Department of Internal Medicine
Division of Respiratory Diseases

Kazuyoshi Kuwano, *Professor*
Katsutoshi Nakayama, *Associate Professor*
Masamichi Takagi, *Assistant Professor*
Hiromichi Hara, *Assistant Professor*
Akira Kojima, *Professor*
Jun Araya, *Associate Professor*
Takanori Numata, *Assistant Professor*

General Summary

We developed clinical and basic research concerning chronic obstructive pulmonary disease (COPD), bronchial asthma, pulmonary infection, pulmonary fibrosis, and lung cancer. Basic research should resolve clinical problems and clinical research should construct novel treatments. We specifically investigated the role of apoptosis, senescence, and autophagy in the pathogenesis of various lung diseases. Basic research focusing on the molecular mechanisms of pulmonary fibrosis, and COPD are in progress. We also collaborate with National Cancer Research Center concerning the detection of mutations of the epidermal growth factor receptor (EGFR) gene (*EGFR*) and exosome research.

Research Activities

Cellular senescence and autophagy in COPD

The cause of COPD is the noxious effects of tobacco smoke. Cigarette smoke-induced accelerated cell senescence has been widely implicated in the pathogenesis of COPD. The accumulation of damaged proteins and organelles are typical manifestations of cellular senescence, indicating the involvement of autophagy in the regulation of cell senescence in COPD. We found the involvement of insufficient p62-mediated selective autophagic clearance of ubiquitinated proteins in accelerated cellular senescence in the pathogenesis of COPD (Fujii S, et al, Oncoimmunology 1: 630-641, 2012).

Mitochondria are dynamic organelles and continuously change their shape through fission and fusion. The proper regulation of mitochondrial dynamics is crucial for the maintenance of functional mitochondria. We found that cigarette smoke extract induced mitochondrial fragmentation and mitochondrial oxidative stress, which were responsible for acceleration of cellular senescence in human bronchial epithelial cells. Mitochondrial fragmentation induced by knockdown of fusion proteins also increased mitochondrial reactive oxygen species production and percentages of senescent cells. Hence, disruption of mitochondrial dynamics may be a part of the pathogenic sequence of COPD development (Hara H, et al. Am J Physiol Lung Cell Mol Physiol 305: L737-746, 2013).

We also investigated the role of exosomes. Exosomes are extracellular vesicles that play important roles in cell-to-cell communication. MicroRNA have potential roles in cellular homeostasis and the pathophysiology of various diseases. We found that microRNA-201 suppresses the autophagy related 7 gene (*ATG7*) and autophagy and that such suppression

leads to fibrogenesis in airway walls of COPD (Fujita Y et al. J Extracellular Vesicles 2015).

Cellular senescence and autophagy in idiopathic pulmonary fibrosis

Recent studies have shown that senescence is a risk factor for the development of idiopathic pulmonary fibrosis (IPF). We have produced evidence that lungs with IPF show enhanced senescence with a concomitant increase of sirtuin 6 (SIRT6) expression in epithelial cells, including aberrantly re-epithelialized bronchial cells. Transforming growth factor β induces senescence by increasing p21 expression and also induces SIRT6 expression, and artificial overexpression of SIRT6 efficiently inhibits transforming growth factor β-induced senescence via proteasomal degradation of p21 in human bronchial epithelial cells. These findings shed light on the accelerated epithelial senescence in the pathogenesis of IPF with a possible regulatory role for SIRT6 (Minagawa S, et al. Am J Physiol Lung Cell Mol Physiol. 300: L391-401, 2011).

Autophagy has been shown to prevent cellular senescence caused by tunicamycin-induced endoplasmic reticulum stress in human bronchial epithelial cells. Conversely, autophagy inhibition was sufficient to induce myofibroblast differentiation in lung fibroblasts. Cellular senescence as measured by p21 expression and senescence-associated β-galactosidase staining was observed in metaplastic epithelial cells covering fibrosing lesions. These findings suggest that insufficient autophagy is a potent underlying pathologic finding of both accelerated cellular senescence and myofibroblast differentiation in a cell-type specific manner and is a promising clue for understanding the molecular mechanisms of IPF (Araya J, Am J Physiol Lung Cell Mol Physiol 304: L56-69, 2013).

Etiologies of acute exacerbation of COPD in adults by real-time polymerase chain reaction

Respiratory infection is a major cause of exacerbation in COPD. Multiplex polymerase chain reaction (PCR) has recently been used to detect effectively both respiratory bacteria and viruses. To evaluate causes of the acute exacerbation of COPD, we used a rapid reliable process based on real-time (RT)-PCR for respiratory samples. We analyzed respiratory tract samples by comprehensive RT-PCR. We prospectively studied 46 patients with COPD and examined nasopharyngeal swabs and sputum samples. We performed RT-PCR designed to detect 6 bacterial species and 11 viruses. Viruses were detected in 17 (34%) of 50 exacerbations. The COPD exacerbations caused by Gram-negative bacilli were significantly associated with prolonged hospitalization for COPD exacerbations. We concluded that RT-PCR is useful for detecting the causes of infection and determining the risk of extended hospitalization (Shimizu K et al. Int J COPD 2015).

Analyzing cell-free plasma DNA by picoliter-droplet digital PCR to detect EGFR *mutations in patients with lung adenocarcinoma*

Resistance develops in patients with adenocarcinoma of the lung who received EGFR-tyrosine-kinase inhibitor (TKI) therapy. Noninvasive monitoring of the secondary Thr790Met mutation in *EGFR* is necessary for precise treatment of lung adenocarcinoma. We examined TKI-sensitive mutations (L858R and inflame exon 19 deletions) and TKI-

resistant mutations (i.e., Thr790Met) in cell-free plasma DNA using picoliter-droplet digital PCR cell-free plasma DNA analysis. We found that picoliter-droplet digital PCR enables noninvasive assessment of *EGFR* mutations that confer resistance to TKIs (Seki Y et al. Oncologist 2015).

Publications

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