 mmH_2O, and the recovery speed was also much slower than that of NHNE cells, indicating that HNPE cells are more sensitive to mechanical pressure than NHNE cells. In addition, changes in the air path by nasal polyp and accumulated mucus can produce complex forms of stress including compression and shear on nasal epithelial cells. Taken together, these results indicate that the nasal polyp epithelium is much more sensitive to compressive stress than normal nasal epithelium, probably due to the cytokines and proteases expressed from nasal mucosal inflammation with nasal polyps. Given that nasal polyp basal cells retain a memory of the initial transcriptional response to chronic inflammatory conditions[28], it is possible that HNPE cells respond to compressive stress more sensitively and rapidly than NHNE cells by inducing the expression of inflammatory cytokines and proteases in a cell-intrinsic fashion when encountering compressive stress again in vitro.

In addition, western blot analysis showed that compressive stress increased the levels of pFAK-Y397, pFAK-Y925, and pSrc, revealing that compression can induce collective migration of HNPE cells through the Src/FAK-dependent pathway.

However, in this study, unexpectedly, mechanical compression induced a modest increase in tight junction proteins and decrease in permeability of HNPE cells. In addition, immunofluorescence analysis revealed that the compressed HNPE cells exhibited more enlarged and elongated shapes compared with the uncompressed controls, while the overall structure of TJs and AJs remained intact. These data indicate that compressive stress does not affect the epithelial barrier integrity and function, but significantly changes cell shape and actin cytoskeleton organisation in HNPE cells. This discrepancy is probably due to our investigation of only the early effect of compressive stress. We cannot exclude the possibility that long-term compression or the late effect of compressive stresses might damage tight junction proteins and increase epithelial permeability. If compressive stresses are maintained for a long time, such as under in vivo conditions, mechanical compression may induce epithelial cell death and release DAMPs, which may lead to tight junction proteins damage. These processes may allow pathogens or allergens to easily approach subepithelial connective tissue as a result of the increased permeability.

Concerning the effect of mechanical stresses on the release of inflammatory cytokines, compressive stress increases tissue factor expression[29], a cellular initiator of coagulation, IL-8, and vascular endothelial growth factor[30], in human bronchial epithelial cells, and activates IKK-NF-κB signalling[31] in MC3T3-E1 cells and nuclear factor-kB in A549 cells[32]. In the gene expression data for differentially expressed genes due to compressive stress, we found that the expression of genes relevant to cell proliferation, cell migration, cell adhesion, and wound healing increased.

All these genes are related to airway remodelling. Previous in vitro studies have shown that bronchial epithelial cells, lung fibroblasts, and smooth muscle cells exposed to compressive stresses exhibit increased expression of genes relevant to airway

remodelling[33]. For instance, compressive stress induces upregulation of the EGFR ligands epiregulin, HB-EGF, and amphiregulin in human bronchial epithelial cells, showing the possibility of involvement of selected EGFR ligands in the mechanically activated EGFR autocrine feedback[34]. In a three-dimensional model of the bronchial wall, compressive strain causes tissue remodelling similar to the remodelling seen in asthma, even in the absence of inflammation[35]. Interestingly, we found for the first time that compressive stress induces CCL20, a dendritic cell chemo-attractant, IL-1A, a proinflammatory mediator, and TSLP, an important epithelial cell-derived cytokine for type 2 innate immunity. Considering that nasal polyps occur frequently in Th2-skewed inflammatory conditions, compressive stresses may augment Th2 mucosal immunity.

Conclusion

The mechanism of how compressive stress induces collective migration still remains unclear. In this study, taken together, the results indicate that nasal mucosa reorganisation can occur in a complex manner under a dynamic condition when both the frequency and magnitude of mechanical stimulation change. In conclusion, we demonstrated that compressive stresses on nasal polyp epithelium due to contact with adjacent nasal mucosa or stagnated mucus is capable of inducing collective migration and induce increased expression of genes related to airway inflammation, innate immunity, and polyp remodelling, even in the absence of inflammatory mediators.

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Authorship contribution

JHY, HL, and SNL designed the studies and experiments. JMC, SGL, JGH, JSN, and JHC performed the experiments. CHK and HJC contributed new analytic tools. JHY, HL, SNL, JMC, and SGL interpreted the data. JHY, HL, SNL, and JSN wrote the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

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