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General Summary

We performed 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 lead to novel treatments. We completed clinical research concerning COPD and manuscript has been submitted. We are also completed the study investigating etiologies of acute exacerbation of bronchial asthma and COPD in adults by real-time polymerase chain reaction. Concerning basic research, we are investigating lung homeostasis, especially aging, apoptosis, necrosis, and senescence. We also interested in autophagy in the pathogenesis of lung diseases.

Research Activities

Cellular senescence and autophagy in COPD

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). The acceleration of cell senescence induced by cigarette smoke has been widely implicated in the pathogenesis of COPD. The accumulation of damaged proteins and organelles are typical manifestations of cell senescence, indicating the involvement of autophagy, a bulk degradation pathway for cellular components, in the regulation of cell senescence in COPD. We found that treatment of human bronchial epithelial cells (HBECs) with cigarette smoke extract (CSE) 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 HBECs from patients with COPD, and levels of both p62 and ubiquitinated protein were increased in lung homogenates from these patients, suggesting the involvement of insufficient p62-mediated selective autophagic clearance of ubiquitinated proteins in accelerated cellular senescence in the pathogenesis of COPD (Fujii S, Oncoimmunology 1: 630-641, 2012).

Mitochondria are dynamic organelles that are essential for cellular metabolic functions and 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 production of ROS, resulting in apopto-

sis and cellular senescence. Accelerated cellular senescence is implicated in the pathogenesis of COPD. Accordingly, we investigated the involvement of mitochondrial dynamics in CSE-induced cellular senescence in HBECs. Treatment with CSE induced both mitochondrial fragmentation and mitochondrial oxidative stress, which were responsible for the acceleration of cellular senescence in HBECs. Both mitochondrial fragmentation and mitochondrial oxidative stress induced by CSE treatment were inhibited in the presence of N-acetylcysteine or Mito-TEMPO. Mitochondrial fragmentation induced by knockdown of fusion proteins also increased mitochondrial ROS production and the percentage of senescent cells. Mitochondrial fragmentation induced by CSE 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 by which COPD develops (Hara H et al, Am J Physiol Lung Cell Mol Physiol 305: L737-746, 2013).

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 have shown that senescence is a risk factor for the development of IPF. Among the SIRT family of class III histone deacetylases, SIRT6 has been shown to antagonize senescence. We examined epithelial senescence as a representative phenotypic alteration in conjunction with SIRT6 expression in IPF. We have produced evidence that IPF lungs show enhanced senescence with a concomitant increase in SIRT6 expression in epithelial cells, including aberrantly re-epithelialized bronchial cells. Transforming growth factor (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 HBECs. Secretion of interleukin β1 from TGF-β-induced senescent HBECs is responsible for myofibroblast differentiation in fibroblasts. 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).

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. To determine if insufficient autophagy is involved in the pathogenesis of IPF, the regulatory role of autophagy in cell senescence and myofibroblast differentiation was tested with *in-vitro* models. We also examined the autophagy status using immunohistochemial evaluation of microtubule-associated protein light chain 3 (LC3), beclin 1, p62, and ubiquitin in the lung. Autophagy has been shown to prevent cellular senescence caused by tunicamycin-induced endoplasmic reticulum stress in HBECs. Conversely, autophagy inhibition was sufficient to induce myofibroblast differentiation in lung fibroblasts. We also demonstrated that metaplastic epithelial cells and fibroblasts in fibroblastic foci expressed both ubiquitinated proteins and p62 in IPF. Cellular senescence, as measured by p21 expression and senescence-associated β-galactosidase staining, was observed in metaplastic epithelial cells

covering fibrosing lesions. Type II alveolar epithelial cells in relatively normal areas of IPF exhibited ubiquitin staining; however, a concomitant increase in LC3, indicating autophagy activation, may explain why p21 expression was not observed in these 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).

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

The microorganisms most commonly associated with acute exacerbation of bronchial asthma (AEBA) are respiratory viruses, such as rhinovirus, and atypical bacteria, such as *Mycoplasma pneumonia*. Causative organisms of AEBA in pediatric populations have been well documented but are rarely reported in adults. Recently, multiplex polymerase chain reaction (PCR) has been used to effectively detect both respiratory bacteria and viruses. To evaluate etiologies in adult AEBA, a rapid, reliable process based on real-time PCR for respiratory samples was used. We prospectively enrolled adult patients with AEBA who satisfied our criteria: 20 years or older, within 7 days of onset, and informed consent. Nasopharyngeal swabs and sputum samples were collected from all patients, and comprehensive real-time PCR was used to detect 6 bacteria and 11 respiratory viruses. Of the 36 patients who satisfied our criteria, 25 (69.4%) had microorganisms, either bacteria or viruses or both, which were detected with PCR. The diagnosis was viral infection in 7 patients (19.4%), bacterial infection in 11 patients (30.6%), atypical bacterial infection in 3 patients (8.3%), and viral/bacterial co-infection in 4 patients (11.1%). The remaining 11 patients (30.6 %) had unknown pathogens. The most common microorganisms were *Haemophilus influenzae*, *M. pneumonia*, and rhinovirus. Our results suggest that real-time PCR analysis of nasopharyngeal swabs and sputum samples is helpful for determining the cause of AEBA in adults. Results of the detection of *M. pneumonia* and rhinovirus were as expected; however, the detection of *H. influenzae* was unexpected. On the basis of these results, we analyzed the association between microorganisms and AEBA. These results were presented at the European Respiratory Society meeting and are being prepared for journal submission.

Publications

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Reviews and Books

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