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EDITORIAL

Stricter definitions of myeloid senescence are needed

Brandt D. Pence[a, *]

[a] *College of Health Sciences, University of Memphis, Memphis, TN, USA.*

Abstract

Cellular senescence is a state of irreversible cell cycle arrest that is associated with age-related diseases. There is considerable interest in studying senescence in immune cells such as macrophages. However, current popular markers of cellular senescence overlap substantially with cellular programs induced in macrophages and related cells during acute inflammation responses, raising questions about the degree to which the current literature truly reflects senescence in the innate immune system.

Keywords: Senescence, macrophage, monocyte, dendritic cell, immune aging

Cellular senescence is classically defined as an irreversible state of cell cycle arrest [1]. Senescence is a hallmark of aging, with increased senescent cell burden in a variety of tissues with advancing age. Senescent cells are thought to contribute to pathology of a variety of age-related diseases including cardiovascular and renal disease, diabetes, and Alzheimer's disease, with the strongest evidence being amelioration or reduction of disease through selective senescent cell removal with various senolytic drugs [1]. Senescent cells mediate disease and propagate senescence to other cells through secretion of a suite of proteins known as the senescence-associated secretory phenotype (SASP) [2], which includes a variety of pro-inflammatory cytokines, growth factors, and chemokines.

Although most work in cellular senescence has examined fibroblasts, endothelial cells, and other major tissue resident cell types, there has been some interest in studying cellular senescence in the immune system. A seminal paper in Science in 2016 purported to link cellular senescence to disease mediated by macrophages, showing that foam cells in atherosclerotic plaques have increased senescence-associated markers and SASP constituents [3]. Senescence in macrophages has been of tremendous interest in recent years, with more than 600 papers per year matching the search term "senescence AND macrophages" since 2020.

The topic of macrophage senescence has been comprehensively reviewed very recently [4], and interested readers can find significantly more information about this topic in that paper than is possible to include in this format. My purpose here is to point out several concerns with the current literature on senescence in macrophages (and similar cell types such as monocytes and dendritic cells). While it is by no means conclusive that the cells described in many of these papers are not senescent, the available evidence is also not sufficient to conclusively state that they are true senescent cells.

The majority of the senescence literature, including that pertaining to immune cells, defines senescent cells on the basis of some combination of (a) increased expression of the enzyme β-galactosidase (β-gal), (b) upregulation of the cell cycle protein $p16^{INK4A}$ or its encoding gene CDKN2A, and/or (c) expression of some interrogated subset of known SASP proteins. This is problematic in innate immune cells for a variety of reasons. In the last case, defined SASP proteins (many being pro-inflammatory cytokines) have substantial overlap with proteins produced by macrophages and other immune cells during acute inflammatory activation. This makes distinguishing acute inflammation responses from cellular senescence difficult, especially in shorter-term *in vitro* experiments.

With respect to other routine senescence markers, a notable paper by Hall *et al*. [5] demonstrated that both β-gal expression and p16 activation are upregulated in a reversible manner in macrophages during acute polarization responses. This conflicts with the definition of senescence in most cell types, where a key facet is its irreversible nature. Many other papers have shown these markers to be activated in immune cells during biological responses which are decidedly not senescence. For example, we have previously shown acute increases in CDKN2A expression in monocytes within hours after activation by the spike protein from SARS-CoV-2 [6]. Relatedly, the over-

* Corresponding author: Brandt Pence
Mailing address: 495 Zach H Curlin St., Memphis, TN 38152, USA.
Email: bdpence@memphis.edu
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lap between senescence and immune activation makers raises the question of whether some effects attributed to senolytic drugs may be due to the nonselective removal or suppression of innate immune responses as well.

As such, traditional markers of senescence are not likely to be sufficient (by themselves) to define innate immune cells as senescent. There are other established markers of senescence including those that reflect DNA damage which might be useful [7], but this will require extensive study. Some effort has been made with single cell sequencing to define unique markers of macrophage senescence (reviewed in [4]), but this area is in its infancy and suffers from the same uncertainty in defining innate immune cells as senescent as in the other literature described above. Irradiation-induced macrophage senescence in *in vitro* studies seems a promising place to start when developing macrophages which are probably senescent rather than acutely activated, and some progress has been made in this area [4].

Finally, from a philosophical (or perhaps teleological) perspective, the purpose of replicative senescence in innate immune cells such as macrophages is rather unclear. Macrophages and other myeloid cells are primarily postmitotic and do not replicate to any great degree (beyond perhaps to permit self-renewal in resident tissue macrophages [8]), so the biological purpose of a senescence program in these cell types is murky. Given all of the above, some care should be taken when defining macrophages and similar cell types as senescent.

Declarations

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