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REVIEW

Elimination of Senescent Cells: Prospects According to the Subtelomere-Telomere Theory

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Abstract—Cell senescence is an artificially reversible condition activated by various factors and characterized by replicative senescence and typical general alteration of cell functions, including extra-cellular secretion. The number of senescent cells increases with age and contributes strongly to the manifestations of aging. For these reasons, research is under way to obtain "senolytic" compounds, defined as drugs that eliminate senescent cells and therefore reduce aging-associated decay, as already shown in some experiments on animal models. This objective is analyzed in the context of the programmed aging paradigm, as described by the mechanisms of the subtelomere-telomere theory. In this regard, positive effects of the elimination of senescent cells and limits of this method are discussed. For comparison, positive effects and limits of telomerase activation are also analyzed, as well of the combined action of the two methods and the possible association of opportune gene modifications. Ethical issues associated with the use of these methods are outlined.

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A senescent cell is not simply an aged cell but a cell in a particular and well-defined condition. In fact, cell senescence is a specific program triggered in normal cells by various factors [1], such as telomere shortening [2], other forms of DNA damage without alterations of telomere status, oxidative stress, cellular stress, suboptimal culture conditions, and activated oncogenes [3, 4].

Cell senescence is characterized by: (i) inhibition of proliferative activity (replicative senescence) [5]; (ii) typical general alteration of cell functions [6-9] ("Senescence-related chromatin remodelling leads to profound transcriptional changes" [10]); (iii) alterations in extracellular secretion (senescence-associated secretory phenotype, SASP) [11, 12]; (iv) resistance to apoptosis [9, 13].

In the past, cell senescence was conceived as a one-step phenomenon. Now, it appears that, at least for cell senescence stimulated by certain types of stress, it first goes through the phase in which it is reversible if the stress factor is reduced or eliminated [10]. After this phase, cell senescence becomes irreversible, although under artificial conditions, for example, upon simultaneous inactivation of p53 and expression of $p16^{Ink4a}$, it appears reversible [14]. This confirms that cell senescence is not a result of accumulation of harmful substances or events but a genetically regulated process that has been defined as a cellular program [1].

The number and fraction of senescent cells (identified as cells expressing $p16^{Ink4a}$) increases with age [15-17]. This increase in the content of senescent cells is clearly related to aging manifestations and age-related diseases [18-20]. Furthermore, elimination of such cells reduces and improves aging manifestations [19-21].

This has generated the motivated firm belief that selective elimination of senescent cells with appropriate (senolytic) drugs is an important and useful therapeutic goal [21-23].

Interesting results have already been obtained using (i) dasatinib + quercetin in mice with idiopathic pulmonary fibrosis [24]; (ii) senolytic compound UBX0101 in transgenic mice with post-traumatic osteoarthritis [25]; (iii) FOXO4 peptide in naturally aged and fast aging XpdTTD/TTD mice and in mice with doxorubicin-induced chemotoxicity to restore fitness, fur density, and renal function or to counteract chemotoxicity, respectively [26]; (iv) siRNAs or the small molecule ABT-737 in

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transgenic p14(ARF) mice with epidermis and lung damage [27].

These results promote continuous search for senolytic drugs and studies of their possible beneficial effects for humans. However, it also seems useful and necessary to analyze the prospects of elimination of senescent cells (in particular, greatest possible results and synergy with other anti-aging treatments) in the framework of a general theory of aging. Here, we focus on this problem in the context of the programmed aging paradigm and, specifically, the subtelomere-telomere theory (see below).

NON-PROGRAMMED AND PROGRAMMED AGING PARADIGMS

Here, aging is defined as "increasing mortality [= decreasing fitness] with increasing chronological age in populations in the wild" [28], which is a synonym for "actuarial senescence" [29] if explicitly restricted to animals in the wild.

Although in an earlier work, aging has been documented for 175 different animal species [30], it should not be considered a universal phenomenon. In fact, in the living world, there is a large variety of age patterns of mortality [31] (e.g., species with no age-related decline in fitness, species that die immediately after reproduction, species that live only few days in the adult stage, and many other types of life tables [31, 32]). Only a few species show aging, but they are among the most known for us, which creates the widespread wrong belief that aging is universal, with only few exceptions.

Many theories have tried to explain the causes of aging [33-36]. All of them use two opposite and mutually incompatible interpretations of aging defined as the "non-programmed aging paradigm" and the "programmed aging paradigm" [36]:

a) In the first paradigm, aging is non-adaptive and represents a cumulative result of action of various damaging factors that natural selection cannot counteract completely. Both in the older theories and in some more recent ones, it is implicitly admitted that natural selection does not have sufficient strength to counteract the damaging factors [36]. In the most recent theories, which try to take into account the mechanisms of natural selection, this is considered to be weakened or limited by (i) the small number of individuals who survive at older ages (mutation accumulation theory [37-40]); (ii) constraints determined by genes with pleiotropic effects (antagonistic pleiotropy theory [41, 42]); (iii) constraints determined by other conflicting physiological needs (disposable soma theory [43, 44]).

b) In the second paradigm, aging is adaptive and represents a physiological phenomenon that, being harmful to an individual, is in some way advantageous in terms of supra-individual natural selection [36]. It is a form of phenoptosis [45, 46], or "programmed death of an individual" [47], a large category of phenomena [32] favored in terms of supra-individual natural selection [46], and it is necessarily the result of specific genetically determined and regulated mechanisms [48, 49].

In short, the main differences between the two paradigms are:

a) In the non-programmed aging paradigm, aging is a failure of natural selection; specific programmed mechanisms that determine aging cannot exist and do not exist.

b) In the programmed aging paradigm, aging is a conquest of natural selection, and specific programmed mechanisms that determine aging must exist and do exist.

Although the first paradigm represents the most widespread opinion [50-55], there exist strong arguments and evidence against it and in support of the second paradigm [36, 56, 57], which will be considered as the working hypothesis in this paper.

Moreover, because efficient discussion needs well-defined and empirically documented mechanisms, here we present the mechanisms that appear to determine aging in accordance with the theoretical assumptions of programmed aging and limit our discussion to the points that are essential for the aims of this work. These mechanisms can be summarized, for brevity, by the term the "telomere theory" [58] or, perhaps, more precisely as we will see, by the term "subtelomere-telomere theory".

Other descriptions of aging mechanisms, which are declared by the authors as consistent with the programmed aging paradigm, have been proposed (e.g., [59-63]). However, in this paper, only the subtelomere-telomere theory will be considered, because the empirical evidence appears to strongly support it.

THE SUBTELOMERE-TELOMERE THEORY

The mechanisms that we here define as the subtelomere-telomere theory have been discussed in greater detail in other works [48, 58, 64, 65]. Only a brief description of these mechanisms will be given in this work together with the concepts essential for our discussion.

Each end of the eukaryotic DNA molecule, the "telomere", is a specific nucleotide sequence repeated many times and very conserved in evolution (TTAGGG for many species, humans included [66, 67]). It was observed that DNA polymerase, the DNA duplicating enzyme, cannot duplicate an entire molecule and so each telomere shortens at each replication [68, 69]. Therefore, the existence of an enzyme that could compensate for this insufficiency had been predicted [70] and 12 years later, such enzyme was found [71]. The enzyme (telomerase) (i) is under genetic regulation [72]; (ii) if active without restrictions, as in germ line cells, allows an unlimited number of replications [58]; (iii) if entirely repressed, allows only a limited number of replications [58]; iv) if

active but with some restrictions, allows a greater but not unlimited number of duplications [58]. Partial or total repression of telomerase is the likely cause of the Hayflick limit [73, 74].

The telomere is covered by protective nucleoproteins [75], the heterochromatin hood [58]. It is necessary to assume that: (a) at each cells duplication, the hood must be duplicated without changes in its length [58, 65]; (b) in the moment defined as the "reset" phase, in the first cell of an organism and before any duplication, the hood is formed according to the initial length of the telomere [65].

At each duplication, if telomerase is inactive, telomeres shorten and the hood, which is assumed to be of a fixed length, slides over the telomere and represses the subtelomeric region of DNA molecule [58] (i.e., the subtelomere).

The subtelomere has a repetitive structure ("unusual structure: patchworks of blocks that are duplicated" [76]; "a common feature associated with subtelomeric regions in different eukaryotes is the presence of long arrays of tandemly repeated satellite sequences" [77]). This might have generated the wrong impression that the subtelomere is a DNA fragment of low significance, while on the contrary, its function is likely very important and should be examined in depth.

Subtelomeric repetitive sequences presumably regulate a whole set of cellular functions as well as stability of the telomere-heterochromatin hood complex. In fact, as the telomere shortens and a growing portion of the subtelomere is covered and repressed by the hood, two effects occur:

1) Cell functions are progressively altered. Cell-specific extracellular secretion is altered too, and this compromises the extracellular fluids and other cells that depend on the altered cells [58]. This phenomenon, known as the "telomere position effect" [78], has been defined, according to its effects, as "gradual cell senescence" [79].

2) The telomere-heterochromatin hood complex oscillates between the two states: (i) in the first state, the telomere is capped and stable; (ii) in the second state, the telomere is uncapped and susceptible to the blocking of its replication capacity [75], namely to the activation of the cell senescence program [1]. An increasing portion of the subtelomere repressed by the hood sliding over it increases the fraction of time during which the telomere is uncapped (i.e., vulnerable to the cell duplication blockage) [75]. Cell senescence determines both the blockage of duplication capacity and a condition in which gradual cell senescence is maximal [58]. Figure 1 illustrates these concepts.

The restriction of cell duplication determined by the gradual shortening of telomeres and the consequent activation of the cell senescence program progressively compromises the turnover capacity of various cell types. In

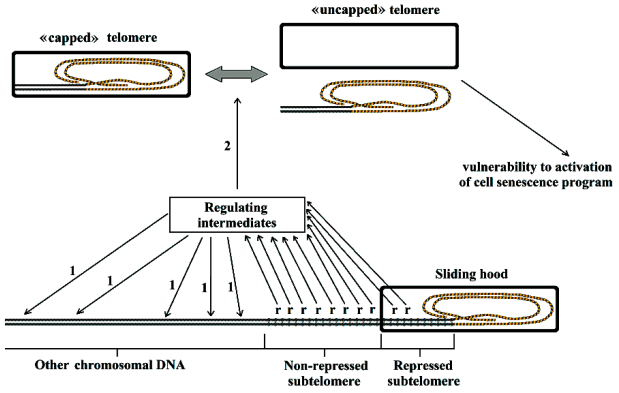

Fig. 1. Telomere shortening causes the sliding of the hood on the subtelomere and this determines a progressive repression of the subtelomeric repeated sequences ("r"), which appear to modulate (likely through regulating intermediate molecules): 1, DNA-regulated cell functions (gradual cell senescence); 2, oscillation between "capped" and "uncapped" telomere states, in particular duration of the "uncapped" phase when there is vulnerability to the activation of cell senescence program (modified from [65]).

vertebrates, cells under normal conditions (i.e., in the absence of accidental events) are physiologically subjected to various types of programmed cell death (PCD), such as: (i) keratinization and detachment of the epidermis and hair cells; (ii) detachment of cells from the mucous membranes of the intestine; (iii), expulsion of the nucleus from erythrocytes and their phagocytosis by macrophages; (iv) elimination of osteocytes by osteoclasts; (v) apoptosis. The last type of PCD had been unknown until its relatively late discovery [80]. In vertebrates, the main function of apoptosis is related to cell turnover in healthy organs [81-83], as documented for many tissues and organs [79].

Elimination of cells by the various types of PCD is compensated by the duplication of specific stem cells that occurs at different rate depending on the cell type. While for the intestinal mucosa "cells are replaced every three to six days" [84], "the heart is replaced roughly every 4.5 years" [85], "bone has a turnover time of about ten years in humans" [84], and other types of cells at replaced at the intermediate rates [86]. It has been estimated that each day, 50 to 70 billion cells are eliminated in an average adult by different types of PCD (a mean value of 690,000 cells per second), and each year "a mass of cells, equal to our entire body weight" is lost and substituted [87].

The limits in cell duplication, clearly genetically determined and modulated, imply a limit on the cell turnover. This, combined with the effects of cell metabolic alterations caused by gradual cell senescence, determines a progressive decline in tissue and organ efficiency [48, 58] (i.e., general fitness decline, or aging).

These mechanisms imply the existence of stem cells allowing cell turnover that also show the age-related

decline. These stem cells, the relationship between their decline and aging, and also the possible use of stem cells to treat aging have been discussed in numerous works [88-90].

For tissues with long-living cells (i.e., without turnover, as many types of nervous cells), their decline can be well explained if these cells depend on satellite cells with the turnover [91]. The decline of non-perennial cells ("direct" aging) causes alterations and death of the long-living cells ("indirect" aging) [92].

It should be pointed out that the definitions "direct" and "indirect" aging are useful to distinguish between the two different ways by which the decline in cell turnover compromises the functionality of tissues and organs. However, in the case of "indirect" aging, the difference between long-living and satellite cells does not imply lesser importance of the satellite cells – even disregarding other possible functions, satellite cells are essential for the tissue viability and functionality.

In short, alterations resulting from the progressive subtelomere repression, determined by telomere shortening and consequent sliding of the heterochromatin hood, cause the following effects that have been described, with some differences, as the "atrophic syndrome" [48, 64]:

a) increase in the fraction of cells with in varying degree of gradual cell senescence;

b) increase in the fraction of senescent cells (i.e., (i) in replicative senescence; (ii) with the greatest alteration of cell functions; (iii) resistant to apoptosis);

c) as a consequence of <a> and <b>:

– alterations of the extracellular fluid;

– metabolic alterations or death of near or distant cells;

d) as a consequence of <b>:

– reduced mean cell duplication capacity;

– slower cell turnover;

e) as a consequence of <d>:

– cell atrophy;

– replacement of missing specific cells with non-specific cells;

– hypertrophy of the remaining specific cells;

f) as a consequence of <e>:

– metabolic alterations or death of cells that depend on the missing cells (indirect aging);

g) as a consequence of <d>, <e>, and <f>:

– irreversible anatomical and functional alterations (e.g., reduced capacity for renewal and recovery of a tissue/organ, deformations of bone and bone-cartilaginous structures, degradation of nerve and cerebral structures, and dilatations of cavities such as in heart failure and emphysema);

h) risk of cancer development because of DNA instability induced by the telomere dysfunction [93].

In every organ or cell apparatus, aging-associated alterations lead to disorders that are generally identified as specific diseases, leaving in the background the fact that they are only different manifestations of a single process. Moreover, the physiological rate of aging can be accelerated by various factors that increase the necessity for cell duplication (e.g., unhealthy lifestyle, harmful substances, particular genetic alterations) and slowed down by factors that reduce or avoid these harmful effects (e.g., healthy lifestyles, "protective drugs") [94]. These concepts are illustrated in Fig. 2.

THE MECHANISMS OF THE SUBTELOMERE-TELOMERE THEORY AND RISK OF CANCER

The existence of mechanisms that compromise organism's fitness is predicted by the programmed aging paradigm, or rather, this is indispensable for the validity of the paradigm. On the contrary, the non-programmed aging paradigm does not predict the existence of such mechanisms: if there is no evolutionary explanation of their existence, this would definitively and completely invalidate the paradigm.

In an attempt to save the non-programmed aging paradigm, the only justification that can be proposed for these mechanisms is that they constitute a general defense against proliferation of cancerous cells [95-97]. Moreover, in the acknowledgment of the damage caused by these mechanisms, they have been considered as an example of antagonistic pleiotropy (i.e., an evolutionary awful trade-off between the advantageous contrast of malignant proliferation and deadly damage [98]).

There are serious objections against this hypothesis [49, 57, 91, 99]:

a) There are animal species without decline in age-related fitness and with noteworthy longevity ("animals with negligible senescence" [32]). Old rainbow trout and lobsters, which are animals of this type, studied in the wild show the same telomerase activity as young individuals [100, 101]; the same is true for rockfish species, other ageless animals [102]. For these species, an age-related increased risk of cancer is unlikely, because there is no age-related increasing mortality [56], and this is against the hypothesis that telomerase action has an intrinsic oncogenic effect.

b) Telomere dysfunction has been proposed as an important cause of cancer at older age, in particular in epithelial cells that have a high turnover rate [93, 103]. Patients with dyskeratosis congenita, a disease caused by genetic defects, suffer from a high rate of cancer [104], and this may be explained by the telomerase deficiency and, as a consequence, unstable chromosomes [105-107].

c) Telomerase activation is common in cancer. However, it appears to follow and not to precede the cancer onset; therefore, it should be considered a feature of cancerous cells that aggravates the disease and not a cause of cancer [58]. "The role of the telomere in chromosomal stability (Blagosklonny, 2001; Campisi et al., 2001;

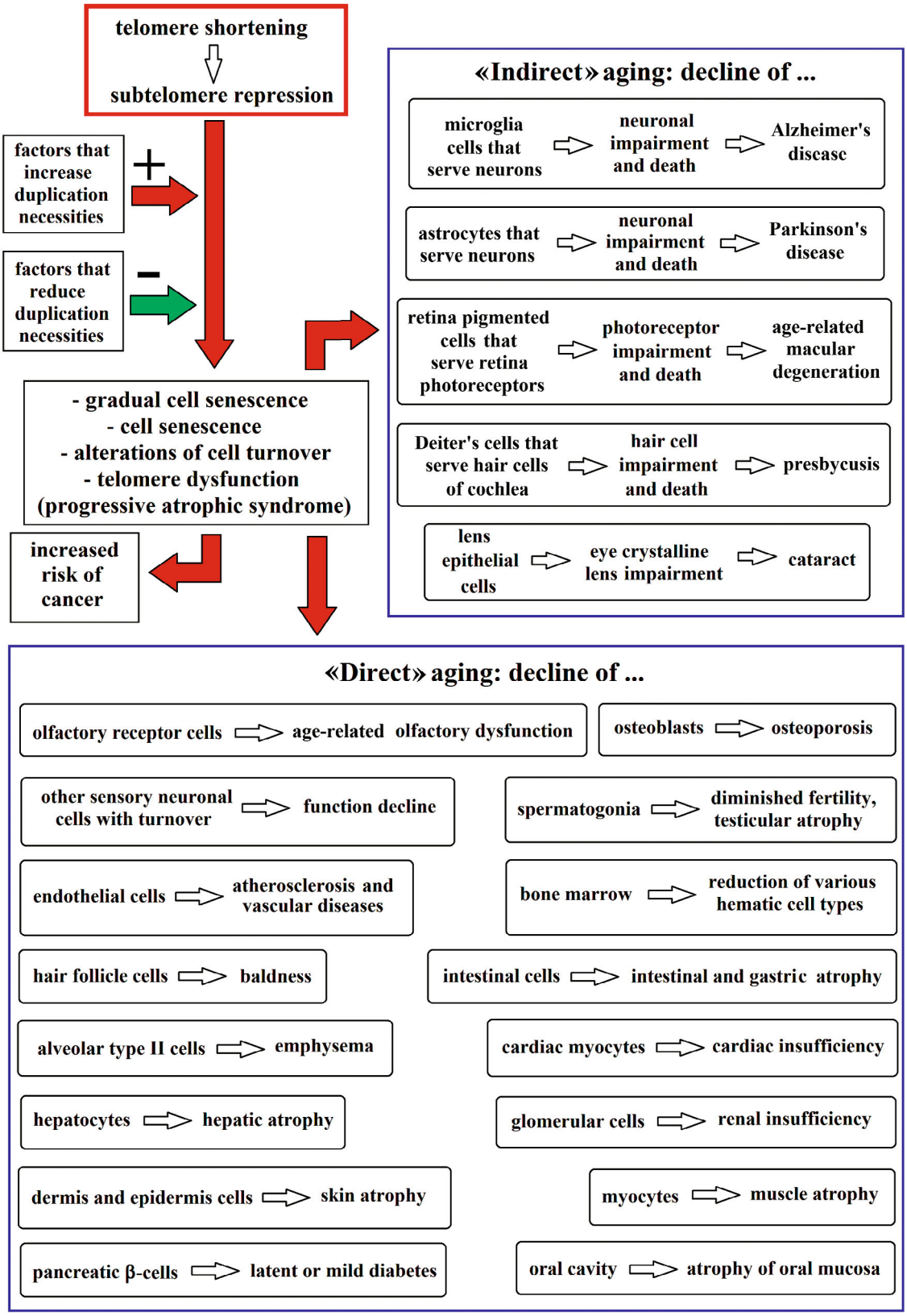

Fig. 2. General scheme of aging. It should be noticed that in this diagram, the risk of cancer and cancer are the outcomes of telomere shortening and not an evolutionary justification for telomere shortening and aging. For the meaning of "direct" and "indirect" aging, see the text.

Hackett et al., 2001) argues that telomerase protects against carcinogenesis (Chang et al., 2001; Gisselsson et al., 2001), especially early in carcinogenesis when genetic stability is critical (Elmore and Holt, 2000; Kim and Hruszkewycz, 2001; Rudolph et al., 2001)... The role of telomerase depends on the stage of malignancy as well as cofactors (Ohmura et al., 2000); expression is late and permissive, not causal (Seger et al., 2002)" [58].

d) Numerous studies in humans have shown a relation between the cancer risk and short telomeres [108, 109]. Moreover, in normal mice, telomerase expression, induced by viruses with the telomerase reverse transcriptase, delays aging and increases longevity without increasing the cancer risk [110].

e) Cell senescence and the decline of cell turnover compromise the efficiency of the immune system [58], which is related to cancer resistance [111].

f) A study on the Ache, a small indigenous population of hunters and gatherers, reported no cases of cancer [112], and only in individuals older than 70 years, when the survival was about 20% [112], sporadic deaths by cancer might be hypothesized. In the same population, the age-related increase in mortality started at the age of about 30 years, which is the same as in non-primitive populations [41]. It appears illogical to propose that the mechanisms causing the age-related increase in mortality and killing about 80% of the population before age 70 could be justified as a defense against a disease sporadically affecting older individuals [99].

g) Alterations in cell metabolism and extracellular secretion in gradual cell senescence have no reasonable anti-cancer meaning. Likewise, the subtelomeric location of essential regulatory DNA regions at the site with the greatest vulnerability to the sliding of the heterochromatin hood upon telomere shortening (a fact that is necessary to explain gradual cell senescence) has no meaning as an anti-cancer defense [65]. However, both phenomena are rational as components of aging mechanisms.

h) Alterations in cell metabolism and extracellular secretion in cell senescence have no plausible anti-cancer meaning. As a rational defense against malignant proliferation, cell senescence should be replicative senescence only. "If cellular senescence is designed to cut off cancerous cell lines, why would senescent cells remain alive and toxic? They could, instead, be programmed to be good citizens and dismantle themselves via apoptosis to facilitate recycling of proteins and nutrients. The fact that senescent cells emit poisons is completely consonant with the theory that cellular senescence is a form of programmed organismal death" [57]. Moreover, "human cells induced to senesce by genotoxic stress secrete myriad factors associated with inflammation and malignancy" [11] and experiments in mice have shown that selective elimination of senescent cells, apart from increasing lifespan and causing fewer age-dependent changes, also delayed cancer progression [17].

i) Yeast, a unicellular organism, in the mother lineage shows gradual cell senescence and propensity for replicative senescence in proportion to the number of previous divisions [113-115]. These phenomena are caused by the accumulation of particular molecules (extrachromosomal ribosomal DNA circles, ERCs) over the subtelomere and not by telomere shortening (telomerase is always active, and telomeres have a fixed length). Moreover, replicative senescence is associated with the cell death by apoptosis and not with increased resistance to apoptosis [113-115]. Notwithstanding these differences, a phylogenetic relationship has been proposed between these phenomena, which cannot have an anti-cancer value in unicellular organisms, and analogous phenomena in multicellular organisms [79].

In short, the hypothesis that limitations of cell duplication and other related phenomena, such as cell senescence, represent a general defense against cancer is untenable. A quite harsh remark appears justified: "The hypothesis that telomerase is restricted to achieve a net increase in lifespan via cancer prevention is certainly false. Were it not for the unthinkability of the alternative – programmed death – the theory would be dead in the water" [57].

In particular, as far as cell senescence is concerned, the well-rooted idea that cell senescence is a defense against cancer is not easily repudiated, even when in the same works that reaffirm this idea, the arguments or the results disavow it:

(i) As stated in [116], cell senescence is a "potent cancer-protective response to oncogenic events", but in the same work, a model is proposed, in which cell senescence is connected with "an inflammatory phenotype and cancer" [116].

(ii) In another work, while the authors declare that "cellular senescence suppresses cancer by irreversibly arresting cell proliferation" [117], it is observed that, in cancer therapies, "several chemotherapeutic drugs induce [cell] senescence" [117] and that "eliminating TIS [therapy-induced senescent] cells reduced several short- and long-term effects of the drugs, including ... cancer recurrence..." [117].

(iii) An interesting fact is that "senescent cells are present in premalignant lesions and sites of tissue damage and accumulate in tissues with age" [118]. Here, to defend the old idea, it would be necessary to explain the compatibility of the hypothesis that senescent cells are a defense against cancer with the fact that, before the development of cancer, aging cells accumulate in tissues and premalignant lesions, causing inflammation and increasing the risk of cancer.

(iv) A very authoritative researcher declared that "the senescence response is widely recognized as a potent tumor suppressive mechanism. However, recent evidence strengthens the idea that it also drives both degenerative and hyperplastic pathologies (i.e., cancer), most likely by

promoting chronic inflammation. Thus, the senescence response may be the result of antagonistically pleiotropic gene action" [119]. In this case, genes determining the sophisticated characteristics of cell senescence are hypothesized as the result of a delicate balance between opposing needs (the need to counteract cancerous proliferation and other needs, not better specified, that lead to promoting cancerous proliferation). Apart from the lack of precise specifications, the existence of such a balance that cannot be resolved in any way by evolution seems unlikely. Moreover, the invocation of the popular antagonistic pleiotropy has the taste of *deus ex machina* that had been used in ancient theatrical presentations to solve situations without any logical solution.

POTENTIAL EFFECTS OF DIFFERENT ANTI-AGING TREATMENTS

In accordance with the subtelomere-telomere theory (and without the prejudice that cell senescence and restrictions in the telomerase activity are general mechanisms of defense against cancer), it is now possible to envisage, from a theoretical point of view, the effects of some types of treatments of aging manifestations:

A) Repeated elimination of senescent cells by means of senolytic drugs. This method can certainly improve many manifestations of aging, generally conceived as particular diseases (see Fig. 2), and the general conditions of the aged organism, with the result of partial rejuvenation. However, there are theoretical limits to the positive effects of senolytic drugs. Apart from the need for repeated interventions over time to eliminate senescent cells formed in the time after each senolytic intervention, senolytic drugs cannot affect other age-related events, such as: (i) increasing number of cells in gradual cell senescence; (ii) increasing failure of cell turnover; (iii) increasing irreversible anatomical and physiological alterations.

Despite these restraints, elimination of senescent cells, repeated over time, would have the effect of greatly improving diseases associated with aging process and, in general, manifestations of aging, as already demonstrated in animal for some manifestations of aging [26] or for artificially created conditions similar to aging [25-27]. However, considering the absence of actions on telomerase and telomeres, these interventions could not prevent the general decline of an organism when the limits imposed by the restrictions on cell duplication capacity are reached. In other words, the health conditions and psychophysical efficiency of the elderly would be greatly improved, but without extension of the maximum longevity.

B) Repeated activation of telomerase. Telomerase stimulation (e.g., reactivation of telomerase in mice [120] or activation of telomerase by reverse transcriptase introduced with an adeno-associated virus [110] or by opportune drugs [121, 122]) reverts the effects of gradual cell senescence and counteracts further decline of the cell turnover.

Apart from the need for repeated interventions in order to prevent the shortening of telomeres during the periods after interventions, telomerase reactivation would not be able to eliminate (i) senescent cells (with possible exception of those at the reversible stage [10]) and (ii) already existing irreversible anatomical and functional alterations.

With this second type of intervention, the maximum longevity should increase significantly, and health conditions and ageing-associated diseases should also show improvement [123], as already proven in experiments on mice [110]. However, progressive accumulation of senescent cells would hold back these improvements.

C) A and B simultaneously and repeatedly, starting from an old age. A rational and more complete approach would be combined and repeated application of the methods A and B. The theoretical limit of this approach is that it could not eliminate irreversible anatomical and functional alterations that must be assumed to be relevant if the first use of the aforementioned methods happens at the old age.

By the combined action of A and B, the limits highlighted for their individual action would be overcome without, however, eliminating the irreversible damage that has already occurred at the age of the first application of the aforementioned methods.

D) A and B simultaneously and repeatedly, starting from a relatively young age. Otherwise, if the methods A and B are applied, in a combined and repeated way, starting from a young age, it is presumable that the irreversible functional and anatomical alterations, on which the aforementioned methods cannot act, are of minor or negligible relevance. In short, the combined action of A and B starting from a relatively young age would overcome the limitations mentioned for C.

E) Subtelomere and telomere elongation, plus A and B repeatedly. A different and more elaborate approach is the combination of method B with the subtelomere and telomere elongation (by adding neutral sequences in the parts adjacent to the subtelomere-telomere junction) [65]. This approach, with the addition of method A, would allow the best results even not started from a relatively young age and with the repetition of methods A and B after not so short intervals. These considerations are summarized in table.

Another possible approach to the treatment of aging alterations starts from the hypothesis that aging derives from alterations of the epigenetic regulation [124], a concept that is fully compatible with the subtelomere-telomere theory. It has been proposed to counteract degenerative diseases, and also aging, by correcting the epigenetic regulation and in particular by reprogramming the cells to a condition similar to that of pluripotent stem cells

Theoretical effects of various types of treatment on aging manifestations

Age-related increasing alterations	Treatments				
	A	B	C	D	E
Number of cells undergoing gradual senescence	–	Y	Y	Y	Y
Number of senescent cells	Y	–	Y	Y	Y
Failure of cell turnover	–	Y	Y	Y	Y
Irreversible anatomical and functional alterations	–	–	–	Y/–	Y

Note: A, B, C, D, E, as described in the text; Y, positive effect; –, null or not relevant effect.

[125, 126], for example by temporary or partial activation of the so-called Yamanaka factors [127, 128].

An attempt to reprogram adult cells in mice with the Hutchinson–Gilford Progeria Syndrome by transient induction of some Yamanaka factors appeared to "ameliorate aging-like phenotypes". The induction of the same factors in middle-aged wild-type mice exposed to specific toxins appeared "to promote youthful regenerative capability". However, these effects were transient and perhaps due to "induction of key homeostatic regulators" and "increased proliferation and enhanced function of adult stem cells" [129].

ETHICAL PROBLEMS

Development of any new therapy always encounters ethical problems related to experimentation. Here, however, we mention the only particular ethical problems that may arise in the development of treatments for aging "troubles".

The use of the word "trouble" instead of disease or illness is not accidental.

– According to the non-programmed aging paradigm, aging is the manifestation of a set of diseases caused by accumulation of various damages over time. For this paradigm, the term "disease" for the manifestations of aging is quite correct. Counteracting aging diseases is a part of the duties of medicine and does not raise any particular ethical problem.

– On the contrary, according to the programmed aging paradigm, aging is a physiological process, and, even if its manifestations cause suffering and death, these manifestations are not definable as "diseases", as well as the disturbances that occur during normal pregnancy and normal childbirth cannot be defined as diseases. According to this second paradigm, counteracting the manifestations of aging is not a cure of a disease but modification of a physiological process.

By considering that manifestations of aging certainly compromise the state of health and are widely perceived as diseases, this distinction may seem irrelevant and perhaps even silly.

But what can be said when, with appropriate treatments, in addition to counteracting the manifestations of aging, a significant increase in the duration of life could be obtained? Moreover, if the aforementioned treatments begin to have as specific aims the non-aging state or the possibility of unlimited longevity, the distinction no longer appears irrelevant.

In short, is it ethically acceptable to pursue the goal of unlimited longevity, even if this aim plausibly involves actions on gene activity or even permanent genetic changes?

An easy answer could be that this is acceptable without reserve. The opposite answer could be that there are strict limits to be observed when human nature is modified in one of its essential characteristics (i.e., its natural cycle of life).

It is obvious that this problem goes beyond the limits of science and enters the fields of religion, philosophy and, in general, ethics.

Here, it is possible to add only some comments. The use of possible efficacious and manageable senolytic drugs in the future would counteract the manifestations of aging without changing the maximum longevity limits. Such use would be considered a form of therapy, even if, strictly speaking, it is something different.

When telomerase activation methods, with or without the senolytic methods, will show the ability to significantly increase longevity, this would bring out the aforementioned ethical problem.

This is true even more in the case of genetic modifications capable of increasing longevity, for which ethical problem would be highlighted to the highest degree.

Geriatrics, if we want to say things without euphemism, is currently the most unsuccessful of medical activities. It has failed to cure aging and, indeed, the incurability of aging is emphasized by the same discipline to justify this failure.

Geriatrics, at best, allows us to mitigate the suffering of the elderly and to compensate for the deficits caused by this suffering. In other cases, it can only apply palliative treatments and psychological support.

Of course, with appropriate preventive measures, various manifestations of aging can be avoided or delayed, but this brings aging back in its physiological tracks and is not a real treatment of aging.

This harsh reality, that occupied the mind of humankind from antiquity, without solutions or only with illusory remedies such as drinking the water from the fountain with the power of granting eternal youth, as narrated in the History of Herodotus (III, 23), could perhaps radically change in a fairly short time.

Senolytic drugs have already offered good results in animal models and there are no theoretical preclusions for the development of effective drugs of this type for use on humans. Senolytic drugs could drastically improve a large part of manifestations of aging.

This would open a way for telomerase activation interventions that, combined with the senolytic drugs, would allow greater longevity without the aging-associated physical decline.

Conflicts of Interest

Authors declare no conflicts of interest.

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