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Review

Biomarkers to identify and isolate senescent cells

Mantas Matjusaitis[a], Greg Chin[b], Ethan Anders Sarnoski[b], Alexandra Stolzing[c,d,*]

[a] Scottish Centre for Regenerative Medicine, The University of Edinburgh, Edinburgh, England, UK
[b] Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT, USA
[c] Institute IZBI, University of Leipzig, Leipzig, Germany
[d] Loughborough University, Loughborough, England, UK

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ABSTRACT

Aging is the main risk factor for many degenerative diseases and declining health. Senescent cells are part of the underlying mechanism for time-dependent tissue dysfunction. These cells can negatively affect neighbouring cells through an altered secretory phenotype: the senescence-associated secretory phenotype (SASP). The SASP induces senescence in healthy cells, promotes tumour formation and progression, and contributes to other age-related diseases such as atherosclerosis, immune-senescence and neurodegeneration. Removal of senescent cells was recently demonstrated to delay age-related degeneration and extend lifespan.

To better understand cell aging and to reap the benefits of senescent cell removal, it is necessary to have a reliable biomarker to identify these cells. Following an introduction to cellular senescence, we discuss several classes of biomarkers in the context of their utility in identifying and/or removing senescent cells from tissues. Although senescence can be induced by a variety of stimuli, senescent cells share some characteristics that enable their identification both in vitro and in vivo. Nevertheless, it may prove difficult to identify a single biomarker capable of distinguishing senescence in all cell types. Therefore, this will not be a comprehensive review of all senescence biomarkers but rather an outlook on technologies and markers that are most suitable to identify and isolate senescent cells.

Contents

* Corresponding author at: Loughborough University, Wolfson School, Epinal Way, LE11 3TU, Loughborough, UK.
E-mail addresses: A.Stolzing@lboro.ac.uk, Stolzing@gmail.com (A. Stolzing).

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1. Introduction

1.1. Aging and cellular senescence

Our society is rapidly aging and the incidence of age-related diseases, such as Alzheimer's, diabetes and cancer is increasing (Christensen et al., 2009). If these trends continue, aging will become a major economic and social burden (Harper, 2014; Kankeu et al., 2013; Wimo et al., 2013). To avert this impending crisis, we must better understand why we age. Aging is a heterogenic process at both the organismal and cellular level. The number of contributing internal and external factors, such as epigenetic changes (Sinclair and Oberdoerffer, 2009) and the environment, make it difficult to categorize and prioritize the importance of each component. Such diversity has given rise to multiple theories regarding the root cause of aging (Harman, 1956; Park and Yeo, 2013; Wei et al., 2001), which sometimes contradict but more often complement one another. The variety of associated causes implies that aging is likely to be multifactorial in nature (Riess and Krüger, 1999; Sheikh et al., 2013).

One known aging factor is cellular senescence. Senescent cells accumulate with age in organisms, albeit at different rates in the various organs (Erusalimsky and Kurz, 2005; Herbig et al., 2006; Jeyapalan et al., 2007; Paradis et al., 2001). Originally, cellular senescence was defined as a loss of replicative capacity (Hayflick, 1965) caused by a progressive shortening of the tandem repeats protecting chromosome ends (telomeres). This eventually leads to chromosomal damage and replicative arrest (Campisi, 1997). Interestingly, cellular senescence can also be induced by stress (Toussaint et al., 2000) and oncogenes (Bartkova et al., 2006), demonstrating that cellular senescence is not only caused by exhaustion of replicative capacity as first thought. Such heterogeneity of cellular senescence, which we will briefly discuss in the next paragraph, has led a field to sometimes unnecessary ambiguity, leaving researchers to disagree what cellular senescence entails (Burton and Faragher, 2015). For the purposes of this review, we define cellular senescence as a permanent (under physiological conditions) cell cycle arrest that is a result of cellular stress or damage, including but not limited to abnormal activation of oncogenes, telomere shortening and macromolecule accumulation (De Cecco et al., 2011). With this description we deliberately exclude developmental senescence, quiescent cells, and post-mitotic cells.

1.2. Senescent cells in health and disease

Cellular senescence is thought to have developed as a safeguard to prevent damaged cells from accumulating and either becoming cancerous or causing cancer (Fig. 1). However, accumulation of senescent cells in tissues is detrimental to the animal (Herbig et al., 2006; Jeyapalan et al., 2007) as these non-functional cells directly and indirectly damage surrounding cells (Salama et al., 2014). Examples of such damage include occupying niches required by competent cells to function (Lynch, 2004), secreting transforming, inflammatory and otherwise damaging components of the SASP (Campisi and d'Adda di Fagagna, 2007; Coppé et al., 2010, 2008), promoting tumour formation (Leikam et al., 2015; Zacarias-Fluck et al., 2015) and contributing to various age-related diseases such as atherosclerosis (Irvine et al., 2014; Wang and Bennett, 2012) (Fig. 2).

While long-term accumulation of senescent cells is harmful to the organism, short-term senescence events prevent cancer (Kuilman et al., 2008), guide development (Muñoz-Espín et al., 2013) and improve tissue repair and wound healing (Demaria et al., 2014; Rodier and Campisi, 2011). One proposed mechanism to negate the long-term detrimental effects of senescent cells, while retaining their short-term beneficial functions, is to periodically purge them from the body. Regular elimination of p16-positive senescent cells from functionally wild-type mice slows time-dependent functional decline and extends median lifespan ~30% (Baker et al., 2016, 2011). Moreover, recent studies have demonstrated clearance of senescent cells from wild-type mice using small molecules which target the BCL-2 protein family (Chang et al., 2016; Zhu et al., 2015a,b), lending credence to this approach as a therapeutic strategy. However, no method currently exists to accomplish this in humans, in large part because senescent cells cannot yet be reliably identified in living tissue. In the following sections, we will discuss biomarkers and their utility in identifying or eliminating senescent cells in a living organism.

1.3. Characteristics of useful biomarkers

A biomarker is a biological signature of a condition which enables one to evaluate if the biological system (organism, cell, etc.) possesses that condition or not. Many molecules, such as proteins, nucleic acids, and lipids, can be used as a biomarker. They can be found within the cell, in the adjacent extracellular area, or even systemically in the circulatory system. Importantly, no single marker currently provides an accurate representation of cellular senescence.

A useful biomarker must display several important features. First, it should be robustly associated with the condition. Although it is likely that (i) context, such as cell type, will be relevant and that (ii) it may not identify all cases of cellular senescence, it is crucial that the presence of the marker strongly correlates with a specific condition. Second, it is essential to know the threshold at which a marker becomes representative of the specific feature. Most proteins are expressed at basal levels in many cells, and simple evaluation of the presence or absence of the protein is not informative. For discrimination purposes, it is imperative to identify a clear threshold value which defines the cellular status. Finally, to be practical, a marker must be quantifiable using current technologies. Even a comprehensive understanding of a marker is not practically helpful unless we are able to monitor its levels or purify cells positive for it.

In this review, we will emphasize markers of cellular senescence that would be practical to assay. Only single-cell markers of senescence will be discussed. Detailed reviews on systemic aging

Table 1
Key cellular senescence phenotypic traits and related mechanisms.

Senescent cell phenotypic traits	Reference
Permanent cell cycle arrest	Shay et al., 1991
Persistent DNA damage response (DDR)	Fumagalli et al., 2012
Senescence-associated heterochromatic foci (SAHFs) and other epigenetic changes	Narita et al., 2003
Senescence associated secretory phenotype (SASP)	Campisi, 2005
Altered metabolism including increased lysosomal and proteosomal activity	García-Prat et al., 2016