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REVIEW

Is cellular senescence an example of antagonistic pleiotropy?

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Summary

It is generally accepted that the permanent arrest of cell division known as cellular senescence contributes to aging by an antagonistic pleiotropy mechanism: cellular senescence would act beneficially early in life by suppressing cancer, but detrimentally later on by causing frailty and, paradoxically, cancer. In this review, we show that there is room to rethink this common view. We propose a critical appraisal of the arguments commonly brought in support of it, and we qualitatively analyse published results that are of relevance to understand whether or not cellular senescence-associated genes really act in an antagonistic-pleiotropic manner in humans.

Key words: aging; antagonistic pleiotropy; cancer; cellular senescence; DNA Damage Response; p53; tumour suppression.

Introduction

In the 19th century, August Weismann suggested that multicellular organisms could become more fragile with age because of an evolved mechanism that limits the proliferative potential of their somatic cells (Weismann, 1889). Such a mechanism would impair the regenerative capacity of damaged tissues in the organisms, thereby promoting their aging. More than one hundred years later, the possible link between the limited proliferative potential of somatic cells and the evolution of aging still engages many scientists.

Somatic cells permanently arresting their proliferation is indeed the defining feature of the cell condition known as cellular senescence (Hayflick & Moorhead, 1961; Hayflick, 1965). To explain its evolutionary origin, some authors have proposed an hypothesis that has become the common view in the literature: cellular senescence could be an example of the antagonistic pleiotropy theory of aging (Wright & Shay, 1995; Campisi, 2005), which is an influential explanation of how organisms could evolve mechanisms that reduce their own chances of survival (Medawar, 1946, 1952; Williams, 1957). The theory posits that mutations that produce deleterious effects late in life could be positively selected during evolution, provided that they act sufficiently beneficially in the first part of life (Williams, 1957).

In this review, after illustrating briefly the antagonistic pleiotropy theory of aging, we aim at understanding, on the ground of different types of evidence and with careful theoretical scrutiny, whether there is room to question the hypothesis that cellular senescence may really be a case of the antagonistic pleiotropy theory of aging. As cellular senescence is a phenomenon best studied in human cell cultures, our focus will necessarily be on humans. In particular, we will draw attention on human genetic data that bear on this issue.

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The antagonistic pleiotropy theory of aging

Aging processes in an organism are those that render it more susceptible to death as it becomes older (Maynard Smith, 1962), even in the absence of variations in external hazards or vital resources. To explain how aging may have evolved, it has been noted that any organism is more likely to survive to age x than to age $x+i$, because survival to $x+i$ is conditional on survival to x. Any allele with deleterious effects confined to late life will then have scarce chances of being negatively selected, as very few organisms will live long enough to experience the detrimental consequences of such an allele – which in the meanwhile may have already been transmitted to the progeny (Medawar, 1946, 1952). Consistent with this principle, the antagonistic pleiotropy theory for the evolution of aging postulates that late-life detrimental gene variants could guarantee overall higher Darwinian fitness (survival and/or fecundity) if they confer sufficiently strong beneficial effects early in life to outweigh their own negative effects (Williams, 1957). These alleles would therefore be pleiotropic and their different effects antagonistic. It should then be noticed that, to contribute to aging, effects on fitness have to take place with the correct timing: advantageous effects should become apparent early in life, while disadvantageous ones should come about later on, independently of when exactly underlying mechanistic causes operate (Leroi *et al.*, 2005). Evidence in favour of this theory, which is firmly grounded in population genetics (Charlesworth, 1994), can be gained through different methods. Given that aging is certainly polygenic and many antagonistic-pleiotropic mutations are supposed to accumulate (Williams, 1957), quantitative genetic methods such as artificial selection experiments and decomposition of genetic variance across ages can be used to detect negative genetic correlations between early- and late-age fitness traits (Moorad & Promislow, 2009). These methods have produced confirming evidence in non-human animals like flies and nematodes (Rose & Charlesworth, 1980; Tatar *et al.*, 2001; Wilson *et al.*, 2006). Despite the expected polygenicity of aging, a few individual genes showing opposite effects on fitness components, such as survival and fertility, at different times (the expected pattern of antagonistic pleiotropy), have been described in animal models too (Leroi *et al.*, 2005; Flatt & Promislow, 2007). Some of these genes belong to the TOR (target of rapamycin) pathway (Blagosklonny, 2010). Similarly, the existence of alternative alleles in humans at single loci that have temporally opposite patterns of effects on survival and are compatible with antagonistic pleiotropy has been reported (Schächter *et al.*, 1994; Charlesworth, 1996; Toupance *et al.*, 1998; Dato *et al.*, 2007). In studies of a mixed population of human individuals with an antagonistic-pleiotropic mutation causing aging and individuals lacking such mutation, the mortality curves of the two types of individuals are expected to cross, and so should their associated survival curves (Hirsch, 1995), thus displaying an early life survival advantage for the mutants followed by a late-life

disadvantage (Toupance *et al.*, 1998). However, antagonistic pleiotropy studies on humans are (and cannot but be) purely observational. Therefore, they are not regarded as clear-cut and informative as those in model organisms because of the potential confounding effect of uncontrolled linked loci and/or environmental factors (Leroi *et al.*, 2005).

Cellular senescence

Cellular senescence was first defined by Leonard Hayflick, who proved the *in vitro* finite proliferative potential of normal human fibroblasts (Hayflick & Moorhead, 1961; Hayflick, 1965). We now know that senescent cells accumulate in the body of long-lived animals (Dimri *et al.*, 1995; Jeyapalan *et al.*, 2007). Patients with progeric syndromes (genetic conditions associated with accelerated aging) and related mouse models bear cells that have a reduced proliferative capacity (Fossel, 2003; Hasty *et al.*, 2003). In the last years, the biological mechanisms underlying cellular senescence have mostly been unravelled, and they largely confirmed Hayflick's seminal intuitions correlating cellular senescence with chromosomal damage (Hayflick, 1965). Cells may become senescent following the detection of DNA damage and the activation of a persistent DNA damage response (DDR) (d'Adda di Fagagna, 2008). This is a collection of cellular actions encompassing cell cycle arrest and attempts to repair damaged DNA (Jackson & Bartek, 2009). When DNA lesions cannot be efficaciously repaired, chronic DDR can lead either to cellular senescence or to programmed cell death (apoptosis).

The best-documented endogenous sources of DNA damage that can lead to cellular senescence are at least two: telomere erosion and oncogene activation (Fig. 1) (Campisi & d'Adda di Fagagna, 2007; d'Adda di Fagagna, 2008). Telomeres are the ends of linear chromosomes. In most somatic cells, telomeres shorten as cells proliferate, and eventually, when too short to fulfil their protective functions, they become recognized as DNA damage and trigger DDR activation. This causes cells to become senescent as first directly observed by Hayflick (Harley *et al.*, 1990; d'Adda di Fagagna *et al.*, 2003; Herbig *et al.*, 2004).

An apparently different known cause of cellular senescence is oncogene activation. Oncogenes drive the uncontrolled cellular proliferation of cancer cells. However, when they become activated in absence of other tumour-suppressor inactivating mutations, cells arrest proliferation and undergo cellular senescence associated with chronic DDR (Serrano *et al.*, 1997; Bartkova *et al.*, 2006; Di Micco *et al.*, 2006; Halazonetis *et al.*, 2008). Consistent with this *in vitro* findings, senescent cells are found in preneoplastic and benign lesions *in vivo* (Braig *et al.*, 2005; Chen *et al.*, 2005; Collado *et al.*, 2005; Michaloglou *et al.*, 2005; Halazonetis *et al.*, 2008) suggesting that, to fully develop, cancer has to overcome the barrier of cellular senescence, which is a potent tumour suppressor restraining the initial steps of cancerogenesis (Collado *et al.*, 2005).

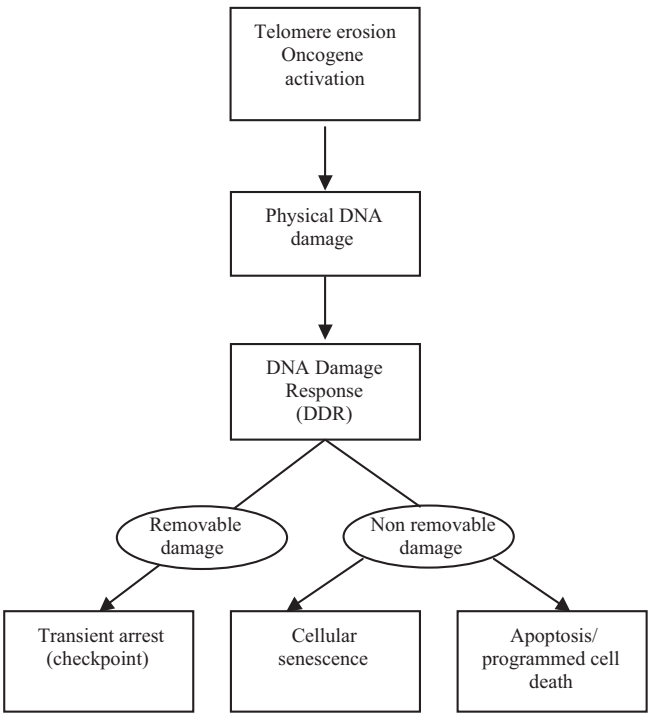

Fig. 1 Cellular senescence as a DNA damage response.

Cellular senescence and antagonistic pleiotropy: the common view

Cellular senescence is thought to contribute to keep organisms relatively free from cancer by limiting cellular division either promptly after oncogene activation or following telomere erosion (Campisi, 2001; Wright & Shay, 2001); indeed, unprotected chromosomes are recombinogenic and can fuse together triggering genome instability, which is an often-observed precondition of cancerogenesis. Some authors have conjectured that this initially beneficial tumour-preventive function may produce, later in life, a decrease of the regenerative capability of the tissues that might outweigh the initial benefit (Wright & Shay, 1995). In their view, the higher the number of cells that undergo senescent arrest, the lower the number of cells that would be available to replace lost cells in damaged tissues resulting in diminished regenerative capacity of body tissues and higher organismal frailty (Wright & Shay, 1995). This conjecture has naturally lead these authors to entertain the hypothesis that, even if cellular senescence is cancer preventive early in life, it may promote aging at a later time in an antagonistic-pleiotropic fashion via the diminished regenerative capability and robustness of body tissues (Wright & Shay, 1995; Campisi, 2003).

More recently, this hypothesis has been more firmly stated, and slightly modified, by other authors on the basis of the *in vitro* observation that senescent cells secrete active peptides (the so-called senescence-associated secretory phenotype – SASP) that can disrupt tissue integrity and, thus, contribute to aging (Krtolica *et al.*, 2001; Campisi, 2005). Notably, this *in vitro* observation appears to be compatible with *in vivo* results from a progeroid mouse model that undergoes delayed tissue dysfunction when senescent cells are experimentally removed (Baker *et al.*, 2011). In addition, factors secreted by senescent cells contribute to maintain the condition of senescence (Kuilman *et al.*, 2008; Acosta *et al.*, 2008), in spite of the fact that they may also fuel the proliferation of cancerous cells both *in vitro* and in mouse xenografts (Krtolica *et al.*, 2001; Coppé *et al.*, 2008) – although *in vivo* evidence is still lacking (Rodier & Campisi, 2011). As an example, interleukin-6 has been reported to contribute to both oncogene-induced cellular senescence entry and maintenance (Kuilman *et al.*, 2008) but also tumorigenesis (Ancrile *et al.*, 2007). Thus, some authors have considered this evidence as compatible with the theoretical possibility that *in vivo* senescent cells could elicit both tissue frailty and/or cancer susceptibility late in life, although these same cells are cancer preventive early in life, thereby representing a case of antagonistic pleiotropy (Wright & Shay, 1995; Campisi, 2003; Rodier & Campisi, 2011). With perhaps few exceptions (Kirkwood, 2002, 2005), this model was embraced by most of the scientific community (Pelicci, 2004; Partridge & Gems,

2006; Adams, 2009; Bartholomew *et al.*, 2009; Kuilman & Peeper, 2009). But is there room to question this common view?

Rethinking the common view

We would like to suggest that the common view on cellular senescence as an example of antagonistic pleiotropy in humans is worth additional consideration and pondering. In fact, the above-described thinking appears to be vulnerable to two main lines of criticism. First, additional evidence is emerging of putative beneficial effects of cellular senescence other than tumour suppression: peptides secreted by senescent cells, which can fuel cancer cell proliferation both *in vitro* and in mouse xenografts (Krtolica *et al.*, 2001; Coppé *et al.*, 2008), can also trigger the innate immune response and contribute to tumour clearance *in vivo* in mouse models (Xue *et al.*, 2007; Kang *et al.*, 2011); cellular senescence can also perform a useful task in tissue repair, where senescent cells can prevent organ degeneration (Krizhanovsky *et al.*, 2008); finally, cellular senescence may be involved in the regulation of wound healing, where fibrosis, and therefore scarring and loss of tissue functioning, is prevented through the senescent arrest of myofibroblasts (Jun & Lau, 2010). This evidence of additional positive effects of cellular senescence and its secretory phenotype makes less linear, although it does not contradict (Campisi, 2011; Rodier & Campisi, 2011) the arguments underpinning the common view. Certainly, it should be recognized that the existence of both positive and negative effects on survival is certainly a necessary condition of the antagonistic pleiotropy theory of aging. However, we would like to stress that it is not a sufficient condition. This consideration leads to the second, and most important, line of criticism to which the common view could be susceptible: no evidence has been brought that the positive effects of cellular senescence on survival prevail at young ages, while the negative effects prevail at late ages, as required by the theory (Williams, 1957). Cellular senescence, especially if established upon oncogene activation, can occur in youth as well as in later years; in addition, senescent cells can accumulate and linger for years in the organism (Michaloglou *et al.*, 2005; Jeyapalan *et al.*, 2007). Given that cellular senescence has both negative and positive effects that are mechanistically intertwined, this situation is in principle compatible with a range of different patterns, only one of which reflects the common view. For instance, the positive effects of cellular senescence could prevail early in life, and the negative ones could prevail later (as the common view presupposes), thereby contributing to aging; negative effects could prevail early in life and positive effects could prevail later, thereby combating aging (in contrast to the common view), which would then be promoted by mechanisms other than cellular senescence; or the resultant of positive and negative effects may be approximately identical at all ages with no separate prevalence of bad and good effects (again, in contrast to the common view), implying no impact on aging at all. Hence, a clearer temporal assessment of the effects of cellular senescence on survival is still needed to establish whether or not the common view is correct.

Mice models of altered DNA-damage response deserve a separate, although brief, discussion, because these models have sometimes been invoked by supporters of the common view as potential *in vivo* evidence in favour of antagonistic pleiotropy (Campisi, 2005). Such models have generated partially contradictory results with tumour-resistant phenotypes not necessarily associated with curtailed lifespan. For instance, the genetic constitutive activation of p53, a key effector of DDR and an enforcer of cellular senescence (Harris & Levine, 2005), in transgenic mice models renders them more tumour resistant than wild type, but also prone to early appearance of aging-related phenotypes and reduced lifespan (Tyner *et al.*, 2002). However, in other studies, it has been shown that when p53 expression is increased, but is kept under normal physiological control instead of constitutive activation, the long-life tumour resistance of mice is not necessarily associated with premature aging and blunted longevity (García-Cao *et al.*, 2002). Similar conclusions can be reached in mice using a different system for maintaining constitutively high p53 activity (Mendrysa *et al.*, 2006). An overall complication of these animal model studies is that, given the variety of mechanisms activated upon an enhanced p53 expression, among which, most notably, apoptosis, it remains difficult to attribute these aging effects exclusively to cellular senescence, as the supporters of the common view do recognize (Campisi, 2003).

In summary, experimental evidence is still complex. Cellular senescence does have both good and bad effects, and senescent cells do accumulate in the organism with age. However, the data we have just surveyed do not constitute clear evidence on whether such effects have the timing predicted by the antagonistic pleiotropy theory of aging. Moreover, the scope of the antagonistic pleiotropy theory is the genetics of aging, while cellular senescence is a complex cellular behaviour, thus any crosstalk between underlying multiple loci is presently theoretically unexplored.

Aside from highlighting the potential shortcomings of the common view, we would like to put forward a constructive criticism too. To this aim, it should be noticed that all data supporting the common view have been drawn from *in vitro* cellular and molecular studies and *in vivo* mice models. Instead, the temporal influence of cellular senescence on human mortality, as well as the genotypic conditions associated with cellular senescence and their evolution, has not received adequate attention, although their study would certainly prove valuable and would also align with analogous research lines on antagonistic pleiotropy in humans, as reviewed previously.

As a first step in the direction of integrating these methodologies, with all the limitations that we have mentioned before, we propose that potentially useful observations of relevance to the hypothesis that cellular senescence is an example of antagonistic pleiotropy can come from the study of codon 72 of p53, which is a crucial genetic component of the DDR and, therefore, of cellular senescence. In the human population, this codon is polymorphic and comes in two variants: proline (Pro) and arginine (Arg) (Ara *et al.*, 1990). In human cell cultures, it has been observed that, upon DNA damage, Pro preferentially elicits cellular senescence, while Arg is more prone to trigger apoptosis (Dumont *et al.*, 2003; Pim & Banks, 2004; Salvioli *et al.*, 2005; Bergamaschi *et al.*, 2006; den Reijer *et al.*, 2008). Fortunately, data on the correlation between p53 polymorphisms at codon 72 and human aging, cancer and evolution are available in the literature. If the common view were correct, then it would be expected that the 'pro-cellular senescence' activity of the Pro allele should lead to decreased mortality in early life because of reduced cancer incidence but to increased late-life mortality because of augmented organismal frailty and/or higher cancer incidence, when compared with the control 'pro-apoptotic' Arg allele. The differences in survival chances induced by either allele should be apparent by comparing the mortality or survival curves of the bearers of the different genotypes at this locus (Toupance *et al.*, 1998). Moreover, this polymorphism should be maintained by natural selection (Rose, 1982; Curtsinger *et al.*, 1994).

Admittedly, the enhanced apoptotic response associated with the Arg allele might be regarded as a potential bias in the proposed method, as apoptosis has also been proposed to be a case of antagonistic pleiotropy for reasons analogous to cellular senescence (Campisi, 2003) – 'early in life, apoptosis is an anticancer mechanism; late in life, as stem cells no longer exist in sufficient quantity to maintain cell populations, it is supposed to contribute to the failure of tissue integrity' (Leroi *et al.*, 2005). However, while cellular senescence and apoptosis are both supposed to

undermine to some extent tissue robustness in the long run, apoptosis exerts its tumour suppressive function by fully removing damaged cells from the body. Therefore, it is much less permissive than cellular senescence in allowing these cells, which are at risk of transformation and secrete cancer-promoting factors, to be long retained in the organisms. This feature, thus, makes apoptosis an ideal control for observing whether or not, as the common view presupposes, the long-term accumulation of senescent cells in the human body really tends to undermine late-life survival by increasing the likelihood of developing a tumour. If the common view were correct, then we would expect long-lived 'pro-apoptotic' individuals to be more cancer-free than long-lived 'pro-cellular senescence' individuals.

Mortality rates for the entire lifespan of the alternative genotypes Pro/Pro, Pro/Arg and Arg/Arg are currently lacking. Therefore, we are not in a position to observe whether or not the corresponding mortality or survival curves cross as would be expected under antagonistic pleiotropy (Toupance *et al.*, 1998). This is the unfortunate case for most genetic association studies trying to detect antagonistic pleiotropy (Leroi *et al.*, 2005). Nonetheless, interesting results have been obtained. A cross-sectional study failed to find a difference in the frequency of these alleles in centenarians versus young controls (Bonafè *et al.*, 1999). In addition, no significant difference in the representation of these genotypes was detected among spontaneously aborted foetuses and newborns, which can be regarded as a form of early life survival advantage (Beckman *et al.*, 1994), as envisaged by Williams himself (Williams, 1957). Most importantly, two independent big cohort studies with a more robust design and higher statistical power to detect potential differences (if any) in survival or gene frequency than the previous cross-sectional studies (Lewis & Brunner, 2004) indicate consistently that late-life mortality rates may be significantly lower for the more cellular senescence-prone individuals than for the more apoptosis-prone ones. These observations are contrary to the common view, which would predict a higher late-life mortality rate associated with cellular senescence. In the first study, 1226 subjects aged 85 or more were followed for survival in 5 or 10 years. Pro/Pro individuals had 1.41-fold increased survival with respect to Arg/Arg, meaning reduced late-life mortality (van Heemst *et al.* 2005). With an even higher statistical power, the second cohort study showed that, in the general population, Pro/Pro (the pro-senescence allele) had the best survival chances, at least from the age of 70 onwards. The study found that the Pro/Pro population had the highest median survival (82 years) and that the Pro allele is found at increasing frequency with increasing age (Ørsted *et al.*, 2007).

The issue of cancer incidence with reference to codon 72 is controversial. From the plethora of studies trying to associate codon 72 polymorphisms and cancer, contradictory data have emerged – in this regard, the case of cervical cancer is emblematic (Helland *et al.*, 1998; Hildesheim *et al.*, 1998; Storey *et al.*, 1998; Zehbe *et al.*, 1999) – to the effect that associations between these p53 variants and cancer are considered largely uncertain by the scientific community (Whibley *et al.*, 2009). In addition, reported risks are rarely sorted out by age class and, therefore, they are of scarce use to assess the validity of the common view. To this aim, age-related risks would be needed to assess the differential impact across a life of cellular senescence on cancer susceptibility, as according to the common view cellular senescence is expected to delay the age of tumour onset at the cost of increased incidence.

Finally, under some rather limited conditions, antagonistic pleiotropy can preserve genetic variability at the locus (Rose, 1982; Curtsinger *et al.*, 1994). Is this the case for the human polymorphism at codon 72 of p53 (Leroi *et al.*, 2005)? The answer could be positive. Variation in the distribution of Arg and Pro alleles among human populations is indicative that this polymorphism may indeed be balanced and maintained by selection (Beckman *et al.*, 1994; Bereir *et al.*, 2003). The previously reported association between Arg and diminished late-life survival (van Heemst *et al.* 2005; Ørsted *et al.*, 2007), together with the observation that this allele is found only in humans, is also in part consistent with antagonistic pleiotropy at the locus. Nonetheless, the common view does not seem to be supported. Arg is prevalent in humans, but novel among primates, which all possess Pro (Puente *et al.*, 2006). Therefore, the 'pro-apoptotic' Arg allele could have risen in frequency at the expenses of the 'pro-senescence' Pro allele by being selectively favoured under certain environmental circumstances, despite limiting longevity. With regard to potential antagonistic effects on fecundity, the Arg allele may be associated with a lower risk of implantation failure in *in vitro* fertilization (Kang *et al.*, 2009), but there may be no differential impact in natural fertility at all (Coulam *et al.*, 2006).

Overall, it should be considered that the data on codon 72 of p53 discussed here were not originally generated to test the presumed antagonistic pleiotropy of cellular senescence and are derived from observational studies and that aging is unquestionably a multifactorial trait likely influenced by several genes and environmental factors. Consequently, their study design may be suboptimal, whereas linked loci and environmental factors could act as confounders. However, this entails the advantage of dealing with a natural polymorphism instead of laboratory-generated mutations that hardly reflect genetic variation in the wild (Zwaan, 2003). Finally, it must be underlined that the polymorphism at codon 72 of p53 is the only one so far studied that occurs in the wild and has a demonstrated impact on cellular senescence establishment. Further evidence on genomic loci that boost (or diminish) the cellular senescence response influencing survival differentially at separate ages would be the most welcome to test more conclusively the hypothesis of antagonistic pleiotropy for the evolution of this cell behaviour.

Conclusions

Current evidence does not seem to fully support the common view according to which cellular senescence is a case of antagonistic pleiotropy in humans. This is mainly because clear evidence is lacking of alleged detriments of cellular senescence specifically at old ages and because its beneficial consequences may play a role throughout life. Our analysis indicates that evolutionary hypotheses on the origin and the adaptive value of cellular senescence may be better understood and put to the test only if, in addition to studying the mechanistic aspects of cellular senescence, a larger-scale perspective is taken on the impact of cellular senescence-influencing genotypes on human survival at different ages. Such a perspective may help us in shedding new and unexpected light on the issue. Finally, we would like to suggest that, along this route, a deeper integration of cell biology and evolutionary theory can be reached for the benefit of improving our understanding of one of the most complex phenotypes of life: aging.

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