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General Summary

We address clinical and basic research concerning COPD, bronchial asthma, pulmonary infection, pulmonary fibrosis, and lung cancer. Basic research should resolve clinical problems and clinical research should construct novel treatments. We investigated COPD and IPF pathogenesis concerning cellular senescence and autophagy, and published several reports. 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 against devastating lung diseases.

Research Activities

Cellular senescence and autophagy in chronic obstructive pulmonary disease (COPD)
Chronic obstructive pulmonary disease (COPD) is caused by the noxious effects of tobacco smoke, which leads to airway epithelial cell injury and the induction of phenotypic changes such as squamous metaplasia and cellular senescence, which are assumed to be part of the adaptive response to toxic components such as reactive oxygen species (ROS). Cigarette smoke–induced accelerated cell senescence has been widely implicated in COPD pathogenesis. The accumulation of damaged proteins and organelles are typical manifestations of cellular senescence, indicating the involvement of autophagy, a bulk degradation pathway for cellular components, in the regulation of cellular senescence in COPD. We found that cigarette smoke extract (CSE) treatment of human bronchial epithelial cells (HBEC) transiently induced activation of autophagy, which was associated with accelerated cellular senescence and concomitant accumulations of p62 and ubiquitinated proteins. Autophagy induction in response to CSE was significantly decreased in HBEC from COPD patients, and both p62 and ubiquitinated protein levels were increased in lung homogenates from COPD patients, suggesting the involvement of insufficient p62-mediated selective autophagic clearance of ubiquitinated proteins in accelerated cellular senescence in COPD pathogenesis (Fujii S, et al, Oncoimmunology 1:630–641, 2012).
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. Accelerated cellular senescence is

implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD). Accordingly, we investigated the involvement of mitochondrial dynamics in cigarette smoke extract (CSE)-induced cellular senescence in human bronchial epithelial cells (HBEC). CSE induced mitochondrial fragmentation and mitochondrial oxidative stress, which were responsible for acceleration of cellular senescence in HBEC. Both mitochondrial fragmentation and mitochondrial oxidative stress induced by CSE treatment were inhibited in the presence of NAC or Mito TEMPO. Mitochondrial fragmentation induced by knockdown of fusion proteins also increased mitochondrial ROS production and percentages of senescent cells. CSE-induced mitochondrial fragmentation is involved in cellular senescence through the mechanism of mitochondrial ROS production. 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 exosome. Exosome is one of extracellular vesicles which have important roles in cell to cell communications. MicroRNA have potential roles in cellular homeostasis and pathophysiology of various diseases. We found that mir201 suppresses ATG7 and autophagy, which leads to fibrogenesis in airway walls of COPD (Fujita Y *et al. J Extracellular Vesicles* 2015).

Cellular senescence and autophagy in idiopathic pulmonary fibrosis
Aberrant re-epithelialization with bronchial epithelial cells is a prominent pathologic finding in idiopathic pulmonary fibrosis (IPF) and is implicated in abnormal epithelial-mesenchymal interactions. Recent studies show senescence as a risk factor for development of IPF. We have produced evidence that IPF lungs show enhanced senescence with a concomitant increase of SIRT6 expression in epithelial cells, including aberrantly re-epithelialized bronchial cells. TGF-β induces senescence by increasing p21 expression and also induces SIRT6 expression, and artificial overexpression of SIRT6 efficiently inhibits TGF-β induced senescence via proteasomal degradation of p21 in HBEC. IL-1β secretion from TGF-β-induced senescent HBEC is responsible for myofibroblast differentiation in fibroblasts. These findings shed light on the accelerated epithelial senescence in IPF pathogenesis with a possible regulatory role for SIRT6 (Minagawa S, et al. Am J Physiol Lung Cell Mol Physiol. 300: L391-401, 2011).
Accelerated epithelial cell senescence accompanied by excessive myofibroblast proliferation has been implicated in the pathogenesis of IPF. Autophagy plays an important regulatory role in cellular senescence and differentiation. Autophagy has been shown to prevent cellular senescence caused by tunicamycin-induced ER stress in human bronchial epithelial cells (HBEC). Conversely, autophagy inhibition was sufficient to induce myofibroblast differentiation in lung fibroblasts. We also demonstrated that metaplastic epithelial cells and fibroblasts in fibroblastic foci (FF) expressed both ubiquitinated proteins and p62 in IPF. Cellular senescence as measured by p21 expression and senescence associated SA-β-Gal staining was observed in metaplastic epithelial cells covering fibrosing lesions. AECII in relatively normal areas of IPF exhibited ubiquitin staining, however a concomitant increase of LC3, indicating autophagy activation, may explain why p21 expression was not observed in those cells. These findings suggest that insufficient autophagy is a potent underlying pathology 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).
Insufficient mitophagy, which is specific autophagy for mitochondria, 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 197: 504-16, 2016).

Etiologies of in community-acquired pneumonia in adults by real-time polymerase chain reaction

Recently multiplex polymerase chain reaction (PCR) has been applied to detect effectively both respiratory bacteria and viruses. To evaluate etiologies in community-acquired pneumonia in adults, a rapid reliable process based on real-time PCR for respiratory samples was used. We analyzed respiratory tract samples by comprehensive real-time PCR. We prospectively studied 92 patients with COPD using nasopharyngeal swab and sputum samples. Streptococcus pneumoniae was most frequently identified, followed by Haemophilus influenzae and Mycoplasma pneumoniae. PCR also identified viral pathogens. Real-time PCR of nasopharygeal and sputum samples could better identify bacterial and viral pathogens in community-acquired pneumonia than conventional methods (Yoshii Y, et al. Infec Dis 48: 782-8, 2016).

Extracellular Vesicles in Chronic Obstructive Pulmonary Disease

Although several mechanisms of COPD pathogenesis have been verified, the precise mechanism remains unknown. Extracellular vesicles (EVs), including exosomes, microvesicles, and apoptotic bodies, are released from almost all cell types and are recognized as novel cell-cell communication tools. They have been shown to carry and transfer a wide variety of molecules, such as microRNAs, messenger RNAs, and proteins, which are involved in physiological functions and the pathology of various diseases. We address EV-mediated COPD pathogenesis and also investigate the usefulness of EVs as biomarkers (Kadota T, et al. Int J Mol Sci, 2016).

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