Nasal lavage fluid-derived exosomal microRNA let-7a-5p suppresses IL-4-induced MUC5AC expression

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Nasal lavage fluid-derived exosomal microRNA let-7a-5p suppresses IL-4-induced MUC5AC expression

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ABSTRACT

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MicroRNAs (miRNAs) are implicated in the pathogenesis and development of allergic rhinitis (AR) caused by goblet cell hyperplasia and chronic inflammation of the nasal mucosa. Specific exosomal miRNAs in the nasal mucus or serum may provide diagnostic or prognostic potential for AR. The therapeutic effect of exosomal miRNAs on AR occurrence and progression is well-studied in association with T helper 2 immunity. However, exosome and/or exosomal miRNA function in regulating mucin-secreting epithelial tissues remains unknown.

Small RNA sequencing was conducted to identify the miRNAs responsible for AR using exosomes isolated from the nasal lavage (NAL) fluid of the control (n=9) and AR patient groups (n=8). Overall, 181 mature miRNAs were identified in the exosomes isolated from NAL fluids, 32 were differentially expressed with more than a two-fold change ($p<0.05$). Mucin5 AC, encoded by the *MUC5AC* gene, is a critical factor in mucus hypersecretion and is highly expressed under allergic conditions. To identify MUC5AC-targeting miRNAs, I assessed down-regulated miRNAs in interleukin-4 (IL-4)-treated human nasal epithelial cells. Among the 32 miRNAs, let-7a-5p was significantly downregulated by IL-

4 treatment. Moreover, the target prediction algorithm indicated that the 3′ untranslated region of *MUC5AC* mRNA harbors a let-7a-5p miRNA recognition element (MRE). Argonaute2 RNA immunoprecipitation and luciferase assays revealed that let-7a-5p suppressed MUC5AC expression by directly binding to the MRE of its mRNA. Upon treatment with IL-4, the decrease in let-7a-5p expression reduced *MUC5AC* mRNA enrichment in the miRNA-induced silencing complex, leading to increased *MUC5AC* expression.

My findings demonstrate that the exosomal miRNA let-7a-5p isolated from NAL fluid can inhibit mucus hypersecretion in airway epithelial cells, I propose it as a potential therapeutic target for AR treatment.

Key words : allergic rhinitis; microRNA; microarray; nasal lavage fluid; exosome; air-liquid interface culture; interleukin-4; MUC5AC; let-7a-5p;

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I. INTRODUCTION

Allergic rhinitis (AR) is a chronic upper respiratory disorder caused by immunoglobulin (IgE)-mediated inflammation. AR is characterized by pruritus, sneezing, rhinorrhea (nasal discharge), and nasal congestion. Although AR is not a life-threatening disease, it seriously impacts the professional and personal lives of patients, leading to increased socioeconomic burden and reduced quality of life[1,2]. Investigating the pathological mechanisms of AR is essential for developing diagnostics and treatments.

Mucus hypersecretion is a common pathological feature of airway inflammatory diseases such as AR, chronic rhinosinusitis (CRS), asthma, and chronic obstructive pulmonary disease (COPD), resulting in mucociliary dysfunction in the upper and lower airways[3-5]. Thus, identifying potential targets is necessary for the pharmacotherapy of mucus hypersecretion. Mucus is secreted by goblet cells, which are specialized secretory epithelial cells that make up the mucosal surfaces of the airways, along with ciliated and basal cells[6,7]. Mucins are the main components of mucus in the nasal mucosa and are largely divided into gel-forming mucins (mucin 5AC (MUC5AC) and MUC5B) and tethered mucins (MUC1, MUC4, MUC16, and MUC20)[7]. In particular, MUC5AC is considered the predominant mucin in the airway and a marker of goblet cell hyperplasia because its secretion increases during nasal allergic inflammation[8]. It has been previously reported that inflammatory cytokines (e.g., tumor necrosis factor-alpha, interleukin (IL)-1β, IL-4, IL-6, IL-9, IL-13, and IL-17) stimulate *MUC5AC* gene expression in airway epithelial cells either *in vitro* or *in vivo*[9-12]. For example, IL-4 and IL-13 has been found to promote mucus production and the secretion of inflammatory cytokines in airway

epithelial cells (NEC)[13]. In addition, IL-1β, IL-6, IL-8 inflammatory molecules produced by mast cell contributed to the influx of immune cells into nasal mucosa tissue of AR, and accelerated inflammatory reactions[14]. Therefore, targeting the regulation of MUC5AC production may be a promising approach for developing treatments for mucus hypersecretion.

MicroRNAs (miRNAs) are evolutionarily conserved small non-coding RNAs of 18-25 nucleotides in length. miRNAs generally suppress the expression of target genes by directly binding to the miRNA recognition element (MRE) present in the 3′ untranslated region (UTR) of target mRNA, thereby lowering the stability and translation of the target mRNA[15]. The inhibitory function of miRNA is attributed to the formation of the miRNA-induced silencing complex (miRISC) by loading miRNA into Argonaute2 (AGO2)[16]. A set of miRNAs that are specifically expressed in multiple allergic inflammatory diseases has been reported; hence, miRNAs are considered to have high potential as diagnostic and therapeutic biomarkers for AR[17]. The following miRNAs have been reported to be associated with AR: upregulated miRNAs, including miR-7, miR-498, and miR1194, and downregulated miRNAs, including miR-15a, miR-16, miR-21, miR-124, miR-125a, miR-141, miR-143, miR-187, miR-224, miR-375, miR-487b, and let-7e[18-28]. Collison et al demonstrated that inhibition of miR-145 significantly attenuated allergic inflammation of the nasal mucosa in mite-induced allergic airways disease[29]. However, there is no direct evidence to show that MUC5AC regulated by miRNA. Here I revealed that an exosomal miRNA let-7a-5p suppressed MUC5AC mRNA expression by binding to the 3'-UTR of it upon IL-4 treatment.

Exosomes are extracellular vesicles (EVs) with an average diameter of approximately 100 nm and are released by almost all cell types[30]. However, exosomes are easily decorated with mucins in human primary airway cells, resulting in the secretion of exosomes larger than typical exosomes (approximately 200 nm)[31]. As exosomes transport cellular cargo, including proteins, lipids, and nucleic acids (e.g., miRNAs), their functions are emphasized in cell-cell crosstalk[32,33]. Exosomes isolated from various biological fluids have been investigated for their potential use as diagnostic biomarkers and therapeutics for various diseases. Studies on exosomes in the lower airways have demonstrated that exosomes isolated from bronchoalveolar lavage (BAL) fluids reduce airway inflammation in an allergic sensitization mouse model[34], and exosomes from human primary cell culture secretions enhance innate mucosal defense[35] and transfer cellular information related to

mucin hypersecretion[36]. In a clinical study, miRNAs in EVs isolated from the nasal mucus of patients with AR were analyzed using miRNA arrays[37]. However, the functional mechanism of exosomal miRNAs isolated from the upper airway, such as nasal lavage (NAL) fluid, in AR remains unclear. Given that miRNAs packed in exosomes are involved in intercellular communication between human tracheobronchial epithelial cells (HTBECs) and an airway epithelial cell line (Calu-3) by transmitting inflammatory stimuli [36], I hypothesized that miRNAs in NAL fluid exosomes might be responsible for the pathological progression of AR. I also assumed the presence of miRNAs that exhibited compatible expression patterns in the nasal epithelium of patients with AR *in vivo* and in human nasal epithelial cells (HNECs) upon the induction of allergic inflammation *in vitro* (e.g., IL-4 treatment).

Little attention has been paid on the evaluation of miRNAs function in NAL fluid exosome. To my knowledge, only one study has previously shown the expression profile of NAL fluid-derived exosomal miRNAs isolated from AR patient, however, there is no in-depth study on their molecular mechanism in nasal epithelium exposed to allergen. Therefore, this study aimed to investigate the potential role of let-7a-5p in regulating mucin hypersecretion in airway epithelial cells and its potential as a therapeutic target for AR.

II. MATERIALS AND METHODS

1. Study design and sample collection

The study was approved by the Institutional Review Board of Yonsei University College of Medicine (4-2016-0902 and 4-2016-1153), and all participants provided informed consent.

Recruitment of healthy and AR patients. A total of 17 patients with allergic rhinitis (AR) were enrolled, and all met the following criteria: *i*) over 18 years of age, *ii*) typically showing pale and edematous inferior turbinate mucosa, watery rhinorrhea, and sneezing when collecting samples, *iii*) receiving no immunotherapy, *iv*) no chronic rhinosinusitis as confirmed using computed tomography scans, *v*) not pregnant or breast-feeding, *vi*) not diagnosed with malignant neoplasm, *vii*) no history of nasal surgery, *viii*) no history of allergic skin diseases or allergic asthma, and *ix*) receiving no medication for the treatment of allergic diseases, including AR, during the previous two months.

Next, the subjects were evaluated to confirm an AR diagnosis. AR was diagnosed when patients fulfilled the following criteria: *i*) presenting with persistent rhinitis symptoms (more than four days per week for more than four weeks) for over one year (according to ARIA), *ii*) a positive skin prick test (wheal diameter 3 mm larger than that of the negative control after 15 min) to at least one allergen, and *iii*) high titer (positivity score of 2; 0.70 IU/ml) of IgE antibodies specific for *Dermatophagoides farina* or *Dermatophagoides pteronyssinus* in the Multiple-antigen simultaneous test (MAST). Control groups *i*) presenting with no symptoms of clinical rhinitis, such as chronic sneezing and watery rhinorrhea, *ii*) negative by the skin prick test, and *iii*) with the lowest levels (positivity score of 1; 0–0.34 IU/mL) of allergen specific-IgE antibodies in the MAST were considered as the control group (Table 1).

Table 1. NAL fluid donor demographic information

Analysis	Allergic status	Donor	Age	Sex	Race	Smoking history
Small RNA Sequencing	Control	1	23	Male	Asian	Non-smoker
		2	27	Male	Asian	Non-smoker
		3	55	Female	Asian	Non-smoker
		4	41	Male	Asian	Current smoker
		5	65	Male	Asian	Current smoker
		6	36	Male	Asian	Non-smoker
		7	36	Male	Asian	Non-smoker
		8	27	Male	Asian	Current smoker
		9	29	Female	Asian	Non-smoker
	Allergic Rhinitis	1	36	Male	Asian	Non-smoker
		2	22	Female	Asian	Non-smoker
		3	40	Male	Asian	Non-smoker
		4	26	Female	Asian	Non-smoker
		5	19	Female	Asian	Non-smoker
		6	19	Male	Asian	Non-smoker
		7	42	Male	Asian	Non-smoker
		8	24	Female	Asian	Non-smoker

Sample collection. Nasal lavage (NAL) fluid was collected as previously described [38]. Briefly, the patient was placed in a sitting position with their neck flexed on a chair at the outpatient clinic, and 10 mL of normal saline was introduced into the middle meatus of the nasal cavity using a syringe with a cut-down needle to prevent injury to the nasal mucosa. The lavage fluid was collected into a sterilized 15-mL conical tube. All procedures were performed under endoscopic visualization to obtain lavage fluid from the middle meatus.

Exosomes preparation. The NAL fluid was centrifuged at 200 ×*g* for 10 min, followed by centrifugation at 5,000 ×*g* for 35 min to remove cells and cell debris. The resulting supernatants were filtered using a 0.8-μm pore syringe filter (25CS080AS; ADVANTEC, Tokyo, Japan) and stored at −80 °C. To accumulate exosomes from large volumes of NAL fluid (10 mL) and reduce the total duration of the isolation process, I established an easy and consistent protocol using a column-based exosomal miRNA isolation kit (Figure E1). Exosomes were isolated from pre-filtered NAL fluid samples using the exoRNeasy Serum/Plasma Midi Kit (77044; QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Briefly, one volume of the exosome-binding buffer was added to the samples and applied to the column. After washing, the column membrane-bound Exosomes were lysed with QIAzol for exosomal RNA extraction and lysed with 5x SDS sample buffer for exosomal protein extraction (Figure 1A).

2. Scanning electron microscopy

For scanning electron microscopy, the membranes of the columns were treated with PBS (negative control) or NAL fluid and fixed with chilled Karnovsky's fixative (2% glutaraldehyde, 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4) for 24 h. After washing twice with 0.1 M PBS for 30 min, the membranes were fixed with 1% osmium tetroxide for 2 h, dehydrated in an ascending gradual series (50–100%) of ethanol using a Critical Point Dryer (Leica EM CPD300; Leica, Wetzlar, Germany), coated with platinum by ion sputter (Leica EM ACE600), and observed using a field emission scanning electron microscope (Merlin; ZEISS, Oberkochen, Germany).

3. Nanoparticle tracking analysis (NTA) of exosomes

NTA was performed using apical secretions of human nasal epithelial cells (HNECs) before or after column binding. After adding 500 μL of PBS to the apical part of the Transwell-clear culture insert, mucus layers were incubated for 15 min at 37 °C in a humidified 5% CO_2 incubator. Apical secretions from the three inserts were collected by pipetting, pooling, and centrifugation to remove the cells and cell debris. The resulting supernatants were filtered using a 0.8-μm pore syringe filter. A NanoSight NS300 particle analyzer (NanoSight Ltd., Amesbury, UK) was used to determine the size distribution of the isolated particles. NTA software was used to determine the concentration and size of the exosomes (version 2.3; NanoSight Ltd.).

4. Western blotting

The extracted exosomal protein samples were separated on 10% SDS-PAGE gels and transferred to polyvinylidene fluoride membranes (IPVH00010; Merck Millipore, Burlington, MA, USA). After blocking with TTBS [5% nonfat milk in Tris-buffered saline (50 mM Tris-Cl, pH 7.5, 150 mM NaCl) containing 0.5% Tween 20], the membranes were incubated overnight at 4 °C with antibodies against CD81 (1:500, sc-166029; Santa Cruz Biotechnology, Dallas, TX, USA) or calnexin (1:500, sc-23954; Santa Cruz Biotechnology), followed by incubation with horseradish peroxidase-conjugated secondary antibodies. Protein bands were detected using ECL Western blotting substrate (34580; Thermo Fisher Scientific, Wilmington, DE, USA).

5. RNA extraction

Exosomes were isolated from pre-filtered NAL fluid samples using the exoRNeasy Serum/Plasma Midi Kit (77044; QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Briefly, one volume of the exosome-binding buffer was added to the samples and applied to the column. After washing, the column membrane-bound exosomes were lysed with QIAzol for exosomal RNA extraction (Figure E1). The assessment of both total RNA and small RNA quality was conducted using the Bioanalyzer RNA Pico chip (25~4,000 bp) and Bioanalyzer Small RNA chip (4 ~ 150 bp) (Agilent Technologies, Waldbronn, Germany).

6. Small RNA library construction & sequencing

The RNA isolated from each sample was used to construct sequencing libraries using the SMARTer smRNA-Seq Kit for Illumina (Illumina, San Diego, CA, USA), following the manufacturer's protocol. Briefly, the Input RNA was polyadenylated to provide a priming sequence for the oligo(dT) primer. cDNA synthesis was primed by the 3′ smRNA dT primer, which incorporates an adapter sequence at the 5′ end of each RNA template to add non-templated nucleotides, which are bound by the SMART smRNA Oligo-enhanced with locked nucleic acid (LNA) technology for greater sensitivity. In the template-switching step, PrimeScript RT incorporates the SMART smRNA Oligo as a template for adding a second adapter sequence to the 3′ end of each first-strand cDNA molecule. In the next step, full-length Illumina adapters (including index sequences for sample multiplexing) were added during polymerase chain reaction (PCR) amplification. The Forward PCR primer bound to the sequence added by the SMART smRNA Oligo, whereas the Reverse PCR primer bound to the sequence added by the 3′ smRNA dT primer. The resulting cDNA library included the sequences required for clustering in an Illumina flow cell. The libraries were gel purified and validated by checking their size, purity, and concentration using an Agilent Bioanalyzer (Agilent). Libraries were quantified using quantitative PCR (qPCR) according to the qPCR Quantification Protocol Guide (KAPA Library Quantification kits for Illumina Sequencing platforms) and qualified using a TapeStation D1000 ScreenTape (Agilent). The libraries were pooled in equimolar amounts and sequenced on an Illumina HiSeq 2500 instrument to generate 51 bases with single-end reads. Image decomposition and quality value calculations were performed using Illumina pipeline modules.

Adaptor trimming. Raw sequencing reads of circulating miRNAs from different experimental samples were preprocessed and analyzed using miRDeep2. Adaptor trimming was performed to eliminate adaptor sequences that existed in the reads attached to the miRNA during smRNA library construction. If a read matched at least the first 8 bp of the 3′ adapter sequence, the sequence was regarded as an adapter sequence and trimmed from the read. Trimmed reads needed to have a minimum of 18 bp to be considered reliable for analysis.

Clustering. Adaptor sequence-trimmed reads were gathered to form a cluster to minimize sequence uniqueness and computational intensity. This cluster contained reads that were 100% matched to the sequence identity and read length and were assigned a temporary cluster ID with the number of reads therein.

Sequence alignment and detection of known and novel miRNAs were performed using miRDeep2 software. Prior to sequence alignment, the *Homo sapiens* reference genome hg19 was retrieved from the UCSC genome browser and indexed using Bowtie (1.1.2), a bowtie for aligning sequencing reads to reference sequences. These reads were then aligned to *H. sapiens* matured and precursor miRNAs obtained from miRBase v21. The miRDeep2 algorithm is based on the miRNA biogenesis model; it aligns reads to potential hairpin structures in a manner consistent with Dicer processing and assigns scores that represent the probability that hairpins are true miRNA precursors. In addition to detecting known and novel miRNAs, miRDeep2 was used to estimate their abundance.

Novel miRNA prediction. Novel miRNAs were predicted from mature, star, and loop sequences according to the RNAfold algorithm using miRDeep2. The RNAfold function uses a nearest-neighbor thermodynamic model to predict the minimum free-energy secondary structure of an RNA sequence. The RNA fold-generated graphic contains the actual *in silico*-folded hairpin, along with the number of reads for each part of the hairpin, score for minimum free energy, score for random folding, and score for the conserved seed sequence.

Proportion of miRNA and other RNA categories. Uniquely clustered reads were then sequentially aligned to the reference genome, miRBase v21, and the non-coding RNA database Rfam 9.1 to identify known miRNAs and other types of RNA.

Statistical analysis of differential miRNA expression. Raw data (reads for each miRNA) were normalized by relative log expression normalization using DESeq2. For pre-processing, miRNAs with zero counts across more than 50% of all samples were excluded, leaving 137 mature miRNAs to be analyzed. I added 1 to the normalized read count of the filtered miRNAs to facilitate log2 transformation and plotted the correlation. For each miRNA, the base mean and log-fold changes were calculated between the AR and control groups. A statistical hypothesis test for comparison of the two groups was conducted using the negative binomial Wald test in DESeq2. Differentially expressed miRNAs between two groups were determined by adjusting |fold change|≥2 and *p*-value < 0.05.

Multidimensional scaling. I used the multidimensional scaling (MDS) method to visualize similarities among the samples. MDS converts a structure in a similarity matrix into a simple geometrical picture as a scatter plot. The larger the dissimilarity between the control and AR samples, the further apart are the points representing the experiments in the picture. I applied Euclidean distance as a measure of dissimilarity.

Hierarchical clustering. Hierarchical clustering analysis was also performed using complete linkage and Euclidean distance as a measure of similarity to display the expression patterns of differentially expressed miRNAs that are satisfied with |fold change|≥2 & *p*-value < 0.05. All data analyses and visualization of differentially expressed genes were conducted using R 3.3.1 (www.r-project.org).

7. Cell culture

Human nasal epithelial cells (HNECs) were cultured as previously described [39,40]. Briefly, passage #2 HNECs were seeded at a density of 1×10^5 cells on a 12-mm, 0.45-μm pore Transwell-clear culture insert (Costar Co, Cambridge, MA, USA) and incubated at 37 °C in a humidified 5% CO_2 incubator. The cells were cultured in a 1:1 mixture of bronchial epithelial cell growth media and DMEM, supplemented with growth factors according to the manufacturer's instructions (Lonza, Basel, Switzerland). After reaching confluence under submerged culture conditions, the cells were maintained in an air-liquid interface (ALI) culture system by removing the medium from the apical chamber. Culture medium was changed every 2 days. Cells were treated with 10 ng/mL of IL-4 on Day 14 of ALI culture (ALI D14) for 7 days.

8. miRNA transduction

For lentiviral miRNA overexpression, miR-overexpressing lentiviruses were purchased from Lugen Science (CS940MR; Puchun, Korea). HNECs were infected with lentiviruses bearing miRNAs at 4 h after seeding, at a multiplicity of infection (MOI) of 10. Lentiviral scrambled miRNA (sc-miR) served as a negative control. The infected cells were incubated for 24 h at 37 °C in a humidified 5% CO_2 incubator, after which the virus-containing medium was replaced with fresh complete medium.

9. Quantitative real-time reverse-transcription-PCR (qRT-PCR)

For *in vitro* sample validation, total RNAs were extracted from HNECs using an RNeasy mini kit (74104; Qiagen) according to the manufacturer's instructions. RNA (500 ng) was converted into cDNA using the PrimeScript™ RT Master Mix (RR036A; Takara, Shiga, Japan). qRT-PCR was performed using the KAPA SYBR® FAST qPCR Master Mix Kit (KK4605; KAPA Biosystems, Wilmington, MA, USA). The following primers were used: human *MUC5AC* (forward, 5′-CCT TCG ACG GAC AGA GCT AC-3′; reverse, 5′-TCT CGG TGA CAA CAC GAA AG-3′), human SPDEF (forward, 5'-ATGAAAGAGCGGACTTCACCT-3'; reverse, 5'- CTGGTCGAGGCACAGTAGTG-3'), human *GAPDH* (forward, 5′-ACA GTT GCC ATG TAG ACC-3′; reverse, 5′-TTT TTG GTT GAG CAC AGG-3′). *GAPDH* was used as a housekeeping gene for normalization. Quantitative real-time PCR of exosomal miRNAs was performed using the QuantiMir RT kit (RA420A-1, SBI) with total RNAs from HNECs and normalized against the level of miR-4516. The following primers were used: has-let-7a-5p (5'-TGAGGTAGTAGGTTGTATAGTT-3'), U6 (5'- CGCAAGGATGACACGCAAATTC-3'), has-miR-4516 (5'- GGGAGAAGGGTCGGGGC-3').

For in vivo sample validation, patient-derived NAL fluid exosomal RNAs were extracted using exoRNeasy Serum/Plasma Midi Kit as described in Materials and Methods. qRT-PCR of exosomal miRNAs was performed using the QuantiMir RT kit (System Biosciences, Palo Alto, CA, USA) and normalized by U6 small nuclear RNA or miR-4516. The primers were used described above.

10. Luciferase reporter assay

The direct interaction between let-7a-5p and *MUC5AC* mRNA was examined using a dual-luciferase vector (pmirGLO dual-luciferase vectors; Promega, Madison, WI, USA) containing the wild-type (WT) and mutant (MT) sequences of the let-7a-5p miRNA recognition element. Following the transfection of HEK293T cells (CRL-3216; ATCC) with negative control miR (nc-miR) or let-7a-5p mimic, an equal number of transfected cells were resuspended in 24-well plates and transfected with either the WT or MT luciferase vector. Luciferase activity was assessed using a Dual-GLO Luciferase Assay System (Promega).

11. Ribonucleoprotein immunoprecipitation (RIP) assay

The enrichment of *MUC5AC* mRNA in miRNA-induced silencing complex (miRISC) was assessed by AGO2 RIP using Dynabeads® Protein G (Thermo Fisher Scientific) as previously described [41]. Briefly, beads were coated with anti-IgG or anti-AGO2 antibody (Sigma, St. Louis, MO, USA) prior to incubation. After cell lysates were prepared using polysome lysis buffer, equal amounts were incubated with antibody-coated Dynabeads for 4 h. Following treatment with DNase I (Thermo Fisher Scientific) and protease K (Bioneer, Daejeon, Korea), RNA was isolated by precipitation with absolute ethanol. The level of RIP mRNA was determined using qRT-PCR.

12. Immunofluorescence confocal microscopy

For immunofluorescence staining, HNECs on the culture insert were fixed with 4% paraformaldehyde for 20 min and permeabilized with 1% Triton X-100 for 5 min at room temperature. The cells were then incubated with 50 mM NH_4Cl for 15 min and blocked with blocking solution (1% bovine serum albumin, 10% normal donkey serum, and 1% Triton X-100 in PBS) for 1 h at room temperature. After incubation in the blocking solution, the cells were stained with mouse anti-MUC5AC antibodies (MA1-21907; Invitrogen, Carlsbad, CA, USA), followed by incubation with goat anti-mouse Alexa568-labeled secondary antibodies, (A11004; Invitrogen). Nuclei were stained with 4,6,-diamidino-2-phenylindole (DAPI, Sigma) for 2 min. Immunofluorescence signals were visualized using an LSM780 laser scanning confocal microscope (Carl Zeiss MicroImaging GmbH, Jena, Germany).

13. Statistical analysis

Data are expressed as the mean ± SEM. Student's *t*-test or one-way analysis of variance was used to compare groups. *P* values <0.05 were considered statistically significant.

III. RESULTS

1. Characterization of exosomes isolated from human NAL fluids

Exosomes were isolated from the NAL fluids of control subjects and patients with AR to identify the exosomal miRNAs responsible for AR. To accumulate exosomes from massive volumes of NAL fluid (10 mL) and reduce the total duration of the isolation process, I established an easy and constant protocol using a column-based exosomal miRNA isolation kit as described in *Methods* (Figure 1A). I first examined the isolated structures as extracellular vesicle-phenotypic exosomes using scanning electron microscopy, NTA analysis and Western blot analysis. Scanning electron microscopy images showed that the structures attached to the membrane surfaces of the columns were globular in shape and similar to mucin-decorated exosomes (approximately 200 nm diameter) (Figure 1B). Nanoparticle tracking analysis (NTA) also showed that the size of the secreted exosomes was approximately 100-350 nm (Figure 1C). I quantified the number of extracellular vesicles (EVs) obtained from nasal fluids by NTA analysis. NTA analysis revealed the detection of approximately 1.1 x 10^9 EVs/mL, and no significant difference was observed between control (n = 5) and AR patients (n = 4) (Figure 1D). Further analysis by western blotting indicated that the structures from all NAL fluid samples (control, $n = 4$ vs. AR, $n = 4$) showed the presence of CD81, a member of the tetraspanin family, used as a marker of exosomal fractions, and the absence of calnexin, an endoplasmic reticulum protein, used as a marker of non-exosomal fractions (Figure 1E). These results demonstrate that the structures isolated using my protocol were exosomes. Further, there were no significant differences in the amount of CD81-enriched exosomes between the control and patients with AR, indicating that the number of exosomes secreted by the two groups did not differ significantly.

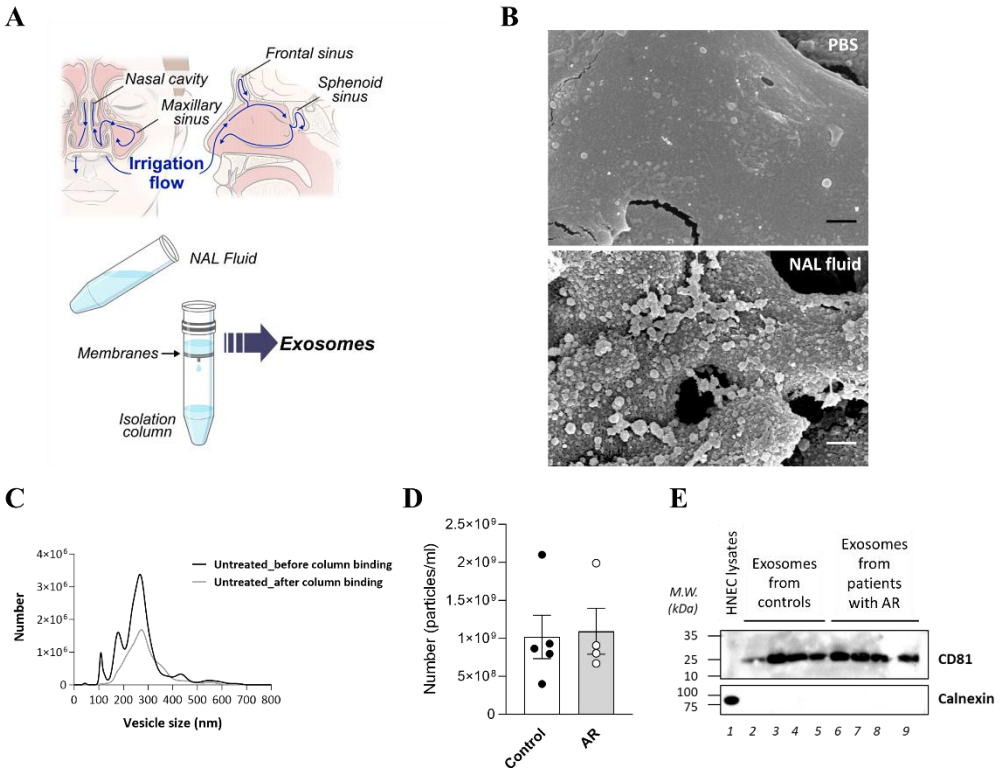


Figure 1. Isolation of exosomes from human NAL fluids (A) Scheme of NAL fluid exosome isolation using isolation column. (B) Scanning electron microscopy images of the isolation column surface after filtering PBS or NAL fluids. Scale bar = 500 nm. (C) Nanoparticle tracking analysis (NTA) of exosomes isolated from human nasal epithelial cells. NTA was performed using apical secretions of human nasal epithelial cells before or after column binding. (D) Numbers of EVs isolated from control (n = 5) and AR patients (n = 4) determined by NTA. (E) Western blot analysis showing the presence of tetraspanin CD81, a marker of exosomal fractions, albeit the absence of the endoplasmic reticulum protein calnexin, a marker of non-exosomal fractions, in NAL fluid-derived exosomes. HNEC cell lysates were used as a negative control. NAL = nasal lavage; HNECs = human nasal epithelial cells. M.W. = molecular weight.

2. Differential expression of miRNAs in NAL fluid exosomes

To identify the miRNAs responsible for AR, I conducted small RNA sequencing (smRNA-seq) using exosomal RNAs isolated from the NAL fluid of control subjects ($n = 9$) or patients with AR ($n = 8$). By analyzing the smRNA-seq data, 181 mature miRNAs were identified (Figure 2A). Among them, 32 miRNAs were differentially expressed in AR with a two-fold change compared to the control ($p < 0.05$); only one miRNA was upregulated, whereas 31 miRNAs were downregulated in AR compared to the control. (Figure 2B).

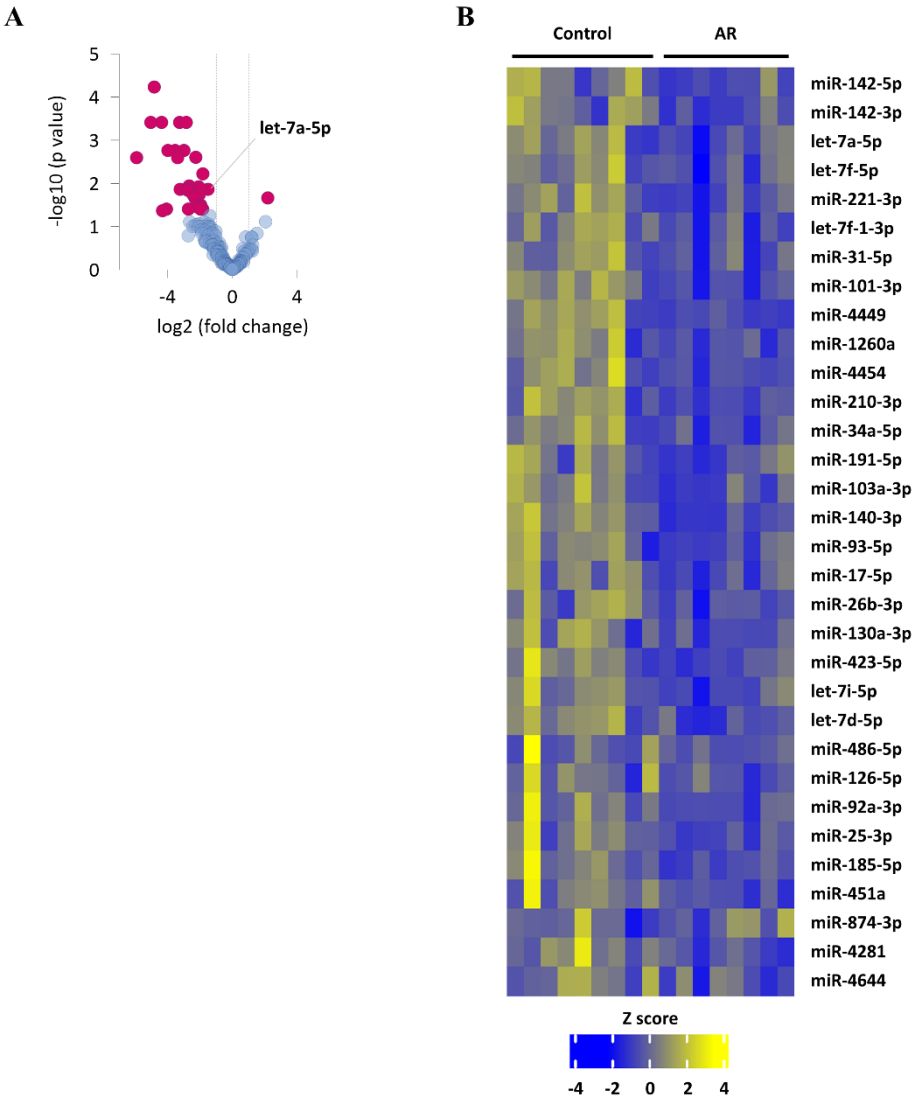
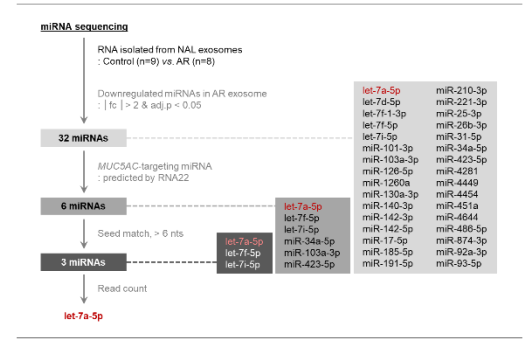


Figure 2. Small RNA sequencing analysis of NAL fluid exosomes. (A) Volcano plot of fold changes (AR/control) in miRNA abundance between control and AR NAL fluid exosomes (n = 9 *vs.* n = 8, respectively). The x-axis represents the log2 ratio; the y-axis represents significant differences (−log10 of p-value). Significantly changed miRNAs are colored in magenta (> 2-fold, $p < 0.05$). (B) Heat map of 32 differentially expressed miRNAs. AR = allergic rhinitis; miRNA = microRNA.

3. Identification of let-7a-5p as a candidate miRNA downregulated in AR exosomes

Given that goblet cell hyperplasia of epithelial cells in inflammatory airway diseases correlates with increased MUC5AC mRNA expression[8], and MUC5AC copy number correlates with AR susceptibility[42], the target miRNA was selected for further study as shown Figure 3A. To determine whether miRNAs may regulate MUC5AC expression, I predicted potential miRNA binding sites within the MUC5AC mRNA 3′UTR using the RNA22 miRNA target discovery tool (https://cm.jefferson.edu/rna22/). Bioinformatics analysis revealed that MUC5AC possessed miRNA recognition elements (MREs) for six of the 32 screened miRNAs (Figure 3B). As let-7a-5p exhibited the most significant folding energy and abundance (baseMean), I evaluated its role in MUC5AC induction and mucus hypersecretion.

A



B

Heteroduplex		Folding energy (in –Kcal/mol)	baseMean
5′ GCTATGGGT-CACCTGCTGCCT 3′ TGTTGGTCGATTCTGTGACGGT	3′UTR of *MUC5AC* mRNA miR-34a-5p	-12.10	8
5′ CCCCGCCTGC-AGCCACCTCTCA 3′ TTTCAGAGCGAGAGACGGGGAGT	3′UTR of *MUC5AC* mRNA miR-423-5p	-14.10	104
5′ GAAGAACCTCACTCCTACCTCA 3′ TTGATATGTTGGATGATGGAGT	3′UTR of *MUC5AC* mRNA let-7a-5p	-15.20	274
5′ GAAGAACCTCACTCCTACCTCA 3′ TTGATATGTTAGATGATGGAGT	3′UTR of *MUC5AC* mRNA let-7f-5p	-15.20	114
5′ GAAGAACCTCACTCCTACCTCA 3′ TTGTCGTGTTTGATGATGGAGT	3′UTR of *MUC5AC* mRNA let-7i-5p	-16.90	11
5′ CAGCCCT--CAGC-CTGCGCTC 3′ TACGGGAAAATTGTAACGTGAC	3′UTR of *MUC5AC* mRNA miR-103a-3p	-15.00	5
5′ TCAGCCTGCGCTC-C-CCTCCTCA 3′ TTTCAGA-GCGAGAGACGGGGAGT	3′UTR of *MUC5AC* mRNA miR-423-5p	-18.40	104

Figure 3. Identification of let-7a-5p as a candidate miRNA downregulated in AR exosomes. (A) Flow chart illustrating the steps in the target miRNA discovery strategy. (B) Using RNA22, six of the 24 miRNAs were predicted to strongly interact with the 3’UTR of *MUC5AC* mRNA. Among these, let-7a-5p was selected for further evaluation based on its folding energy and abundance. AR = allergic rhinitis; miRNA = microRNA. UTR = untranslated region.

4. IL-4 down-regulates let-7a-5p in HNECs

For *in vitro* validation of let-7a-5p, HNECs were treated with IL-4 to mimic allergic conditions (Figure 4A). Consistent with previous studies[43,44], MUC5AC expression was increased by more than 20-fold by IL-4 treatment in fully differentiated HNECs (Figure 4B). The expression level of let-7a-5p was examined using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). I found that the expression levels of let-7a-5p was significantly reduced in IL-4-treated HNECs (Figure 4C).

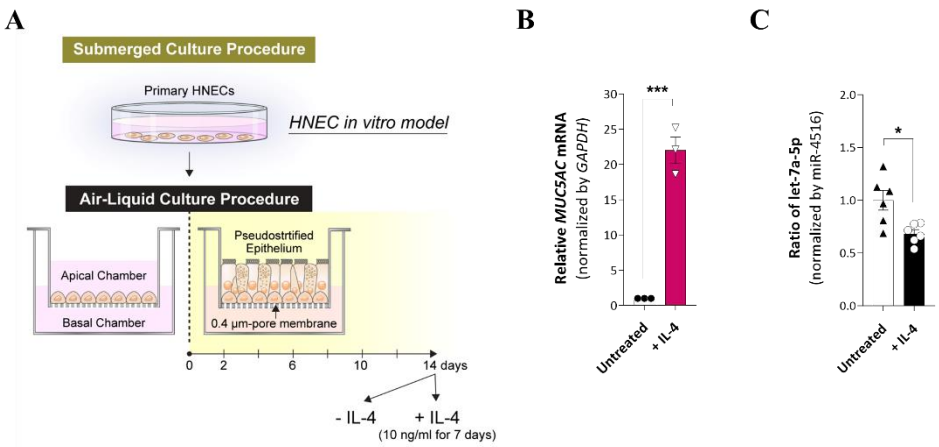


Figure 4. IL-4-induced increase in *MUC5AC* mRNA expression and decrease in let-7a-5p expression in HNECs. (A) Scheme of *in vitro* validation. Timeline of HNEC differentiation from submerged culture to air-liquid interface culture (for 14 days) and IL-4 treatment (for 7 days). (B) *MUC5AC* expression without or with IL-4 treatment for 7 days as determined in HNECs by qRT-PCR ($n = 3$). (C) Expression of let-7a-5p in the IL-4-treated HNECs ($n = 6$) as analyzed by qRT-PCR on Day 21. Results represent the means ± SEM; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ by student's *t*-tests. HNECs = human nasal epithelial cells; IL = interleukin; miR = microRNA; qRT-PCR, quantitative real-time reverse-transcription-polymerase chain reaction.

5. Overexpressed let-7a-5p inhibits IL-4-induced *MUC5AC* expression

To define the functional roles of let-7a-5p in AR, I tested whether let-7a-5p blocked the upregulation of MUC5AC expression induced by IL-4. To obtain a higher transduction efficiency, HNECs were infected with lentiviruses expressing the let-7a-5p at a multiplicity of infection of 10 before IL-4 treatment. I confirmed that let-7a-5p was overexpressed and maintained in the presence of IL-4 until 21 days post-infection (Figure 5A). Overexpression of let-7a-5p significantly inhibited the upregulation of *MUC5AC* by IL-4 compared with scrambled miRNA (sc-miR) (40.9 ± 8.00%) (Figure 5B). Spearman's correlation analysis showed that the level of let-7a-5p was negatively correlated with that of *MUC5AC* mRNA (p = 0.0003; Figure 5C).

Similar to the mRNA levels, MUC5AC protein levels were increased by IL-4 treatment, and IL-4-mediated MUC5AC induction was blocked by let-7a-5p overexpression (Figure 6A and 6B). Additionally, let-7a-5p overexpression did not affect the expression of SAM-pointed domain-containing ETS transcription factor (*SPDEF)*, an upstream regulator of *FOXA3*, *AGR2*, and *MUC5AC*[45], indicating that the inhibitory effect of let-7a-5p is specific to *MUC5AC* mRNA (Figure 6C).

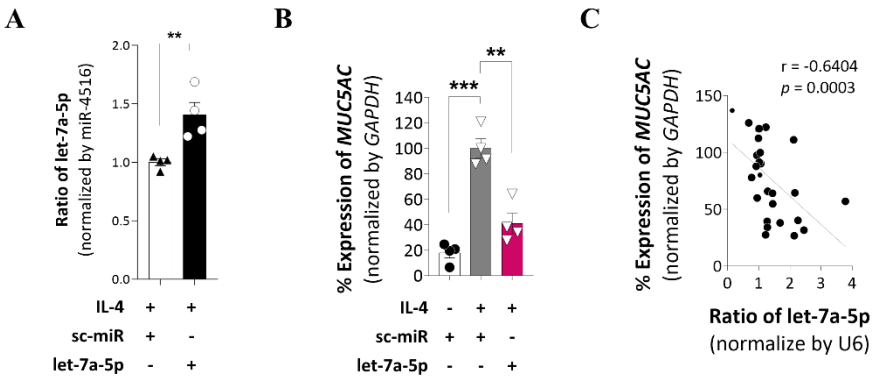


Figure 5. Exosomal miRNA let-7a-5p suppresses *MUC5AC* mRNA expression. (A-C) Scrambled miRNA (sc-miR) or let-7a-5p was overexpressed in HNECs via lentiviral transduction, followed by IL-4 treatment. (A) Levels of let-7a-5p overexpressed in HNECs using a lentiviral expression system at a multiplicity of infection of 10, as analyzed by qRT-PCR with or without IL-4 treatment for 7 days (n = 4). (B) *MUC5AC* gene expression as analyzed by qRT-PCR (n = 4). (C) Spearman linear correlation between *MUC5AC* and let-7a-5p expression in HNECs (n = 27). Results represent the means ± SEM; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001 by student's t-tests or one-way analysis of variance with Turkey's multiple comparisons test. HNEC = human nasal epithelial cells; IL = interleukin; qRT-PCR = quantitative real-time reverse-transcription-polymerase chain reaction; *n.s.* = not significant

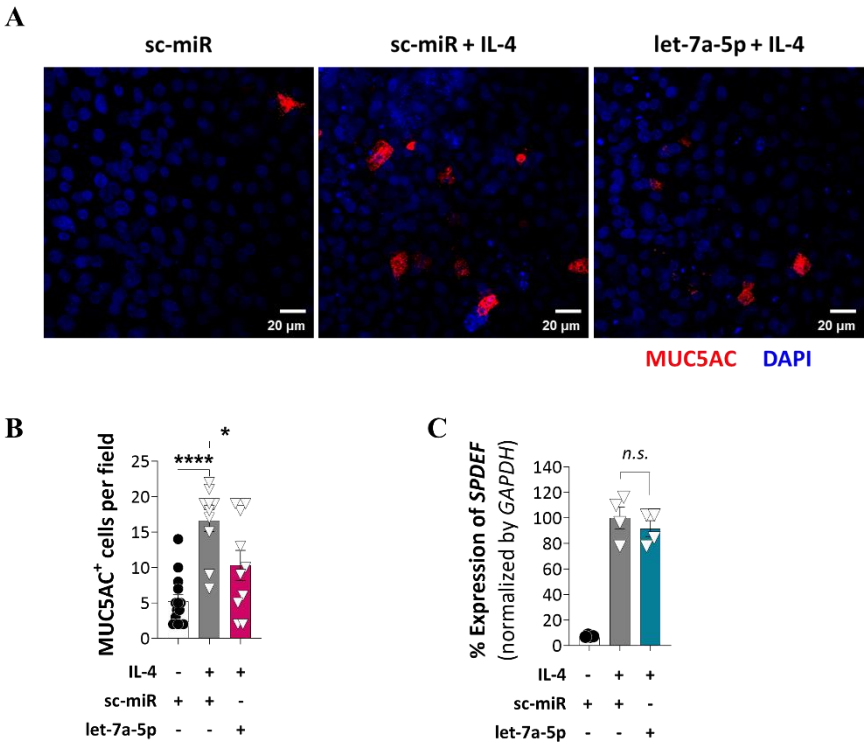


Figure 6. Exosomal miRNA let-7a-5p suppresses MUC5AC protein level. (A-C) Scrambled miRNA (sc-miR) or let-7a-5p was overexpressed in HNECs via lentiviral transduction, followed by IL-4 treatment. (A) MUC5AC protein expression as analyzed by immunofluorescence staining with antibodies against MUC5AC (*red*) and with DAPI (*blue*). (B) MUC5AC-positive ($MUC5AC^+$) cells were counted and presented as bar graphs; sc-miR-overexpressed and untreated group (*sc-miR*, n = 14), sc-miR-overexpressed and IL-4-treated group (*sc-miR* + *IL-4*, n = 10), and let-7a-5p-overexpressed and IL-4-treated group (*let-7a-5p* + *IL-4*, n = 10). (C) *SPDEF* gene expression as analyzed by qRT-PCR (n = 4). Results represent the means ± SEM; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001 by student's t-tests or one-way analysis of variance with Turkey's multiple comparisons test. HNEC = human nasal epithelial cells; IL = interleukin; qRT-PCR = quantitative real-time reverse-transcription-polymerase chain reaction; *n.s.* = not significant

6. let-7a-5p regulates *MUC5AC* mRNA expression

The miRNA target prediction algorithm RNA22 (https://cm.jefferson.edu/rna22/) predicted a direct interaction between let-7a-5p and the 3′ UTR of *MUC5AC* mRNA. There is one MRE in the sequence of the 3′ UTR of *MUC5AC* mRNA (Figure 7A). To validate whether the inhibitory effect of let-7a-5p on *MUC5AC* expression was dependent on the MRE, I constructed two luciferase reporter vectors containing the wild-type (WT) or mutant (MUT) sequence of the let-7a-5p MRE (Figure 7A). Since the transfection efficiency was not sufficient to investigate the effect of let-7a-5p on luciferase expression in HNECs, this assay was conducted in HEK293 cells. The luciferase assay indicated that overexpression of let-7a-5p significantly suppressed the expression of luciferase in the WT reporter vector but not in the MUT reporter vector (Figure 7B).

I hypothesized that the dramatic decrease in let-7a-5p expression was responsible for the increased expression of *MUC5AC* in IL-4-treated HNECs. As depicted in Figure 7C, the suppressive effect of let-7a-5p may be attributed to the formation of miRISC, which occurs when let-7a-5p is loaded onto AGO2 and binds to the 3′ UTR of *MUC5AC* mRNA. To investigate whether miRISCs are involved in the increase in MUC5AC expression, the enrichment of *MUC5AC* mRNA in miRISCs was examined. The AGO2 RNA immunoprecipitation (RIP) assay showed that *MUC5AC* mRNA was more enriched in AGO2 IP than in control IgG IP (2.0 ± 0.34-fold) (Figure 7C). However, upon IL-4 treatment, the enrichment of *MUC5AC* mRNA in the AGO2 IP was significantly reduced (Figure 7D). These results suggest that IL-4 significantly lowered the expression level of *MUC5AC* mRNA-targeting let-7a-5p, which led to the upregulation of MUC5AC.

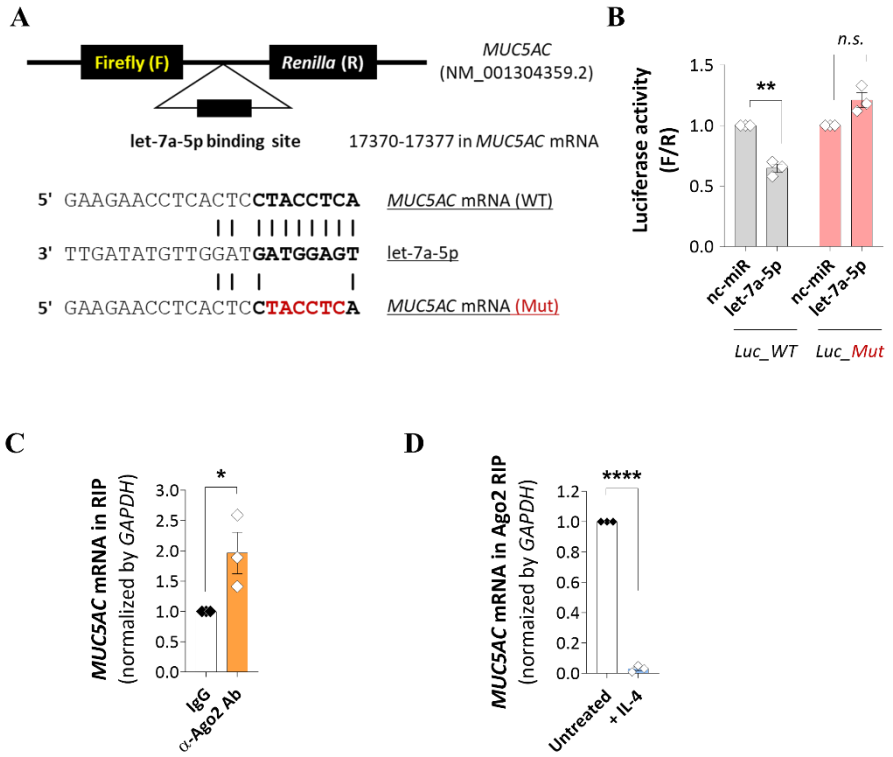


Figure 7. *MUC5AC* is a novel target of let-7a-5p. (A) Results of bioinformatics analyses showing a miRNA-recognition element (MRE) of let-7a-5p in the *MUC5AC* mRNA 3′UTR (nt 17,370–17,377). Luciferase reporter vectors containing wild-type (*WT*) or mutant (*Mut*) sequences of let-7a-5p MRE were constructed. (B) Assessment of luciferase activity in HEK293T cells following let-7a-5p overexpression, presented as a ratio of the firefly (F) to *Renilla* (R) luciferase activities (F/R). (C) RNA immunoprecipitation (RIP) of HNEC lysates with α-AGO2 antibodies. The amount of *MUC5AC* mRNA in AGO2 RIP complexes is presented as a ratio to that in control IgG RIP complexes ($n = 3$). (D) The amount of *MUC5AC* mRNA in IL-4-treated AGO2 RIP complexes is presented as a ratio to that in untreated AGO2 RIP complexes ($n = 3$). Results represent the means ± SEM; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ by student's *t*-tests or one-way analysis of variance with Turkey's multiple comparisons test. nc-miR = negative control miR; WT = Wild type; Mut = Mutant; AGO2 = Argonaute2; miRISC = miRNA-induced silencing complex.

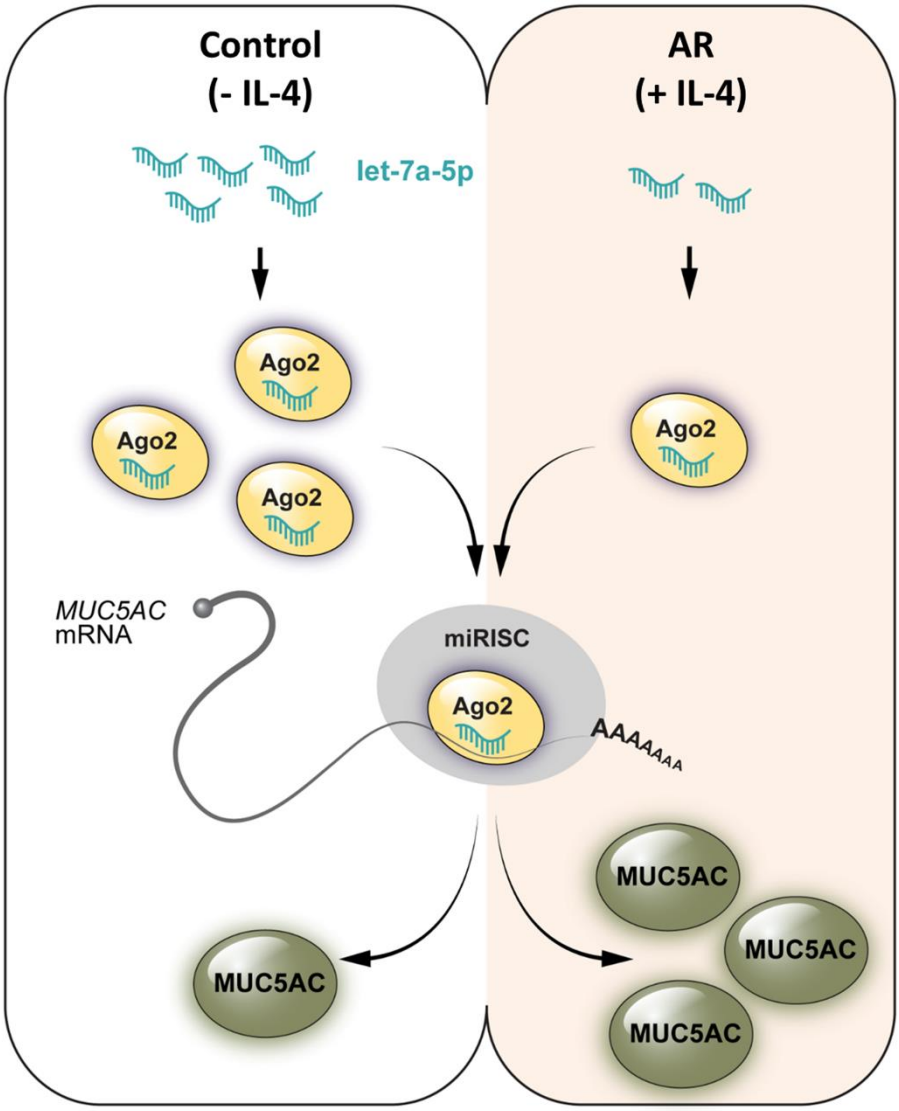


Figure 8. Schematic diagram of *MUC5AC* gene regulation by let-7a-5p-containing miRISC. miRISC, miRNA-induced silencing complex.

7. let-7a-5p is down-regulated in AR NAL fluid exosomes

To investigate the potential of exosomal let-7a-5p as a new therapeutic target for AR, I examined the expression level of let-7a-5p in NAL fluid exosomes using a larger sample size of controls (n = 8) and patients with AR (n = 15). My findings verified that let-7a-5p was present at very low levels in the exosomes isolated from the NAL fluid of patients with AR compared with control (49.6 ± 7.10%) (Figure 9A). The receiver operating characteristic (ROC) curve demonstrated that let-7a-5p differentiated AR from the control, with an area under the curve of 0.783 (p = 0.028) (Figure 9B). These results suggest that targeting let-7a-5p in exosomes isolated from the NAL fluid may be a promising approach for AR treatment by reducing mucus production.

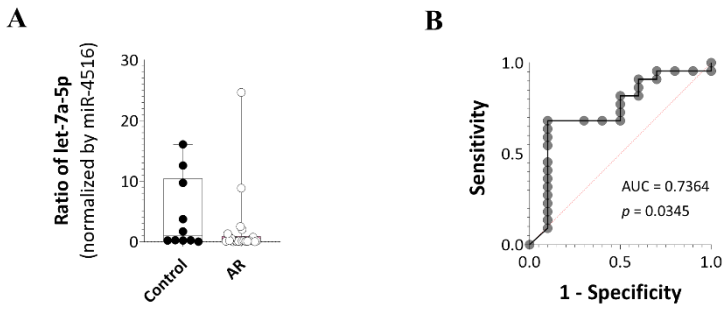


Figure 9. Down-regulation of let-7a-5p in NAL fluid exosomes of patients with AR. (A) The amount of let-7a-5p was determined by qRT-PCR in NAL fluid exosomes of control (n = 10) and AR (n = 22) patients. (B) Receiver operating characteristic curve showing performance of let-7a-5p expression in NAL fluid between control and AR. Results are the means ± SEM; *p < 0.05; ****p < 0.0001 by student's t-tests or one-way analysis of variance with Turkey's multiple comparisons test. AR = allergic rhinitis; NAL = nasal lavage; qRT-PCR = real-time quantitative reverse transcription polymerase chain reaction.

IV. DISCUSSION

Since the discovery of lethal-7 (let-7) as an essential gene in *Caenorhabditis elegans* development[46], let-7 and its family members have been recognized as some of the first miRNAs. The let-7 family members are highly conserved across species; however, let-7h, let-7j, and let-7k are not expressed in humans[47]. Several members of the let-7 family have been reported to be associated with allergic diseases. Polikepahad et al.[48] reported that *IL-13* is a direct target of let-7a, and the inhibition of let-7a by locked nucleic acid (LNA) anti-miRNAs significantly upregulates *IL-13* mRNA expression. Furthermore, intravenous injection of anti-let-7a LNA decreased allergic airway inflammation in an ovalbumin (OVA)-induced asthma mouse model. In a combined study using *in silico* approaches and *in vitro* human primary cell cultures, seven members of the let-7 family (let-7a, let7b, let-7c, let-7d, let7f, let-7g, and let-7i) were downregulated in OVA-challenged asthmatic lungs, and intranasal administration of a let-7 mimic ameliorated asthmatic phenotypes, including inflammatory cell infiltration and mucus hypersecretion[49]. In contrast, let-7b was upregulated in an HDM-induced asthma mouse model; however, no effect of anti-let-7b on mucus secretion or eosinophil accumulation was observed[50]. A recent study revealed that let-7a-5p is downregulated in chronic rhinosinusitis with nasal polyps (CRSwNP) tissues, suggesting *IL-6* is a target gene of let-7a-5p[51]. However, none of the miRNAs, including let-7 family miRNAs, are known to target *MUC5AC*. In this study, I identified *MUC5AC* as a direct target of let-7a-5p and suggested that the delivery of exosomal let-7a-5p to the site of nasal mucosal inflammation may be a useful strategy to reduce mucus hypersecretion and consequently ameliorate the allergic symptoms of various airway inflammatory diseases.

Although I showed that let-7a-5p is downregulated in AR NAL fluid (*in vivo*) and IL-4-treated HNECs (*in vitro*), the underlying mechanism of the decrease in let-7a-5p has not been fully elucidated. Long non-coding RNAs and circular RNAs have been recently reported to participate in AR pathogenesis by functioning as competitive endogenous RNA. For example, linc00632 and CDKN2B antisense RNA1 (ANRIL) suppress the expression of *MUC5AC* by mitigating the inhibitory functions of miR-498[24] and miR-15a[52], respectively. Additionally, circARRDC3 contributes to the development of AR by regulating the miR-375/KLF4 pathway[25]. These reports indicate that miRNAs are intricately involved in AR, either alone or through interactions with other non-coding RNAs, and suggest that targeting miRNAs may offer a new approach to diagnose and treat

patients with AR.

As IL-4 and IL-13 are encoded by adjacent genes and share a functional receptor complex, Type II IL-4R (which consists of IL-4Rα and IL-13Rα1), it was originally assumed that they would serve analogous functions in the pathogenesis of allergic disease. Although a series of *in vivo* functional experiments conducted in mice have shown that IL-4 and IL-13 play distinct roles in allergic inflammation, it remains controversial whether these two cytokines are involved in distinct signaling pathways of the allergic response and/or whether they work alone or in concert to induce pathophysiological manifestations of the disease[53]. Previous studies have shown that IL-4 successfully induces MUC5AC expression at the mRNA and protein levels in *in vivo* mouse models[54,55] and *in vitro* primary epithelial cells[10,38,43,44]. Recently, Cho et al.[56] demonstrated that the majority of differentially expressed genes in scRNA-seq analysis of HNECs treated with either IL-4 or IL-13 overlapped and that only a few biological processes were different. I examined the suppressive effects of let-7a-5p on *MUC5AC* expression in IL-13-treated HNECs, and let-7a-5p was overexpressed and maintained in the presence of IL-13 for 21 days after lentiviral infection (Figure E6A). Similar to IL-4 treatment, *MUC5AC* was highly induced by IL-13 (16.3 ± 3.48-fold), and the upregulation of *MUC5AC* was abolished by overexpression of let-7a-5p (Figure E6B). These results indicate that let-7a-5p has an inhibitory effect on *MUC5AC* expression following both IL-4 and IL-13 stimulation.

Recently, several studies have been conducted on the role of exosomes in AR. Specific exosomal miRNAs in the nasal mucus or serum may provide diagnostic or prognostic potential for AR detection[37,57]. In particular, serum exosomal miR-4669 has been associated with the efficacy of subcutaneous immunotherapy in children with AR[57]. Additionally, the therapeutic application of exosomes is promising because they have been found to be well-tolerated *in vitro*[58-60] and *in vivo* [61]. The therapeutic effect of exosomal miRNAs on the occurrence and progression of AR has been intensively investigated in association with T helper 2 (Th2) immunity[62-66]. However, the function of exosomes and/or exosomal miRNAs in regulating mucin-secreting epithelial tissues remains unknown. Having shown that the exosomal miRNA let-7a-5p isolated from NAL fluid can inhibit mucus hypersecretion in airway epithelial cells, I propose it as a potential therapeutic target for AR treatment.

V. CONCLUSION

My study reveals previously unknown role of exosomal miRNA isolated from NAL fluid. Using a small RNA sequencing (smRNA-seq) assay, I found that thirty-two exosomal miRNAs were significantly differentially expressed in NAL fluid exosomes from AR patients compared with those from non-AR patients, suggesting that miRNA may be involved in the pathologic process of AR. Among them, let-7a-5p suppresses allergic responses by directly down-regulation of MUC5AC expression. Furthermore, my findings suggest that let-7a-5p is a potential therapeutic target for AR treatment.

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ABSTRACT(IN KOREAN)

비강 세척액 유래 엑소좀 내의 마이크로 RNA인 let-7a-5p의 인터류킨 4에 의해 유도되는 MUC5AC 발현 억제 기전 연구

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정 다 은

마이크로 RNA는 술잔세포의 과형성 및 비강 점막의 만성 염증을 포함하는 알레르기성 비염의 병인 및 발달과 연관되어 있습니다. 그러나 마이크로 RNA가 코점막 상피세포에서 표적 유전자를 조절하여 점액의 과분비를 유도하는 자세한 기전은 연구된 바가 미비한 실정입니다. 이에 저는 알레르기성 비염 환자의 비강세척액에서 분리한 엑소좀 마이크로 RNA인 let-7a-5p가 *MUC5AC* 발현을 직접적으로 조절하고, 이는 알레르기성 비염과 부적 상관관계인 것을 확인하였습니다.

알레르기성 비염 환자의 비강 세척액에서 분리된 엑소좀을 사용하여 차별적으로 발현되는 마이크로 RNA를 small RNA 시퀀싱을 통하여 확인하였습니다 (정상대조군, n = 9 vs. 알레르기성 비염 환자, n = 8). 32개의 마이크로 RNA가 2배 이상의 차이로 발현되었습니다($p < 0.05$). 인터류킨 4를 처리하여 알레르기 상태를 유도한 사람 코점막

상피세포에서 let-7a-5p의 발현만이 정상보다 유의미하게 감소한다는 것을 확인하였습니다. 뿐만 아니라, let-7a-5p가 과발현된 사람 코점막 상피세포에 인터류킨 4를 처리하여 알레르기 상황을 유도하였을 때, *MUC5AC*의 발현이 유의미하게 감소함을 확인하였습니다. 이에 let-7a-5p의 과발현이 인터류킨 4에 의해 유도되는 *MUC5AC*의 발현을 억제한다는 것을 확인하였고, 그 기전을 확인하기 위한 실험을 진행하였습니다. *MUC5AC* mRNA의 3'-UTR에서 let-7a-5p가 결합할 가능성이 있는 부위를 확인하였고, 실험을 통해 let-7a-5p가 *MUC5AC* mRNA의 3'-UTR에 직접적으로 결합한다는 것을 확인하였습니다. 더 나아가 정상 사람과 알레르기성 비염 환자의 비강 세척액 내의 엑소좀에서 let-7a-5p의 발현양을 확인한 결과, let-7a-5p는 정상 사람 ($n \geq 8$) 에 비해 알레르기성 비염 환자 ($n \geq 15$) 에서 유의미하게 감소한다는 것을 확인하였습니다.

이러한 결과는 let-7a-5p가 *MUC5AC* 발현양을 감소시켜 점액의 과분비를 억제한다는 기전을 확인하였을 뿐 아니라, 비침습적으로 얻을 수 있는 비강 세척액에서 let-7a-5p의 발현양 확인이 알레르기성 비염의 새로운 치료 후보물질이 될 수 있음을 의미합니다.

핵심되는 말 : 알레르기성 비염; 마이크로 RNA; 비강세척액; 엑소좀; 기체-액체 간 계면 배양; 인터류킨 4; MUC5AC; let-7a-5p