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Editorial

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# SARS-CoV-2-induced senescence as a potential therapeutic target

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The global COVID-19 pandemic has caused major morbidity, mortality, and socioeconomic disruption on an individual and collective level. Over six million COVID-related deaths have been reported, with total case numbers now well over 500 million worldwide [1]. Whilst the prompt and efficient design of effective vaccines has restored varying degrees of normal activity to some parts of world, the effects of the pandemic will be long in duration and far-reaching.

Our knowledge of the mechanisms of action of the pandemic's causative agent, the human pathogenic coronavirus, SARS-CoV-2, has progressed at great speed since the onset of the pandemic, with resultant implications for clinical care and vaccination strategies. In this issue of the *European Respiratory Journal, Evangelou et al.* outline the role of cellular senescence in the pathogenesis of severe clinical forms of COVID-19 [2].

Cellular senescence is a state of stable and generally irreversible cell cycle arrest characterised by altered cell morphology and metabolism, in conjunction with generation of a potent secretory pro-inflammatory environment – the senescence associated secretory phenotype (SASP) [3]. Originally identified in the setting of replicative senescence whereby cultured cells cease to replicate after a set number of cell divisions [4], senescence has since been found to arise in response to a wide number of stressors including oncogene activation, genotoxic stress and hypoxia among others [3]. This expansion in the understanding of senescence has led to it being widely implicated in a range of diseases, including cancer, fibrosis, neurodegenerative diseases and atherosclerosis among many others [5]. Its role in diseases of aging and how it may contribute to the frailty, multi-morbidity and enhanced clinical vulnerabilities seen in older populations is an area of increasing focus [6], including within this pandemic [7].

Virus-induced senescence (VIS) is less well understood than other senescence-inducing mechanisms, but as recently summarised by *Kohli et al.*, senescence may be induced both in response to viruses themselves, as an antiviral defence mechanism, and in response to antiviral therapies [8, 9]. To date the most significant exploration in this area has related to Human Immunodeficiency Virus (HIV) where senescence has been implicated as a potentially detrimental outcome of anti-retroviral therapy contributing to premature ageing phenotypes [8, 10, 11] and as recently summarised by *Sánchez-Díaz et al.* a number of senolytic and senomorphic agents are being investigated in this setting [12].

In their paper *Evangelou et al.* use autopsy lung tissue samples from COVID-19 patients to establish the presence of senescence and demonstrate evidence of increased expression of the key SASP cytokines IL-1$\beta$ and IL-6. These findings provide a link for how VIS and the SASP can be implicated in the cytokine storm and immune activation that characterises severe clinical forms of COVID-19, which feature significant mortality rates and a wide range of systemic complications causing notable morbidity for those patients that survive [13, 14]. IL-6 has already been in particular focus within the pandemic, including as a therapeutic target, with the use of the monoclonal antibody Tocilizumab which is recommended as a treatment option in certain sets of hospitalised patients based on the results of a number of clinical trials [15]. As a comparison the authors describe that SARS-CoV-2 infected AT2 cells from the lungs of patients with severe COVID-19 displayed significantly higher levels of senescence than those of an age-matched control group including patients with acute pneumonia and other respiratory pathologies.
Particularly noteworthy given the multi-system nature of COVID-19, *Evangelou et al.* also demonstrate the presence of senescence in extra-pulmonary sites including the kidneys and liver. They correlate their findings *in vitro* by demonstrating the establishment of senescence in Vero-E6 epithelial kidney cells infected with SARS-CoV-2, with dramatically reduced implementation of senescence in response to treatment with a selective ATM inhibitor, strongly suggesting DNA damage as an initiator of senescence in this setting. Interestingly, *Tsuji et al.* recently proposed virus-induced cytokine production as an inducer of senescence in COVID-19 infections, having demonstrated that human lung diploid fibroblasts infected with SARS-CoV-2 can induce a senescent-like phenotype in neighbouring uninfected cells [16]. The senescent cells then continue to express high levels of SASP factors even once SARS-CoV-2 infection is no longer detectable, a finding corroborated by re-analysis of single-cell transcriptomic data from lung tissue of patients with severe and prolonged COVID-19 [17]. The authors provide evidence supporting the involvement of virus-induced cytokines - in particular TNF-$\alpha$ - as mediators of senescence induction, suggesting that there may be multiple potential inducers of senescence in COVID-19.

These findings complement recent work published by *Lee et al.* [18] and *Camell et al.* [19] on this subject.
*Lee et al.* demonstrated increased markers of senescence in the airway mucosa of COVID-19 patients in addition to elevated concentrations of SASP factors in patient serum [18]. An association of clinical case severity with high pro-inflammatory and pro-coagulant SASP factor levels (IL-6 and SERPINA3 respectively) and coagulation activity was also demonstrated and correlated *in vitro* with supernatant from virus-induced senescent cells promoting platelet activation, reduced clotting times and increased neutrophil extracellular trap formation,

consistent with the pro-thrombotic phenotype seen clinically. Both *Lee et al*. and *Camell et al*. [19] have also demonstrated improved outcomes with senolytic therapies including navitoclax and a combination of dasatinib plus quercetin, using *in vivo* models with both SARS-CoV-2 and a closely-related β-coronavirus. *Camell et al.* focused particularly on an aged mouse model, establishing an increased senescent cell burden with age as a potential mechanism for the enhanced vulnerability of the elderly and frail to COVID-19.

In a novel finding *Evangelou et al.* also detail the potential implications of senescence on viral mutagenesis. They explain that senescent cells' ability to host the virus for longer periods increases the probability of host mediated editing of the viral genome [20]. Building on earlier work that demonstrated high expression of APOBEC enzymes in senescent cells [21], and using bioinformatic analyses and next generation sequencing of senescent cell progenies they demonstrate the prominence of the APOBEC enzymes in the mutational signature of SARS-CoV-2 variants. Characterisation of the Omicron (B.1.1.529) variant identified the APOBEC signature as the main mutational profile, consistent with previous observations of a prominent role for APOBEC-mediated editing in the current pandemic [22]. Interestingly, *Evangelou et al.* also observed significantly lower mutational burdens in oncogenic viruses including human papilloma virus (HPV) and Epstein-Barr Virus (EBV) than in SARS-CoV-2 and HIV.
Such insights into the mutagenesis of SARS-CoV-2 are of significant value as despite the positive impacts generated by COVID-19 vaccine development, the risk of further disruption and morbidity from new variants remains. New observations on viral mutagenesis in the context of this pandemic may therefore have significant implications for subsequent vaccination strategies.

Furthermore, it is also, as yet, not fully clear to what extent vaccine efficacy is achieved or maintained in clinically vulnerable groups, for example cancer patients [23], meaning that insights into mechanisms and therefore potential therapeutic targets remain important. The promising results of senolytic therapies *in vivo* continue to be explored in a number of clinical trials with some encouraging early results [24–26]. The particular promise of flavonoids such as fisetin and quercetin in recently published work relating to COVID-19 is noteworthy due to their low toxicity profile, as other senolytic agents such as the Bcl-2/Bcl-xl dual inhibitor, navitoclax, have been limited in clinical tolerability due to significant toxicities [27]. High rates of thrombocytopaenia have been a particular concern with navitoclax use previously [27], though modification using prodrugs and drug adaptation with proteolysis-targeting chimera technology have shown promise in reducing the incidence of thrombocytopaenia *in vivo* [28, 29]. Platelet toxicity is of relevance in COVID-19 (and other coronaviruses) as mild thrombocytopaenia is present in approximately 30-60% of cases, and cases of severe immune thrombocytopenia have been reported [30, 31]. Of note, *Lee et al.* found that in *in vivo* models emulating a severe COVID-19 phenotype, the animals treated with navitoclax had significantly worse outcomes in terms of clinical impairment and weight loss, whilst those treated with quercetin (in combination with the tyrosine kinase inhibitor Dasatinib) displayed improved survival and clinical course [18].

In their recent review *Kholi et al*. summarised key unanswered questions on the subject of virus-induced senescence, with two of those being of particular relevance here [8]. Firstly, the question of whether senolytics or SASP modulators (termed as senomorphics or senostatics, such as rapamycin or metformin) could be used clinically in the setting of acute and chronic viral infections. Secondly, they also questioned the role of senescence in COVID-19 pathology and potential links to the higher mortality rates in older patients, building on the hypotheses proposed in *Nehme et al.*[7].

The work of *Evangelou et al*.,[2] and others [16, 18, 19] contribute towards answering these questions, providing a significant contribution to understanding the role of senescence in the cytokine storm and severe multi-organ morbidity that has characterised severe clinical forms of COVID-19.
The insights gleaned are valuable both in the current pandemic and for future pandemic preparedness, with first generation senolytics already in clinical trials providing potential new therapeutic avenues. Challenges remain, and more refined tools to assess for the presence of senescence *in vivo* and in clinical settings are still required but in conclusion, cellular senescence should be considered as a key factor and potential target in the pathogenesis of severe clinical forms of COVID-19, and the impact of senescence actively considered in other viral pathologies.

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