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General Summary

The number of patients with lung diseases are increasing along with the aging. We address clinical and basic research concerning COPD, bronchial asthma, pulmonary infection, pulmonary fibrosis, and lung cancer, which are closely associated with aging. We intensively investigated the pathophysiology of lung diseases associated with aging, especially COPD and IPF pathogenesis concerning cellular senescence and autophagy. We also collaborate with National Cancer Research center concerning EGFR mutation detection and exosome research in the field of lung cancer treatment. We will further extend our research to develop novel treatments targeting devastating lung diseases.

Research Activities

Chronic obstructive pulmonary disease (COPD)
Chronic obstructive pulmonary disease (COPD) is caused by the noxious inhalation such as tobacco smoke, which leads to airway epithelial cell injury and inflammation, and the phenotypic changes such as squamous metaplasia and cellular senescence of epithelial cells, which are assumed to be part of the adaptive response to toxic components. Cigarette smoke effects have been widely implicated in COPD pathogenesis. Autophagy is a bulk degradation pathway for cellular components and essential for the maintenance of cellular homeostasis. When autophagy is impaired, damaged proteins and organelles accumulate, which leads to cellular senescence. Cellular senescence has been considered to be involved in COPD pathogenesis.
Mitochondria are dynamic organelles that are essential for cellular metabolic functions, which continuously change their shape through fission and fusion. The proper regulation of mitochondrial dynamics is crucial for the maintenance of functional mitochondria and hence disruption of dynamics induces excessive reactive oxygen species (ROS) production, resulting in apoptosis and cellular senescence.
We have recently reported several papers concerning the roles of autophagy and cellular senescence in COPD pathogenesis. We are now trying to verify molecular mechanisms of COPD to further extend our understanding of COPD pathogenesis and finally to develop novel therapies.

Bronchial Asthma (BA)
Respiratory tract infection is a major cause of acute exacerbation of bronchial asthma. However, few epidemiologic analysis of infectious pathogens including atypical bacteria

and viruses has been performed. We addressed to detect pathogens during acute exacerbation of BA by using real-time polymerase chain reaction (PCR) in comparison to conventional methods. Infectious pathogens collected in nasopharyngeal swab and sputum samples were examined in each patient by conventional methods and real-time PCR. In the results, Real-time PCR was more useful than conventional methods to detect infectious pathogens in patients with acute exacerbation of BA. Accurate detection of pathogens with real-time PCR may enable the selection of appropriate anti-bacterial/viral agents as a part of the treatment for BA exacerebations (Yoshii Y, et al. BMC Pulm Med. 2017).
We also evaluated perioperative management using new inhalant drugs in patients with COPD and asthma, since most evidence regarding postoperative pulmonary complications (PPC) has been established more than 10 years ago. We retrospectively evaluated physiological backgrounds, surgical factors and perioperative specific treatment for COPD and asthma. History of smoking or severe asthma is a risk factor of PPC in patients with asthma, and age, upper abdominal surgery, or long operation time is a risk factor of PPC in patients with COPD. Adequate inhaled corticosteroids treatment in patients with eosinophilic asthma and introducing treatment for COPD in patients with COPD could reduce PPC (Numata T, et al. BMC Pulm Med. 2018).

Idiopathic pulmonary fibrosis (IPF)
We have produced evidence that IPF lungs show enhanced epithelial cell senescence with a concomitant increase of SIRT6 expression, including aberrantly re-epithelialized bronchial cells. TGF-β induces senescence by increasing p21 expression and also induces SIRT6 expression. TGF-β-induced senescent HBEC is responsible for myofibroblast differentiation in fibroblasts. Autophagy plays an important regulatory role in cellular senescence and differentiation. We also found that insufficient autophagy is a potent underlying pathology of both accelerated cellular senescence and myofibroblast differentiation in IPF. Insufficient mitophagy leads to the accumulation of injured mitochondria, which produce excessive reactive oxygen species (ROS). Excessive ROS activate PDGFR, which results in augmentation of AKT-mTOR pathway. Activation of mTOR induces fibroblatst to omyofibroblats differentiation, and also inhibits autophagy (Kobayashi K et al. J Immunol 2016).
Accumulation of profibrotic myofibroblasts is involved in the process of fibrosis development during IPF pathogenesis. TGF-β is one of the major profibrotic cytokines for myofibroblast differentiation and NOX4 (NADPH oxidase 4) has an essential role in TGF-β-mediated cell signaling. Azithromycin (AZM) has a pleiotropic effect on cellular processes including proteostasis. TGF-β-induced NOX4 and myofibroblast differentiation were clearly inhibited by AZM treatment in fibroblasts. AZM suppresses NOX4 by promoting proteasomal degradation, resulting in inhibition of TGF-β-induced myofibroblast differentiation and lung fibrosis development. AZM may be a candidate for the treatment of the fibrotic lung disease IPF (Tsubouchi K, et al. Autophagy 2017).
Pirfenidone (PFD) is an anti-fibrotic agent used to treat idiopathic pulmonary fibrosis (IPF), but its precise mechanism is not clear. Mitophagy has been implicated in myofibroblast differentiation through regulating mitochondrial reactive oxygen species (ROS)-mediated platelet-derived growth factor receptor (PDGFR) activation. We found that PFD

induced autophagy/mitophagy activation via enhanced PARK2 expression. PFD inhibited the myofibroblast differentiation induced by PARK2 knockdown by reducing mitochondrial ROS and PDGFR-PI3K-Akt activation. BLM-treated PARK2 KO mice demonstrated augmentation of lung fibrosis and oxidative modifications, which were efficiently attenuated by PFD, which may at least partly explain the anti-fibrotic mechanisms of PFD for IPF treatment (Kurita Y, et al. Respir Res 2017).

Alveolar epithelial cell (AEC) injury leading to cell death is involved in the process of fibrosis. The excessive apoptosis of AECs has been widely implicated in IPF pathogenesis. Necroptosis is a type of regulated/programmed necrosis. A multiprotein complex composed of receptor-interacting protein kinase-1 and -3 (RIPK1/3) plays a key regulatory role in initiating necroptosis. We found that RIPK3 expression levels were increased in IPF lungs and both apoptosis and necroptosis were detected mainly in AECs. BLM treatment induced RIPK3 expression in AECs and increased High Mobility Group Box 1 (HMGB1) and interleukin 1β (IL-1β) levels in mouse lungs. BLM-induced lung inflammation and fibrosis was attenuated in RIPK3 knockout mice with a concomitant reduction in HMGB1 and IL-1β. Therefore, we concluded that RIPK3-regulated necroptosis in AECs is involved in the mechanism of lung fibrosis development through the release of DAMPs as the pathogenesis of IPF (Yoshida M, et al, Am J Respir Cell Mol Biol. 2018).

Lung cancer

Non-invasive monitoring of epidermal growth factor receptor (EGFR) mutations conferring sensitivity and resistance to tyrosine kinase inhibitors (TKIs) is vital for efficient therapy of lung adenocarcinoma. Although plasma circulating cell-free tumor DNA (ctDNA) is detectable at an early stage, the size of the tumor does not strongly correlate with concentration of whole cell-free DNA (cfDNA), including normal leucocyte DNA. cfDNA analysis from patients with acquired TKI-resistance disease or extrathoracic disease progression correlated with a high detection rate of TKI sensitive mutations. We concluded that cfDNA in patients with EGFR-TKI-resistance or extrathoracic disease progression may be useful for analysis of cancer genomics (Seki Y, et al. ESMO Open 2018).

Publications

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