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Review

The role of cellular senescence in aging through the prism of Koch-like criteria

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ABSTRACT

Since Hayflick's discovery of cellular senescence (CS), a great volume of knowledge in the field has been accumulated and intensively discussed. Here, we attempted to organize the evidence "for" and "against" the hypothesized causal role of CS in aging. For that purpose, we utilized robust Koch-like logical criteria, based on the assumption that some quantitative relationships between the accumulation of senescent cells and aging rate should exist. If so, it could be expected that (i) the "CS load" would be greater in the premature aging phenotype and lesser in longevity phenotype; (ii) CS would promote age-related diseases, and (iii) the interventions that modulate the levels of senescent cells should also modulate health/lifespan. The analysis shows that CS can be considered a causal factor of aging and an important player in various age-related diseases, though its contribution may greatly vary across species. While the relative impact of senescent cells to aging could overall be rather limited and their elimination is hardly expected to be the "fountain of youth", the potential benefits of the senolytic strategy seems a promising option in combating age-related diseases and extending healthspan.

1. Introduction

Since Hayflick's discovery (Hayflick and Moorhead, 1961; Hayflick, 1965) of cellular senescence (CS), a great volume of knowledge in the field has been accumulated and intensively discussed. Up to date, over 6000 articles dealing with CS are deposited in PubMed, of them, 1280 are reviews. An important point is that the field is growing in an exponential rate, indicating both its ongoing interest and the emergence of new ideas (Fig. 1).

Now, it is clear that CS is not only an aging-associated phenomenon (as suggested by the name itself) but rather reflects a basic cellular response to a milieu of stress insults (de Magalhaes and Passos, 2017; Rodier and Campisi, 2011) and is also an essential component of normal physiological processes such as development (Munoz-Espin et al., 2013) and wound healing (Demaria et al., 2014). Yet, the links between CS, aging, and age-related diseases (ARDs) remain a major issue, mirrored by the notion that CS is a "hallmark of aging" (Lopez-Otin et al., 2013). Despite this wide consensus, there is still no definite answer to the question of whether CS is an obligatory (causal) or subsidiary factor of aging. In other words, to what extent is CS essential to the aging process, and if so, to what extent does it contribute?

We attempted to answer these questions using robust logical criteria, without going into mechanistic details. We have previously shown the power of this "Koch-like" approach in analyzing the role of DNA damage and repair in aging (Moskalev et al., 2013). Here, we specifically evaluated whether the current evidence in the field meets the following criteria, divided into two major groups:

A. Relationships between aging and CS:

Criterion 1: Senescent cells accumulate with age.

Criterion 2: There are functional links between CS and ARDs.

Criterion 3: The premature aging phenotype is associated with an increased "CS load".

B. Relationships between longevity and CS:

Criterion 4: (i) The longevity phenotype is associated with lower levels of senescent cells; (ii) pro-longevity interventions reduce the accumulation of senescent cells.

Criterion 5: Efficient elimination of senescent cells increases lifespan.

In 1890, at the Tenth International Congress of Medicine in Berlin, Koch presented four criteria ("Koch postulates") that, when met, establish a causal role of a given pathogen in an infectious disease (Koch, 1891; see also Appendix A). We believe that this approach could be widely adopted to answer a variety of biomedical questions. In this study, we adopted the Koch concept to evaluate whether CS plays a causal role in aging. We postulate that fulfillment of all the criteria

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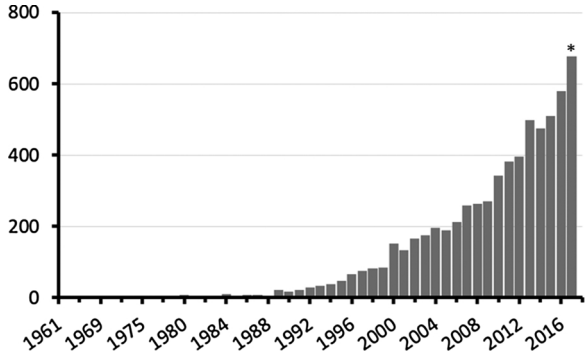

Fig. 1. The number of publications on PubMed containing the words "cellular senescence" and/or "replicative senescence" per year. * Predicted from the number of publication during Jan-June 2017.

above is sufficient to state that CS is a causal factor of aging, without stating the relative weight of its contribution or excluding the role of other factors. Lack of data on a specific criterion would place the whole hypothesis under question and insufficiency to meet even a single criterion would greatly diminish the possible role of CS in aging.

2. Relationships between aging and cellular senescence

2.1. Do senescent cells accumulate with age?

In order for senescent cells to play a part in aging, they must at least be physically present and apparently increase in number with advancing age. This criterion is one of the most investigated, and an accumulation of CS markers with age has been shown in a vast variety of tissues/cells and diverse species (roundworm, *C. elegans*; fish, *D. rerio*; rodents, *M. musculus, R. norvegicus*; monkeys, *P. papio, M. mulatta*; and *H. sapiens*) (Table 1). This was not however shown for all tissues in a reproducible manner. For example, Martin and Buckwalter (2003) reported an increase in CS markers for chondrocytes from old donors, while Price et al. (2002) found that SA-β-Gal only increases in chondrocytes from donors with osteoarthritis, without a correlation with age. Another example includes the mouse testes, where Sedelnikova et al. (2004) reported an accumulation of senescent cells, based on the CS marker γH2AX-foci, whereas Wang et al. (2009a, 2009b) failed to observe such an accumulation using the same marker (Table 1). While an age-related increase in the number of SA-β-Gal positive cells has been observed in the skin of 4 species examined, there are also reports indicating that such an increase is not mandatory (Severino et al., 2000).

An important point in *in vivo* studies of CS is that there are still no perfect methods to unequivocally identify senescent cells. The problem lies in the fact that the major hallmark of CS—an irreversible growth arrest—is hard to measure *in vivo*. With this in mind, numerous attempts have been made in the past to ascertain the replicative power of primary cells *ex vivo* as a function of donor age. Both a reduction (Bruce and Deamond, 1991; Schneider and Mitsui, 1976) and the absence of a decline (Cristofalo et al., 1998; Goldstein et al., 1978; Tesco et al., 1998) in replicative power of cultured fibroblasts have been reported. These discrepancies could result from changes accompanying the transition of cells from *in vivo* to *in vitro* conditions (e.g. stress, selection, etc.); it is also not fully clear to what extent is the *in vivo* phenotype preserved. In a very recent study, Biran et al. (2017) used ImageStreamX analysis combined with SA-β-Gal staining and a battery of other markers for the identification of senescent cells in a single-cell suspension, and showed the high efficiency of this approach. This new approach could overcome many of the existing obstacles in the identification of senescent cells, especially from *in vivo* samples.

All in all, despite some contradictory data and the technical difficulties, the sheer amount of evidence (Table 1) makes it reasonable to state that *senescent cells do accumulate with age*, at least in various tissues of mammalian species. Whether this accumulation is the result of cellular intrinsic or extrinsic factors that promote entry to CS, or a failure of the immune system to sufficiently clear senescent cells is yet to be established.

2.2. Are there associations between CS and age-related diseases?

It is becoming increasingly clear that age-related diseases (ARD) are an inevitable and integral part of the aging process (Budovsky et al., 2007; Dilman, 1971). If so, it is reasonable to postulate that if CS essentially contributes to aging, it must also contribute to late-onset pathologies. In other words, some accumulation of senescent cells should be observed in at least some ARDs. The links between CS and ARDs have been previously addressed by Munoz-Espin and Serrano (2014). The updated summary of the relevant studies is presented in Table 2, and briefly discussed in light of *Criterion 2* in Sections 2.2.1–2.2.12.

2.2.1. Cancer

The links between CS and cancer have been proven beyond reasonable doubt and have previously been intensively discussed (Campisi, 2005; Campisi and d'Adda di Fagagna, 2007; Campisi, 2013; Ovadya and Krizhanovsky, 2014; Tacutu et al., 2011). In short, CS is believed to be one of the tumor-suppressor mechanisms, but if senescent cells are not timely eliminated from the tissue, they could promote cancerogenesis via SASP. This concept, elegantly described by Campisi (2005) as "Good citizens, bad neighbors", clearly reflects the antagonistic (dual) nature of CS. With this complexity in mind, the induction of CS in tumor cells has been demonstrated to be a viable anti-tumor strategy (Lujambio et al., 2013; Roberson et al., 2005), while escaping from CS could result in a more aggressive tumor phenotype (Yang et al., 2017). Further complicating the cancer-CS relationships is a recently discovered mechanism by which the cancer cells may induce CS in normal cells that, in turn, form a cancer-promoting microenvironment, with subsequent cancer progression (Mikula-Pietrasik et al., 2016, 2017). At least for some tumors, the presence of senescent cells could be of prognostic value (Caliò et al., 2015).

2.2.2. Atherosclerosis

The link between atherosclerosis and CS is based on several lines of evidence (Table 2), ranging from an accumulation of senescent markers in human atherosclerotic lesions *in vivo* (Minamino et al., 2003; Ragnauth et al., 2010; Vasile et al., 2001; Wang et al., 2015) to CS-induced promotion of vascular inflammation in the arteries (Minamino et al., 2003) and alleviation of vasomotor dysfunction in atherosclerotic mice by senolytic treatment (Roos et al., 2016). Overall, it can be stated that CS is functionally meaningful for atherosclerosis, both in humans and in several animal models (mice, rats, rabbits).

2.2.3. Osteoarthritis

Strong association with CS was also shown for osteoarthritis (Table 2). This ARD was accompanied by an accumulation of senescent cells, and functional studies demonstrated a beneficial effect for CS clearance (Jeon et al., 2017).

2.2.4. Osteoporosis

To date, only one experimental study directly demonstrated a link between CS and osteoporosis, focusing specifically on increased CS in osteoprogenitors from old mice (Kim et al., 2017). Other evidence are mostly indirect (reviewed by Kassem and Marie, 2011; Marie, 2014).

2.2.5. Fibrosis

Fibrosis is considered to be one of the major aging-associated conditions (Rockey et al., 2015). CS has been studied in the context of several types of tissue fibrosis including renal, cardiac, liver, and