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Review
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TELOMERES CONTROL CELL AGING

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Summary

It has been well established that various mammalian cells demonstrate a limited growth capacity in culture referred to as cellular or replicative senescence. There is also some evidence in support of the idea that this is the basis for organismal aging. Recent studies have revealed the molecular mechanisms of telomere involvement in cell senescence, cell cycle control, genome (in)stability and immortalization. This has provided an explanation of various phenomena related to process of aging and carcinogenesis. In this review we aim to describe some of the most important features of normal cell aging and the that result in malignant transformation.

Keywords: telomeres; cell senescence; aging; telomere shortening; human fibroblasts

INTRODUCTION

Most normal mammalian cell lines demonstrate a common growth pattern during proliferation in vitro. Young cultures initially have a period of rapid growth during which the majority of the cells divide vigorously. As the culture accumulates more divisions the growth potential declines due to the gradual increase in the fraction of nondividing, senescent cells. Eventually, the entire culture enters the terminally nondividing state referred to as replicative senescence [1,2]. Upon growth cessation, senescent cells do not die but remain viable in culture, undergoing various morphological and biochemical changes, e. g. cell size enlargement, lack of ^{3}H-thymidine incorporation, endogenous senescence associated (SA)-β-gal staining at pH6 [2-4] as well as altered gene expression [5]. However, the introduction of various viral proteins that impair p53

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and pRB functions results in an extended lifespan followed by entry into crisis and occasionally the emergence of immortal clones. It has been proposed that these and other phenomena have their origins in processes related to the maintenance of telomeres, which are repetitive sequences at the end of all eukaryotic chromosomes. Such processes include telomere shortening, telomere extension or conformational changes of telomere DNA through changes in interactions of telomere associated proteins. Here we point out some important features of cell senescence, cell immortalization and factors that are involved in these events:

GRADUAL ACCUMULATION OF SENESCENT CELLS IN CULTURE

Classic analyses of ^{3}H-thymidine incorporation into cells have revealed that the limited population doubling (PD) potential of the culture is due to the gradually increasing fraction of nondividing cells, rather than all the cells that comprise a culture simultaneously entering senescence at the end of the in vitro lifespan [6]. Because of this accumulation of nondividers, dividing cells have to compensate for them to accomplish additional PD. This result in the divisions of individual cycling cells, known as cell generations (CG) [7, 8], constantly increases over PD. Maximum CG represents the maximum dividing potential of the culture. Previousely, we have calculated that at the end of the average human fibroblast culture growth, CGs are at least twice the value of PD [7].

SENESCENT CELLS APPEAR IN THE CULTURE IN SUDDEN AND STOCHASTIC FASHION

Fact that individual senescent cells appear in the culture unexpectedly, in a sudden and stochastic fashion due to Sudden Senescence Syndrome (SSS) [7, 9, 10] has been unjustly overlooked. In twin sisters experiment, Smith and Whitney demonstrated that the degree of difference in doubling potentials between two cells arising from a single mitotic event can vary anywhere from between 0 and >8 PDs and even more CGs [9]. Thus, SSS cause cells to senesce within one cell division, long before the terminal phase of the growth of the culture as a whole [9-11]. Continuous presence of SSS in the proliferating culture has been demonstrated through consecutive subcloning experiments. These experiments have shown that individual subclones of the same culture constantly generate heterogeneity in proliferative potentials resulting in their distinct bimodal distribution. This heterogeneity is an intrinsic property of the culture so that a single clone produces a new bimodal distribution when it is subcloned again [9].

The frequency of SSS increases almost exponentially as the culture approaches its maximum number of divisions [9,10].

EXTENDED LIFESPAN, CRISIS AND IMMORTALIZATION.

Another challenge for molecullar models based on telomere biology is to explain the phenomenon of extended lifespan and crisis observed in various human cells upon expression of SV40 large T antigen (Tg) [12-14] which has been in the focus of aging research for many years because it demonstrates that the biological clock can be reset, a strong argument for the genetic mechanism(s) of cell senescence. It is found that SV40 Tg cause this effect through binding and inactivation of both p53 and pRB [15-18]. In contrast, combinations of both type 5 adenovirus proteins E1A (binds pRB) + E1B (binds p53) and types 16 or 18 of human papilloma virus proteins E6 (binds p53) + E7 (binds pRB) are required to perform such alterations [12]. Although these data strongly suggest the involvement of p53 and pRB in normal cell senescence and extended lifespan, one can argue that the viral proteins mentioned above may also interact with some other proteins in the cell. This argument was eliminated when specific antisense-p53/pRB oligomers were used to specifically suppress these proteins resulting in the same effect as viral agents [19]. At the end of the lifespan extension cells enter crisis, a period when massive cell death occurs due to severe chromosome instability, e. g. end to end fusions, generation of dicentric, ring or other unstable forms [20]. From this highly unstable state a colonies of immortalized cells appear at frequency of ~$3x10^{-7}$ as in human diploid fibroblast cultures [21]. To escape from crisis and stabilize immortal state all cell lines must constantly maintain telomeres above minimum length through expression of telomerase or alternative lengthening of telomeres (ALT).

CAN TELOMERE SHORTENING AND/OR EXTENSION EXPLAIN ALL THESE PHENOMENA?

First idea that incomplete chromosome end replication can cause replicative senescence come from Olovnikov in 1973 [22]. Progresive telomere shortening with advancing cell senescence has been confirmed by C. Greider in 1990 [23]. Today, most authors agree that, as a consequence of telomere shortening, a single chromosome denuded of telomeric repeats produces a DNA-damage signal which than induces p53/p21/pRB dependent cell-cycle arrest [10, 24, 25].

However, our previous results indicate that this single telomere damage, which occurs only through gradual telomere shortening, cannot explain the rapid development of intraclonal variation or the large differences in doubling potentials between the products of single mitotic events driven by SSS [9,10].

Unlike other models that are based exclusively on gradual telomere shortening predicted by the Theory of Marginotomy [22], we proposed a recombination model and performed computer simulation based on a combination of both, gradual telomere shortening (GTS) and abrupt telomere shortening (ATS) in order to explain the observed stochastic phenomena [10]. GTS occurs as a consequence of the inability of DNA polymerase to replicate the very end of chromosomal DNA [20, 26] as well as exonuclease degradation of the 5'-strand at telomere ends [27] and is responsible for the gradual decline in proliferative potential of a culture. ATS is predicted to occur through telomere 3'-self invasion at the telomere/subtelomere border region and subsequent recombination resulting in a deletion of distal repeats through circularization. This abrupt telomere shortening is than detected as DNA damage resulting in sudden and stochastic cell cycle arrest or SSS. It should be noted that in this model abrupt shortening could equally occur at any telomere. Our model matches well with experimental data [9, 10]. More importantly it has been shown by electron microscopy that during in vivo remodeling of linear telomeric DNA into large duplex telomere loops (t-loop) and self-invasion of the 3'-telomeric overhang at the telomere-subtelomere border region, predicted by the model, occurs in human cells [28, 29], as well as in ciliated protozoa [30]. Our model is additionally strengthened by the findings that double-strand break proteins are required to cap the ends of mammalian chromosomes [31], and that there is cell-cycle dependent direct association of the RAD50/MRE11/NBS1 complex with TRF2 and human telomeres [32]. Thus, several recombination proteins are present at the 3'-invasion site which may initiate a Holliday structure and recombination event. Each recombination of the crossover type (splice) would results in deletion of distal fragments through circularization causing ATS.

GTS and ATS are mutual events so that GTS controls the frequency at which ATS occurs. This is based on the observation that human and mouse cells with long telomeres (14-150 kb) display stable and more canonical chromatin structure with extensive arrays of tightly packed nucleosomes, in contrast to unusual less stable chromatin structure, characterized by diffuse micrococcal nuclease patterns found in various human cells with relatively short telomeres (2-7 kb) [33]. According to our model, long telomeres are tightly packed and stable so that they are resistant to various enzymatic activities such as nuclease degradation, telomerase or recombination proteins and therefore less likely to be involved in

recombination events. But as telomeres shorten through GTS, these structures decrease and becomes more susceptible to recombination shortening or ATS, a key event in the occurrence of SSS. This would explain the gradual and stochastic appearance of senescent cells as well as accelerated transition of clones from higher to lower PD potentials as normal cell culture approach their maximum division potential [9,10].

Obviously, impairment of SSS would have serious consequences on cell culture dynamics. Indeed, our results demonstrate that there must be a strong inhibition of SSS in normal cultures expressing SV40 Tg so that the ratio of senescent cells is reduced and maintained at around 5%. As a consequence of these conditions, the vast majority of cells successfully divide during each doubling cycle resulting in (i) an increase in the number of PDs to the number of CGs, as well as (ii) an increase in the growth rate of the culture. It has been calculated that an average normal culture would double its PD and equalize it to CGs if there is no SSS [7]. The same increase was determined when we calculated lifespan extension by SV40 Tg. Additionally, inhibition of SSS affects the average telomere length of the culture in that it eliminates interference of telomeres of nondividing cells that are constantly being transferred from previous passages, in normal cultures, and provides a more realistic picture of the average telomere lengths of actively cycling cells.

Thus, it should be pointed out that, regardless of potential mechanisms [10, 7,25,34,35], we have established the fact that SSS plays a crucial role in senescence of individual cells as well as in cell culture dynamics and this should be considered in interpretation of various phenomena that result from its impairment.

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Sažetak

Telomere kontroliraju staničnо starenje

Dobro je poznato da razni stanični tipovi sisavaca imaju ograničen rast kad se uzgajaju u kulturi, što nazivamo staničnim starenjem. Brojni dokazi upućuju na to da je takav ograničen stanični rast osnova procesa starenja. Nedavna otkrića pokazala su da su telomere ključne za navedene procese i za kontrolu staničnog ciklusa, (ne)stabilnosti genoma i imortalizaciju. U ovom pregledu bit će riječi o telomerama koje su pružile objašnjenja za razne fenomene procesa starenja i karcinogeneze.

Ključne riječi: Telomere; stanična senescencija; starenje; skraćivanje telomera; ljudski fibroblasti