Experimental
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AT A CROSSROADS OF CANCER RISK AND AGING: THE ROLE OF TELOMERES

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The risk of overall cancer inevitably increases with advancing age. The cancer incidence rate is not constant within the human life span (it exponentially increases with advancing age). Aging itself is a complex biological process with a poorly understood mechanism of its regulation. The aging process, as evidenced from the survey of the chances for death for the large cohort of people of various age groups, manifests probably due to a progressive accumulation of diverse adverse changes that increase the risk of death. While an increase of cancer risk due to aging cannot be fully explained, the length of telomeres (biomarker of aging) appears to be important to predict this risk. Cellular senescence, which is believed to be associated with dysfunctional (shortened) telomeres, may contribute to the aging of a whole organism. Here, based on recent literature data, we investigate the possible link between telomere dysfunction associated cellular senescence and tumorigenesis.
***Key words*: telomeres, aging, cancer incidence rate, cellular senescence, TIF.**

CANCER INCIDENCE RATE AND AGING

In 1825, Gompertz [1] reported that the mortality increases exponentially with age (Gompertz law of mortality). At present, this law is still true. Fig. 1 shows the recent data on death rates for specified age groups (taking into account both sexes of all races) that were obtained from the survey of the chances for death in 2006 for the entire population of the United States (these data are from the National Center for Health Statistics, USA [2]). The fact that the mortality increases exponentially with age indicates that the progressive accumulation of diverse adverse changes within the life span (these changes increase the risk of death) is likely to predetermine the rate of aging. As Harman [3] stated, "the rate of aging is low early in life, but rapidly increases with age due to the exponential nature of the process". Obviously, the aging process depends upon two components of its regulation: 1) intrinsic (i.e., genetic); 2) extrinsic, which includes lifestyle factors and environmental exposures.

Advancing age is known as a high risk factor for cancer. The relevance of aging to the risk of overall cancer is likely to be supported by the fact that the cancer risk exponentially increases with age despite the age-specific occurrence of some kinds of cancer. According to the data from the U.S. National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results (SEER) Program [4, 5], the cancer risk increases exponentially up to a certain age (70–74 years), then decelerates (75–84 years) and eventually declines (≥ 85 years) (Fig. 2). It should be noted that ≈ 60% of newly diagnosed malignancies and ≈ 70% of all cancer deaths occur in persons aged ≥ 65 years [4, 5]. More than 70% of the mortality associated with many cancers including prostate, bladder, colon,

uterus, pancreas, stomach, rectum and lung occurs in persons aged ≥ 65 years [4–6]. Deceleration and decline of the cancer incidence rate at older ages is an interesting but not fully understood phenomenon. It seems that the large amount of age-associated changes accumulated by older ages (≥ 75 years; see Fig. 2) somehow reduces the probability of cancer occurrence and/or its detection. Driver *et al.* [7] have recently proposed that the decrease in incidence of cancer late in life is largely due to a substantial amount of undiagnosed disease. On the other hand, it has been reasonably hypothesized that the age-related decline of the rate of cell proliferation together with accumulation of senescent (non-proliferating) cells may lead to the reduction in the number of newly transformed cells at older ages, thereby contributing to this aforementioned phenomenon [8]. A late-life mortality rate plateau that has been theoretically predicted [9, 10] may be relevant to deceleration and decline of the cancer incidence rate at older ages.
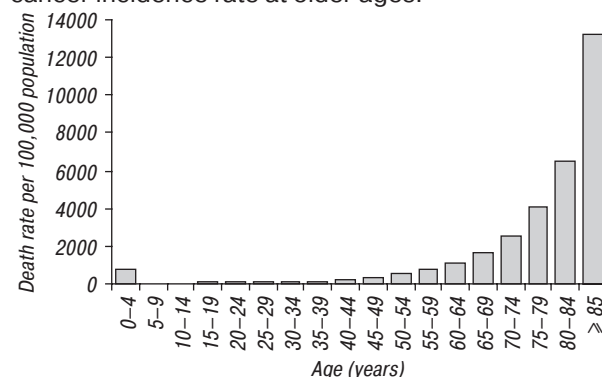
Fig. 1. Death rates for specified age groups (both sexes of all races) obtained from the survey of the chances for death in 2006 for the entire population of the United States. Elevated mortality rate in children of the age group of 0–4 years can be explained by the fact that children under 1 year die quite frequently (death rate: 690.7) [2]

It should be pointed out that in the United States, for instance, among the causes of death in the current millennium, malignant neoplasms remain to be second

after cardiovascular disease (in 2006, cancers were 23.1%, while cardiovascular disease was 26.0% [2]). Interestingly, according to the annual reports of the U.S. National Center for Health Statistics [2], the mortality rate for cardiovascular disease steadily declines (≈ 0.5% every year), while the mortality rate for cancers remains unchanged, so one can predict that cancers by 2013 will be the number one cause of death.
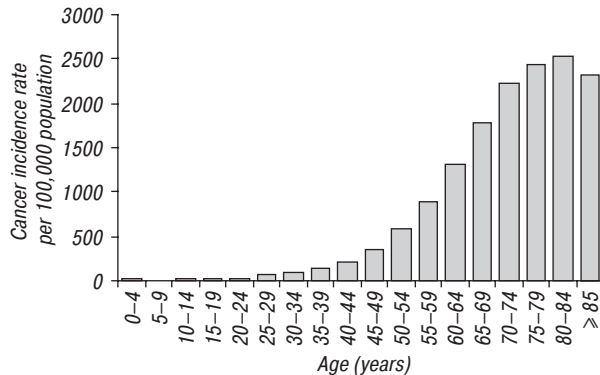
Fig. 2. Cancer incidence rates for specified age groups (both sexes of all races) based on NCI SEER program data of 1994–1998 [4, 5]

TELOMERES: THEIR ROLE IN CELLULAR SENESCENCE AND TUMORIGENESIS

The fact that the cancer incidence rate progresses with advancing age indicates that the mechanisms of tumorigenesis and aging somehow intersect. Dysfunctional (shortened) telomeres appear to play a key role at the aging-cancer interface. In the beginning of 1990th it has been reported that the length of telomeres markedly decreases with advancing age and increasing passage of cell culture [11, 12]. At present, attrition of telomeres is a significant molecular biomarker of aging. Telomeres are known as nucleoprotein structures at the extreme ends of linear chromosomes, whose length (telomeric length) is a critical factor in maintaining genomic stability. Telomeric DNA, which does not contain protein-encoding genes, is composed of G-rich hexanucleotide repeats that have (TTAGGG)$_n$ sequence. Incomplete and inefficient (due to accumulation of telomeric DNA damage) end replication may contribute to telomere shortening [13, 14]. Repair of DNA (including telomeric DNA) appears to decline with aging [14, 15]. Telomeres shorten to a certain critical size, and this event signals cells to enter an irreversible growth arrest (i.e., cellular senescence) that may contribute to the aging of a whole organism. In most instances cells become senescent before they accumulate enough mutations to be cancerous, (this, in part, can explain deceleration and decline of the cancer incidence rate at older ages). The growth arrest induced by shortened telomeres may be a potent anti-cancer mechanism. It has been reasonably noted that the starting point for telomere length in somatic cells is the length of telomeres in germ line cells of the individual or species, and this parameter is and must be strictly conserved in order to maintain viability of the species [16]. The rate of telomere shortening is not necessarily a constant function of cell division. In *in vitro* maintained normal human cells the rate of telomere shortening may vary from 50 to 100 bp per cell division ("normative" telomere loss [16]) [17]. A permanent loss of telomeric

DNA with each cell division is primarily because of the lagging strand of DNA synthesis cannot replicate the extreme 3′ end of the chromosome (the "end replication problem") [13]. Short telomeres in peripheral blood leucocytes have been reported to be associated not only with risks of cancer and cardiovascular disease, but also with mortality from them [18−20].

The integrity of telomeres is maintained, at least in part, by the ribonucleoprotein enzyme telomerase [21, 22]. Telomerase activity is present in almost all human tumors but not in adjacent normal cells [23, 24]. It is growth-regulated, since it correlates with cell proliferation in both normal tissues and tumors [25]. The expression of telomerase activity does not necessarily correlate with the length of telomeres. In some mitotically active normal somatic cells telomeres become shorter with each cell division cycle even though telomerase activity is still present [26]. Although most tumors do express telomerase activity, their telomeres are usually not as long as in normal tissues with lack of cell proliferation [25]. Activation of telomerase that is important in maintaining telomere length stability may be necessary for the sustained growth of most tumors. Telomerase activity appears to be a promising diagnostic and prognostic marker of cancer [25, 27−29]. Nevertheless, telomerase expression alone is not the inciting event in the transformation to neoplasia. This is based on the fact that introduction and expression of telomerase do not induce a transformed phenotype [30, 31].

Although telomerase as well as telomeres plays an important role at the aging-cancer interface [32, 33], the mechanism that can trigger tumorigenesis due to aging remains largely unknown. Most normal cells respond to dysfunctional telomeres by mounting a senescence response that requires the function of both pRb and p53, but another possible consequence of telomere dysfunction (absence of the senescence checkpoint and p53 function) is genomic instability, which is believed to be an early event in tumorigenesis [33, 34]. Thus, cellular senescence may be a "double-edged sword" in this regard (i.e., pro- and anti-tumorigenic roles). Interestingly, dysfunctional telomeres can induce a DNA damage response that involves their association with DNA damage response factors (53BP1, γ-H2AX, Rad17, ATM, and Mre11) [35]. The domain of telomere-associated DNA damage factors is often referred as a telomere dysfunction-induced focus (TIF). TIFs containing multiple DNA damage response factors have been found to be assembled in a subset of senescent cells and signaled through ATM to p53, upregulating p21 and causing G1-phase arrest [36]. Senescent cells displaying dysfunctional telomeres (i.e., TIF-positive cells) have been found to accumulate with increasing age in dermal fibroblasts of skin biopsies of aging baboons [37], which like humans have a relatively long life span and show age-dependent telomere shortening [38]. It should be pointed out that the number of TIF-positive cells in this study accumulated exponentially with increasing age, reaching a value of 15−20% in very old (25−30 yr)

animals [37]. Probably, this finding is the first strong evidence of progressive (exponential) accumulation of age-related changes at the cellular level. The exponential character of telomere dysfunction associated cellular senescence that was observed in primates is likely to take place in humans and contribute to an exponential increase of mortality and cancer incidence rates with advancing age (see Fig. 1 and 2, respectively). In senescent cells, in addition to the damage to telomeric DNA, the damage may also occur to non-telomeric main DNA that contains protein-encoding genes. According to another finding by Herbig *et al.* [37], in senescent dermal fibroblasts ≈ 30% of nuclear foci containing 53BP1 (one of the markers of DNA double-strand break) do no co-localize with telomeric DNA. If the double-strand break misrepaired or left unrejoined, it can cause transformation or cell death [39]. Telomere dysfunction has been reported to impair DNA repair [40], a finding that could help explain the persistence of DNA damage nuclear foci associated with non-telomeric DNA [37].

Finally, cellular senescence may not be caused due exclusively to replicative exhaustion. Oxidative stress appears to increase the rate of telomere shortening as evidenced from telomere-specific accumulation of DNA damage induced by reactive oxygen species [41]. Interestingly, accelerated telomere shortening has been found to be a function of response to life stress, namely to psychological stress, which is significantly associated with oxidative damage [42].

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