

# Relationships between cancer and aging: a multilevel approach

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Received: 6 October 2008 / Accepted: 16 December 2008 / Published online: 21 January 2009  
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**Abstract** The incidence of cancer increases with age in humans and in laboratory animals alike. There are different patterns of age-related distribution of tumors in different organs and tissues. Aging may increase or decrease the susceptibility of various tissues to initiation of carcinogenesis and usually facilitates promotion and progression of carcinogenesis. Aging may predispose to cancer in two ways: tissue accumulation of cells in late stages of carcinogenesis and alterations in internal homeostasis, in particular, alterations in immune and endocrine systems. Increased susceptibility to the effects of tumor promoters is found both in aged animals and aged humans, as predicted by the multistage model of carcinogenesis. Aging is associated with a number of events at the molecular, cellular and physiological levels that influence carcinogenesis and subsequent cancer growth. An improved

understanding of age-associated variables impacting on the tumor microenvironment, as well as the cancer cells themselves, will result in improved treatment modalities in geriatric oncology.

**Keywords** Aging · Carcinogenesis

## Introduction

It is well documented that the incidence of malignant tumors increases progressively with age, both in animals and humans (Anisimov 1987; Dix and Cohen 1999; Parkin et al. 2001; Balducci and Ershler 2005). Three major hypotheses have been proposed to explain the association of cancer and age. The first hypothesis holds that this association is a consequence of the duration of carcinogenesis. In other words, the high prevalence of cancer in older individuals simply reflects a more prolonged exposure to carcinogens (Peto et al. 1985). The second hypothesis proposes that age-related progressive changes in the internal milieu of the organism may provide an increasingly favorable environment for the induction of new neoplasia and for the growth of already existent, but latent malignant cells (Anisimov 1983, 2003a, b, c; Miller 1991; Dilman 1994; Simpson 1993). These mechanisms may also include proliferative senescence, as the senescent cells lose their ability to undergo apoptosis and produce some

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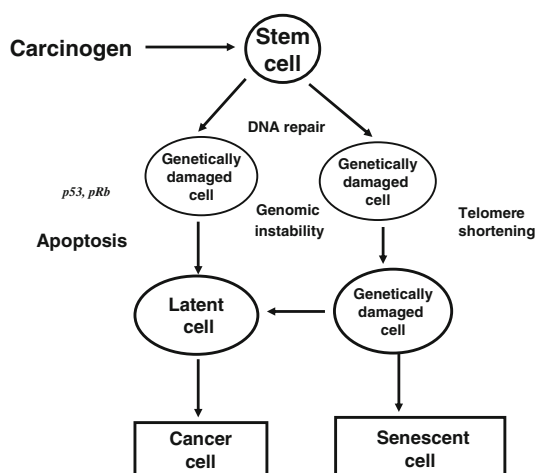
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factors which stimulate epithelial cells with oncogenic mutations (Campisi 2000, 2005). The third hypothesis proposes that the cancer-prone phenotype of older humans might reflect the combined effects of cumulative mutational load, increased epigenetic gene silencing, telomere dysfunction and altered stromal cell milieu (DePinho 2000). Aging is associated with a number of events at the molecular, cellular and physiological levels that influence carcinogenesis and subsequent cancer growth (Anisimov 2003a, b, c; Balducci and Ershler 2005) such that individualized treatment of each patient's tumor will require taking this into account. The elucidation of causes of an age-related increase in cancer incidence may thus be the key to a strategy for primary cancer prevention and improved treatment in geriatric oncology.

### Multistage model of carcinogenesis and aging

There are two pathways which a stem cell can follow in the adult organism (Fig. 1). One is physiological cell proliferation, differentiation and aging, resulting in its individual death (apoptotic or necrotic) (von Wangenheim and Peterson 1998). When the stem cell reserve reaches some limit to compensatory capacity to support tissue repair and functional homeostasis, the death of the organism as a whole take place once a critical organ or critical mass of dysfunctional tissue



**Fig. 1** Two pathways that stem cells may follow after exposure to carcinogens

has been reached. The second stem cell pathway under the influence of exogenous or endogenous harmful factors leads to pathological dedifferentiation, immortalization and possibly formation of a clone of neoplastic cells (von Wangenheim and Peterson 1998; Reya et al. 2001). Both pathways comprise multi-stage processes, many steps of which are well-characterized in relation to the process of development, maintenance and carcinogenesis (Anisimov 1983, 2003a, b, c; Moolgavkar et al. 1999; Hahn and Weinberg 2002; Weinberg 2008). However, the multi-stage pattern of aging needs serious studies and formalization (Butov et al. 2002; Anisimov 2000c). It is worthy of note that multistage models of cellular aging and immortalization have been developed in an attempt to explain delayed genomic instability, in which initiation of carcinogenesis is linked not only to a direct increase in chromosomal aberrations and mutations of oncogenes and tumor suppressor genes, but also to enhanced levels of aberrations and mutations in distant progeny and a predisposition to immortalization (Butov et al. 2002). Carcinogenesis is a multistage process: neoplastic transformation implies the engagement of a cell through sequential stages, and different agents may affect the transition between contiguous stages in different ways (Berenblum 1975; Hahn and Weinberg 2002; Weinberg 2008; Hu and Polyak 2008).

The process of neoplastic development is frequently divided into three operationally defined stages—initiation, promotion and progression. During the first stage of carcinogenesis (initiation) irreversible changes in the genotype of the normal target stem cell leading to its re-acquisition of immortality take place. It is worthy of note that initiation may involve changes leading to conditions such as atypical hyperplasia and carcinoma in situ. Some of these changes may be epigenetic, and in theory reversible with appropriate treatment. During the second stage of carcinogenesis (promotion) an initiated (latent, immortalized) cell acquires phenotypic features of a transformed (malignant) cell, and under the influence of microenvironmental factors can proceed to tumor progression. Carcinogens affect not only the target cell itself but also influence many microenvironmental factors to create conditions for the promotion of immortalized cell growth (growth factors, cytokines, immunodepression, biogenic amines, hormonal and metabolic imbalance). Carcinogens affect both stages (initiation and promotion) whereas tumor promoters

affect only the second stage. Unlike initiation, promotion requires prolonged exposure to the carcinogen and may be reversible to a large extent. A carcinogen that is able to act as both initiator and promoter is referred to as a full carcinogen. Although the dissection of carcinogenesis into initiation, promotion, and progression is useful as a frame of reference, it should not be assumed that only three carcinogenic stages exist: each stage can be subdivided into multiple sub stages. Promotion may involve the activation of several enzymes, such as protein kinase C and ornithine decarboxylase; enhanced hexose transport; increased polyamine production, prevention of cell differentiation; and inhibition of cell-to-cell communication (Weinberg 2008). Discovery of oncogenes and of their function has provided new insights into the carcinogenic process. One may view carcinogenesis as a “cascade” phenomenon, resulting in serial activation of multiple cellular oncogenes and/or inactivation of tumor-suppressing genes (e.g. p53) (Hanahan and Weinberg 2000).

Both experimental and epidemiologic studies illustrate the interaction of aging and carcinogenesis. The malignant transformation of normal cells involves both quantitative and qualitative changes (Hahn and Weinberg 2002; Weinberg 2008). Carcinogenic agents not only cause genomic transformation of the cell, but also create the conditions that facilitate proliferation and clonal selection in the cell microenvironment (Anisimov 1987, 2003a, b, c; Vijg and Campisi 2008).

### Population aging and carcinogenesis

There is a large literature on spontaneous tumor incidence in experimental animals of various species and strains. It is well documented that the rate of malignant tumors increases progressively with age, both in rodents and humans (Dix et al. 1980; Anisimov 1987; Parkin et al. 2001; Napalkov 2004). Age-related increases of spontaneous tumor incidence have been observed in other species, too: amphibians, fish, hamsters, guinea pigs, dogs and other domestic animals. There is also evidence of an increase in spontaneous neoplasm incidence with age in invertebrate animals (Ponten 1977).

The term “spontaneous tumors” can be misleading, as the majority of these neoplasia in humans are

caused by environmental and lifestyle-related factors, including tobacco smoking, diet, alcohol consumption, sexual promiscuity, industrial carcinogens, ultraviolet radiation, some drugs, and oncogenic viruses (Tomatis 1990). Some strains of mice and rats have a high and low incidence of tumors, characterized by the selective development of one or two tumor localizations (Anisimov 1987; Staats 1980; Ward 1983). Tumor incidence, site, and type in experimental animals vary greatly depending on both endogenous (genetic) and exogenous factors. Even under standard dietary and housing conditions, animals demonstrated a wide range of spontaneous tumor incidence (Anisimov 1987; Ward 1983). Genetic factors seem to determine the site (localization) and age at tumor onset. There is evidence of increased cancer risk in humans with some progeria syndromes as well as in mice with accelerated aging induced by certain genetic manipulations (Anisimov 2003a, b, c, 2006).

It is evident that the incidence of both total and fatal tumors decreases in the oldest groups of males and females. An analysis of data on tumor incidence in ad libitum versus calorie restricted mice (Lipman et al. 1999a, b) shows the same. This result corresponds with observations on a decrease in cancer incidence in centenarians (Bordin et al. 1999; Miyashi et al. 2000).

### Aging and carcinogenesis: relationship at the systemic/organ level

There is significant similarity between aging and carcinogenesis at the level of systemic regulation of integration at the physiological level. Desynchronization in circadian rhythms of melatonin and some other hormones is common both for aging and carcinogenesis in rodents and humans (Anisimov et al. 1995; Anisimov 2003a, b, c; Bartsch et al. 2001). Both the levels of serum melatonin and its metabolite, 6-sulfatoxymelatonin excretion, decrease during normal aging and in cancer patients (Touitou 2001; Bartsch et al. 2001). Hypothalamic levels of biogenic amines (catecholamines and serotonin) significantly decrease in aging animals and humans (Anisimov 2003a, b, c). A number of chemical carcinogens as well as exposure to ionizing radiation also cause decreases and/or disturbances in biogenic amines in

the hypothalamus (Anisimov 1987; Arutjunyan et al. 2001). The hypothalamic threshold of the sensitivity to feedback inhibition is a key mechanism of age-related switching-off of reproductive function in female rodents and women (Dilman and Anisimov 1979a; Dilman 1994). We have found that exposure to various chemical carcinogens leads to a similar phenomena (Anisimov 1987).

The potential link between aging and insulin/IGF-1 signaling has attracted substantial attention during the last few years, on the basis of evidence including age-related increased incidence of insulin resistance and type 2 diabetes in accelerated aging syndromes and life span extension by caloric restriction in rodents, primates, and arguably, humans. Concomitant reduction in plasma insulin and plasma glucose levels, which implies increased sensitivity to insulin, is emerging as a hallmark of increased longevity (Bartke et al. 2003; Tatar et al. 2003). Hyperglycemia is an important aging factor involved in the generation of advanced glycation end-products (AGEs) (Facchini et al. 2000; Ulrich and Cerami 2001). There is a great deal of evidence that hyperinsulinemia favors accumulation of oxidized proteins by reducing their degradation as well as facilitating protein oxidation by increasing the steady-state level of oxidative stress (Facchini et al. 2000). Untreated diabetics with elevated glucose levels suffer many manifestations of accelerated aging, such as impaired wound healing, obesity, cataracts, vascular and microvascular damage (Dilman 1994). It was shown that centenarians have a preserved glucose tolerance and sensitivity to insulin as well as a lower degree of oxidative stress as compared to other aged persons (Barbieri et al. 2003). It is perhaps not unrelated to these findings that hyperinsulinemia is also known to be an important factor in the development of cancer as well (Dilman 1994; Colangelo et al. 2002; Gupta et al. 2002).

The intensive investigations in the nematode worm model organism *C. elegans* since the 1990s, which have identified insulin signaling components including *daf-2*, *age-1* and *daf-16* as the genes whose mutations lead to life span extension, have shed new light on the molecular mechanisms underlying aging (Kenyon 2001; Bartke et al. 2003; Tatar et al. 2003). Also in the fruit fly *D. melanogaster*, mutations of genes operating in signal transduction from the insulin receptor to transcription factor *daf-16* (*age-1*, *daf-2*,

CHICO, InR) show that these same genes are closely associated with longevity in other organisms as well (Kenyon 2001; Dillin et al. 2002). It was demonstrated that FKHR, FKHRL1 and AFX, which are mammalian homologues of the *daf-16* fork head transcription factor, function downstream of insulin signaling and akt/PKB (Richards et al. 2002). Thus, these pathways may be conserved throughout evolution and also have relevance for human aging.

*Daf-2* and InR are structural homologues of tyrosine kinase receptors in vertebrates that include the insulin receptor and the insulin-like growth factor type 1 receptor (IGF-1R); the insulin receptor regulates energy metabolism, whereas IGF-1R promotes growth. At least three genes (*Pit1<sup>dw</sup>*, *Prop1<sup>dw</sup>*, *Ghr*) have been identified which, when knocked out, lead to dwarfism with reduced levels of IGF-1 and insulin, and at the same time result in increased longevity (Flurkey et al. 2001; Coschigano et al. 2000). In Snell and Ames dwarf mice, sexual maturation is delayed, and few males are fertile, while females are invariably sterile. These animals thus have a longer but presumably more miserable life. These mice as well as *Ghr<sup>-/-</sup>* knockout mice have significantly reduced glucose levels and fasting insulin levels, decreased tolerance to glucose and increased sensitivity to insulin which appears to be combined with reduced ability to release glucose in response to acute challenge (Bartke et al. 2003). Further strong support for the role of the insulin/IGF-1 signaling pathway in the control of mammalian aging and for the involvement of this pathway in longevity of IGF-1-deficient mice comes from the experiments of Hsieh et al. (2002a, b). They showed that in the Snell dwarf mice, GH deficiency would lead to reduced insulin secretion and alterations in insulin signaling via InR $\beta$ , IRS-1 or IRS-2 and that P13K affects genes influencing longevity. They concluded that the *Pit1* mutation may result in physiological homeostasis that favors longevity.

Reduction in both glucose and insulin levels, as well as an increase in insulin sensitivity is a well-documented response to life-extending caloric restriction in rodents and monkeys (Weindruch and Sohal 1997; Lane et al. 2000). It was shown that improved sensitivity to insulin in calorie-restricted animals is specifically related to reducing visceral fat (Barzilai and Gupta 1999). It is worthy of note that *Ghr<sup>-/-</sup>* mice have a major increase in the level of insulin receptors (Dominici et al. 2000), while Ames dwarf

mice have a smaller increase in insulin receptors and substantially increased amounts of insulin receptor substrates IRS-1 and IRS-2 (Dominici et al. 2002). The development of tumors in Ames dwarf mice is postponed and incidence reduced compared to controls (Ikeno et al. 2003). The crucial mechanism responsible for the longevity-promoting and anti-carcinogenic effects of caloric restriction is thought to be the maintenance of these low levels of insulin and IGF-1 and also an increase in insulin sensitivity in rodents (Chiba et al. 2002) as well as in monkeys (Mattison et al. 2003). Many characteristics of the long-lived mutants and GH-receptor knockout mice resemble those of normal animals under caloric restriction. These characteristics include reduced plasma levels of IGF-1, insulin and glucose, with the consequent reductions in growth and body size, delayed puberty, and significantly increased sensitivity to insulin action.

To directly test some of these ideas, Holzenberger et al. (2003) inactivated the *Igf1r* gene by homologous recombination in mice. Although *Igf1r*<sup>-/-</sup> mice died early in the life, heterozygous *Igf1r*<sup>+/-</sup> mice lived on average 26% longer than their wild-type littermates. These mice did not manifest dwarfism, and their energy metabolism was normal. Food intake, physical activity, fertility and reproduction were also unaffected. These mice and embryonic fibroblasts derived from them were more resistant to oxidative stress than controls. However, spontaneous tumor incidence in the aging cohort of *Igf1r*<sup>+/-</sup> mice was similar to wild-type controls. At the molecular level, insulin receptor substrates and the *p52* and *p66* isoforms of *Shc*, both main substrates of the IGF-1 receptor, showed decreased tyrosine phosphorylation. Two main pathways—the extracellular-signal regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K)-Akt pathway—were downregulated in *Igf1r*<sup>+/-</sup> mice. Longevity extension was also reported in fat-specific insulin receptor knockout (FIRKO) mice (Bluher et al. 2003). These animals have reduced fat mass and were protected against age-related obesity and its subsequent metabolic abnormalities including deterioration of glucose tolerance, despite normal food intake. Both male and female FIRKO mice were found to have an increase in mean life span (by 18%) with parallel increases in maximum life span. Extended longevity in FIRKO mice

was associated with a shift in the age at which age-dependent increase in mortality risk becomes appreciable and a decreased rate of age-related mortality, especially after 36 months of age. In FIRKO mice, resistance to developing obesity, despite normal food intake, suggests that metabolic rate is increased, rather than decreased (Bluher et al. 2003). It was suggested that decreased fat mass could lead to a decrease in oxidative stress in FIRKO mice. Another possibility is that the increased longevity in these mice is the direct result of altered insulin signaling. Also along this line of investigation, Shimokawa et al. (2002) created a transgenic strain of rats whose GH gene was suppressed by an anti-sense GH transgene. Male rats homozygous for the transgene (*tg/tg*) had a reduced number of pituitary GH cells, a lower plasma concentration of IGF-1, and a dwarf phenotype. Heterozygous rats (*tg/-*) had a phenotype in plasma IGF-1, food intake, and body weight intermediate between *tg/tg* and control (*-/-*) rats. The life span of *tg/tg* rats was 5–10% shorter than *-/-* rats. In contrast, the life span of *tg/-* rats was 7–10% longer than *-/-* rats. It was found that it was enhanced tumorigenesis that caused earlier death in *tg/tg* rats; in contrast, *tg/-* rats had reduced non-neoplastic diseases and a prolonged life span. Immunological analysis revealed a smaller population and lower activity of splenic natural killer cells in homozygous *tg/tg* rats. These results provided evidence that an optimal level of function of the GH-IGF-1 axis is required for longevity in mammals and implicates innate immunity in this process.

Recently it was shown that the incidence of mutations in insulin regulatory region (IRE) of APO C-III T-455 C directly correlates with longevity in humans. This is the first evidence showing that mutations located downstream of *daf-16* in insulin signal transduction system is associated with longevity in humans (Anisimov et al. 2001). As already mentioned above, it is worth stressing again that centenarians display a lower degree of resistance to insulin and lower degree of oxidative stress as compared with the average elderly person <90-year-old (Barbieri et al. 2003). It was suggested that centenarians may have been selected for appropriate insulin regulation as well as for the appropriate regulation of tyrosine hydroxylase (TH) gene, the product of which is rate limiting in the synthesis of the stress-response mediators catecholamines. It was

shown that catecholamine may increase free radical production through enhancing the metabolic rate and auto-oxidation in diabetic animals (Singal et al. 1983). Recent studies on aging parameters of young (up to 39) and old (over 70) humans having similar IGF-1 serum levels provides evidence of an important role of this peptide for longevity in people too (Ruiz-Torres and Soares de Melo Kirzner 2002). Roth et al. (2002) analyzed data from the Baltimore Longitudinal Study of Aging and found that survival was greater in men who maintained lower insulin levels.

Immunodepression is a common feature of both aging and carcinogenesis (Dilman 1994; Pawelec et al. 2006; Derhovanessian et al. 2008). Some of the characteristic dysfunctional attributes of T cell immunity observed in the elderly (“immune exhaustion”) may be shared by tumor-specific T cells in younger cancer patients (Walter et al. 2005). Thus, alterations of immune surveillance accompanying immunosenescence may contribute to age-associated increased cancer rates. That tumour regression following immunotherapy can be observed in immunocompetent hosts but not in old hosts support this contention in animal models (Gravekamp et al. 2008). Decreased specific T cell responses to tumor antigens due to immune exhaustion may be part of the explanation (Pawelec et al. 2006). This T cell clonal exhaustion may itself lead to the development of T-regulatory cells which suppress anti-tumor responses (Derhovanessian et al. 2008). The unbalanced pro-inflammatory response in relation to the relatively conserved innate immune response might also favour cancer development due to the production of free radicals (Derhovanessian et al. 2008). However, more studies are required to define the role of immunosenescence in cancer in the elderly, about which little can be currently stated with any certainty.

### **Aging and carcinogenesis: relationship at the cellular level**

The term “cellular senescence”, originally coined to describe the many cellular changes associated with aging, now refers more commonly to a signal transduction program leading to irreversible arrest of cell growth, accompanied by a distinct set of changes in the cellular phenotype and genetic program (Shay and Roninson 2004). Senescence is

proposed to have evolved as a potent anticarcinogenic mechanism, with the process of neoplastic transformation requiring a series of events allowing cells to bypass senescence (Campisi 2003, 2005; Shay and Roninson 2004). Thus, cellular senescence is controlled by the tumor suppressor proteins p53 and pRb (Campisi 2005). Inactivation of these proteins results in bypassing senescence; due to its essentially irreversible growth arrest and the requirement for p53 and pRb function, cellular senescence is therefore considered a potent tumor suppressor mechanism (Campisi et al. 2001; Kim et al. 2003; Campisi 2003; Itahana et al. 2004). This may be true for rodents, but not proved for humans. Reactivation of telomerase is considered rate-limiting to bypass senescence and necessary for many human carcinomas (as distinct from rodents which express telomerase and may have long telomeres), and is not accomplished simply by inactivation of RB and p53 (Rangarajan and Weinberg 2003). Although the relationships between cellular senescence and aging *in vivo* is not very clear yet, senescent cells do accumulate in aging tissues, at least in human skin and liver (Dimri et al. 1995; Paradis et al. 2001; Dimri 2005) and in primate retina and skin (Mishima et al. 1999; Herbig et al. 2006). The contribution of the growth arrest and anti-tumor protein p16 has been implicated in studies on human skin (Ressler et al. 2006). Senescent cells were shown to be capable of stimulating the malignant progression of premalignant keratinocytes and mammary gland epithelial cells (Krtolica et al. 2001). Senescent cells have also been detected at sites of age-related pathology, including benign hyperplastic prostate (Choi et al. 2000) and atherosclerotic lesions (Vasile et al. 2001). Nonetheless, some published observations have failed to support the existence *in vivo* of a significant number of cells with the phenotype observed during replicative aging. Thus, Cristofalo (2005) was unable to demonstrate any donor age-specific increase in senescence-associated beta-galactosidase (SA- $\beta$ -gal) activity staining in human skin samples.

The natural history of spontaneous tumors in humans (the rate of tumor doubling, metastazing potential) and on the survival of cancer patients newly diagnosed at different ages provides information on the effects of age on tumor growth in humans. Available data both in experimental animals and in humans are contradictory and support different

effects of age on tumor development (Anisimov 2006). In general, an “age effect” may be recognized both in experimental and in human malignancies. Mikhlin et al. (2004) have studied the growth rate and the volume doubling in 150 malignant melanomas of the skin in patients aged from 16 to 85 years. Regardless of the pattern of melanoma growth (superficial and/or nodular) there was no influence of age on the kinetics of tumor growth. This observation is not easily reconciled with the idea that accumulation of senescent cells promotes tumor growth in humans. Tissue origin (histogenesis) and immunogenicity of the tumor are the principal factors determining age-related differences in tumor growth. Notwithstanding the above observations, there is increasing evidence that age-related changes in the tumor microenvironment might play a significant role, perhaps mostly in tumors other than melanoma, which is perhaps less dependent upon stromal support. In our own experiments, lung-affine cells of rat rhabdomyosarcoma RA-2 were intravenously inoculated into rats of different ages (Anisimov et al. 1988). It was observed that the number of lung tumor colonies was highest in 1- and 15-month-old animals and lowest in 3- and 12-month-old animals. A positive correlation was found between the number of tumor lung colonies and somatomedine activity in the lung. In another experiment, RA-2 cells from a 3-month-old donor were inoculated into 2–3 or 21- to 23-month-old recipients and 3 weeks later were separately taken from “young” and “old” hosts and transplanted into 3-month-old recipients. The number of lung colonies was significantly decreased in 3-month-old recipients injected with RA-2 cell passed via “old” host. The results obtained suggest the critical role of host and donor microenvironment in lung colony forming potential of RA-2 cells.

McCullough et al. (1994) have observed that transformed rat hepatocytic cells lines were only weakly tumorigenic following transplantation into the livers of young adult rats. The tumorigenicity of these cell lines increased progressively with the age of the tumor recipients. These results suggest strongly that the tissue microenvironment is an important determinant in the age-related tumorigenic potential of transformed cells. Thus, available data show that some changes in structure and function of DNA are evolving with natural aging. The character of these changes could vary in different tissues and might

cause a mosaic of tissue aging. In turn, this may lead to both age-related increases in spontaneous tumor incidence and age-related changes in susceptibility to carcinogens in various organs (Anisimov 2003a, b, c).

### **Aging and carcinogenesis: relationship at molecular level**

One of the most popular theories of aging is the free radical theory proposed in 1956 by Denham Harman (Harman 1998). This theory postulated that various oxidative reactions occurring in the organism (mainly in mitochondria) generate free radicals as byproducts which cause multiple lesions in macromolecules (nucleic acids, proteins and lipids), leading to their damage and aging. This theory explains not only the mechanism of ageing per se but also a wide variety of age-associated pathology, cancer included (Harman 1998; Hamilton et al. 2001; von Zglinicki et al. 2001). Recent evidence suggests that key mechanisms of both aging and cancer are linked via endogenous stress-induced DNA damage caused by reactive oxygen species. These include oxidative nuclear and mitochondrial DNA damage and repair, telomere shortening and telomere-driven cellular senescence and have been intensively discussed in a number of comprehensive reviews (Hamilton et al. 2001; Kawanishi et al. 2001; von Zglinicki et al. 2001). It is worthy of note that free radical damage in chemically and radiation-induced carcinogenic processes is critically related to cell transformation (Kawanishi et al. 2001; Anisimov 2003a, b, c). Epidemiological observations have shown that oxidative stress is one of the major facilitators of carcinogenesis. Recent data suggest that common molecular mechanisms exist in oxidative stress-induced carcinogenesis, including p16INK4A inactivation (Toyokuni 2008). Using novel technique based on DNA immunoprecipitation it was shown that the localization of oxidative DNA damage is not random in vivo (Toyokuni 2008).

The intensity of natural damage to DNA is very high, e.g. in a human cell, spontaneous depurination takes place at a rate of up to 10,000 events per day and spontaneous deamination of adenine and cytosine at a rate of hundreds of events per day (Singer and Grunberger 1983). As a result, permanently active mechanisms of DNA repair have evolved. It transpires that in both the most intensive natural mutation

processes (depurination and deamination), thymine is not present (mutations related to it are significantly more rare (Singer and Grunberger 1983), and therefore repair mechanisms for thymine may have evolved less intensively. Hence, if we want to induce uniformly distributed point mutations (and simultaneously to minimize damage to other structures) in laboratory animals then it is appropriate to use analogues of thymine as a mutagen.

Some “in vitro” and “in vivo” effects of the thymidine analogue, 5-bromodeoxyuridine (BrdUrd), suggest that BrdUrd can be profitably used to investigate the role of selective DNA damage both in carcinogenesis and in aging. BrdUrd is incorporated into replicating DNA in place of thymine, and this effect is mutagenic (Morris 1991). Assuming a fairly even level of BrdUrd incorporation into the DNA of various tissues of neonatal rats and long-term persistence therein (Ward et al. 1991), cells with the highest proliferative activity would be more likely to undergo malignant transformation. Exposure to BrdUrd has dramatic effects on cellular functions including cell differentiation, inactivation of regulatory genes or master switching, and proliferation (Anisimov 1994). These changes in cellular function may favor tumor development.

In a series of experiments (Napalkov et al. 1989; Ward et al. 1991; Anisimov and Osipova 1992) rats received subcutaneous injections of BrdUrd at 1, 3, 7 and 21 days of postnatal life at the single dose of 3.2 mg per rat. Exposure to BrdUrd caused a decrease in the mean life-span of the animals of 38% in males and 27% in females by increasing the rate of aging (calculated according to the Gompertz equation). Monitoring estrus showed an earlier natural age-related switching-off of reproductive function in female rats, due to disturbances in central regulation of gonadotropic function in the pituitary. The exposure of rats to BrdUrd was accompanied by signs of immunodepression, increased incidence of chromosome aberrations and decreased latency of spontaneous tumors. Moreover, in offspring of rats neonatally treated with BrdUrd, there was an increased incidence of congenital malformation and of spontaneous tumors, and accelerated aging was observed. Neonatal exposure of rats or mice to BrdUrd was followed by the initiation of the neoplastic process and, consequently, by increased tissue susceptibility to “late stage” carcinogens such as NMU, X-irradiation, urethane, estradiol-benzoate and TPA (Napalkov et al.

1989; Anisimov and Osipova 1992; Anisimov 1994). Our data thus provided evidence that perturbation of DNA induced by BrdUrd exposure contributed substantially to the initiation of tumorigenesis and to the acceleration of aging not only in exposed animals but also their offspring.

In vitro, BrdUrd was found to induce flat and enlarged cell shapes, characteristic of senescent cells, and SA- $\beta$ -gal expression in mammalian cells regardless of cell type or species. In immortal human cells, fibronectin, collagenase I, and *p21(waf1/sdi-1)* mRNAs were immediately and very strongly induced, and the mortality marker mortalin changed to the mortal type from the immortal type. Human cell lines lacking functional *p21(waf1/sdi-1)*, *p16(ink4a)*, or *p53* behaved similarly. The protein levels of *p16(ink4a)* and *p53* did not change uniformly, while the level of *p21(waf1/sdi-1)* was increased by varying degrees in positive cell lines. Telomerase activity was suppressed in positive cell lines, but accelerated telomere shortening was not observed in tumor cell lines (Michishita et al. 1999; Suzuki et al. 2001; Minagawa et al. 2005). These results suggest that BrdUrd induced senescence-like phenotypic resemblance in both mortal and immortal cultured mammalian cells and, possibly, activated a common senescence pathway present in both types of cells (Suzuki et al. 2001). The level of gene expression in HeLa cells and normal human diploid fibroblasts (TIG7 cells) exposed to BrdUrd has been examined by others (Suzuki et al. 2001). BrdUrd was found to induce expression of various known and novel genes in addition to several senescence-marker genes in HeLa cells, and more than half of these genes were found to be induced in normally senescent human fibroblasts. The affected genes in BrdUrd-treated HeLa cells include those involved in remodeling of the extracellular matrix, cell cycle progression, and metabolism of intracellular compounds essential for normal cell growth. These observations reflect features characteristic of normal senescent cells, e.g. specific morphological changes and the cell cycle arrest at the G1/S boundary, and support the view that BrdUrd induces a senescence-like phenomenon. In another set of in vitro experiments, it was shown that BrdUrd activates a silenced transgene integrated in HeLa cells (Suzuki et al. 2001), supporting the idea that similar mechanisms may operate in the regulation of BrdUrd-inducible genes and the senescence-associated genes. It is important to stress that BrdUrd immediately

induces premature senescence in normal cells and the senescence-like phenomenon in any type of immortal cells (Suzuki et al. 2001). Recently, Minagawa et al. (2004) have shown that BrdUrd immediately and dramatically induces senescence-associated genes in human cells. Together, these data imply shared pathways of “normal” ageing and the response to carcinogens/mutagens.

Mathematical modeling of processes of aging and carcinogenesis in tissues based on the experimental data alluded to above (Napalkov et al. 1989; Anisimov and Osipova 1992; Anisimov 1994, 1995) has been performed (Butov et al. 2002) on the basis of the recurrent algorithms constructed on the stochastic equations in terms of semi-Martingale characteristics of these processes. The results support the conclusion that BrdUrd treatment causes accelerated aging in tissues with proliferating cells, as well as a mortality increment directly due to tumorigenesis. Thus, mathematical modeling further supports the hypothesis that levels of tissue damage resulting from mutagenesis and oxidative stress accelerate aging.

### Senescence of cancer cells

Senescence, like apoptosis, is a barrier to tumorigenesis and SA- $\beta$ -gal-positive cells have been detected in early, but not late stages of carcinogenesis (Narita and Lowe 2005). However, escaping the senescence program during cancer progression does not shut it off completely as many tumor cells retain the ability to undergo senescence in vitro (Han et al. 2002; Kim et al. 2003; Roninson 2003; Eom et al. 2005; Xu et al. 2005; Ota et al. 2006; Rebbaa et al. 2006; Sliwiska et al. 2008). Thus, it seems that the phenomenon of inducing senescence of immortal/cancer cells is not restricted to BrdUrd and even not to genotoxic stress as inhibition of histone deacetylation can give the same effect (e.g. Rebbaa et al. 2006). However, the majority of data showing senescence of cancer cells were obtained using DNA-damaging agents. Indeed, it was shown that cancer cells exposed to DNA-damaging agents, such as many of those commonly used in chemotherapy, undergo permanent cell cycle arrest and acquire a phenotype similar to that observed in replicative or premature senescence of normal human cells (Chang et al. 1999; Roninson 2003; Sliwiska et al. 2008; Eom et al. 2005; Jackson

and Pereira-Smith 2006). This includes an enlarged, flattened morphology, positive staining for SA- $\beta$ -gal and induction of p21. Recently, this capacity of cancer cells was documented in vivo as well. The presence of SA- $\beta$ -gal-positive cells has been reported in specimens from breast cancer (te Poele et al. 2002) and lung cancer patients who received chemotherapy (Roberson et al. 2005) as well as in an animal cancer model (Puig et al. 2008). It is believed that inducing the senescence of cancer cells is an important outcome for the successful treatment of cancers—especially those resistant to apoptosis—with many chemotherapeutic agents (Berns 2002).

Interestingly, it is commonly believed that normal human fibroblasts are blocked in the G1 phase of the cell cycle when senescent, whilst cancer cells induced to senescence with DNA-damaging agents can be blocked also in G2 or even bypass cell cycle checkpoints and undergo endoreplication by multiplying their DNA content several times. Senescence-like growth arrest upon treatment with the DNA-damaging agent doxorubicin has been shown to be associated with polyploidy in several human cell lines (Han et al. 2002; Kim et al. 2003; Roninson 2003; Eom et al. 2005; Xu et al. 2005; Ota et al. 2006; Rebbaa et al. 2006; Sliwiska et al. 2008) but not in breast cancer (Jackson and Pereira-Smith 2006). Using the same concentration and time course of doxorubicin treatment we have in fact shown that en route to senescence, colon HCT116 cells became polyploid with a DNA content reaching 16C, but breast MCF-7 cells were arrested in the G1 and G2 phases of the cell cycle (Mosieniak and Sikora, unpublished data). This implies that the genetic background can influence the molecular pathways leading to the terminal growth arrest of cancer cells. It is believed that polyploidization can be prevented by a p53-dependent G1 checkpoint, the G2 checkpoint, and the mitotic spindle checkpoint (Storchova and Pellman 2004; Vogel et al. 2004). Interestingly, HCT 116 cells, like MCF-7, activate p53 upon doxorubicin-treatment and have properly functioning mitotic checkpoint controls, as revealed by nocodazole treatment. Despite this, the majority of doxorubicin-treated HCT 116 do not enter mitosis but endoreduplicate, suggesting that active p53 is not enough to stop them in G1 or tetraploid G1 (Sliwiska et al. 2008). It cannot be excluded that another tumor suppressor, namely pRb, is critical in blocking DNA replication and preventing endoreduplication (Niculescu et al. 1998; Srinivasan

et al. 2007). Indeed, we have identified several differences between HCT 116 and MCF-7 cells regarding expression of pRB family members (Mosieniak and Sikora, not shown).

Polyploid cells are potentially dangerous as they can undergo aberrant mitoses, giving rise to chromosomally unstable progeny (Storchova and Pellman 2004). One cannot exclude the possibility that on their route to senescence, polyploid cells can infrequently escape senescence and divide. Indeed, this was recently documented in several laboratories (Sundaram et al. 2004; Erenpreisa and Cragg 2007; Puig et al. 2008; Sliwinska et al. 2008). Although Bataller et al. (2008) did not document senescence-like characteristics of HCT 116 cells after treatment with mithramycin-SK, they did find that a small number of polyploid cells are able to divide and give rise to aneuploid progeny. In contrast, MCF-7 cells which show senescence-like growth arrest without endoreduplication are permanently arrested and even rare cell division events are never observed (Mosieniak and Sikora, unpublished results).

In the view of these findings, the question is whether senescence of cancer cells is actually a desirable outcome of cancer treatment? Although this can be true for some types of cancer cells (e.g. breast cancer), this might not be the case for all (e.g. colon cancer). The capacity for endoreplication en route to senescence induced with anticancer agents may be a valuable for prediction the outcome of cancer treatment.

### **Interventions in aging and carcinogenesis: the case of antidiabetic biguanides**

Several years ago, it was suggested that biguanide antidiabetics may act as a potential anti-aging intervention (Dilman 1971). The antidiabetic drugs, phenformin (1-phenylethylbiguanide), buformin (1-butylbiguanide hydrochloride) and metformin (*N,N*-dimethylbiguanide) reduce hyperglycemia, improve glucose utilization, reduce free fatty acid utilization, gluconeogenesis, serum lipids, insulin, somatomedin, reduce body weight and decrease metabolic immunodepression both in humans and rodents (Muntoni 1999; Dilman 1994). Thus, according to the above arguments, they should decrease cancer occurrence and enhance longevity. Although phenformin is no longer

used in clinical practice due to its side effects (mainly lactic acidosis) in patients with non-compensated diabetes, a >10-year-long experience of treating patients without advanced diabetes did not reveal problems with lactic acidosis or any other side effects (Dilman 1994). Thus, analysis of the results of long-term administration of this drug as well as other antidiabetic biguanides (buformin and metformin) to non-diabetic animals will be important for understanding of links between insulin and longevity, and could have potential application in humans. In this regard, it was reported that treatment with antidiabetic biguanides prolonged the mean life span of female mice and rats (Dilman and Anisimov 1980; Anisimov et al. 2003, 2005a, b, 2008) and that metformin also significantly increases rat life span (G. S. Roth, personal communication).

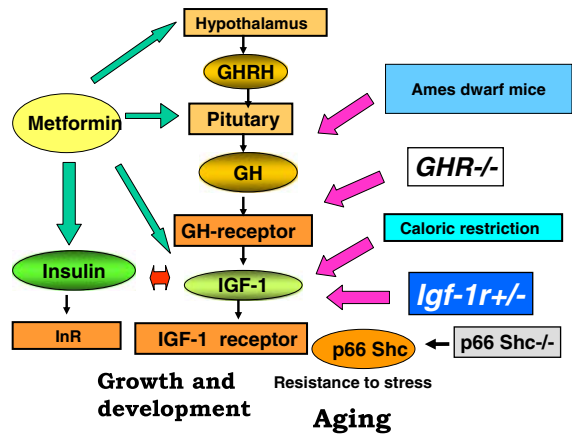
Paralleling the above findings, the anticarcinogenic effect of antidiabetic biguanides has been demonstrated in several models of spontaneous and induced carcinogenesis. Treatment with phenformin normalized glucose tolerance and serum insulin and IGF-1 levels in rats given intravenous injections of *N*-nitrosomethylurea (NMU). Moreover, mammary carcinogenesis was inhibited in these animals (Anisimov 1987). Treatment of rats with 1,2-dimethylhydrazine (DMH) caused a decrease in the level of biogenic amines, particularly of dopamine in the hypothalamus, as well as decreased glucose tolerance and increased insulin and triglyceride levels in blood. Administration of phenformin restored immunological indices and inhibited DMH-induced colon carcinogenesis (Anisimov 1987). Growth of colon 38 adenocarcinoma was significantly slowed in liver-specific IGF-1-deficient mice, whereas injections of IGF-1 promoted tumor growth and metastasis (Wu et al. 2002).

Postnatal treatment with biguanides started from the age of 2 months significantly inhibited the development of malignant neurogenic tumors in rats transplacentally exposed to NMU or NEU (Alexandrov et al. 1980). In hamsters fed a high fat diet, treatment with *N*-nitrosobis-(2-oxopropyl)amine caused development of pancreatic malignancies in 50% of the animals, whereas no tumors were found in the hamsters exposed to the carcinogen but also treated with metformin (Schneider et al. 2001). Thus, anticarcinogenic effects of antidiabetic biguanides have been demonstrated in relation to spontaneous carcinogenesis in mice and rats, in different models of chemical

carcinogenesis in mice, rats and hamsters, and in radiation carcinogenesis models in rats (Anisimov 2008). Finally, phenformin administered orally to rodents was also found to potentiate the antitumor effect of cytostatic drugs on transplantable tumors (Dilman and Anisimov 1979b).

Monitoring cancer patients over 10 years has shown that metabolic rehabilitation in (including restricted fat and carbohydrate diets and treatment with antidiabetic biguanides) resulted in a significant survival benefit in breast and colorectal cancer sufferers, associated with an increased cancer-free duration, and decreased incidence of metastasis (Berstein et al. 2004; Berstein 2005). Consistent with this, it was reported that in type 2 diabetics, metformin treatment may be associated with reduced cancer risk (Evans et al. 2005; Bowker et al. 2006).

The anti-diabetic biguanides inhibit fatty acid oxidation, inhibit gluconeogenesis in the liver, increase the availability of insulin receptors, inhibit monoamine oxidase (Muntoni 1999), increase sensitivity of hypothalamo-pituitary complex to negative feedback inhibition, and reduce the excretion of glucocorticoid metabolites and dehydroepiandrosterone-sulfate (Dilman 1994). These drugs have been proposed for the prevention of the age-related increase of cancer and atherosclerosis, and for retardation of the aging process (Dilman 1971, 1994). It has been shown that administration of antidiabetic biguanides to patients with hyperlipidemia lowers the level of blood cholesterol, triglycerides, and  $\beta$ -lipoproteins. It also inhibits the development of atherosclerosis, reduces hyperinsulinemia in men with coronary artery disease. It increases hypothalamo-pituitary sensitivity to inhibition by dexamethasone and estrogens, causes restoration of estrous cycle in persistent-estrous old rats, improves cellular immunity in atherosclerotic and cancer patients, lowers blood IGF-1 levels in cancer and atherosclerotic patients with Type IIb hyperlipoproteinemia (Dilman 1994). There are also data on the antioxidative effect of biguanides (Mattson et al. 2001; Gargiulo et al. 2002) and their neuroprotective activity (Lee et al. 2002). It was shown that biguanides inhibit complex I of the respiratory chain in mitochondria that leads to an activation of physiological intracellular inhibition of mitochondrial respiration (El Mir et al. 2000). Biguanides stimulate a protein kinase cascade inhibiting expression of the transcription factor SREBP-1. Activation of this factor with cholesterol



**Fig. 2** The effect of metformin on insulin/IGF-1 signaling in rodents

leads to an increase in transcription of genes coding lipogenesis enzymes and to suppression of free fatty acid oxidation. Thus, stimulation of uptake of glucose in tissues by biguanides inhibits lipogenesis and activates oxidation of FFA (Zhou et al. 2001). It was also shown that in vivo biguanides reduce appetite (Paolisso et al. 1998) and serum levels of leptin and IGF-1 (Fruehwald-Schultes et al. 2002). It was suggested that biguanides regulate energy balance of the organism at the fat tissue level (Mick et al. 2000). In general, results of biguanide treatment effects seem very similar to those of calorie restriction (Fig. 2). Their use as mimetics may therefore be preferable to the hard discipline of calorie restriction itself and increase compliance for optimal anti-aging and anti-cancer results.

**Conclusions**

Thus, data available in the literature and obtained in our own experiments suggest that many of the hormonal and metabolic shifts in the organism, as well as alterations to immunity, and dysregulation at the tissue and cellular levels are common to both natural aging and different types of carcinogenesis in vivo. Carcinogens could be supposed to initiate transformation of a normal cell, interacting with its elements on the molecular level, on the one hand, and to produce diverse changes in the organism facilitating promotion and progression of tumor growth, on the

other. The logical consequences of this hypothesis are that what we observe as “normal” or “natural” aging is in fact also a product of environmental exposures throughout life, at least to some degree.

**Acknowledgments** This paper evolved from discussions initiated at the The European Conference on Cancer and Aging—SeneCa, senescence and cancer—which took place in Warsaw, Poland between 4th and 6th October 2007, supported by the European Commission (contract LSSM-CT-2006-037312). For a summary of the main presentations at the conference, see Pawelec and Solana (2008). VA is supported by grant NS-5054.2006.4 from the President of The Russian Federation. ES was supported by the Ministry of Science and Higher Education (grant N301 008 32/0549).

## References

- Alexandrov VA, Anisimov VN, Belous NM et al (1980) The inhibition of the transplacental blastomogenic effect of nitrosomethylurea by postnatal administration of bufornin to rats. *Carcinogenesis* 1:975–978
- Anisimov VN (1983) Carcinogenesis and aging. *Adv Cancer Res* 40:265–324
- Anisimov VN (1987) Carcinogenesis and aging, vol 1 and 2. CRC, Boca Raton
- Anisimov VN (1994) The sole DNA damage induced by bromodeoxyuridine is sufficient for initiation of both aging and carcinogenesis in vivo. *Ann N Y Acad Sci* 719:494–501. doi:10.1111/j.1749-6632.1994.tb56854.x
- Anisimov VN (1995) Effect of aging and interval between primary and secondary treatment in carcinogenesis induced by neonatal exposure to 5-bromodeoxyuridine and subsequent administration of N-nitrosomethylurea in rats. *Mutat Res* 316:173–187
- Anisimov VN (2003a) Insulin/IGF-1 signaling pathway driving aging and cancer as a target for pharmacological intervention. *Exp Gerontol* 38:1041–1049. doi:10.1016/S0531-5565(03)00169-4
- Anisimov VN (2003b) Molecular and physiological mechanisms of aging. Nauka, St. Petersburg
- Anisimov VN (2003c) The relationship between aging and carcinogenesis: a critical appraisal. *Crit Rev Oncol Hematol* 45:277–304. doi:10.1016/S1040-8428(02)00121-X
- Anisimov VN (2006) Effect of host age on tumor growth rate in rodents. *Front Biosci* 11:412–422. doi:10.2741/1808
- Anisimov VN (2008) Antidiabetic drugs in aging and cancer: results and perspectives. *Open Aging J* 2:36–48
- Anisimov VN, Osipova GY (1992) Effect of neonatal exposure to 5-bromo-2'-deoxyuridine on life span, estrus function and tumor development in rats—an argument in favor of the mutation theory of aging? *Mutat Res* 275:97–110
- Anisimov VN, Zhukovskaya NV, Loktionov AS, Vakhtin YB (1988) Influence of host age on lung colony forming capacity of injected rat rhabdomyosarcoma cells. *Cancer Lett* 40:77–82. doi:10.1016/0304-3835(88)90264-9
- Anisimov VN, Zhukovskaya NV, Loktionov AS, Kaminskaya E, Vakhtin YB (1995) Host and donor age dependency of colony forming capacity of lung-affine rat rhabdomyosarcoma RA-2 cells. Abstracts of the international conference on tumor microenvironment: progression, therapy and prevention. Tiberias, Israel:6
- Anisimov SV, Volkova MV, Lenskaya LV et al (2001) Age-associated accumulation of the Apolipoprotein C-III gene T-455C polymorphism C allele in a Russian population. *J Gerontol Biol Sci* 56A:B27–B32
- Anisimov VN, Semenchenko AV, Yashin AI (2003) Insulin and longevity: antidiabetic biguanides as geroprotectors. *Biogerontology* 4:297–307. doi:10.1023/A:1026299318315
- Anisimov VN, Berstein LM, Egorin PA, Piskunova TS, Popovich IG, Zabezhinski MA, Kovalenko IG, Poroshina TE, Semenchenko AV, Provinciali M, Re F, Franceschi C (2005a) Effect of metformin on life span and on the development of spontaneous mammary tumors in HER-2/neu transgenic mice. *Exp Gerontol* 40:685–693. doi:10.1016/j.exger.2005.07.007
- Anisimov VN, Ukrainitseva SV, Yashin AI (2005b) Cancer in rodents: does it tell us about cancer in humans? *Nat Rev Cancer* 5:807–819. doi:10.1038/nrc1715
- Anisimov VN, Berstein LM, Egorin PA, Piskunova TS, Popovich IG, Zabezhinski MA, Tyndyk ML, Yurova MV, Kovalenko IG, Poroshina TE, Semenchenko AV (2008) Metformin slows down aging and extends life span of female SHR mice. *Cell Cycle* 7:2769–2773
- Arutjunyan AV, Kerkeshko GO, Anisimov VN et al (2001) Disturbances of diurnal rhythms of biogenic amines contents in hypothalamic nuclei as an evidence of neurotropic effects of enterotropic carcinogen 1,2-dimethylhydrazine. *Neuroendocr Lett* 22:229–237
- Balducci L, Ershler WB (2005) Cancer and ageing: a nexus at several levels. *Nat Rev Cancer* 5:655–662. doi:10.1038/nrc1675
- Barbieri M, Rizzo MR, Manzella D et al (2003) Glucose regulation and oxidative stress in healthy centenarians. *Exp Gerontol* 38:137–143
- Bartke A, Chandrashekar V, Dominici F, Turyn D, Kinney B, Steger R, Kopchick JJ (2003) Insulin-like growth factor 1 (IGF-1) and aging: controversies and new insights. *Biogerontology* 4:1–8. doi:10.1023/A:1022448532248
- Bartsch C, Bartsch H, Blask D, Cardinali DP, Hrushevsky WJM, Mecke D (eds) (2001) The pineal gland and cancer. Springer, Berlin
- Barzilai N, Gupta G (1999) Interaction between aging and syndrome X: new insights on the pathophysiology of fat distribution. *Ann N Y Acad Sci* 892:58–72. doi:10.1111/j.1749-6632.1999.tb07785.x
- Bataller M, Mendez C, Salas JA, Portugal J (2008) Mithramycin SK modulates polyploidy and cell death in colon carcinoma cells. *Mol Cancer Ther* 7:2988–2997. doi:10.1158/1535-7163.MCT-08-0420
- Berenblum I (1975) Sequential aspects of chemical carcinogenesis: skin. In: Becker J (ed) *Cancer—a comprehensive treatise*. Plenum, New York, pp 323–344
- Berns A (2002) Senescence: a companion in chemotherapy? *Cancer Cell* 1:309–311. doi:10.1016/S1535-6108(02)00063-6
- Berstein LM (2005) Clinical usage of hypolipidemic and antidiabetic drugs in the prevention and treatment of cancer. *Cancer Lett* 224:203–212. doi:10.1016/j.canlet.2004.11.011

- Berstein LM, Kvatchevskaya JO, Poroshina TE, Kovalenko IG, Tsyrlina EV, Zimarina TS, Ourmantcheeva AF, Ashrafian L, Thijssen JH (2004) Insulin resistance, its consequences for clinical course of the disease and possibilities of correction in endometrial cancer. *J Cancer Res Clin Oncol* 130:687–693
- Bluhner M, Kahn BB, Kahn CR (2003) Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299:572–574
- Bordin P, Da Gol PG, Peruzzo P et al (1999) Causes of death and clinical diagnostic error in extreme aged hospitalized people: a retrospective clinical-necropsy survey. *J Gerontol Med Sci* 54A:M554–M559
- Bowker SL, Majumdar SR, Veugelers P, Johnson JA (2006) Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. *Diabetes Care* 29:254–258. doi:10.2337/diacare.29.02.06.dc05-1558
- Butov AA, Volkov MA, Anisimov VN, Sehl ME, Yashin AI (2002) A model of accelerated aging induced by 5-bromo-deoxyuridine. *Biogerontology* 3:175–182. doi:10.1023/A:1015647225196
- Campisi J (2000) Cancer, aging, and cellular senescence. *In Vivo* 14:183–188
- Campisi J (2003) Cellular senescence and apoptosis: how cellular responses might influence aging phenotypes. *Exp Gerontol* 38:5–11. doi:10.1016/S0531-5565(02)00152-3
- Campisi J (2005) Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* 120:513–522. doi:10.1016/j.cell.2005.02.003
- Campisi J, Kim S, Lim CS, Rubio M (2001) Cellular senescence, cancer and aging: the telomere connection. *Exp Gerontol* 36:1619–1637. doi:10.1016/S0531-5565(01)00160-7
- Chang BD, Xuan Y, Broude EV, Zhu H, Schott B, Fang J, Roninson IB (1999) Role of p53 and p21waf1/cip1 in senescence-like terminal proliferation arrest induced in human tumor cells by chemotherapeutic drugs. *Oncogene* 18:4808–4818. doi:10.1038/sj.onc.1203078
- Chiba T, Yamaza H, Higami Y, Shimokawa I (2002) Anti-aging effects of caloric restriction: involvement of neuroendocrine adaptation by peripheral signaling. *Microsc Res Tech* 59:317–324. doi:10.1002/jemt.10211
- Choi J, Shendrik I, Peacocke M (2000) Expression of senescence-associated beta-galactosidase in enlarged prostates from men with benign prostatic hyperplasia. *Urology* 56:160–166. doi:10.1016/S0090-4295(00)00538-0
- Colangelo LA, Gapstur SM, Gann PH (2002) Colorectal cancer mortality and factors related to the insulin resistance syndrome. *Cancer Epidemiol Biomarkers Prev* 11:385–391
- Coschigano KT, Clemmons D, Bellush LL, Kopchick JJ (2000) Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. *Endocrinology* 141:2608–2613
- Cristofalo VJ (2005) SA  $\beta$  Gal staining: biomarker or delusion. *Exp Gerontol* 40:836–838. doi:10.1016/j.exger.2005.08.005
- DePinho RA (2000) The age of cancer. *Nature* 408:248–254. doi:10.1038/35041694
- Derhovanessian E, Solana R, Larbi A, Pawelec G (2008) Immunity, ageing and cancer. *Immun Ageing* 15:11
- Dillin A, Crawford DK, Kenyon C (2002) Timing requirements for insulin/IGF-1 signaling in *C. elegans*. *Science* 298:830–834. doi:10.1126/science.1074240
- Dilman VM (1971) Age-associated elevation of hypothalamic threshold to feedback control and its role in development, aging and disease. *Lancet* 1:1211–1219. doi:10.1016/S0140-6736(71)91721-1
- Dilman VM (1994) Development, aging, and disease. A new rationale for an intervention strategy. Harwood, Chur
- Dilman VM, Anisimov VN (1979a) Hypothalamic mechanisms of ageing and of specific age pathology-I. Sensitivity threshold of hypothalamo-pituitary complex to homeostatic stimuli in the reproductive system. *Exp Gerontol* 14:161–174. doi:10.1016/0531-5565(79)90015-9
- Dilman VM, Anisimov VN (1979b) Potentiation of antitumor effect of cyclophosphamide and hydrazine sulfate by treatment with the antidiabetic agent, 1-phenylethylbiguanide (phenformin). *Cancer Lett* 7:357–361. doi:10.1016/S0304-3835(79)80066-X
- Dilman VM, Anisimov VN (1980) Effect of treatment with phenformin, diphenylhydantoin or L-DOPA on life span and tumor incidence in C3H/Sn mice. *Gerontology* 26:241–245
- Dimri GP (2005) What has senescence got to do with cancer? *Cancer Cell* 7:505–512. doi:10.1016/j.ccr.2005.05.025
- Dimri GP, Lee X, Basile G, Acosta M (1995) A novel biomarker identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci USA* 92:9363–9367. doi:10.1073/pnas.92.20.9363
- Dirks AJ, Leeuwenburgh C (2005) Caloric restriction in humans: potential pitfalls and health concerns. *Mech Ageing Dev* 127:1–7. doi:10.1016/j.mad.2005.09.001
- Dix D, Cohen P (1999) On the role of aging in carcinogenesis. *Anticancer Res* 19:723–726
- Dix D, Cohen P, Flannery J (1980) On the role of aging in cancer incidence. *J Theor Biol* 83:163–173
- Dominici FP, Arosegui Diaz G, Bartke A (2000) Compensatory alterations of insulin signal transduction in liver of growth hormone receptor knockout mice. *J Endocrinol* 166:579–590. doi:10.1677/joe.0.1660579
- Dominici FP, Hauck S, Argenton DP (2002) Increased insulin sensitivity and upregulation of insulin receptor, insulin receptor substrate (ISR)-1 and IRS-2 in liver of Ames dwarf mice. *J Endocrinol* 173:81–94. doi:10.1677/joe.0.1730081
- El Mir MY, Nogueira V, Fontaine E (2000) Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. *J Biol Chem* 275:223–228. doi:10.1074/jbc.275.1.223
- Eom YW, Kim MA, Park SS, Goo MJ, Kwon HJ, Sohn S, Kim WH, Yoon G, Choi KS (2005) Two distinct modes of cell death induced by doxorubicin: apoptosis and cell death through mitotic catastrophe accompanied by senescence-like phenotype. *Oncogene* 24:4765–4777. doi:10.1038/sj.0nc.1208627
- Erenpreisa J, Cragg MS (2007) Cancer: a matter of life cycle? *Cell Biol Int* 31:1507–1510. doi:10.1016/j.cellbi.2007.08.013
- Evans JMM, Donnely LA, Emslie-Smith AM (2005) Metformin and reduced risk of cancer in diabetic patients. *BMJ* 330:1304–1305. doi:10.1136/bmj.38415.708634.F7
- Facchini FS, Hua NW, Reaven GM, Stoohs RA (2000) Hyperinsulinemia: the missing link among oxidative stress and age-related diseases? *Free Radic Biol Med* 29:1302–1306. doi:10.1016/S0891-5849(00)00438-X

- Flurkey K, Papaconstantinou J, Miller RA, Harrison DE (2001) Life-span extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc Natl Acad Sci USA* 98:6736–6741
- Fruehwald-Schultes B, Oltmanns KM, Toschek B (2002) Short-term treatment with metformin decreases serum leptin concentration without affecting body weight and body fat content in normal-weight healthy men. *Metabolism* 51:531–536. doi:10.1053/meta.2002.31332
- Gargiulo P, Caccese D, Pignatelli P et al (2002) Metformin decreases platelet superoxide anion production in diabetic patients. *Diabetes Metab Res Rev* 18:156–159
- Gravekamp C, Kim SH, Castro F (2008) Cancer vaccination: manipulation of immune responses at old age. *Mech Ageing Dev*. doi:10.1016/j.mad.2008.05.003
- Gupta K, Krishnaswamy G, Karnad A, Peiris AN (2002) Insulin: a novel factor in carcinogenesis. *Am J Med Sci* 323:140–145
- Hahn WC, Weinberg RA (2002) Modeling the molecular circuitry of cancer. *Nat Rev Cancer* 2:331–341. doi:10.1038/nrc795
- Hamilton ML, Van Remmen H, Drake JA (2001) Does oxidative damage to DNA increase with age? *Proc Natl Acad Sci USA* 98:10469–10474. doi:10.1073/pnas.171202698
- Han Z, Wei W, Dunaway S, Darnowski JW, Calabresi P, Sedivy J, Hendrickson EA, Balan KV, Pantazis P, Wyche JH (2002) Role of p21 in apoptosis and senescence of human colon cancer cells treated with camptothecin. *J Biol Chem* 277:17154–17160. doi:10.1074/jbc.M112401200
- Hanahan D, Weinberg RA (2000) The hallmarks of cancer. *Cell* 100:57–70
- Harman D (1998) Extending functional life span. *Exp Gerontol* 33:95–112. doi:10.1159/000028983
- Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM (2006) Cellular senescence in aging primates. *Science* 311:1257. doi:10.1126/science.1122446
- Holzenberger M, Dupond J, Ducos B (2003) IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 421:182–187. doi:10.1038/nature01298
- Hsieh CC, DeFord JH, Flurkey K (2002a) Effects of the Pit1 mutation on the insulin signaling pathway: implications on the longevity of the long-lived Snell dwarf mouse. *Mech Ageing Dev* 123:1245–1255. doi:10.1016/S0047-6374(02)00037-4
- Hsieh CC, DeFord JH, Flurkey K et al (2002b) Implications for the insulin signaling pathway in Snell dwarf mouse longevity: a similarity with the *C. elegans* longevity paradigm. *Mech Ageing Dev* 123:1229–1244
- Hu M, Polyak K (2008) Microenvironmental regulation of cancer development. *Curr Opin Genet Dev* 18:27–34. doi:10.1016/j.gde.2007.12.006
- Ikeno Y, Bronson RT, Hubbard GB (2003) Delayed occurrence of fatal neoplastic diseases in Ames dwarf mice: correlation to extended longevity. *J Gerontol A Biol Sci Med Sci* 58:B291–B296
- Itahana K, Campisi J, Dimri GP (2004) Mechanisms of cellular senescence in human and mouse cells. *Biogerontology* 5:1–10. doi:10.1023/B:BGEN.0000017682.96395.10
- Jackson JG, Pereira-Smith OM (2006) Primary and compensatory roles for RB family members at cell cycle gate promoters that are deacetylated and downregulated in doxorubicin-induced senescence of breast cancer cells. *Mol Cell Biol* 26:2501–2510. doi:10.1128/MCB.26.7.2501-2510.2006
- Kawanishi S, Hiraki Y, Oikawa S (2001) Mechanism of guanine-specific DNA damage by oxidative stress and its role in carcinogenesis and aging. *Mutat Res* 488:65–67. doi:10.1016/S1383-5742(00)00059-4
- Kenyon C (2001) A conserved regulatory system for aging. *Cell* 105:165–168
- Kim JH, Kim JH, Lee GE, Kim SW, Chung IK (2003) Identification of a quinoxaline derivative that is a potent telomerase inhibitor leading to cellular senescence of human cancer cells. *Biochem J* 373:523–529. doi:10.1042/BJ20030363
- Krtolica A, Parinello S, Lockett S, Campisi J (2001) Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc Natl Acad Sci USA* 98:12072–12077. doi:10.1073/pnas.211053698
- Lane MA, Tilmont EM, De Angelis H (2000) Short-term calorie restriction improves disease-related markers in older male rhesus monkeys (*Macaca mulatta*). *Mech Ageing Dev* 112:185–196. doi:10.1016/S0047-6374(99)00087-1
- Lee J, Chan SL, Lane MA, Mattson MP (2002) Phenformin suppresses calcium responses to glutamate and protects hippocampal neurons against excitotoxicity. *Exp Neurol* 175:161–167. doi:10.1006/exnr.2002.7864
- Lipman RD, Dallal GE, Bronson RT (1999a) Lesion biomarkers of aging in B6C3F1 hybrid mice. *J Gerontol Med Sci* 54A:466–477
- Lipman RD, Dallal GE, Bronson RT (1999b) Effect of genotype and diet on age-related lesions in ad libitum fed and calorie-restricted F344, BN, and BNF3F1 rats. *J Gerontol Med Sci* 54A:478–491
- Mattson MP, Duan W, Lee J et al (2001) Progress in the development of calorie restriction mimetic dietary supplements. *J Anti-Aging Med* 4:225–232
- McCullough KD, Coleman WB, Smith GJ, Grisham JW (1994) Age-dependent regulation of the tumorigenic potential of neoplastically transformed rat liver epithelial cells by the liver micro-environment. *Cancer Res* 54:3668–3671
- Michishita E, Nakabayashi K, Suzuki T (1999) 5-bromodeoxyuridine induces senescence-like phenomena in mammalian cells regardless of cell type or species. *J Biochem* 125:1052–1059
- Mick GJ, Wang X, Ling FC, McCormick KL (2000) Inhibition of leptin secretion by insulin and metformin in cultured rat adipose tissue. *Biochim Biophys Acta* 1502:426–432
- Mikhlin AE, Barchuk AS, Wagner RI (2004) Kinetics of visual growth of skin melanoma. *Russ Oncol J* 2:29–32
- Miller RA (1991) Gerontology as oncology. *Cancer* 68:2496–2501. doi:10.1002/1097-0142(19911201)68:11+<2496::AID-CNCR2820681503>3.0.CO;2-B
- Minagawa S, Nakabayashi K, Fujii M et al (2004) Functional and chromosomal clustering of genes responsive to 5-bromodeoxyuridine in human cells. *Exp Gerontol* 39:1069–1078
- Minagawa S, Nakabayashi K, Fujii M (2005) Early BrdU-responsive genes constitute a novel class of senescence-

- associated genes in human cells. *Exp Cell Res* 304:552–558. doi:[10.1016/j.yexcr.2004.10.036](https://doi.org/10.1016/j.yexcr.2004.10.036)
- Mishima K, Handa JT, Aotaki-Keen A (1999) Senescence-associated beta-galactosidase histochemistry for the primate eye. *Investig Ophthalmol Vis Sci* 40:1590–1593
- Moolgavkar S, Krewski D, Zeise L (eds) (1999) Quantitative estimation and prediction of human cancer risk. IARC Sci Publ No 131. IARC, Lyon
- Morris SH (1991) The genetic toxicology of 5-bromodeoxyuridine in mammalian cells. *Mutat Res* 258:161–188
- Muntoni S (1999) Metformin and fatty acids. *Diabetes Care* 22:179–180
- Napalkov NP (2004) Cancer and demographic transition. *Vopr Onkol* 50:127–144
- Napalkov NP, Anisimov VN, Likhachev AJ, Tomatis L (1989) 5-bromodeoxyuridine-induced carcinogenesis and its modification by persistent estrus syndrome, unilateral nephrectomy, and X-irradiation in rats. *Cancer Res* 49:318–323
- Narita M, Lowe SW (2005) Senescence comes of age. *Nat Med* 11:920–922. doi:[10.1038/nm0905-920](https://doi.org/10.1038/nm0905-920)
- Niculescu AB, Chen X, Smeets M, Hengst L, Prives C, Reed SI (1998) Effects of p21(Cip1/Waf1) at both the G1/S and the G2/M cell cycle transitions: pRb is a critical determinant in blocking DNA replication and in preventing endoreduplication. *Mol Cell Biol* 18:629–643
- Ota H, Tokunaga E, Chang K, Hikasa M, Iijima K, Eto M, Kozaki K, Akishita M, Ouchi Y, Kaneki M (2006) Sirt1 inhibitor, Sirtinol, induces senescence-like growth arrest with attenuated Ras-MAPK signaling in human cancer cells. *Oncogene* 25:176–185
- Paolisso G, Amato L, Eccellente R et al (1998) Effect of metformin on food intake in obese subjects. *Eur J Clin Invest* 28:441–446. doi:[10.1046/j.1365-2362.1998.00304.x](https://doi.org/10.1046/j.1365-2362.1998.00304.x)
- Paradis V, Youssef N, Dargere D (2001) Replicative senescence in normal liver, chronic hepatitis C, and hepatocellular carcinomas. *Hum Pathol* 32:327–332. doi:[10.1053/hupa.2001.22747](https://doi.org/10.1053/hupa.2001.22747)
- Parkin DM, Bray FI, Devesa SS (2001) Cancer burden in the year 2000. The global picture. *Eur J Cancer* 37(Suppl 8):S4–S66. doi:[10.1016/S0959-8049\(01\)00267-2](https://doi.org/10.1016/S0959-8049(01)00267-2)
- Pawelec G, Solana R (2008) Are cancer and ageing different sides of the same coin? Conference on cancer and ageing. *EMBO Rep* 9:234–238. doi:[10.1038/embor.2008.12](https://doi.org/10.1038/embor.2008.12)
- Pawelec G, Koch S, Griesemann H, Rehbein A, Hähnel K, Gouttefangeas C (2006) Immunosenescence, suppression and tumour progression. *Cancer Immunol Immunother* 55:981–986. doi:[10.1007/s00262-005-0109-3](https://doi.org/10.1007/s00262-005-0109-3)
- Peto R, Parish SE, Gray RG (1985) There is no such thing as ageing, and cancer is not related to it, 58. In: Likhachev A, Anisimov V, Montesano R (eds) Age-related factors in carcinogenesis. IARC, Lyon, pp 43–53
- Ponten J (1977) Abnormal cell growth (neoplasia) and aging. In: Finch CE, Hayflick L (eds) Handbook of the biology of aging. van Nostrand Reinhold Co, New York, pp 536–560
- Puig PE, Guilly MN, Bouchot A, Droin N, Cathelin D, Bouyer F, Favier L, Ghiringhelli F, Kroemer G, Solary E, Martin F, Chauffert B (2008) Tumor cells can escape DNA-damaging cisplatin through DNA endoreduplication and reversible polyploidy. *Cell Biol Int* 32(9):1031–1043
- Rangarajan A, Weinberg RA (2003) Comparative biology of mouse versus human cells: modelling human cancer in mice. *Nat Rev Cancer* 3:952–959. doi:[10.1038/nrc1235](https://doi.org/10.1038/nrc1235)
- Rebbaa A, Zheng X, Chu F, Mirkin BL (2006) The role of histone acetylation versus DNA damage in drug-induced senescence and apoptosis. *Cell Death Differ* 13:1960–1967. doi:[10.1038/sj.cdd.4401895](https://doi.org/10.1038/sj.cdd.4401895)
- Ressler S, Bartkova J, Niederegger H, Bartek J, Scharffetter-Kochanek K, Jansen-Dürr P, Wlaschek M (2006) p16INK4A is a robust in vivo biomarker of cellular aging in human skin. *Aging Cell* 5:379–389. doi:[10.1111/j.1474-9726.2006.00231.x](https://doi.org/10.1111/j.1474-9726.2006.00231.x)
- Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells. *Nature* 414:105–111. doi:[10.1038/35102167](https://doi.org/10.1038/35102167)
- Richards JS, Russell DL, Ochsner S (2002) Novel signaling pathways that control ovarian follicular development, ovulation, and luteinization. *Recent Prog Horm Res* 57:195–220. doi:[10.1210/rp.57.1.195](https://doi.org/10.1210/rp.57.1.195)
- Roberson RS, Kussick SJ, Vallieres E, Chen SY, Wu DY (2005) Escape from therapy-induced accelerated cellular senescence in p53-null lung cancer cells and in human lung cancers. *Cancer Res* 65:2795–2803. doi:[10.1158/0008-5472.CAN-04-1270](https://doi.org/10.1158/0008-5472.CAN-04-1270)
- Roninson IB (2003) Tumor cell senescence in cancer treatment. *Cancer Res* 63:2705–2715
- Roth GS, Lane MA, Ingram D et al (2002) Biomarkers of caloric restriction may predict longevity in humans. *Science* 297:811
- Ruiz-Torres A, Soares de Melo Kirzner M (2002) Ageing and longevity are related to growth hormone/insulin-like growth factor-1 secretion. *Gerontology* 48:401–407
- Schmitt CA, Fridman JS, Yang M (2002) A senescent program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. *Cell* 109:335–346. doi:[10.1016/S0092-8674\(02\)00734-1](https://doi.org/10.1016/S0092-8674(02)00734-1)
- Schneider MB, Matsuzaki H, Harorah J (2001) Prevention of pancreatic cancer induction in hamsters by metformin. *Gastroenterology* 120:1263–1270. doi:[10.1053/gast.2001.23258](https://doi.org/10.1053/gast.2001.23258)
- Shay JW, Roninson IB (2004) Hallmarks of senescence in carcinogenesis and cancer therapy. *Oncogene* 23:2919–2933. doi:[10.1038/sj.onc.1207518](https://doi.org/10.1038/sj.onc.1207518)
- Shimokawa I, Higami Y, Utsuyama M et al (2002) Life span extension by reducing in growth hormone-insulin-growth factor-1 axis in a transgenic rat model. *Am J Pathol* 160:2259–2265
- Simpson AJG (1993) A natural somatic mutation frequency and human carcinogenesis. *Adv Cancer Res* 71:209–240. doi:[10.1016/S0065-230X\(08\)60100-1](https://doi.org/10.1016/S0065-230X(08)60100-1)
- Singer B, Grunberger D (1983) Molecular Biology of Mutagens and Carcinogens. Plenum, New York
- Sliwinka MA, Mosieniak G, Wolanin K, Babik A, Piwocka K, Magalska A, Szczepanowska J, Fronk J, Sikora E (2008) Induction of senescence with doxorubicin leads to increased genomic instability of HCT116 cells. *Mech Ageing Dev* (in press)
- Spindler SR (2006) Use of microarray biomarkers to identify longevity therapeutics. *Aging Cell* 5:39–50. doi:[10.1111/j.1474-9726.2006.00194.x](https://doi.org/10.1111/j.1474-9726.2006.00194.x)

- Srinivasan SV, Mayhew CN, Schwemberger S, Zagorski W, Knudsen ES (2007) RB loss promotes aberrant ploidy by deregulating levels and activity of DNA replication factors. *J Biol Chem* 282:23867–23877. doi:[10.1074/jbc.M700542200](https://doi.org/10.1074/jbc.M700542200)
- Staats J (1980) Standardized nomenclature for inbred strains of mice: seventh listing. *Cancer Res* 40:2083–2128
- Storchova Z, Pellman D (2004) From polyploidy to aneuploidy, genome instability and cancer. *Nat Rev Mol Cell Biol* 5:45–54. doi:[10.1038/nrm1276](https://doi.org/10.1038/nrm1276)
- Sundaram M, Guernsey DL, Rajaraman MM, Rajaraman R (2004) Neosis: a novel type of cell division in cancer. *Cancer Biol Ther* 3:207–218
- Suzuki T, Minagawa S, Michishita E et al (2001) Induction of senescence-associated genes by 5-bromodeoxyuridine in HeLa cells. *Exp Gerontol* 36:465–474. doi:[10.1016/S0531-5565\(00\)00223-0](https://doi.org/10.1016/S0531-5565(00)00223-0)
- Tatar M, Bartke A, Antebi A (2003) The endocrine regulation of aging by insulin-like signals. *Science* 299:1346–1351
- te Poele RH, Okorokov AL, Jardine L, Cummings J, Joel SP (2002) DNA damage is able to induce senescence in tumor cells in vitro and in vivo. *Cancer Res* 62:1876–1883
- Tomatis L (ed) (1990) *Cancer: causes, occurrence and control*. IARC Sci Publ No100. IARC, Lyon
- Touitou Y (2001) Human aging and melatonin. Clinical relevance. *Exp Gerontol* 36:1083–1100
- Toyokuni SM (2008) Molecular mechanisms of oxidative stress-induced carcinogenesis: from epidemiology to oxygenomics. *IUBMB Life* 60:441–447. doi:[10.1002/iub.61](https://doi.org/10.1002/iub.61)
- Ulrich P, Cerami A (2001) Protein glycation, diabetes, and aging. *Recent Progr Horm Res* 56:1–21
- Vasile E, Tomita Y, Brown LF (2001) Differential expression of thymosin beta-1- by early passage and senescent vascular endothelium is modulated by VPF/VEGF: evidence for senescent endothelial cells in vivo at sited of atherosclerosis. *FASEB J* 15:6449–6465. doi:[10.1096/fj.00-0051com](https://doi.org/10.1096/fj.00-0051com)
- Vijg J, Campisi J (2008) Puzzles, promises and a cure for ageing. *Nature* 454:1065–1071
- Vogel C, Kienitz A, Hofmann I, Muller R, Bastians H (2004) Crosstalk of the mitotic spindle assembly checkpoint with p53 to prevent polyploidy. *Oncogene* 23:6845–6853. doi:[10.1038/sj.onc.1207860](https://doi.org/10.1038/sj.onc.1207860)
- von Wangenheim KH, Peterson HP (1998) Control of cell proliferation by progress in differentiation: clues to mechanisms of aging, cancer causation and therapy. *J Theor Biol* 193:663–678. doi:[10.1006/jtbi.1998.0731](https://doi.org/10.1006/jtbi.1998.0731)
- von Zglinicki T, Burkle A, Kirkwood TBL (2001) DNA damage and ageing—an integrative approach. *Exp Gerontol* 36:1049–1062. doi:[10.1016/S0531-5565\(01\)00111-5](https://doi.org/10.1016/S0531-5565(01)00111-5)
- Walter S, Boley G, Bühring H-J, Koch S, Wernet D, Zippelius A, Pawelec G, Romero P, Stevanović S, Rammensee H-G, Gouttefangeas C (2005) High frequencies of functionally impaired cytokeratin 18-specific CD8<sup>+</sup> T cells in healthy HLA-A2<sup>+</sup> donors. *Eur J Immunol* 35:2876–2885. doi:[10.1002/eji.200526207](https://doi.org/10.1002/eji.200526207)
- Ward JM (1983) Background data and variations in tumor rates of control rats and mice. *Prog Exp Tumor Res* 26:241–264
- Ward JM, Henneman JR, Osipova GY, Anisimov VN (1991) Persistence of 5-bromo-2'-deoxyuridine in tissues of rats after exposure in early life. *Toxicology* 70:345–352. doi:[10.1016/0300-483X\(91\)90008-0](https://doi.org/10.1016/0300-483X(91)90008-0)
- Weinberg RA (2008) Mechanisms of malignant progression. *Carcinogenesis* 29:1092–1095. doi:[10.1093/carcin/bgn104](https://doi.org/10.1093/carcin/bgn104)
- Weindruch R, Sohal RS (1997) Caloric intake and aging. *N Engl J Med* 337:986–994. doi:[10.1056/NEJM199710023371407](https://doi.org/10.1056/NEJM199710023371407)
- Wu Y, Yakar S, Zhao L, Hennighausen L et al (2002) Circulating insulin-like growth factor-I levels regulate colon cancer growth and metastasis. *Cancer Res* 62:1030–1035
- Xu WS, Perez G, Ngo L, Gui CY, Marks PA (2005) Induction of polyploidy by histone deacetylase inhibitor: a pathway for antitumor effects. *Cancer Res* 65:7832–7839. doi:[10.1158/0008-5472.CAN-04-4313](https://doi.org/10.1158/0008-5472.CAN-04-4313)
- Zhou G, Myers R, Li Y (2001) Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 108:1167–1174