Involvement of Autophagy in Cigarette Smoking Extract-induced Cellular Senescence in Human Bronchial Epithelial Cells

H. Hara, MD[1], S. Nojiri, M.D[1], J. Araya, M.D[1], Y. Yumino, M.D[1], S. Minagawa, M.D.[1], J. Kojima, M.D[1], T. Numata, M.D[1], M. Kawaishi, M.D[1], M. Odaka, M.D[1], K. Nakayama, MD, PhD[2], T. Morikawa, M.D[1], K. Kuwano, MD, PhD[3]
[1]Jikei University School of Medicine - Tokyo/JP, [2]Jikei University / School of Medicine - Tokyo/JP, [3]Jikei University School of Medicine / - Tokyo/JP

Introduction: Accelerated cellular senescence has been implicated in the pathogenesis of COPD and cigarette smoke, an abundant source of oxidative stress, has been demonstrated to induce cellular senescence partly via post-translational modification of cellular proteins. Autophagy, a tightly regulated degradation process of cytoplasmic proteins, plays an important role in the elimination of abnormal proteins. Accumulation of damaged and dysfunctional proteins is a characteristic feature of cellular senescence, indicating the possible association between autophagy and accelerated cellular senescence by cigarette smoke for COPD development. Indeed increased autophagy has been reported in COPD lung tissues in association with apoptosis induction and, in in vitro model, cigarette smoking extract (CSE) induced both autophagy and apoptosis in airway epithelial cells. The purpose of this study was to elucidate the protective role of increased autophagy in CSE-induced accelerated cellular senescence in human bronchial epithelial cells (HBEC).

Methods: Airways were collected from lobectomy specimens from resections performed for primary lung cancer. HBEC isolation was performed with protease treatment. To characterize autophagy, fluorescence microscopic detection of LC3-EGFP dot formation, RT-PCR, and western blotting of LC3 were performed. Autophagy was inhibited by 3-methyladenine (MA), a specific inhibitor of autophagy, treatment or knock down of LC3 by siRNA tranfection. Rapamycin and torin1, mammalian target of rapamycin inhibitors, were used to induce autophagy. Senescence associated beta-galactosidase (SA-Î²-gal) staining, a representative marker of cellular senescence and western blotting of p21 were performed to evaluate cellular senescence.

Results: CSE induced cellular senescence demonstrated by increased SA-Î²-gal staining and p21 expression levels, and while CSE also induced autophagy shown by formation of LC3-EGFP puncta and increased conversion from LC3-I to -II, it was not sufficient to inhibit cellular senescence in HBEC. However, induction of autophagy by torin1 significantly suppressed CSE-induced senescence, while inhibition by knock down with LC3 siRNA and 3MA treatment further increased CSE -induced senescence.

Conclusions: These results suggested a potential inhibitory role of autophagy in CSE-induced accelerated cellular senescence by preventing the accumulation of damaged and dysfunctional proteins, indicating the novel therapeutic potential by regulation of autophagy in COPD, which remains to be determined in the future study.