Interleukin-8 is a potential senescence inducer in lung fibroblasts

C. Bernardelli[1], P. Selvaggio[1], S.Rosa[1], M. Lazzari[1], L. Casati[2], R. Chiaramonte[2], E. Lesma[1]

[1] Laboratory of Pharmacology, Department of Health Sciences, Università degli Studi di Milano, Italy

[2] Laboratory of Pathology, Department of Health Sciences, Università degli Studi di Milano, Italy

In aging, lung parenchyma remodeling leads to a progressive deterioration of lung function, with the impairment of gas exchange and immunologic changes that prompt infections. Even if it is well established that the risk of primary lung cancer, respiratory infections, obstructive and fibrotic lung diseases increase with age, the drivers of lung aging remain largely unknown. Being the interface with the external environment, lung cells must respond to increasing chemical, mechanical, biological, immunological and xenobiotic solicitations, so they must strongly rely on stress response pathways over the lifetime. Cellular senescence is a stress-response process characterized by the irreversible inhibition of cell proliferation and by the acquisition of a senescence-associated secretory phenotype (SASP). SASP consists in the secretion of a complex mixture of factors, including proinflammatory cytokines, chemokines, growth factors and proteases, as matrix metalloproteases (MMPs). SASP is evolutionally conserved and reinforces senescence in a cell-autonomous manner. However, it can boost a proinflammatory microenvironment with a systemically defective immunosurveillance in many age-related diseases. Moreover, the upregulation of the cell cycle inhibitors p16 and p21 in senescent progenitor cells limits tissue regeneration, further damaging tissue architecture through MMPs.

As a paradigm of the lung impairment driven by senescence, we developed an *in vitro* model of human pulmonary microenvironment by using healthy primary lung fibroblasts (PLFs) and primary LAM/TSC cells, isolated from a chylothorax, characterized by the constitutive activation of mTOR due to the lack of its regulatory protein tuberin. Knowing that the activation of mTOR contributes to cellular senescence, we firstly demonstrated that LAM/TSC cells have senescent features depending on mTOR hyperactivation, since their high positivity to SA-β galactosidase and to phospho-histone H2A.X are reduced by inducing tuberin expression and by inhibiting mTOR with rapamycin. Furthermore, the LAM/TSC cells have the capability to induce senescence in PLFs through factors contained in their conditioned medium (CM). To explore the communication in lung microenvironment, we assessed that LAM/TSC cells secrete extracellular vesicles (EVs), which can be carriers of senescence-inducing insoluble mediators. Both tuberin expression and and rapamycin treatment reduced LAM/TSC cell-mediated EVs secretion, implying a modulation of EVs release by mTOR pathway. Intriguingly, senescent-induced PLFs enhance the expression and secretion of IL-8, a potent neutrophil chemotactic factor that also mediates the spreading of the SASP response in the microenvironment.

This evidence suggests that senescence spreading in lung might reduce the proliferation of lung resident cells and, at the same time, might promote a pro-inflammatory microenvironment that ultimately has detrimental effects on the lung tissue.

To deepen this, the CM of PLFs was supplemented with IL-8, observing a significant increase of senescence, while blocking IL-8 receptor modulated the senescent features of cells. This suggests that, through SASP, senescent cells might establish an effective communication within lung microenvironment with a possible direct involvement of IL-8. Indeed, the high levels of this chemokine might induce senescence and promote a pro-inflammatory/senescent milieu, ultimately causing the disruption of lung parenchyma. Taken together, these results make senescence, and its modulation, an intriguing field of study to understand the mechanisms underlying lung disruption in pulmonary chronic diseases. Moreover, dissecting the pathological communication in the senescent lung microenvironment might allow to identify targets for early diagnosis and novel therapies.