Neuroendocrinology Letters Volume 42 No. 5 2021
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

ATG16L1 regulated IL-22 induced IFN level in Pseudomonas aeruginosa Lung Infection via cGAS signal passage

Yuanbin Sun, Hongxia Li, Hongyu Kang, Longxian Zhang, Jiahao Zhang

Department of Blood Transfusion, The Second People's Hospital, Nanyang 473000, China

Correspondence to: Yuanbin Sun
Department of Blood Transfusion, The Second People's Hospital, No.66 Jianshe East Road, Henan Province, Wancheng District, Nanyang 473000, China
tel.: 0377-61609163; e-mail: yuanbinsun@protonmail.com

Submitted: 2021-06-04 Accepted: 2021-06-24 Published online: 2021-06-24

Key words: **Pseudomonas aeruginosa Lung Infection; ATG16L1; IL-22; cGAS; IFN**

Neuroendocrinol Lett 2021; **42**(5):408–416 **PMID:** 34713687 NEL420521A05 © 2021 Neuroendocrinology Letters • **www.nel.edu**

Abstract

OBJECTIVE: This study explored that the possible effects and mechanism of ATG16L1 in pseudomonas aeruginosa lung infection.
METHODS: C57BL/6J mice were anesthetized with isoflurane and intratracheally (I.T.) inoculated with 5×10^6 CFU of *Pseudomonas aeruginosa* strain PA14. RAW264.7 macrophages were stimulated with 0.1 mg/ml of lipopolysaccharide (LPS). RAW264.7 macrophages were stimulated with 0.1 mg/ml LPS. Hematoxylin-Eosin (H&E), Immunofluorescence, sample acquisition, qPCR validation, Enzyme linked immunosorbent assay (ELISA) and immunofluorescence analysis were used this experiment.
RESULTS: ATG16L1 mRNA and protein expressions in mice with pseudomonas aeruginosa lung infection were also suppressed. ATG16L1 gene reduced inflammation and INF-γ levels in vitro model. On the other hand, ATG16L1 protein presented lung injury and inflammation levels in mice of pseudomonas aeruginosa lung infection. ATG16L1 regulated cGAS/IL-22 signal passage in model of pseudomonas aeruginosa lung onfection.
CONCLUSION: These findings indicate that ATG16L1 reduced IL-22 induced IFN level in pseudomonas aeruginosa lung infection via cGAS signal passage, which may provide a new therapeutic scheme for viral diseases or inflammatory diseases and its associated complications.

INTRODUCTION

Pseudomonas aeruginosa is a common opportunistic bacterial pathogen, which is widespread in nature and is prone to appear acquired drug resistance (Beswick *et al.* 2020). Pulmonary infections caused by Pseudomonas aeruginosa are becoming increasingly common (Carugati *et al.* 2020). According to the 2018 CHINET (China Antimicrobial Surveillance Network) data, the clinical isolation rate of Pseudomonas aeruginosa was 13.97% (Jurado-Martín 2021). With the extensive application of broad-spectrum antibiotics, the number of multi-drug resistant strains and the resistance rate of the bacteria have been increasing, which primarily cause respiratory infections (Deshpande and Zou 2020).

Interleukin (IL) is a member of the interleukin-10 subfamily, whose biological function is associated with the cytokine milieu (Koné *et al.*

2020). It plays varying roles in different diseases and different disease stages. Clinically, it has been found that IL-22 acts on the epithelial cells that communicating with the outside world, affecting their regeneration and barrier function (Michaudel *et al.* 2020). Research has shown close associations of IL-22 with the occurrence, development and prognosis of autoimmune diseases, skin diseases, as well as digestive and respiratory system diseases (Witte *et al.* 2020). Given its complex and changeable functions, it has become a hot research topic in recent years. Regarding respiratory system, IL-22 is related to idiopathic pulmonary fibrosis, asthma, tuberculosis, acute lung injury and so on (Renwick *et al.* 2021).

Cyclic gmp-amp synthase (CGAs) is a kind of dsDNA recognition receptor located in the cytoplasm, which mediates the cytoplasmic DNA-induced innnate immune response together with the downstream stimulator of interferon genes (STING) (Benmerzoug *et al.* 2019a; Benmerzoug *et al.* 2019b). Activation of DNA-cGASSTING signaling pathway promotes the expression and secretion of intracellular interferon I and TNF-α And IL-6 (Comish *et al.* 2021). More and more studies have shown that cGAS-STING signaling pathway plays an important role in pathogen infection, tumor and autoimmune diseases (Ma *et al.* 2020).

Autophagy associated protein 16 like protein 1 (Atg16L1) regulates cell autophagy, and abnormal Atg16L1 level can cause abnormal inflammatory reactions (Chesney *et al.* 2021). Among numerous genes involved in autophagy, the atg16L1 autophagy gene is closely associated with the immune inflammation (Gao *et al.* 2021). Studies have found that Atg16L1 gene is mainly responsible for sustaining intestinal humoral immunity (Boukhalfa *et al.* 2021; Finetti *et al.* 2021).

Studies have found that the atg16L1 is mainly responsible for sustaining intestinal humoral immunity. Its selective deletion in the mouse T cells leads to the down-regulation of pyroptosis-related inflammatory factors in negative feedback regulation, thereby resulting in the development of intestinal immune inflammatory diseases (Ma *et al.* 2021). This study explored that the possible effects and mechanism of ATG16L1 in pseudomonas aeruginosa lung infection.

MATERIALS AND METHODS

Patient samples

Patients with Pseudomonas aeruginosa Lung Infection (N = 12) and normal healthy volunteers (N = 12) were obtained from the Second People's Hospital of Nanyang City from 2018 to 2019. None of the patients received chemotherapy, radiotherapy or immunotherapy before surgery. All the samples were obtained following patient consent and approval by the Ethics Committee of Second People's Hospital of Nanyang City. Serum of all patients and normal healthy volunteers were collected after sign the informed consent form.

Mice model

C57BL/6J mice were originally obtained from Animal experiment center of Henan University and maintained in the animal facility of Second People's Hospital of Nanyang City. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the Second People's Hospital of Nanyang City. *Pseudomonas aeruginosa* strain PA14 was grown in Luria-Bertani (LB). C57BL/6J mice were anesthetized with isoflurane (50 mg/kg) and intratracheally (i.t.) inoculated with 5×10^6 CFU of *Pseudomonas aeruginosa* strain PA14 in 100 μl for 24 h.

Hematoxylin-Eosin (H&E) Staining

After mice sacrificed, lung tissue was collected and fixed with 4% paraformaldehyde for 24 h at room temperature. Lung tissue samples fixed with paraformaldehyde were paraffin-embedded. Samples were cut into 5 μm sections using a paraffin slicing machine and stained with hematoxylin and eosin. Light microscopy (BH3-MJL; Olympus Corporation, Tokyo, Japan) observed lung tissues.

Immunofluorescence

After mice sacrificed, lung tissue was collected and fixed with 4% paraformaldehyde for 24 h at room temperature. Lung tissue samples fixed with paraformaldehyde were paraffin-embedded. Samples were cut into 5 μm sections using a paraffin slicing machine and stained with Triton X-100. Lung tissue samples were determined by immunofluorescence staining using anti-ATG16L1 antibody (1:200, Cell Signaling Technology, Danvers, MA, US) overnight at 4°C. Alex 594 conjugated secondary antibody (1:200, Thermo Fisher) was used to obtain fluorescence images (LSM 510 Meta; Carl Zeiss) after DAPI staining.

Sample acquisition, RNA extraction and qPCR validation

Total cellular RNA or serum samples were extracted using Trizol (Thermo Fisher Scientific) from lung tissue or cells. RNA was then reverse transcribed into cDNA usingMoloney murine leukemia virus reverse transcriptase. The mRNA expression were quantified using 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) and SYBR® Premix Ex TaqTM II (Takara, Japan). Relative gene expression was normalized to GAPDH.

Enzyme linked immunosorbent assay (ELISA)

IL-1β, IL-6, INF-γ, IL-10 and IL-22 levels in cultured supernatants or serum samples were quantified using an ELISA kit according to the manufacturer's instructions.

Cell culture and Cell transfection

Human ATG16L1 were amplified by standard PCR based on the sequence from GenBank and the amplified

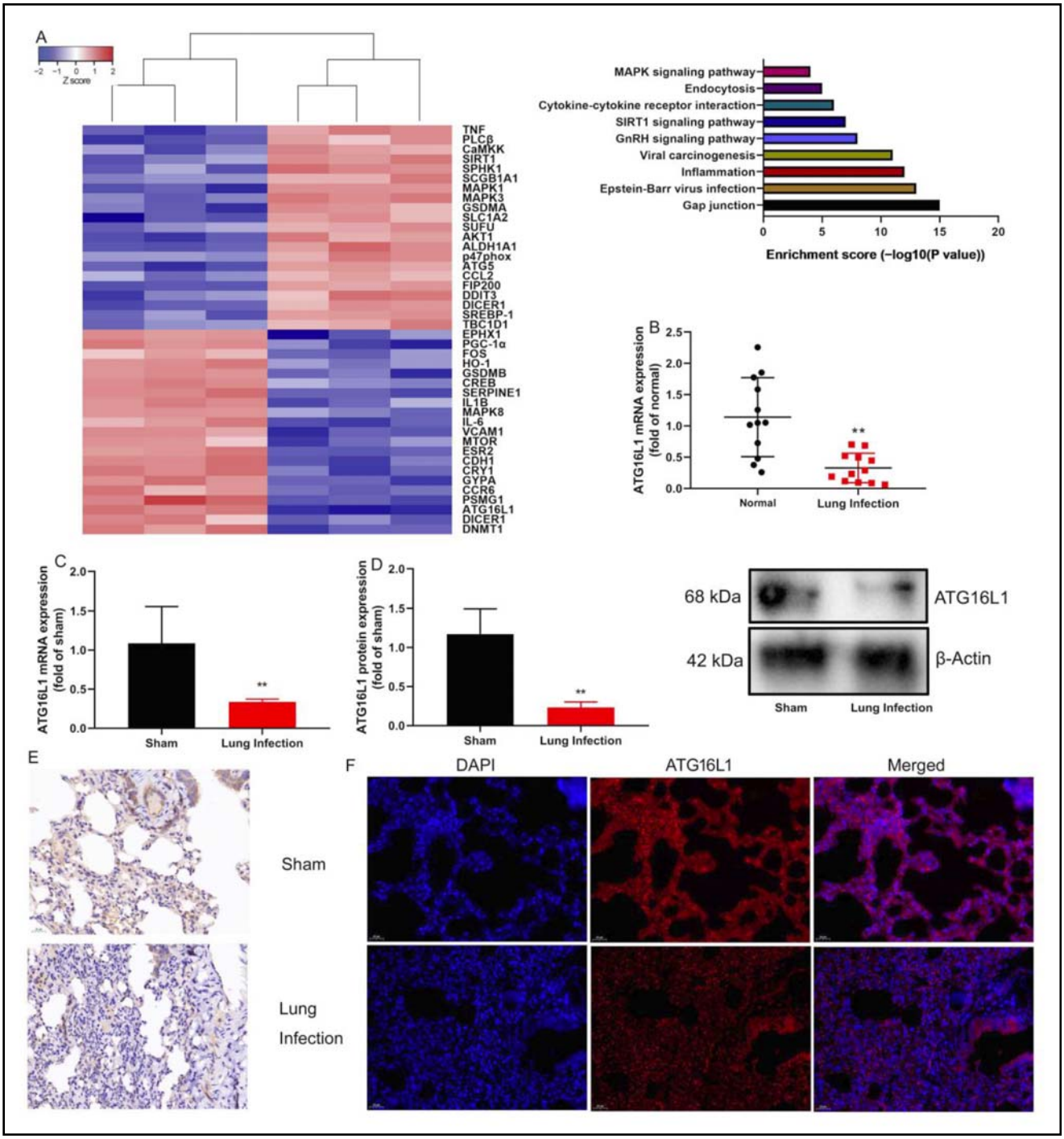

Fig. 1. The expression of ATG16L1 in Pseudomonas aeruginosa Lung Infection

Heat map (A), serum mRNA of ATG16L1 (B) in patients with pseudomonas aeruginosa lung infection; Lung tissue of ATG16L1 mRNA expression (C), lung tissue of ATG16L1 protein expression (D), ATG16L1 protein expression (Immunohistochemistry, E), ATG16L1 protein expression (Immunofluorescence, F) in mice of lung infection.

Normal, normal volunteers group; Lung Infection, Patients with pseudomonas aeruginosa lung infection; Sham, sham control group; Lung Infection, mice with pseudomonas aeruginosa lung infection.

**$p<0.01$ compared with normal volunteers group or sham control group.

fragment was inserted into the pGL3-Basic vector (Promega). Si-ATG16L1 minics was purchased Santa Cruz Biotechnology. RAW264.7 cells were cultured at 37°C, 5% CO_2 in DMEM containing 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 mg/ml).

RAW264.7 macrophages were grown to 80% confluence and transfected with plasmids (ATG16L1, si-ATG16L1, negative control and si-negative control) by Lipofectamine 2000. After transfection at 48 h, RAW264.7 macrophages were stimulated with 0.1 mg/ml LPS or PBS for 4 h, and added to the culture medium.

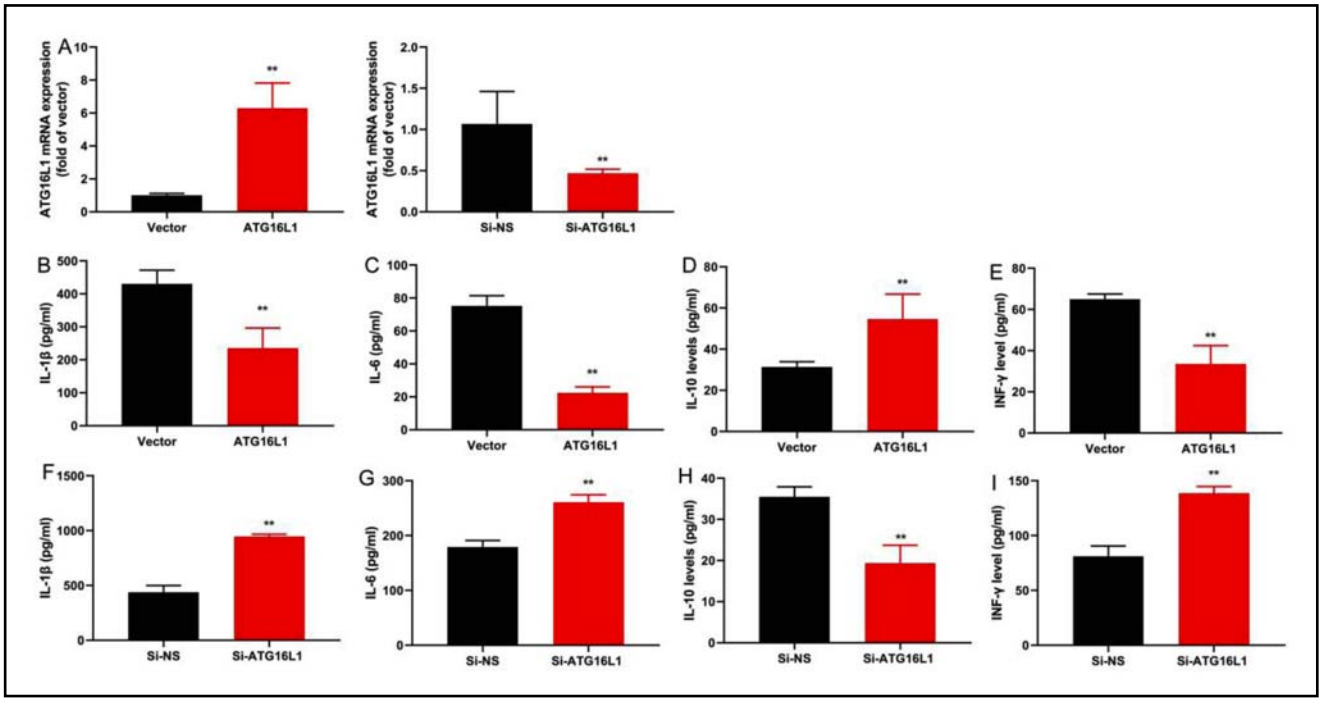

Fig. 2. ATG16L1 gene reduced inflammation and INF-γ levels in vitro model

The expression mRNA of ATG16L1 (A) in vitro model; The expression levels of IL-1β (B), IL-6 (C), IL-10 (D) and INF-γ (E) in vitro model by over-expression of ATG16L1; The expression levels of IL-1β (B), IL-6 (C), IL-10 (D) and INF-γ (E) in vitro model by down-regulation of ATG16L1.

Vector, negative control group; ATG16L1, over-expression of ATG16L1; Si-NS, Si-negative control group; Si- ATG16L1, down-regulation of ATG16L1 group.

**$p<0.01$ compared with control group or Si-negative control group

Western blotting

Cell protein was extracted from Lung tissue or cells samples using radioimmunoprecipitation lysis buffer on ice. Protein concentrations were determined by BCA protein quantitative kit. Total proteins were separated by 10 % SDS-PAGE and blotted on PVDF membrane. Membranes were blocked with 10 % non-fat milk powder at room temperature for 2 h and incubated with primary antibodies: ATG16L1 (ab195242, 1:1000, abcam), cGAS (ab252416, 1:1000, abcam), IL-22 (ab133545, 1:1000, abcam) and β-Actin (sc-8432, 1:5000, abcam) at 4 °C over-night. Bound antibodies were detected using horseradish peroxidase–labeled secondary IgG (dilution 1:1000) and a Chemiluminescence Kit. β-Actin was used as an internal control. Reactive bands were detected using ECL western blotting detection reagent (GE Healthcare, USA).

Immunofluorescence analysis

Cells were washed with PBS, were fixed with 4% paraformaldehyde for 10 min. Coverslips were washed with PBS and incubated 5 min with 0.1% Triton X-100 following fixation. Coverslips were washed with PBS, and after 20 min incubation with 5% BSA for 1 h, coverslips were stained overnight at 4°C with ATG16L1 (ab195242, 1:100, abcam), cGAS (ab252416, 1:100, abcam). Coverslips were incubated with secondary antibodies: goat antimouse-Alexa Fluor 488 (Sigma-Aldrich; 1:200), and goat antirabbit-Alexa Fluor 555 (Sigma-Aldrich; 1:200). Coverslips were stained with DAPI (Vector Labs) for 15 min, and imaged on a Zeiss LSM710.

Statistics

Statistical tests were performed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA). Difference was considered significant at values of $P < 0.05$. Continuous variables were compared one-way analysis of variance (oneway ANOVA) were compared by Chi-square test.

RESULTS

The expression of ATG16L1 in pseudomonas aeruginosa lung infection

Firstly, in order to investigate the expression of ATG16L1 in pseudomonas aeruginosa lung infection, this experiment measured the regulation gene expression levels in serum samples of patients with pseudomonas aeruginosa lung infection using microarray analysis (Figure 1A). Then, qRT-PCR was performed to detect the serum expression of ATG16L1 in patients with pseudomonas aeruginosa lung infection were down-regulation, in comparison to normal group (Figure 1B). In lung tissue samples, ATG16L1 mRNA and protein expressions in mice with pseudomonas aeruginosa

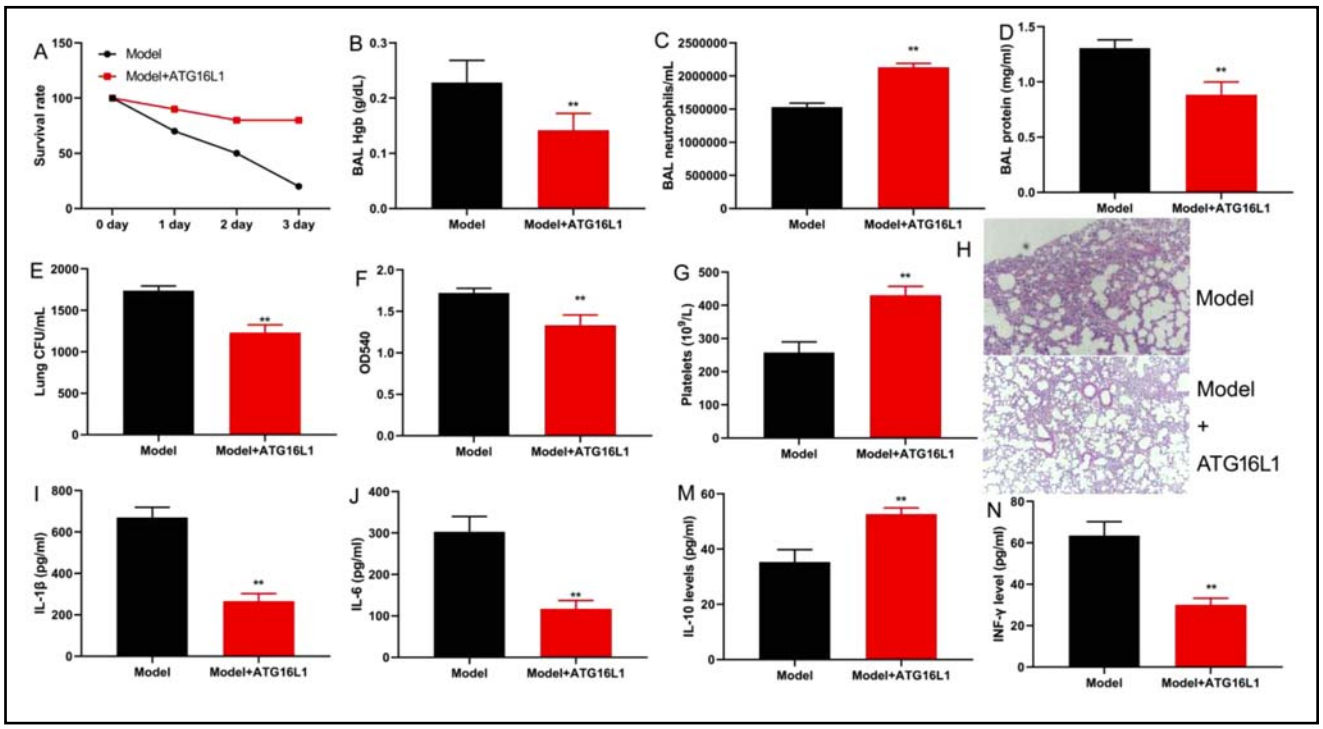

Fig. 3. ATG16L1 protein presents lung injury and inflammation levels in mice of Pseudomonas aeruginosa Lung Infection
Survival rate (A), BAL fluid hemoglobin (Hgb) (B), BAL neutrophils (C), BAL protein concentrations (D), lung bacterial CFU (E), BAL fluid OD540 (F), platelets in BAL fluid (G), lung injury (HE staining, H), IL-1β, IL-6, IL-10 and INF-γ levels (I, J, M and N).
Model, Pseudomonas aeruginosa Lung Infection model mice group; Model + ATG16L1, Pseudomonas aeruginosa Lung Infection model mice with human ATG16L1 recombinant protein (200 ng/mice) group.
**$p<0.01$ compared with Pseudomonas aeruginosa Lung Infection model mice group.

lung infection were also suppressed (Figure 1C-1D). Immunohistochemical showed ATG16L1 expression level were reduced in lung tissue of mice with pseudomonas aeruginosa lung infection (Figure 1E-1F).

ATG16L1 gene reduced inflammation and INF-γ levels in vitro model

To analyze the pre-inflammation function of ATG16L1 gene in lung cell of pseudomonas aeruginosa lung infection, the regulation of ATG16L1 expression by plasmid in vitro model were used in this experiment. ATG16L1 plasmid increased the mRNA expression of ATG16L1, and si-ATG16L1 mimics decreased ATG16L1 mRNA expression in vitro model of pseudomonas aeruginosa lung infection (Figure 2A). Indeed, theover-expression of ATG16L1 reduced IL-1β, IL-6 and INF-γ levels, and increased IL-10 level in vitro model of pseudomonas aeruginosa lung infection (Figure 2B-2E). At the same time, the down-regulation of ATG16L1 promoted IL-1β, IL-6 and INF-γ levels, and decreased IL-10 level in vitro model of pseudomonas aeruginosa lung infection (Figure 2F-2I).

ATG16L1 protein presented lung injury and inflammation levels in mice of pseudomonas aeruginosa Lung Infection

To test the role of ATG16L1 in mice with pseudomonas aeruginosa lung infection, mice with pseudomonas aeruginosa lung infection were treated with human ATG16L1 protein. It could be found that human ATG16L1 protein increased survival rate and BAL neutrophils, reduced BAL fluid hemoglobin(Hgb) and protein concentrations in mice with pseudomonas aeruginosa lung infection (Figure 3A-3D). Meanwhile, Human ATG16L1 protein could reduce lung bacterial CFU and BAL fluid OD540, increased platelets in BAL fluid, and inhibited lung injury of mice with Pseudomonas aeruginosa Lung Infection (Figure 3E-3H). Apart from that, human ATG16L1 protein also reduced IL-1β, IL-6 and INF-γ levels, and increased IL-10 level in lung tissue of mice with Pseudomonas aeruginosa Lung Infection (Figure 3I-3N).

ATG16L1 regulated cGAS signal passage in model of pseudomonas aeruginosa lung infection

Moreover, we examined the mechanism of ATG16L1 in model of pseudomonas aeruginosa lung infection using microarray analysis (Figure 4A). Additionally, our data showed that cGAS and IL-22 protein expressions were suppressed in mice with Pseudomonas aeruginosa Lung Infection by human ATG16L1 protein (Figure 4B). Over-expression of ATG16L1 not only increased ATG16L1 protein expression, but also suppressed cGAS and IL-22 protein expressions in vitro model (Figure 4C). IL-22 increased INF-γ levels in vitro model by ATG16L1 over-expression (Figure 4D).

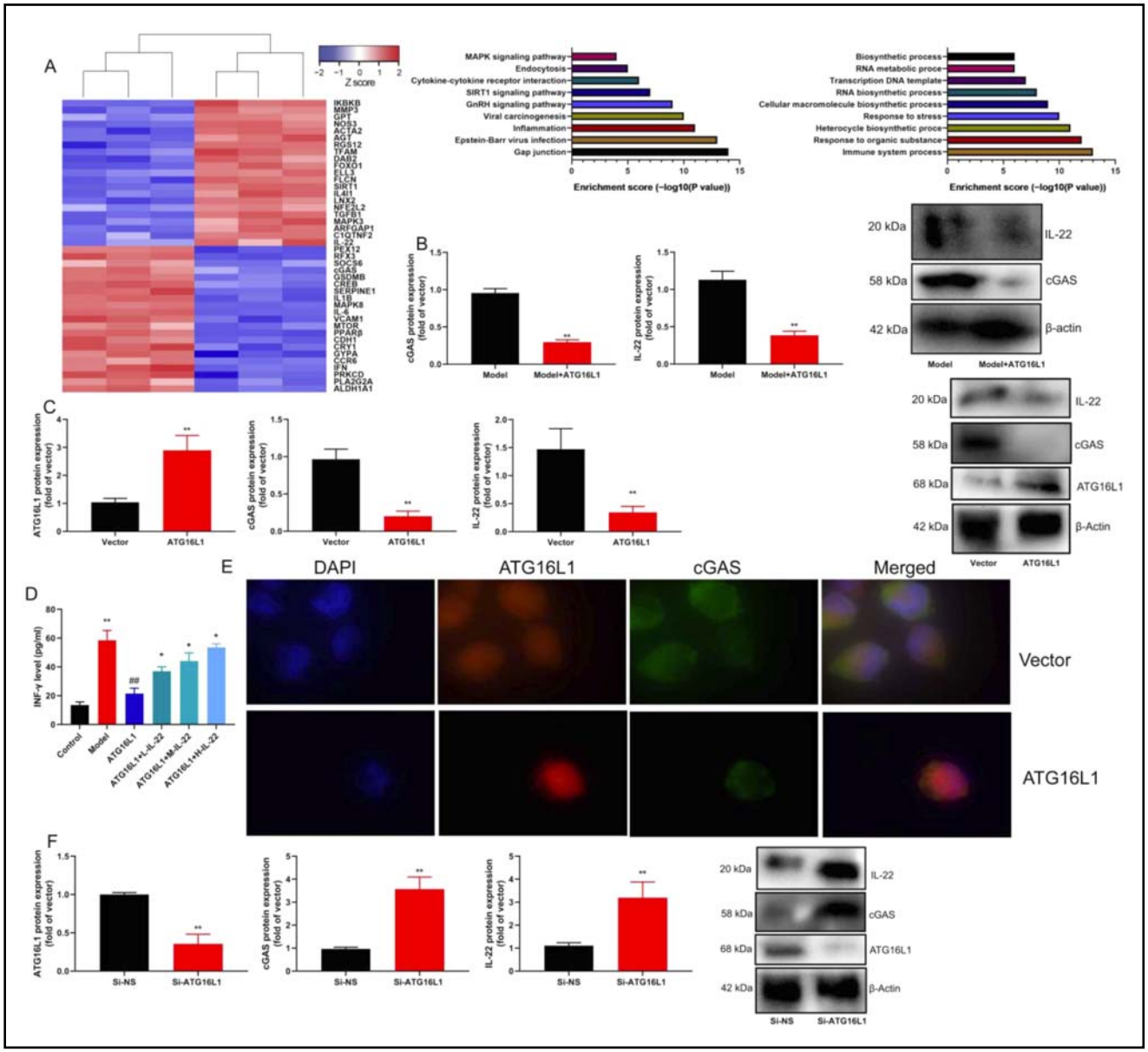

Fig. 4. cGAS signal passage is a target spot for ATG16L1 in model of Pseudomonas aeruginosa Lung Infection

Heat map (A), cGAS and IL-22 protein expressions (B) in Pseudomonas aeruginosa Lung Infection model mice; ATG16L1, cGAS and IL-22 protein expressions (C) in vitro model by over-expression of ATG16L1; INF-γ levels (D) in vitro model by over-expression of ATG16L1; ATG16L1 and cGAS expression in vitro model (E) by over-expression of ATG16L1 using immunofluorescence; ATG16L1, cGAS and IL-22 protein expressions (F) in vitro model by down-regulation of ATG16L1.

Model, Pseudomonas aeruginosa Lung Infection model mice group; Model + ATG16L1, Pseudomonas aeruginosa Lung Infection model mice with human ATG16L1 recombinant protein (200 ng/mice) group; Vector, negative control group; ATG16L1, over-expression of ATG16L1; Si-NS, Si-negative control group; Si- ATG16L1, down-regulation of ATG16L1 group.

**$p<0.01$ compared with Pseudomonas aeruginosa Lung Infection model mice group, control group or Si-negative control group

Over-expression of ATG16L1 suppressed cGAS expression in vtiro model (Figure 4E). Down-regulation of ATG16L1 suppressed ATG16L1 protein expression, and induced cGAS and IL-22 protein expressions in vitro model (Figure 4F).

As shown by cGAS plasmid reversed the effects of ATG16L1 on the inhibition of cGAS and IL-22 protein expressions, and the suppression of IL-22 levels in vitro model (Figure 5A-5C). cGAS inhibitor (50 nM of RU.521) suppressed cGAS and IL-22 protein expressions, and reduced IL-22 levels in vitro model following of ATG16L1 down-regulation (Figure 5D-5F). cGAS plasmid increased IL-1β, IL-6 and INF-γ levels, and reduced IL-10 levels in vitro model by ATG16L1 over-expression (Figure 5G-5J). Additionally, cGAS inhibitor suppressed IL-1β, IL-6 and INF-γ levels, but increased IL-10 levels in vitro model by ATG16L1 down-regulation (Figure 5K-5N).

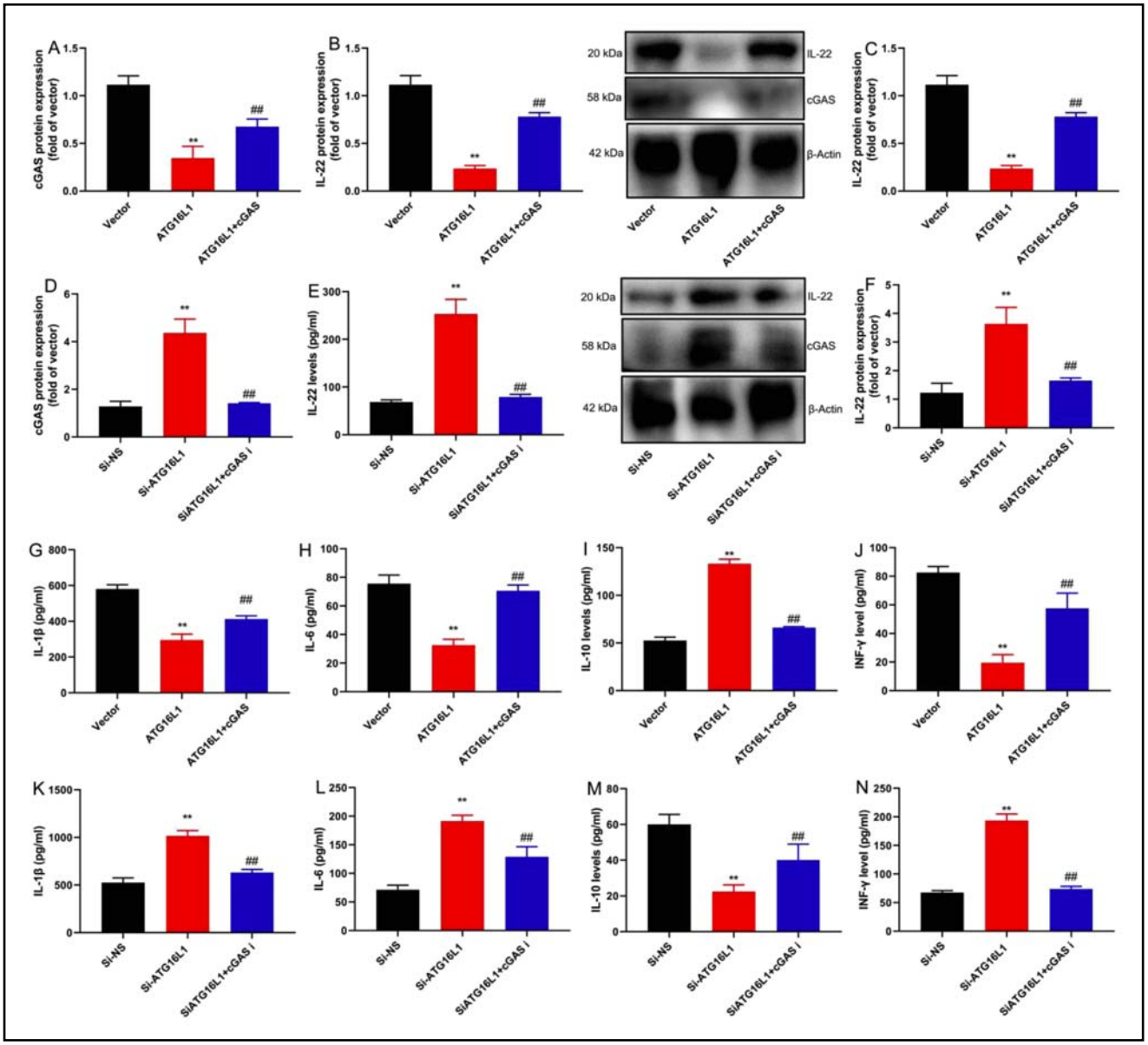

Fig. 5. ATG16L1 regulated cGAS signal passage in model of Pseudomonas aeruginosa Lung Infection

cGAS and IL-22 protein expressions (A and B), IL-22 levels (C) in vitro model of over-expression of ATG16L1 and cGAS; cGAS and IL-22 protein expressions (D and E), IL-22 levels (F) in vitro model of down-regulation of ATG16L1 and cGAS; IL-1β, IL-6, IL-10 and INF-γ levels (G, H, I and J) in vitro model of over-expression of ATG16L1 and cGAS; IL-1β, IL-6, IL-10 and INF-γ levels (K, L, M and N) in vitro model of down-regulation of ATG16L1 and cGAS.

Vector, negative control group; ATG16L1, over-expression of ATG16L1; ATG16L1+ cGAS, over-expression of ATG16L1 and cGAS; Si-NS, Si-negative control group; Si- ATG16L1, down-regulation of ATG16L1 group; Si-ATG16L1+cGAS i, down-regulation of ATG16L1 and cGAS inhibitor group.

**$p<0.01$ compared with control group or Si-negative control group; ##$p<0.01$ compared with over-expression of ATG16L1 group or down-regulation of ATG16L1 group.

DISCUSSION

Pseudomonas aeruginosa is a k conditional pathogen colonized on the human respiratory tract, skin or medical equipment (Langendonk *et al.* 2021). Upon decline in the patients' immunity, it becomes pathogenic, which is one of the major pathogenic bacteria responsible for nosocomial infections (Moser *et al.* 2021). ICU patients often need to stay in bed for a long time, or use tracheal intubation, mechanical ventilation and other operations, which significantly increase the probability of concurrent Pseudomonas aeruginosa pulmonary infection. (Riquelme and Prince 2020). However, due to the unreasonable antibiotic application, the incidence of carbapenem antibiotic-resistant Pseudomonas aeruginosa is getting increasingly higher, which is characterized by high resistance (Rossi *et al.* 2021). Our data indicate that the serum expression of ATG16L1 in patients with pseudomonas aeruginosa lung infection or mice model of pseudomonas

aeruginosa lung infection were down-regulation. ATG16L1 gene reduced inflammation and INF-γ levels in vitro model. ATG16L1 protein presents lung injury and inflammation levels in mice of Pseudomonas aeruginosa lung infection. Li *et al.* indicated that ATG16L1 is associated with decreased risk of non-small cell lung cancer (Li *et al.* 2017). Li *et al.* showed that Mir223 promotes central nervous system by targeting ATG16L1 (Li *et al.* 2019). Moreover, ATG16L1 may be an important factor for disease progression of pseudomonas aeruginosa lung infection.

IL-22, as a proinflammatory cytokine, can induce inflammation by binding to specific receptors (Alcorn 2020). Some studies suggest that the value of IL-22 in the clinical diagnosis of pulmonary infections (Brao *et al.* 2020). Inflammation plays an important role in the occurrence and development of chronic heart failure, and the serum IL-22 level in patients with pulmonary infections may be further elevated after infection (Koné *et al.* 2020; Das *et al.* 2020). We here provide evidence that Over-expression of ATG16L1 increased ATG16L1 protein expression, and suppressed cGAS and IL-22 protein expressions in vitro model. IL-22 increased INF-γ levels in vitro model by ATG16L1 over-expression. Aden *et al.* demonstrated that ATG16L1 orchestrates IL-22 in the intestinal epithelium (Aden *et al.* 2018). So, these results showed that ATG16L1 reduced INF-γ levels in model of pseudomonas aeruginosa lung infection by the regulation of IL-22.

The cGAS-STING pathway promotes the expression of such proinflammatory cytokines as type I IFNs, TNF-α and IL-6 (Nandakumar *et al.* 2019). While capable of inducing cellular production of substantial interferon-stimulated genes (ISGs), it can also promote dendritic cells to present antigens to T cells (Marinho *et al.* 2018). In this study, we found that ATG16L1 regulated cGAS signal passage in model of Pseudomonas aeruginosa Lung Infection. Fischer *et al.* indicate that STING induces LC3B lipidation via ATG16L1-WD40 domain (Fischer *et al.* 2020). Furthermore, ATG16L1 reduced IL-22/ INF-γ levels in model of pseudomonas aeruginosa lung infection by cGAS signaling pathway.

In conclusion, we find that ATG16L1 reduced IL-22 induced IFN level in Pseudomonas aeruginosa Lung Infection via cGAS signal passage. Together, we identified an important role and regulation mechanism of ATG16L1 in maintaining pseudomonas aeruginosa lung infection by IL-22/INF-γ levels, which may provide a new therapeutic scheme for viral diseases or inflammatory diseases and its associated complications.

REFERENCES

1 Aden K, Tran F, Ito G, Sheibani-Tezerji R, Lipinski S, Kuiper JW, Tschurtschenthaler M, Saveljeva S, et al (2018). ATG16L1 orchestrates interleukin-22 signaling in the intestinal epithelium via cGAS-STING. J Exp Med. 2018 Nov 5; **215**(11): 2868–2886. doi: 10.1084/jem.20171029.

2 Alcorn JF (2020): IL-22 Plays a Critical Role in Maintaining Epithelial Integrity During Pulmonary Infection. Front Immunol. **11**: 1160.

3 Benmerzoug S, Bounab B, Rose S, Gosset D, Biet F, Cochard T, Xavier A, Rouxel N et al (2019). Sterile Lung Inflammation Induced by Silica Exacerbates Mycobacterium tuberculosis Infection via STING-Dependent Type 2 Immunity. Cell Rep. 28; **27**(9): 2649–2664.e5. doi: 10.1016/j.celrep.2019.04.110.

4 Benmerzoug S, Ryffel B, Togbe D and Quesniaux VFJ (2019): Self-DNA Sensing in Lung Inflammatory Diseases. Trends Immunol. **40**: 719–734.

5 Beswick E, Amich J, Gago S (2020). Factoring in the Complexity of the Cystic Fibrosis Lung to Understand Aspergillus fumigatus and Pseudomonasaeruginosa Interactions. Pathogens. 6; **9**(8): 639. doi: 10.3390/pathogens9080639.

6 Boukhalfa A, Roccio F, Dupont N, Codogno P, Morel E (2021). The autophagy protein ATG16L1 cooperates with IFT20 and INPP5E to regulate the turnover of phosphoinositides at the primary cilium. Cell Rep. Apr 27; **35**(4): 109045. doi: 10.1016/j.celrep.2021.109045.

7 Brao KJ, Wille BP, Lieberman J, Ernst RK, Shirtliff ME, Harro JM (2020). Scnn1b-Transgenic BALB/c Mice as a Model of Pseudomonas aeruginosa Infections of the Cystic Fibrosis Lung. Infect Immun. 19; **88**(9): e00237–20. doi: 10.1128/IAI.00237-20.

8 Carugati M, Piazza A, Peri AM, et al (2020): Fatal respiratory infection due to ST308 VIM-1-producing Pseudomonas aeruginosa in a lung transplant recipient: case report and review of the literature. BMC Infect Dis. **20**: 635.

9 Chesney KL, Men H, Hankins MA, Bryda EC. The Atg16l1 gene: characterization of wild type, knock-in, and knock-out phenotypes in rats. Physiol Genomics. 2021 Jun 1; **53**(6): 269–281. doi: 10.1152/physiolgenomics.00114.2020.

10 Comish PB, Liu MM, Huebinger R, Carlson D, Kang R, Tang D (2021). The cGAS-STING pathway connects mitochondrial damage to inflammation in burn-induced acute lung injury in rat. Burns. **17**: S0305–4179 (21)00088-7. doi: 10.1016/j.burns.2021.04.007.

11 Das S, St Croix C, Good M, Chen J, Zhao J, Hu S, Ross M, Myerburg MMet al (2020). Interleukin-22 Inhibits Respiratory Syncytial Virus Production by Blocking Virus-Mediated Subversion of Cellular Autophagy. iScience. 2020 Jul 24; **23**(7): 101256. doi: 10.1016/j.isci.2020.101256.

12 Deshpande R, Zou C (2020). Pseudomonas Aeruginosa Induced Cell Death in Acute Lung Injury and Acute Respiratory Distress Syndrome. Int J Mol Sci. 28; **21**(15): 5356. doi: 10.3390/ijms21155356.

13 Finetti F, Cassioli C, Cianfanelli V, Zevolini F, Onnis A, Gesualdo M, Brunetti J, Cecconi F, Baldari CT (2021). The Intraflagellar Transport Protein IFT20 Recruits ATG16L1 to Early Endosomes to Promote Autophagosome Formation in T Cells. Front Cell Dev Biol. 2021 Mar 22; **9**: 634003. doi: 10.3389/fcell.2021.634003.

14 Fischer TD, Wang C, Padman BS, Lazarou M, Youle RJ. STING induces LC3B lipidation onto single-membrane vesicles via the V-ATPase and ATG16L1-WD40 domain. J Cell Biol. **219**(12): e202009128. doi: 10.1083/jcb.202009128.

15 Gao X, Du Y, Lau WB, Li Y, Zhu S, Ma XL (2021). Atg16L1 as a Novel Biomarker and Autophagy Gene for Diabetic Retinopathy. J Diabetes Res. 2021 Mar 18; **2021**: 5398645. doi: 10.1155/2021/5398645.

16 Jurado-Martín I, Sainz-Mejías M, McClean S (2021). Pseudomonas aeruginosa: An Audacious Pathogen with an Adaptable Arsenal of Virulence Factors. Int J Mol Sci. 18; **22**(6): 3128. doi: 10.3390/ijms22063128.

17 Koné B, Pérez-Cruz M, Porte R, et al (2020): Boosting the IL-22 response using flagellin prevents bacterial infection in cigarette smoke-exposed mice. Clin Exp Immunol. **201**: 171–186.

18 Langendonk RF, Neill DR, Fothergill JL (2021). The Building Blocks of Antimicrobial Resistance in Pseudomonas aeruginosa: Implications for Current Resistance-Breaking Therapies. Front Cell Infect Microbiol. 2021 Apr 16; **11**: 665759. doi: 10.3389/fcimb.2021.665759.

19 Li QX, Zhou X, Huang TT, et al (2017): The Thr300Ala variant of ATG16L1 is associated with decreased risk of brain metastasis in patients with non-small cell lung cancer. Autophagy. **13**: 1053–1063.
20 Li Y, Zhou D, Ren Y, Zhang Z, Guo X, Ma M, Xue Z, Lv J et al (2019). Mir223 restrains autophagy and promotes CNS inflammation by targeting ATG16L1. Autophagy. **15**(3): 478–492. doi: 10.1080/15548627.2018.1522467.
21 Ma C, Storer CE, Chandran U, LaFramboise WA, Petrosko P, Frank M, Hartman DJ, Pantanowitz L et al (2021). Crohn's disease-associated ATG16L1 T300A genotype is associated with improved survival in gastric cancer. EBioMedicine. 2021 May; **67**: 103347. doi: 10.1016/j.ebiom.2021.103347.
22 Ma R, Ortiz Serrano TP, Davis J, Prigge AD and Ridge KM (2020): The cGAS-STING pathway: The role of self-DNA sensing in inflammatory lung disease. FASEB J. **34**: 13156–13170.
23 Marinho FV, Benmerzoug S, Rose S, et al (2018): The cGAS/STING Pathway Is Important for Dendritic Cell Activation but Is Not Essential to Induce Protective Immunity against Mycobacterium tuberculosis Infection. J Innate Immun. **10**: 239–252.
24 Michaudel C, Bataille F, Maillet I, et al (2020): Ozone-Induced Aryl Hydrocarbon Receptor Activation Controls Lung Inflammation via Interleukin-22 Modulation. Front Immunol. **11**: 144.
25 Moser C, Jensen PØ, Thomsen K, Kolpen M, Rybtke M, Lauland AS, Trøstrup H, Tolker-Nielsen T (2021). Immune Responses to Pseudomonas aeruginosa Biofilm Infections. Front Immunol. 2021 Feb 22; **12**: 625597. doi: 10.3389/fimmu.2021.625597.
26 Nandakumar R, Tschismarov R, Meissner F, Prabakaran T, Krissanaprasit A, Farahani E, Zhang BC, Assil S et al (2019). Intracellular bacteria engage a STING-TBK1-MVB12b pathway to enable paracrine cGAS-STING signalling. Nat Microbiol. **4**(4): 701–713. doi: 10.1038/s41564-019-0367-z.
27 Renwick J, Reece E, Walsh J, et al (2021): Early Interleukin-22 and Neutrophil Proteins Are Correlated to Future Lung Damage in Children With Cystic Fibrosis. Front Pediatr. **9**: 640184.
28 Riquelme SA and Prince A (2020): Airway immunometabolites fuel Pseudomonas aeruginosa infection. Respir Res. **21**: 326.
29 Rossi E, La Rosa R, Bartell JA, et al (2021): Pseudomonas aeruginosa adaptation and evolution in patients with cystic fibrosis. Nat Rev Microbiol. **19**: 331–342.
30 Witte K, Koch E, Volk HD, Wolk K and Sabat R (2020): The herbal extract EPs® 7630 increases the antimicrobial airway defense through monocyte-dependent induction of IL-22 in T cells. J Mol Med (Berl). **98**: 1493–1503.