

Contents lists available at [ScienceDirect](http://ScienceDirect)

## International Journal of Pediatric Otorhinolaryngology

journal homepage: [www.elsevier.com/locate/ijporl](http://www.elsevier.com/locate/ijporl)

# The effects of physical exercise and smoking habits on the expression of SPLUNC1 in nasal lavage fluids from allergic rhinitis subjects

K. Irander<sup>a</sup>, M.P. Borres<sup>b,c</sup>, B. Ghafouri<sup>d,e,\*</sup><sup>a</sup> Allergy Center, ENT Section, University Hospital, Linköping, Sweden<sup>b</sup> Department of Women's and Children's Health, Uppsala University, Sweden<sup>c</sup> Thermo Fisher Scientific, Uppsala, Sweden<sup>d</sup> Department of Medical and Health Sciences, Division of Community Medicine Rehabilitation Medicine, Faculty of Health Sciences, Linköping University, and Pain and Rehabilitation Centre, County Council of Östergötland, Linköping, Sweden<sup>e</sup> Occupational and Environmental Medicine, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, and Centre of Occupational and Environmental Medicine, County Council of Östergötland, Linköping, Sweden

## ARTICLE INFO

## Article history:

Received 15 November 2013

Received in revised form 11 January 2014

Accepted 14 January 2014

Available online 23 January 2014

## Keywords:

SPLUNC1 protein

Nasal lavage fluid

Allergic rhinitis

Smoking habits

Physical exercise

Acoustic rhinometry

## ABSTRACT

**Objective:** Palate lung nasal epithelial clone (PLUNC) is a family of proteins, which are proposed to participate in the innate immune defense against infections in the upper aero-digestive tract. The aim of this study was to investigate the expression of SPLUNC1 in allergic rhinitis subjects with considerations taken to the mucosal function and smoking habits.

**Methods:** The participants, recruited from a cohort followed from infancy, were re-examined at the age of 18 years regarding allergy development. Based on medical histories and skin prick tests the participants were classified into groups with persistent allergic rhinitis ( $n = 18$ ), intermittent allergic rhinitis ( $n = 8$ ) and healthy controls ( $n = 13$ ). Seven subjects (3, 2 and 2 in each group, respectively) reported smoking habits. The SPLUNC1 levels in nasal lavage fluids were analyzed by Western blot. Changes in the volume of the proper nasal cavity before and after physical exercise ( $\text{Vol2}^{\text{increase}}$ ) were analyzed by acoustic rhinometry.

**Results:** Compared to the control group the SPLUNC1 level was significantly lower in the persistent allergy group ( $3.8 \pm 3.4$  OD vs.  $1.3 \pm 1.5$  OD;  $p = 0.02$ ), but not in the intermittent allergy group without current exposure to allergens ( $3.6 \pm 4.7$  OD). No differences were found in  $\text{Vol2}^{\text{increase}}$  between any of the allergy groups and controls. In smokers  $\text{Vol2}^{\text{increase}}$  was significantly reduced ( $p < 0.01$ ) and the SPLUNC1 levels were lower compared to non-smokers. A significant correlation was found between SPLUNC1 and  $\text{Vol2}^{\text{increase}}$  ( $p < 0.01$ ;  $r = 0.53$ ) in non-smokers.

**Conclusions:** Current allergen exposure has an impact on SPLUNC1 expression in nasal lavage fluid, why allergy ought to be considered in study populations where analyses of SPLUNC1 levels are included in the reports. The normal nasal decongestion after exercise was not affected by allergy in contrast to smoking habits. The correlation between SPLUNC1 levels and  $\text{Vol2}^{\text{increase}}$  in non-smokers may indicate involvement of SPLUNC1 in the regulation of the normal function of the nasal mucosa. Complementary studies are needed to confirm the smoke-related reduction of SPLUNC1 expression and to analyze the possible participation of SPLUNC1 in the nasal mucosa regulation.

© 2014 The Authors. Published by Elsevier Ireland Ltd. Open access under [CC BY-NC-SA license](http://creativecommons.org/licenses/by-nc-sa/4.0/).

## 1. Introduction

Increasing attention is being paid to a group of proteins called palate lung and nasal epithelial clone (PLUNC), a family of related gene products including three short proteins (SPLUNC1–3) and five long proteins (LPLUNC1–4, 6) [1]. The SPLUNC1 protein, also known as BPI fold-containing family A member 1 (BPIFA1) [2], is a highly abundant secreted protein in upper airways and has been the most studied one among the family members [3]. According to

\* Corresponding author at: Rehabilitation Medicine, Department of Medicine and Health Sciences (IMH), Faculty of Health Sciences, University of Linköping, SE-581 85 Linköping, Sweden. Tel.: +46 101034657.

E-mail address: [bijar.ghafouri@liu.se](mailto:bijar.ghafouri@liu.se) (B. Ghafouri).

the immunohistochemical analyses SPLUNC1 is predominantly localized in mucous cells and ducts of submucosal glands, but is also found in some epithelial cells, and coats the surface epithelial cell lining [4].

The functions of the PLUNC proteins are partly defined, but new information is continuously obtained. Great interest has been focused on their function as a part of the innate immune defense, which is presumed to be due to the structural homology between the PLUNC proteins and mediators with known effects against Gram-negative bacteria, i.e. lipopolysaccharide-binding and bactericidal/permeability-increasing proteins [5,6]. The marked hydrophobicity and surfactant properties of the PLUNC proteins interfere with biofilm formations by airway pathogens, and these properties are suggested to contribute to host defense [7]. The role of antibacterial defense is supported by in vitro studies and animal studies [8–13], as well as in human in vivo studies [14–17]. The function and expression of PLUNC proteins have also been studied in other upper airway disorders. In cystic fibrosis [18,19] the levels are increased. Their potential to serve as cancer biomarkers has been evaluated [4,20–24]. Furthermore, SPLUNC1 expressions have been analyzed in relation to exposure to airborne industrial pollutants with known irritating effects in the airways, e.g. epoxy chemicals [25] and carbon nanotubes. [26]. Reduced levels have been reported in tobacco smokers [25,27].

Up to now knowledge of nasal SPLUNC1 expression in allergic rhinitis subjects is limited. In a pilot study, including subjects with intermittent allergic rhinitis due to pollen allergy, we previously found reduced SPLUNC1 levels in NLF during the pollen season compared to their levels out of season and to normal controls [28]. The aim of this report, based on results from participants in a cohort study, was to gain further knowledge of SPLUNC1 expressions in NLF from allergic rhinitis subjects. In a previous report based on this cohort, we found smoking habits to have an impact on the nasal mucosal function, as the normal decongestion after physical exercise was reduced in smokers [29]. For this reason we found an interest to include analysis of SPLUNC1 levels in relation to physical exercise in non-smokers and smokers in this report.

## 2. Materials and methods

### 2.1. Subjects and allergy diagnoses

The participants were recruited from a cohort followed from infancy to the age of 18 years regarding allergy development [30]. Diagnoses of allergy at the 18-year follow-up were based on the histories of allergic symptoms and careful clinical examinations, all of which were performed during winter time out of pollen season. The participants had to be free from airway infections for at least 10 days prior to the examination. As this report is focused on SPLUNC1 expression in NLF in relation to nasal allergy, only subjects suffering from allergic rhinitis were included, whereas atopic subjects with dermatitis but no airway symptoms were excluded. Thus, allergic rhinitis with or without concurrent bronchial or skin symptoms was diagnosed in twenty-six subjects.

### 2.2. Skin prick test and allergy sub-groups

The diagnosis of allergic rhinitis was verified by a skin prick test with ALK extracts (ALK, Sweden AB) including pollen allergens (birch, timothy, mugwort) and perennial allergens (horse, cat, dog, D pteronyssinus, D farinae, Alternaria, Cladosporium). Based on these results the subjects were separated into a persistent allergic rhinitis sub-group sensitized to perennial allergens with or without sensitization to pollens (PAR group;  $n = 18$ ) and an intermittent allergic rhinitis sub-group sensitized to pollens only

**Table 1**

SPLUNC1 levels in smokers and non-smokers in the allergy and control groups. The number of subjects with smoking habits is shown ( $n$ ). Occasional (occ); moderate (mod); optical density (OD).

	SPLUNC1 (OD)		SPLUNC1 (OD)	
	Smokers	Smoking habits occ/low/mod ( $n$ )	Non-smokers	( $n$ )
PAR group	0.0 ± 0.0	2/1/0	1.6 ± 1.5	15
IAR group	3.0 ± 4.3	0/1/1	3.8 ± 5.1	6
Control group	1.9 ± 1.2	2/0/0	4.1 ± 3.6	11
Overall group	1.4 ± 2.3	4/2/1	2.9 ± 3.3	32

(IAR group;  $n = 8$ ). Healthy and prick test negative subjects served as controls ( $n = 13$ ).

### 2.3. Smoking habits

The subjects were asked to report active smoking habits as occasional (1–2 cigarettes per week), low (1–9 cigarettes per day), moderate (10–20 cigarettes per day) and heavy (>20 cigarettes per day). A number of subjects with smoking habits are shown in Table 1.

### 2.4. Symptom scores

Nasal symptoms experienced on the day of examination were registered by the participants on visual analog scales, scoring from 0 (no symptoms) to 10 (disabling symptoms). The combined scores of four rhinitis symptoms (itching, sneezing, secretion and obstruction) were calculated.

### 2.5. Acoustic rhinometry and the physical exercise test

Exercise is known to result in an increased volume of the proper nasal cavity due to decongestion of the nasal mucosa. This function was evaluated by acoustic rhinometry before and immediately after a physical exercise test (refused by two subjects in the PAR group). The individuals had to run on a treadmill for 6 min to achieve a pulse rate of  $\geq 160$  beats per minute. Acoustic rhinometry was performed using Rhin 2000 (S.R. Electronics A.S., Lynge, Denmark). The mean values from three recordings and the sum from both nasal cavities were calculated using the computerized program with pre-determined calculations of volumes and minimal nasal cross-sectional areas [31]. The value of Vol2 corresponds to the volume in the anterior part of the proper nasal cavity (the distance between 2.20 and 5.40 cm from the nostrils, as calculated in Rhin 2000), and the increase after the exercise (Vol2<sup>increase</sup>) was calculated and chosen for statistical analysis.

### 2.6. Nasal lavage sampling

Nasal lavage was performed using saline pre-warmed to 37 °C. The subject held the head bent forward with the face horizontally, while the left nasal cavity was filled with saline, using a 10 ml syringe connected to the nostril via a short tube and a nasal olive. After 5 min approximately 5 ml of the saline could be recovered by aspiration. The samples were centrifuged to remove cellular debris and aliquots of the supernatants were stored at –20 °C in Eppendorf tubes until analysis.

### 2.7. Total protein concentrations

Total protein concentrations in NLF were determined with Bio-Rad protein assays according to Bradford [32].

## 2.8. Western blot analysis of SPLUNC1 levels

Iodoacetamide, dithiothreitol (DTT), sodium dodecyl sulfate (SDS) and CHAPS were acquired from Sigma (Steinheim, Germany). TEMED, Tween-20, 40% acrylamide solution, 2% bis acrylamide solution and ammonium persulfate were purchased from Bio-Rad (Hercules, CA, USA). Urea (pro analysis) was from Fluka (Buchs, Switzerland), and acetonitrile and acetic acid from Riedel-de Haën (Seelze, Germany). All other chemicals were of analytical grade.

Proteins from nasal lavage fluid were separated using SDS-PAGE, with a gradient gel range T: 5–20% and C: 1.5% and a stacking gel with T: 5% and C: 5% on Mini-Protean II electrophoresis cell from Bio-Rad Laboratories. Samples, 0.3 µg of protein, were mixed 1:1 with cocktail (10% (w/v) SDS, 150 mM DTT, 1% (w/v) bromophenol blue, 0.5 mM Tris-HCl pH 6.8, glycerol). As positive control nasal lavage fluid, known to contain PLUNC, was used. The samples were boiled 3 min before loaded in the wells on the SDS-PAGE and run in electrode buffer (0.16% (w/v) Tris, 0.72% (w/v) glycine, 0.05% (w/v) SDS). The SDS-PAGE was run for approximately 30 min in 100 V, 60 mA and then elevated to 200 V until finished.

SDS-PAGE gels were blotted on Immun-Blot PVDF Membrane using Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad Laboratories). Membranes were blocked in Tris-buffered saline (40 mM Tris-HCl, 500 mM NaCl, pH 7.5) with 5% non-fat dried milk over night. Membranes were washed with Tween-20 Tris-buffered saline (TTBS: 40 mM Tris-HCl, 500 mM NaCl, 0.05% Tween-20) and incubated with primary antibody against SPLUNC1 (goat polyclonal, R&D Systems, MN, USA) in TTBS with 2% non-fat dried milk over night. The membranes were washed with TTBS and followed by incubation with HRP-conjugated secondary antibody (anti-goat/sheep IgG, SIGMA, MI, USA) for 1 h. The latter wash procedure was repeated once pursued by detection of antigen/antibody conjugate with ECL (GE Healthcare) and developed on X-ray film. The X-ray films were visualized using a cooled CCD (charged-coupled device) camera digitizing at 1340 × 1040 pixels resolution (Fluor-S Multi-Imager, Bio-Rad Laboratories, CA, USA) in combination with analysis software Quantity One Version 4.3.1 (Bio-Rad Laboratories). The amount of protein in a band was assessed as optical density (OD).

## 2.9. Statistical analyses

Statistics were analyzed by using the Graph Pad Prism software program. The non-parametric method of Mann-Whitney U test was used in calculations of differences between two groups. Results are presented as mean values ± 1 standard deviation. Spearman rank correlation test was used in the analyses of correlation between two parameters. A two-tailed *p*-value of ≤0.05 was regarded as significant.

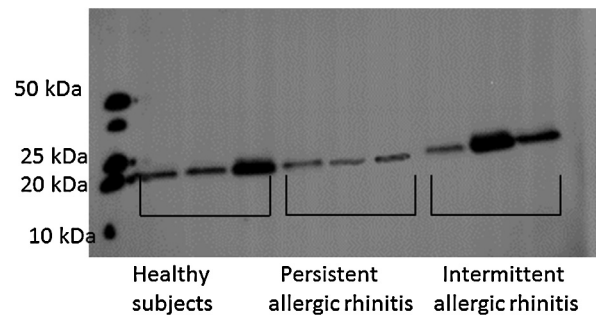
## 2.10. Ethical considerations

The study was approved by the Ethical committee at the University Hospital in Linköping, Sweden (03694). A written informed consent was obtained from each of the participants. The study was performed according to the principles in the Declaration of Helsinki.

## 3. Results

### 3.1. Protein concentrations in nasal lavages

The total protein concentrations in the NLF were 250 ± 190 µg/ml (PAR group), 330 ± 260 µg/ml (IAR group) and 180 ± 100 µg/ml (control group). No significant statistical differences were found between groups.



**Fig. 1.** A representative western blot image for SPLUNC1 in nasal lavage fluid from healthy controls, subjects with persistent allergic rhinitis and subjects with intermittent allergic rhinitis. The quantification data from the allergy group (persistent; *n* = 18 and intermittent; *n* = 8) and healthy controls (*n* = 13) are shown in Fig. 2.

### 3.2. Symptom scores

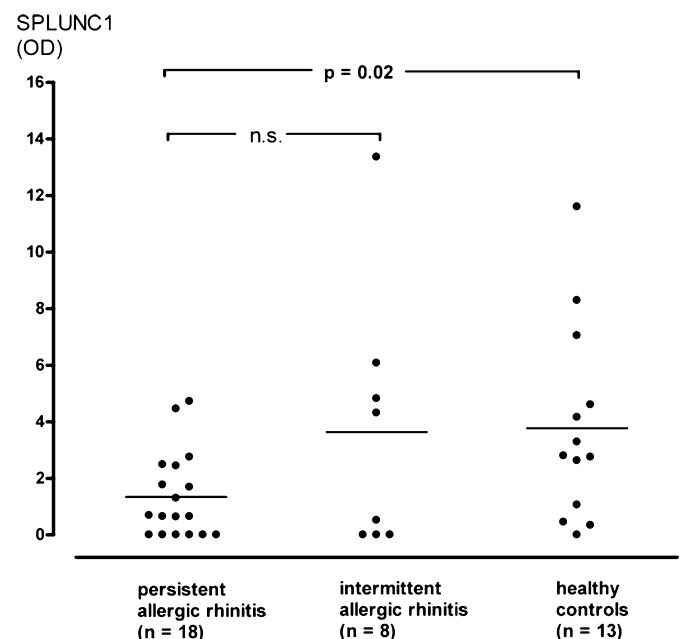
The combined rhinitis symptom scores were low, and the score value of 2.8 ± 4.1 in the PAR group was not significantly high compared to the values of 1.2 ± 1.7 in the IAR group and 1.6 ± 2.8 in the control group.

### 3.3. SPLUNC1 levels in relation to allergic rhinitis

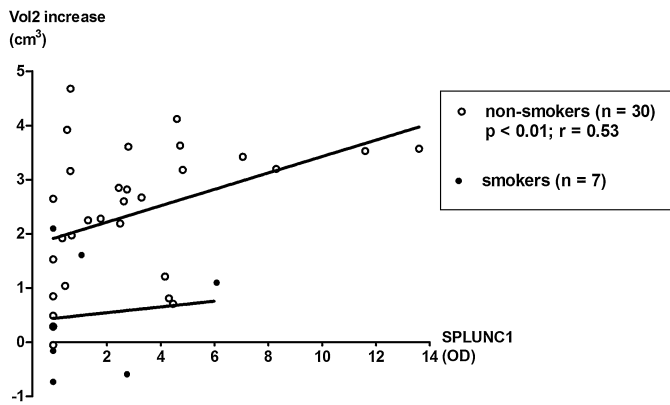
The SPLUNC1 protein could be detected by the Western blot analysis as a distinct band at 25 kDa (Fig. 1). The mean level of SPLUNC1, analyzed in the same amount of total protein (0.3 µg) from each of the NLF samples, was significantly lower in the PAR group compared to the control group (1.3 ± 1.5 OD vs. 3.8 ± 3.4 OD; *p* = 0.02). The mean level in IAR group (3.6 ± 4.7 OD) was not statistically different from the level in the control group (Fig. 2).

### 3.4. SPLUNC1 levels in relation to smoking habits

The smokers were equally distributed between the three groups. The levels of SPLUNC1 were in general numerically lower



**Fig. 2.** SPLUNC1 levels in the allergy groups and the control group. Optical density (OD); not significant difference (n.s.).



**Fig. 3.** The correlation between the increase of the nasal volume after exercise ( $\text{Vol2}^{\text{increase}}$ ) and the SPLUNC1 levels in non-smokers and smokers. Optical density (OD).

in smokers compared to non-smokers in all groups (Table 1). The number of smokers in the separate allergy groups was too low for statistical calculations.

### 3.5. $\text{Vol2}^{\text{increase}}$ in relation to allergy, smoking habits and SPLUNC1 levels

In the overall group the  $\text{Vol2}^{\text{increase}}$  was  $2.1 \pm 1.4 \text{ cm}^3$ . Allergy was not found to have any impact on this increase in contrast to smoking habits. The level of  $\text{Vol2}^{\text{increase}}$  was significantly lower in the smoking group compared to the non-smoking group ( $0.5 \pm 1.1 \text{ cm}^3$ ;  $n = 7$  smokers vs.  $2.4 \pm 1.3 \text{ cm}^3$ ;  $n = 30$  non-smokers;  $p < 0.01$ ). A significant correlation was found between  $\text{Vol2}^{\text{increase}}$  and the SPLUNC1 levels in non-smokers ( $p < 0.01$ ;  $r = 0.53$ ;  $n = 30$ ) (Fig. 3).

## 4. Discussion

This study showed that allergy has an impact on SPLUNC1 expression. The levels of SPLUNC1 were significantly lower in the PAR group, being currently exposed to airway allergens, as compared to the level in the healthy control group. The SPLUNC1 level in the IAR group, being out of their pollen season with no current allergen exposure, was not different from the controls. It is of interest to notice, that this influence on SPLUNC1 levels is found despite quite modest symptoms of nasal allergy with scores only slightly and not significantly higher in the PAR group compared to the IAR group and the controls. The result of reduced levels of SPLUNC1 in currently allergen exposed allergic subjects are in accordance with the results in our previous pilot study, where SPLUNC1 levels in NLF were analyzed by proteomic techniques [28], showing significantly reduced SPLUNC1 levels in pollen allergic subjects during pollen season, but normalized values out of season as compared to controls. Thus, two different methods of SPLUNC1 analysis have verified significant reductions of nasal SPLUNC1 levels in allergic rhinitis subjects during periods of current allergen exposure. A relation between the severity of rhinitis symptoms and the level of SPLUNC1 would increase the acceptance of SPLUNC1 involvement in allergic rhinitis. A relation was supported according to the results in our previous study [28], where the significantly higher symptom score level during allergy season in rhinitis subjects was associated with a significant reduction of the SPLUNC1 level in these subjects as compared to healthy controls. This relation was not found in this study, probably due to the modest levels of symptoms in the PAR subjects.

Adverse effects on the nasal mucosa due to smoke exposure were found, even though the smoking habits were modest. The normal exercise related increase of the nasal cavity volume was

significantly lower in smokers compared to non-smokers; this is previously reported [29]. Analysis of SPLUNC1 expression in relation to smoking habits showed numerically lower levels of SPLUNC1 in smokers compared to non-smokers in line with other studies [25,27]. The difference did not reach statistical significance, probably due to the low number of participants, which were recruited from a cohort designed for longitudinal follow-ups and not permitting substitution of subjects excluded for various reasons. The impact of smoking on the nasal mucosa was expressed in a more obvious way in the analysis of SPLUNC1 in relation to  $\text{Vol2}^{\text{increase}}$ . These two parameters were found to correlate significantly, but only in non-smoking individuals. This relation was not detected in smokers, apparently due to the reduction of SPLUNC1 levels as well as of the  $\text{Vol2}^{\text{increase}}$  values. The association between SPLUNC1 and the normal decongestion of the nasal mucosa has to our knowledge not been described previously and further studies are needed in order to explain the mechanisms behind the results. We can only speculate on the implication of this correlation, whether it is an indication of a participation of SPLUNC1 in the normal function of the nasal mucosa, or whether the elasticity of the mucous membrane as well as the level of SPLUNC1 is affected in parallel by some common factor. Such a factor might be neutrophil elastase, which is described to reduce SPLUNC1 [33].

In conclusion, our analyses of SPLUNC1 expressions in NLF have shown new and valuable information. Current allergen exposure, even at low levels causing modest clinical symptoms, has a significant impact on SPLUNC1 levels in allergic rhinitis subjects. Thus, it could be of importance, even in non-allergic upper airway disorders, to consider current respiratory allergy in study populations, where nasal SPLUNC1 levels are compared inter-individually. However, SPLUNC1 cannot be regarded as an adequate biomarker of allergy in single subjects, due to overlapping values between allergic and healthy individuals.

Smoking habits were found to have adverse effects on the SPLUNC1 levels and mucosal function. The possible involvement of SPLUNC1 in the normal function of the nasal mucosa, indicated by the significant correlation in non-smokers between SPLUNC1 levels and the increase in nasal volumes, needs further analyses including the way it is impaired by tobacco smoke.

### Competing interests

No competing financial interests exist.

### Conflicts of interest statement

The authors have no conflicts of interest to report.

### Acknowledgments

Special thanks also to Lena Lindell, Nina Timelin and Lisbeth Hjälle, Linköping University, for excellent assistance.

Financial support was provided by Stiftelsen Astma- och Allergiförbundets Forskningsfond and Swedish Association of Otorhinolaryngology, Head and Neck Surgery.

### References

- [1] C.D. Bingle, E.E. LeClaire, S. Havard, L. Bingle, P. Gillingham, C.J. Craven, Phylogenetic and evolutionary analysis of the PLUNC gene family, *Protein Sci.* 13 (2004) 422–430.
- [2] C.D. Bingle, R.L. Seal, C.J. Craven, Systematic nomenclature for the PLUNC/PSP/BSP30/SMGB proteins as a subfamily of the BPI fold-containing superfamily, *Biochem. Soc. Trans.* 39 (2011) 977–983.
- [3] L. Bingle, C.D. Bingle, Distribution of human PLUNC/BPI fold-containing (BPIF) proteins, *Biochem. Soc. Trans.* 39 (2011) 1023–1027.

- [4] L. Bingle, S.S. Cross, A.S. High, W.A. Wallace, D.A. Devine, S. Havard, et al., SPLUNC1 (PLUNC) is expressed in glandular tissues of the respiratory tract and in lung tumours with a glandular phenotype, *J. Pathol.* 205 (2005) 491–497.
- [5] C.D. Bingle, S.-U. Gorr, Host defense in oral and airway epithelia: chromosome 20 contributes a new protein family, *Int. J. Biochem. Cell Biol.* 36 (2004) 2144–2152.
- [6] Y.P. Di, Functional roles of SPLUNC1 in the innate immune response against Gram-negative bacteria, *Biochem. Soc. Trans.* 39 (2011) 1051–1055.
- [7] L. Gakhar, J.A. Bartlett, J. Penterman, D. Mizrahi, P.K. Singh, R.K. Mallampalli, et al., PLUNC is a novel airway surfactant protein with anti-biofilm activity, *PLoS ONE* 5 (2010) e9098.
- [8] H.W. Chu, J. Thaikootathil, J.G. Rino, G. Zhang, Q. Wu, T. Moss, et al., Function and regulation of SPLUNC1 protein in mycoplasma infection and allergic inflammation, *J. Immunol.* 179 (2007) 3995–4002.
- [9] H.-D. Zhou, X.-L. Li, G.-Y. Li, M. Zhou, H.-Y. Liu, Y.-X. Yang, et al., Effects of SPLUNC1 protein on the *Pseudomonas aeruginosa* and Epstein–Barr virus, *Mol. Cell. Biochem.* 309 (2008) 191–197.
- [10] L. Lukinskiene, Y. Liu, S.D. Reynolds, C. Steele, B.R. Stripp, G.D. Leikauf, et al., Antimicrobial activity of PLUNC protects against *Pseudomonas aeruginosa* infection, *J. Immunol.* 187 (2011) 382–390.
- [11] D. Jiang, S.E. Wenzel, Q. Wu, R.P. Bowler, C. Schnell, H.W. Chu, Human neutrophil elastase degrades SPLUNC1 and impairs airway epithelial defense against bacteria, *PLoS ONE* 8 (2013) e64689.
- [12] Y. Liu, J.A. Bartlett, M.E. Di, J.M. Bomberger, Y.R. Chan, L. Gakhar, et al., SPLUNC1/BPIFA1 contributes to pulmonary host defense against *Klebsiella pneumoniae* respiratory infection, *Am. J. Pathol.* 182 (2013) 1519–1531.
- [13] S. Sayeed, L. Nistico, C. St Croix, Y.P. Di, Multifunctional role of human SPLUNC1 in *Pseudomonas aeruginosa* infection, *Infect. Immun.* 81 (2013) 285–291.
- [14] L. Fornander, B. Ghafouri, E. Kihlström, B. Åkerlind, T. Schön, C. Tagesson, et al., Innate immunity proteins and a new truncated form of SPLUNC1 in nasopharyngeal aspirates from infants with respiratory syncytial virus infection, *Proteomics Clin. Appl.* 5 (2011) 513–522.
- [15] L.M. Teran, S. Rüggeberg, J. Santiago, F. Fuentes-Arenas, J.L. Hernández, A.R. Montes-Vizuet, et al., Immune response to seasonal influenza A virus infection: a proteomic approach, *Arch. Med. Res.* 43 (2012) 464–469.
- [16] S. Seshadri, D.C. Lin, M. Rosati, R.G. Carter, J.E. Norton, L. Suh, et al., Reduced expression of antimicrobial PLUNC proteins in nasal polyp tissues of patients with chronic rhinosinusitis, *Allergy* 67 (2012) 920–928.
- [17] Y.A. Tsou, M.T. Peng, Y.F. Wu, C.H. Lai, C.D. Lin, C.J. Tai, et al., Decreased PLUNC expression in nasal polyps is associated with multibacterial colonization in chronic rhinosinusitis patients, *Eur. Arch. Otorhinolaryngol.* (2013) (Epub ahead of print).
- [18] L. Bingle, F.A. Barnes, S.S. Cross, D. Rassl, W.A. Wallace, M.A. Campos, et al., Differential epithelial expression of the putative innate immune molecule SPLUNC1 in cystic fibrosis, *Respir. Res.* 8 (2007) 79.
- [19] A.L. Garland, W.G. Walton, R.D. Coakley, C.D. Tan, R.C. Gilmore, C.A. Hobbs, et al., Molecular basis for pH-dependent mucosal dehydration in cystic fibrosis airways, *Proc. Natl. Acad. Sci. U.S.A.* 110 (2013) 15973–15978.
- [20] S. Beniloch, J.M. Galbis-Caravajal, C. Alenda, F.M. Peiró, M. Sanches-Ronco, J.M. Rodriguez-Paniagua, et al., Expression of molecular markers in mediastinal nodes from resected stage 1 non-small-cell lung cancer (NSCLC): prognostic impact and potential role as markers of occult micrometastasis, *Ann. Oncol.* 20 (2009) 91–97.
- [21] P.A. Vargas, P.M. Speight, C.D. Bingle, A.W. Barrett, L. Bingle, Expression of PLUNC family members in benign and malignant salivary gland tumours, *Oral Dis.* 14 (2008) 613–619.
- [22] A. Vidotto, T. Henrique, L.S. Raposo, J.V. Maniglia, E.H. Tajara, Salivary and serum proteomics in head and neck carcinomas: before and after surgery and radiotherapy, *Canc. Biomarkers* 8 (2011) 95–107.
- [23] W.A. González-Arriagada, A.R. Santos-Silva, F.A. Ito, P.A. Vargas, P.M. Speight, L. Bingle, et al., Expression pattern of PLUNC proteins as an auxiliary tool for the diagnosis of high-grade mucoepidermoid carcinoma of the salivary gland, *J. Oral Pathol. Med.* 41 (2012) 589–597.
- [24] P. Chen, X. Guo, H. Zhou, W. Zhang, Z. Zeng, Q. Liao, et al., SPLUNC1 regulates cell progression and apoptosis through the miR-141-PTEN/p27 pathway, but is hindered by LMP1, *PLoS ONE* 8 (2013) e56929.
- [25] B. Ghafouri, E. Kihlström, B. Ståhlbom, C. Tagesson, M. Lindahl, PLUNC (palate, lung and nasal epithelial clone) proteins in human nasal lavage fluid, *Biochem. Soc. Trans.* 31 (2003) 810–814.
- [26] Y.P. Di, A.V. Tkach, N. Yanamala, S. Stanley, S. Gao, M.R. Shurin, et al., Dual acute pro-inflammatory and anti-fibrotic pulmonary effects of SPLUNC1 after exposure to carbon nanotubes, *Am. J. Respir. Cell Mol. Biol.* 49 (2013) 759–767.
- [27] K. Steiling, A.Y. Kadar, A. Bergerat, J. Flanigon, S. Sridhar, V. Shah, et al., Comparison of proteomic and transcriptomic profiles in the bronchial airway epithelium of current and never smokers, *PLoS ONE* 4 (2009) e5043.
- [28] B. Ghafouri, K. Irander, J. Lindbom, C. Tagesson, M. Lindahl, Comparative proteomics of nasal fluid in seasonal allergic rhinitis, *J. Proteome Res.* 5 (2006) 330–338.
- [29] K. Irander, M.P. Borres, J.P. Palm, Acoustic rhinometry, spirometry and nitric oxide in relation to airway allergy and smoking habits in an adolescent cohort, *Int. J. Pediatr. Otorhinolaryngol.* 75 (2011) 177–181.
- [30] K. Irander, M.P. Borres, An 18-year follow-up of allergy development related to nasal metachromatic cell findings during infancy, *Allergol. Int.* 59 (2010) 193–200.
- [31] E. Millqvist, M. Bende, Reference values for acoustic rhinometry in subjects without nasal symptoms, *Am. J. Rhinol.* 12 (1998) 341–343.
- [32] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.* 72 (1976) 248–254.
- [33] D. Jiang, S.E. Wenzel, Q. Wu, R.P. Bowler, C. Schnell, et al., Human neutrophil elastase degrades SPLUNC1 and impairs airway epithelial defense against bacteria, *PLoS ONE* 8 (2013) e64689.