



Ettore "Ted" DeGrazia. *Three Navajo Women*. Watercolor, 13" × 16½". Courtesy of DeGrazia Foundation.

Aging is associated with a number of events at the molecular, cellular, and physiologic levels that influence carcinogenesis and subsequent cancer growth.

Biology of Aging and Cancer

Vladimir N. Anisimov, MD

Background: *The incidence of cancer increases with age in both humans and laboratory animals. A clear understanding of the causes of the age-related increase in cancer incidence is needed to develop a strategy for primary cancer prevention.*

Methods: *We summarized the data available in the literature and our own experience in hormonal metabolic shifts in organisms and disturbances at tissue and cellular levels observed in natural aging and in different types of carcinogenesis in vivo.*

Results: *There are incongruent 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 by at least two mechanisms: tissue accumulation of cells in late stages of carcinogenesis and alterations in internal homeostasis, in particular, alterations in immune and endocrine system. Increased susceptibility to the effects of tumor promoters is found in both aged animals and aged humans, as predicted by the multistage model of carcinogenesis.*

Conclusions: *Aging is associated with a number of events at the molecular, cellular and physiologic levels that influence carcinogenesis and subsequent cancer growth. A clearer understanding of these events will help in predicting and treating cancer more effectively.*

From the Department of Carcinogenesis and Oncogerontology at the N.N. Petrov Research Institute of Oncology, St. Petersburg, Russia. Submitted March 29, 2006; accepted November 1, 2006.

Address correspondence to Vladimir N. Anisimov, MD, Department of Carcinogenesis and Oncogerontology at the N.N. Petrov Research Institute of Oncology, Pesochbny-2, St. Petersburg 197758, Russia. E-mail: aging@mail.ru

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Abbreviations used in this paper: BrdUrd = 5-bromodeoxyuridin.

Introduction

It is well documented that the incidence of malignant tumors increases progressively with age, in both animals and humans.¹⁻⁶ 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.⁷ 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 neoplasms and the growth of already existent but latent malignant cells.^{1,2,8-11} These mechanisms may also include proliferative senescence, as the senescent cells lose their ability to undergo apoptosis and produce some factors that stimulate epithelial cells with oncogenic mutations.¹² The third hypothesis, which practically joins these two hypotheses, 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 milieu.¹³ The elucidation of the causes of the age-related increase in cancer incidence may be the key to a strategy for primary cancer prevention.

Aging is associated with a number of events at the molecular, cellular, and physiologic levels that influence carcinogenesis and subsequent cancer growth.^{5,8}

Interaction of Aging and Carcinogenesis: Molecular Level

Oxydative Damage

One of the more developed theories of aging is the free radical theory proposed in 1954 by D. Harman.¹⁴ This theory postulates that various oxidative reactions occurring in the organism (mainly in mitochondria) generate free radicals as a byproduct. This causes multiple lesions in macromolecules (nucleic acids, proteins, and lipids), leading to their damage and aging. This theory explains not only the mechanism of aging per se, but also a wide variety of age-associated pathologies, including cancer.¹⁴⁻¹⁶ Recent evidence suggests that key mechanisms of both aging and cancer are linked via endogenous stress-induced DNA damage caused by reactive oxygen species. The links include oxydative nuclear and mitochondrial DNA damage and repair, telomere shortening, and telomere-driven cellular senescence. These have been discussed in a number of comprehensive reviews.¹⁵⁻¹⁷ It is worthy to note that free radical processes also play a critical role in chemical- and radiation-induced carcinogenesis.^{8,17}

DNA Damage and Repair

The intensity of natural damages to DNA is high. 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.¹⁸ As a result, permanently active mechanisms of DNA repair have evolved. However, both of the most intensive natural mutation processes (depurination and deamination) rarely involve thymidine,¹⁸ and therefore the reparation schemes for thymidine may have evolved less intensely.

Hence, if we want to induce uniformly distributed point mutations (and simultaneously minimize damages in other structures) in laboratory animals, it is suitable to use analogs of thymidine as a mutagen.

Some in vitro and in vivo effects of the thymidine analog 5-bromodeoxyuridine (BrdUrd) suggest that BrdUrd be used to investigate the role of selective DNA damage in both carcinogenesis and aging. BrdUrd is incorporated into replicating DNA in place of thymidine, and this effect is mutagenic.¹⁹ Assuming a fairly even level of BrdUrd incorporation into the DNA of various tissues of neonatal rats and long-term persistence in them,²⁰ 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 switch and proliferation.²¹ These changes in cellular function may favor tumor development.

In our series of experiments, 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.²⁰⁻²³ The exposure to BrdUrd was followed by a decrease in the mean lifespan of the animals of 38% in males and 27% in females and by an increase in the rate of aging (calculated according to the Gompertz equation) in comparison to controls. The monitoring of estrus showed an acceleration of natural age-related switching-off of reproductive function in female rats due to disturbances in the central regulation of gonadotropic function in the pituitary. The exposure of rats to BrdUrd was followed by signs of immunodepression and by an increase in the incidence of chromosome aberrations and spontaneous tumors. The latency of these tumors was decreased. In the offspring of rats neonatally treated with BrdUrd, an increased incidence of congenital malformations and spontaneous tumors and accelerated aging were both 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 *N*-nitrosomethylurea (NMU), X-irradiation, urethane, estradiol-benzoate, persistent estrus syndrome, and 12-O-tetradecanoylphorbol-13-acetate (TPA).²¹⁻²³ Our data provided evidence that a sole perturbation of DNA induced by BrdUrd contributed substantially to the initiation of tumorigenesis and the acceleration of aging.

BrdUrd was found to induce in vitro a flat and enlarged cell shape characteristic of senescent cells and senescence-associated beta-galactosidase accumulation 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 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 beta-galactosidase cell lines, but accelerated telomere shortening was not observed in tumor cell lines.^{24,25} These results suggest that BrdUrd induced senescence-like phenotypic resemblance in both mortal and immortal mammalian cells and, possibly, activated a common senescence pathway present in both types of cells.²⁵

The level of gene expression in HeLa cells and normal human diploid fibroblasts, TIG7 cells, exposed to BrdUrd has been examined.²⁵ BrdUrd induced 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 extracellular matrix, cell cycle progression, and metabolism of intracellular compounds essential for normal cell growth. The authors believe that this observation can explain features characteristic of normally senescent cells, eg, specific morphological changes and the cell cycle arrest at the G₁/S boundary, and it supports their view that BrdUrd induces a senescence-like phenomenon. In more recent in vitro experiments, it was shown that BrdUrd clearly activates a silenced transgene integrated in HeLa cells.²⁵ The authors suggest that similar mechanisms may operate in the regulation of the 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 phenotype in any type of immortal cells. Recently, Minagawa et al²⁶ showed that BrdUrd immediately and dramatically induces senescence-associated genes in human cells.

Mathematical modeling of the processes of aging and carcinogenesis in tissues based on the experimental data on in vivo exposure to BrdUrd^{22,23,27} has been considered.²⁸ Modeling was carried out on the basis of recurrent algorithms constructed on stochastic equations in terms of semimartingale characteristics of the processes. The results confirm the conclusion that under BrdUrd treatment, there is an accelerated aging in tissues with proliferating cells and an increment of death caused by tumor growth. These results can serve as an indirect validation of the hypothesis that the level of tissue damage during mutagenesis and oxidative stress influence both the rate of aging and the rate of carcinogenesis. The above-mentioned observation on in vitro-inducing cellular senescence effect of BrdUrd^{24,26} is in agreement with this conclusion.

Interaction of Aging and Carcinogenesis: Cellular Level

The term “cellular senescence,” originally defined as a series of 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.²⁹ Senescence is a potent anticarcinogenic program, and the process of neoplastic transformation involves a series of events that allow cells to bypass senescence.^{12,29-31} Although the relationships between cellular senescence and aging in vivo is not yet clear, senescent cells were reported to accumulate in aging tissues: in human skin and liver³²⁻³⁴ and in primate retina.³⁵ Senescent cells were demonstrated capable of stimulating the malignant progression of premalignant keratinocytes and mammary gland epithelial cells.³⁶ Senescent cells have also been detected at sites of age-related pathology, including benign hyperplastic prostate³⁷ and atherosclerotic lesions.³⁸

Cellular senescence is controlled by the tumor suppressor proteins p53 and pRb.^{12,31} Inactivation of these proteins results in bypass of senescence. Due to its essentially irreversible growth arrest and the requirement for p53 and pRb function, cellular senescence is considered a potent tumor suppressor mechanism.^{12,30,31,39,40} Numerous studies, primarily in human fibroblasts, suggest that telomere shortening is the primary cause of replicative senescence.^{12,41}

Cellular senescence can be induced by a variety of extrinsic factors, such as X-ray irradiation, UV-irradiation, H₂O₂, ectopic expression of certain oncogenes (Ras, Raf, ets2, E2F1) and tumor suppressors (p16, p14, p53, PPML).⁴¹ Recent reports suggest that cellular senescence program controlled by p53 and p16^{INK4a} may be one of the mechanisms by which cancer chemotherapy drugs work in vivo.^{29,42}

Some observations failed to support the existence in vivo of a significant number of cells with the phenotype observed during replicative aging.⁴³ Cristofalo⁴³ was unable to demonstrate any donor age-specific increase in senescence-associated beta-galactosidase activity staining. He believes that it is likely that this senescent phenotype either does not exist in individuals of any age or quickly dies in vivo.

The natural history of spontaneous tumors in humans (the rate of tumor doubling, the potential for metastasis) and the survival of cancer patients newly diagnosed at different ages provide information on the effects of age on tumor growth in humans. Available data in both experimental animals and humans are contradictory and support different effects of age on tumor development.⁴⁴ In general, an “age effect” may be recognized both in experimental and in human malignancies. Mikhlin et al⁴⁵ have studied the growth rate and

the volume doubling in 150 malignant melanomas of the skin in patients 16 to 85 years of age. Regardless of the pattern of melanoma growth (superficial and/or nodular), there was no influence of age on the kinetics of the tumor growth. This observation contradicts the suggestion that accumulation of senescent cells promotes the tumor growth in humans regardless of target tissue. Rather, tissue origin (histogenesis) and immunogenicity of tumor are the principal factors determining age-related differences in tumor growth.

There is increasing evidence, however, that age-related changes in tumor microenvironment might also play a significant role. In our experiments, lung-affine cells of rat rhabdomyosarcoma RA-2 were intravenously inoculated into rats of different ages.⁴⁶ 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 somatomedin activity in the lung. In another experiment, RA-2 cells from a 3-month-old donor were inoculated into 2- to 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” hosts.⁴⁷ The results suggest the critical role of host and donor microenvironment in lung colony forming potential of RA-2 cells.

McCullough et al⁴⁸ 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 strongly suggest that the tissue microenvironment is an important determinant in the age-related tumorigenic potential of transformed cells.

Thus, the data available 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 uneven tissue aging. Cellular senescence is suggested to be an important mechanism protecting the organism from cancer at a young age, but it could be a factor of tumor promotion in old age.¹² Thus, cellular senescence may lead to both age-related increases in spontaneous tumor incidence and age-related changes in susceptibility to carcinogens in various organs.⁸

Interaction of Aging and Carcinogenesis: Physiologic Level

The potential link between aging and insulin/IGF-1 signaling has attracted substantial attention during recent years based on evidence such as the age-related

increase in the incidence of insulin resistance and type 2 diabetes in accelerated aging syndromes or lifespan extension by caloric restriction in rodents. Concomitant reduction in plasma insulin and plasma glucose levels, which implies increased sensitivity to insulin, emerges as a hallmark of increased longevity.⁴⁹ Hyperglycemia is an important aging factor involved in the generation of advanced glycosylation endproducts (AGEs).⁵⁰ There is evidence that hyperinsulinemia favors the accumulation of oxidized proteins by reducing their degradation as well as facilitating protein oxidation by increasing the steady-state level of oxidative stress.⁵⁰ Untreated diabetics with elevated glucose levels suffer many manifestations of accelerated aging such as impaired wound healing, obesity, cataracts, and vascular and microvascular damage.¹⁰ Hyperinsulinemia is an important factor not only in aging but also in the development of cancer.^{10,51}

Intensive investigations in *Caenorhabditis 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 lifespan extension, have shed new light on the molecular mechanisms underlying aging.⁴⁹ In *Drosophila melanogaster*, the mutations of genes operating in the signal transduction from insulin receptor to transcription factor *daf-16* (*age-1*, *daf-2*, CHICO, InR, etc) are strongly associated with longevity.⁵² It was demonstrated that FKHR, FKHL1 and AFX, which are mammalian homologs of *daf-16* forkhead transcription factor, function downstream of insulin signaling and akt/PKB under cellular conditions.⁵³

InR and *daf-2* are structural homologs of tyrosine kinase receptors in vertebrates that include the insulin receptor and the insulin-like growth factor type 1 receptor (IGF-1R). It was shown that in vertebrates, the insulin receptor regulates energy metabolism whereas IGF-1R promotes growth. At least three genes (*Pit1^{dw}*, *Prop1^{dw}*, and *Ghr*) have been identified in which knockout led to dwarfism with reduced levels of IGF-1 and insulin and to increased longevity.⁴⁹ In Snell and Ames dwarf mice, sexual maturation is delayed, and few males are fertile while females are invariably sterile. These mice as well as *Ghr^{-/-}* 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 an acute challenge.⁴⁹

Hsieh et al^{54,55} recently provided 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 the longevity of IGF-1 deficient mice.^{54,55} It was shown that in the Snell dwarf mice, growth hormone deficiency led to reduced insulin secretion and alterations in insulin signaling via InR β , IRS-1, or IRS-2, and P13K affects genes involved in the

control longevity. The authors concluded that the Pit1 mutation might result in physiologic homeostasis that favored longevity.

Reduction in both glucose and insulin levels as well as an increase in the sensitivity to insulin are well-documented responses to caloric restriction in rodents and monkeys.^{56,57} It was shown that improved sensitivity to insulin in calorie-restricted animals is specifically related to reduced visceral fat.⁵⁸ *Gbr*^{-/-} mice have a major increase in the level of insulin receptors,⁵⁹ while Ames dwarf mice have a smaller increase in insulin receptor and substantially increased amount of insulin receptor substrates IRS-1 and IRS-2.⁶⁰ The development of tumors in Ames dwarf mice was postponed and the incidence was reduced as compared to the control.⁶¹

The crucial mediators of the effect of caloric restriction are low levels of insulin and IGF-1 and an increase insulin sensitivity in rodents⁶² and monkeys.⁶³ Many characteristics of these long-lived mutants and growth hormone receptor knockout mice resemble those of normal animals exposed to 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.

Holzenberger et al⁶⁴ inactivated the *Igf1r* gene by homologous recombination in mice. *Igf1r*^{-/-} mice died early in life, whereas heterozygous *Igf1r*^{+/-} mice lived on average 26% longer than their wild-type littermates. These mice did not develop dwarfism, and their energy metabolism was normal. Food intake, physical activity, fertility, and reproduction were also unaffected in *Igf1r*^{+/-} mice. These mice and the embryonal fibroblasts derived from them were more resistant to oxidative stress than controls. The spontaneous tumor incidence in the aging cohort of *Igf1r*^{+/-} mice was similar to that in wild-type controls. At the molecular level, insulin receptor substrate and the *p52* and *p66* isoforms of *Sbc*, both main substrates of the IGF-1 receptor, showed decreased tyrosine phosphorylation *p66*^{Sbc}-mediated cellular responses to oxidative stress. 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*^{+/-} mice.

Several years ago, the use of biguanide antidiabetics was suggested as a potential antiaging treatment.⁶⁵ The antidiabetic drugs phenformin (1-phenylethylbiguanide), buformin (1-butylbiguanide hydrochloride), and metformin (N,N-dimethylbiguanide) reduced hyperglycemia, improved glucose utilization, reduced free fatty acid utilization, gluconeogenesis, serum lipids, insulin, and somatomedin, reduced body weight, and decreased metabolic immunodepression in both humans and rodents.^{10,66,67} Phenformin is not used in clinical practice today due to its side effects (mainly lac-

tic acidosis) observed in patients with noncompensated diabetes. During more than 10 years of experience of phenformin administration to patients without advanced diabetes, Dilman¹⁰ observed no cases of lactic acidosis or any other side effects. Nevertheless, we believe that the analysis of results of long-term administration of this drug as well as other antidiabetic biguanides (buformin and metformin) to nondiabetic animals is important in understanding the links between insulin and longevity.

Treatment with antidiabetic biguanides prolonged the mean lifespan of female mice and rats.⁶⁸⁻⁷¹ It was found that metformin significantly increases the lifespan of rats (G. S. Roth, PhD, personal communication, 2001). Spindler⁷² found that metformin treatment reproduced the specific changes in gene expression produced by long-term caloric restriction.

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 exposed to intravenous injections of NMU, and it inhibited mammary carcinogenesis in these animals.^{69,71} Treatment of rats with 1,2-dimethylhydrazine (DMH) caused a decrease in the level of biogenic amines, particularly of dopamine in the hypothalamus, a decrease in glucose tolerance, and an increase in the blood level of insulin and triglycerides. Administration of phenformin restored immunologic indices and inhibited DMH-induced colon carcinogenesis.^{69,71,73}

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.^{69,71,73} In hamsters fed high fat, treatment with *N*-nitrosobis-(2-oxopropyl)amine was followed by the development of pancreatic malignancies in 50% of cases, whereas no tumors were found in the hamsters treated with both the carcinogen and the metformin.⁷⁴

Thus, an anticarcinogenic effect of antidiabetic biguanides has 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 model in rats. Phenformin administered orally to rodents potentiated the antitumor effect of cytostatic drugs on transplantable tumors.⁷⁵

The comparative study of 10-year results of metabolic rehabilitation (including a restricted fat and carbohydrate diet and treatment with antidiabetic biguanides) of cancer patients has shown significant increase in the survival of breast and colorectal cancer patients, an increase in the length of cancer-free period, and a decrease in the incidence of metastasis compared with control patients.^{10,67} In humans with type 2 dia-

betes, taking metformin may be associated with reduced cancer risk.^{76,77}

Antidiabetic biguanides inhibit fatty acid oxidation, inhibit gluconeogenesis in the liver, increase the availability of insulin receptors, inhibit monoamine oxidase, increase sensitivity of hypothalamopituitary complex to negative feedback inhibition, and reduce excretion of glucocorticoid metabolites and dehydroepiandrosterone-sulfate.¹⁰ These drugs have been proposed for the prevention of the age-related increase of cancer and atherosclerosis and for retardation of the aging process.^{10,67} It has been shown that administration of antidiabetic biguanides into patients with hyperlipidemia lowers the level of blood cholesterol, triglycerides, and β -lipoproteins. It also inhibits the development of atherosclerosis and reduces hyperinsulinemia in men with coronary artery disease. It increases hypothalamopituitary 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, and lowers blood IGF-1 levels in cancer and atherosclerotic patients with type IIb hyperlipoproteinemia.¹⁰ Data on the antioxidative effect of biguanides⁷⁸ and their neuroprotective activity are available.⁷⁹ It was shown that

biguanides inhibit complex I of the respiratory chain in mitochondria, which leads to an activation of physiologic intracellular inhibition of mitochondrial respiration.⁸⁰ Biguanides stimulate a protein kinase cascade inhibiting an expression of transcription factor SREBP-1. An activation of this factor with cholesterol leads to an increase in transcription of genes coding lipogenesis enzymes and to suppression of free fat acids oxidation. Thus, stimulation of uptake of glucose in tissues by biguanides inhibits lipogenesis and activates oxidation of FFA.⁸¹ It was shown also that in vivo biguanides inhibits appetite⁸² and serum levels of leptin and IGF-1.⁸³ It was suggested that biguanides regulate energy balance of the organism at the fat tissue level.⁸⁴ In general, the effects of biguanides seem similar to those of calorie restriction, and similar mechanisms are involved in the effects of calorie restriction and biguanides on lifespan and tumorigenesis.

Multistage Model of Aging and Carcinogenesis

There are two paths of development of the stem cell that can be realized in an organism. One is cellular dif-

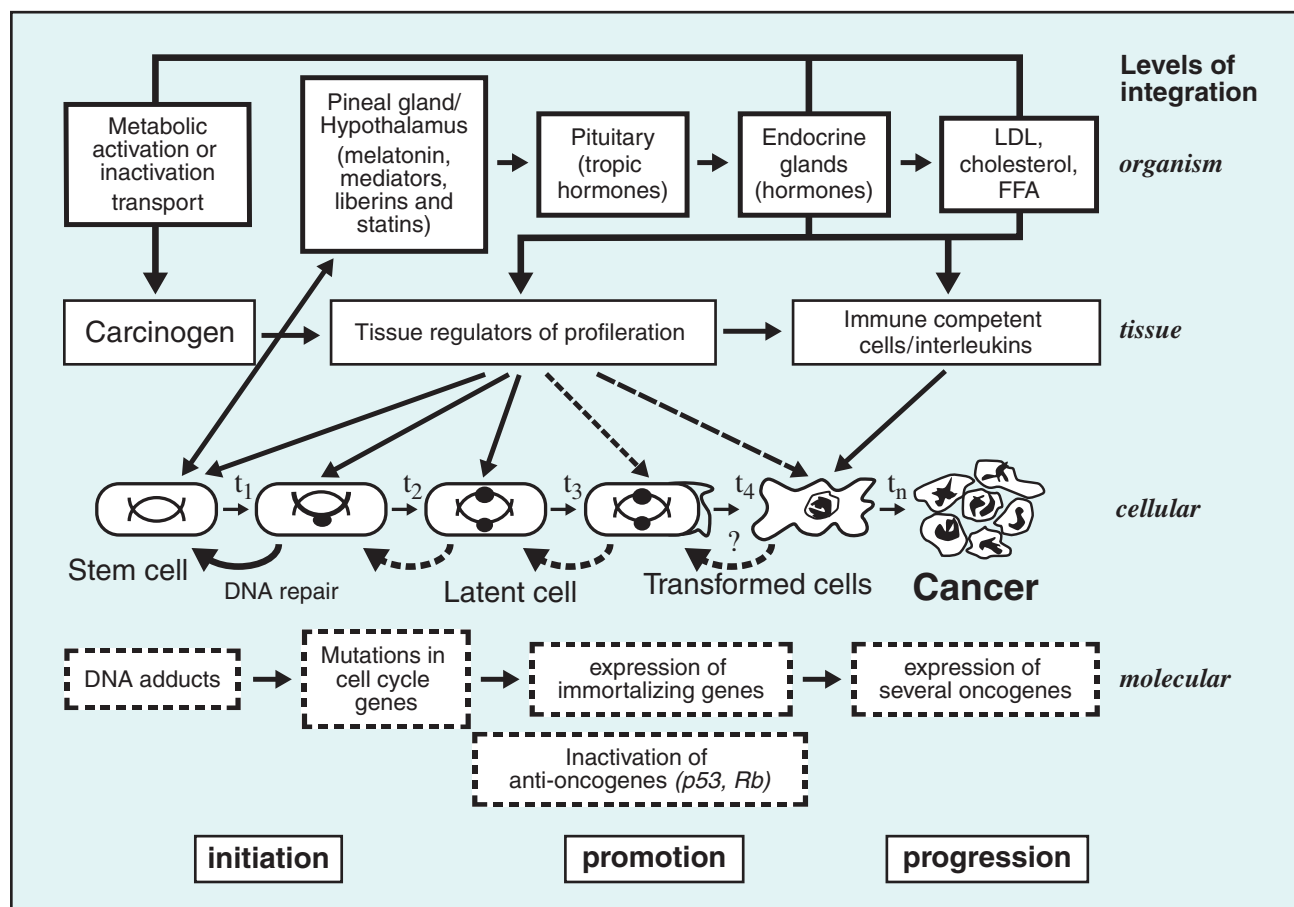


Figure. — Integral scheme of multistage carcinogenesis. From Anisimov VN. The relationship between aging and carcinogenesis: a critical appraisal. *Crit Rev Oncol Hematol.* 2003;45:277-304. Reprinted with permission from Elsevier.

ferentiation and aging and lastly, its individual death (apoptotic or necrotic).⁸⁵ When antiaging factors reach some limit in their compensatory ability to support tissue and functional homeostasis in life-important organs, the death of an organism as a whole takes place. Another possibility is that the influence of harmful exogenous or endogenous factors could lead to cell dedifferentiation, immortalization, and formation of a clone of neoplastic cells.^{85,86} Both strategies are multistage processes, many steps of which are well characterized in relation to the process of carcinogenesis.^{1,2,8,87} However, the multistage pattern of aging needs serious studies and formalization.^{8,28} 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 mutation of oncogenes and tumor suppressor genes, but also to enhanced levels of aberrations and mutation in distant progeny of initiated cells and is a factor of predisposition to immortalization.²⁸ 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.^{88,89}

The process of neoplastic development is often divided into three operationally defined stages: initiation, promotion, and progression. During the first stage of carcinogenesis (initiation), irreversible changes occur in the genotype of the normal target stem cell, leading to its immortality. During the initiation, the carcinogen or its active metabolites (derived by simple degradation or by active enzymatic process) interact with nucleic acids leading to mutations in oncogenes and in antioncogenes. During the second stage of carcinogenesis (promotion), an initiated (latent, immortalized) cell acquires phenotypic features of transformed (malignant) cell, and under microenvironmental factors can evolve to tumor progression. A carcinogen not only affects the target cell but also influences many factors in the microenvironment of the target cell, creating conditions for the promotion of the immortalized cell (eg, growth factors, cytokines, immunodepression, biogenic amines, hormonal and metabolic imbalance). A complete carcinogen affects both stages of carcinogenesis (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. The dissection of carcinogenesis into initiation, promotion, and progression is useful as a frame of reference. It should not be assumed, however, that only three carcinogenic stages exist; each stage can be subdivided into multiple substages. Promotion may involve the

activation of several enzymes (such as protein kinase C and ornithine decarboxylase), enhancement of hexose transport, increase in polyamine production, prevention of cell differentiation, and inhibition of cell-to-cell

Table. — Similarity of Changes Developing in Organisms During Natural Aging and Carcinogenesis.

Parameters	Aging	Carcinogenesis
Molecular Level		
Free radical generation	Increases	Increases
DNA adducts formation	Increases	Increases
DNA repair efficacy	Decreases	Decreases
DNA hypomethylation	Increases	Increases
Genomic instability	Increases	Increases
Telomere length	Decreases	Increases*
Error protein synthesis	Increases	Increases
Mutation rate	Increases	Increases
Oncogene expression	Increases	Increases
p53 mutations	Increases	Increases
Cell/Tissue Level		
Oxidative stress	Increases	Increases
Chromosome aberrations	Increases	Increases
Growth factor production	Decreases	Increases*
Proliferative activity	Decreases	Clonal proliferation*
Focal hyperplasia	Increases	Increases
Apoptosis	Increases	Decreases*
Angiogenesis	Decreases	Increases*
Bioenergetics	Decreases	Anaerobic glycolysis*
Cell-to-cell communication	Decreases	Decreases
Latent (dormant) cells number	Increases	Increases
Systemic/Organism Level		
Melatonin circadian rhythm	Damaged	Damaged
Serum melatonin level	Decreases	Decreases
Hypothalamic biogenic amines level	Decreases	Decreases
Hypothalamic threshold of sensitivity to homeostatic inhibition by steroids	Increases	Increases
Tolerance to glucose	Decreases	Decreases
Serum insulin level	Increases	Increases
Susceptibility to insulin	Decreases	Decreases
LDL and cholesterol level	Increases	Increases
Serum glucocorticoid level	Increases	Increases
Fertility	Decreases	Decreases
T-cell immunity	Decreases	Decreases
Cancer risk	Increases	Increases
Population Level		
Cancer incidence	Exponential pattern	Exponential pattern
Progeria	Accelerates	Increases
Exposure to 5-bromodeoxyuridine	Accelerates	Increases
Exposure to ionizing radiation	Accelerates	Increases
Treatment with geroprotectors	Postponed	Decreases or latency increases
Rate at the oldest age	Decreases in mortality	Decreases in incidence

* Related to clonally proliferating malignant cells.

communication.⁸⁸ TPA, a well-known skin tumor promoter, was found to cause free-radical-mediated DNA alterations such as sister chromatid exchanges and expression of proviruses and retroviruses.⁸⁸

The discovery of oncogenes and their function has provided new insights into the carcinogenic process. Carcinogenesis can be viewed as a “cascade” phenomenon, resulting in serial activation of multiple cellular oncogenes and/or inactivation of tumor-suppressing genes (eg, p53).⁹⁰

Both experimental and epidemiologic studies illustrate the interaction of aging and carcinogenesis. The malignant transformation of normal cells involves both quantitative and qualitative changes. The Figure shows an integrated scheme of multistage carcinogenesis. 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.^{2,8}

The Table summarizes the data available in the literature and obtained in our experiments on some hormonal metabolic shifts in the organism and disturbances at tissue and cellular levels observed in natural aging and in different types of carcinogenesis in vivo. Despite incomplete data, it can be seen that there is a similarity between the shifts in aging and carcinogenesis. On one hand, carcinogens could be thought to initiate a normal cell, interacting with its elements on the molecular level, while on the other hand, they could be thought to produce diverse changes in the organism facilitating promotion and progression of tumor growth.

Thus, three major mechanisms, not mutually exclusive, might explain the association of cancer and age:

- Carcinogenesis is a time-consuming process in which the final product — cancer — is more likely to occur in persons of advanced age, depending on cumulative exposure to environmental carcinogens.
- Aging tissues undergo molecular changes that parallel early carcinogenic changes and prime these tissues to the effects of carcinogens.
- Age-related changes in body microenvironment, including proliferative and immune senescence, may favor cancer development and growth.

We believe that age-related changes in an organism developing at all levels of integration — molecular, cellular, tissue, organ, and physiologic/systemic — contribute to the mechanism of age-related increase in cancer incidence.

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