

Chapter 2

BIOLOGICAL INTERACTIONS OF AGING AND CARCINOGENESIS

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It is well documented that the incidence of malignant tumors increases progressively with age, both in animals and humans ¹⁻³. The relationship between aging and cancer is not clear. Considerable controversy surrounds the mechanisms that lead to increased incidence of cancer in the aged. Three major hypotheses have been proposed to explain the association of cancer and age.

The first hypothesis holds 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 ⁴. 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 for the growth of already existent, but latent malignant cells ⁵⁻⁹. These mechanisms may also include proliferative senescence, as the senescent cells loses their ability to undergo apoptosis and produce some factors which stimulate epithelial cells with oncogenic mutations ¹⁰. 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 milieu ¹¹. The elucidation of causes of an age-related increase in cancer incidence may be the key to a strategy for primary cancer prevention.

1. AGING AND MULTISTAGE MODEL OF CANCER

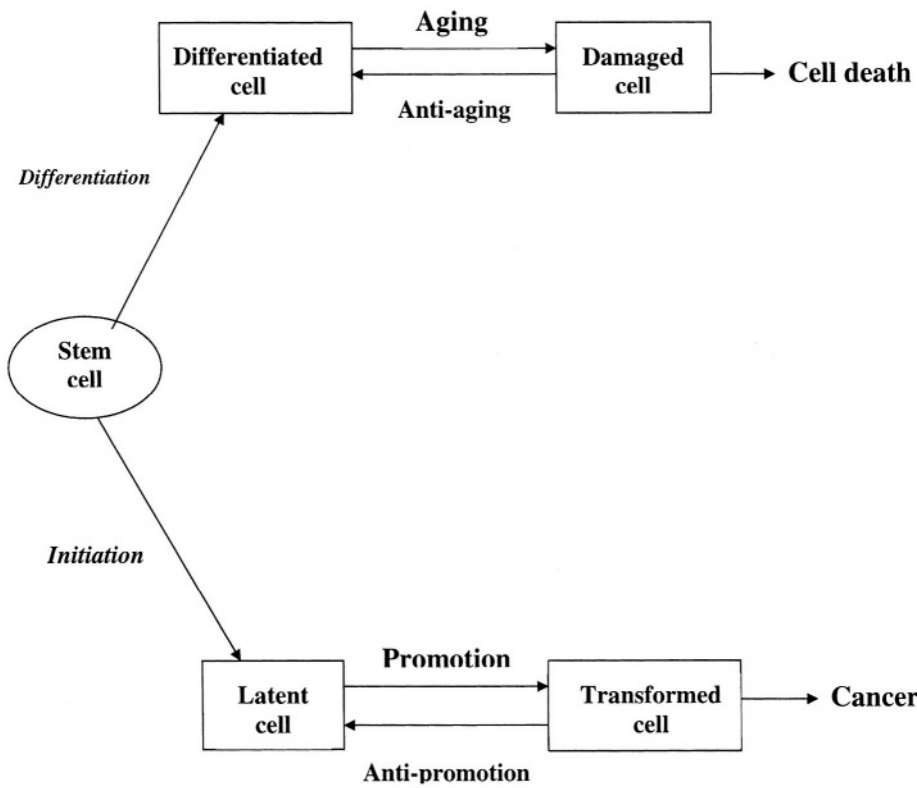
The homeostasis of most tissues is maintained thanks to a pool of stem cells able to reproduce themselves and to differentiate. Cell differentiation is followed by cell death and aging maybe construed as a progressive loss of stem cells to differentiation and death¹². Another possibility involves the immortalization of the stem cell that is associated with a loss of differentiation and apoptosis. These immortalized stem cells may give origin to a clonal population with a survival advantage over the remaining tissues: this process is carcinogenesis^{12,13}. (Figure 1). Both differentiation and death, and immortalization are multi-stage processes. Many steps of carcinogenesis are well-characterized^{5,6,14,15} whereas the steps of aging need better recognition and definition^{6,16}. Both models of cellular aging and immortalization involve delayed genomic instability that is a transmission of genomic aberrations to distant cellular progenies, accompanied by the occurrence of new aberrations. In one case this process results in cellular death; in the other, in cellular immortalization, and some steps may be shared by the two¹⁶.

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^{17,18}. Several lines of evidence support this conclusion¹⁹:

- Histopathology of tumors reveals multiple stages of tumor progression, such as dysplasia and carcinoma *in situ*
- The two-stage model of chemical carcinogenesis in mouse skin shows that different chemicals affect qualitatively different stages in the carcinogenic process
- The existence of individuals with genetic traits manifested by an early occurrence of cancer (e.g., familial retinoblastoma, colon and rectum adenomatosis) suggests that one of the carcinogenic steps is a germ-line mutation, but additional somatic effects are required for neoplastic development
- Mathematical models based on age-specific tumor incidence curves are consistent with the hypothesis that three to seven independent hits (effects of independent carcinogens) are required for tumor development
- Studies with chemical carcinogens in cell cultures reveal that different phenotypic properties of a tumor cell are required for tumor development
- Studies with viral and tumor-derived oncogenes in cell cultures show that neoplastic conversion of normal cells generally requires multiple cooperating oncogenes.

- Transgenic mice that carry activated proto-oncogenes in their germ-line develop focal tumors, which are apparently monoclonal in origin, suggesting that additional somatic events are required for full malignant progression.

Figure 1. Two strategies of stem cell



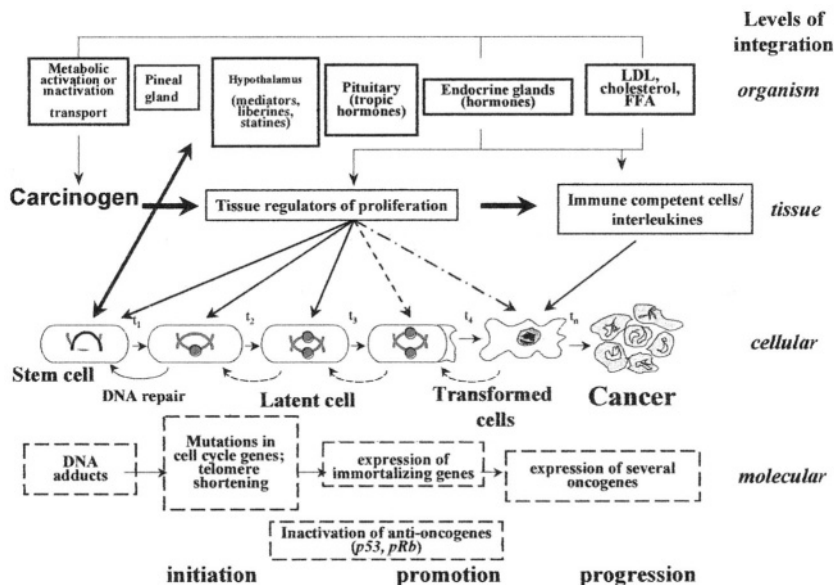
The process of neoplastic development is frequently divided into three operationally defined stages - initiation, promotion and progression. During the first stage of carcinogenesis (initiation) irreversible changes in the genotype of the normal target stem cell leading to its immortality take place. During the initiation the carcinogen or its active metabolite(s) (derived by simple degradation or by active enzymatic process) interacts with nucleic acids leading to mutations in oncogenes and in anti-oncogenes. During the second stage of carcinogenesis (promotion) initiated (latent, immortalized) cell acquires phenotypic features of transformed (malignant) cell, and under the exogenous influence, some of which at least are provided by the neoplastic stroma, tumor progression may occur. A carcinogen affects not only target cells but also influence a lot of factors in the microenvironment of the target cell creating the conditions for promotion of immortalized cell (growth factors, cytokines, immunodepression, biogenic amines, hormonal and metabolic imbalance). Some carcinogens, such as tobacco smoke may effect multiple carcinogenic steps.

Unlike initiation, promotion requires prolonged exposure to the carcinogen and may be reversible to a large extent. 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; enhanced hexose transport; increased polyamine production, prevention of cell differentiation; and inhibition of cell-to-cell communication²⁰⁻²¹. It was found that 12-O-tetradecanoylphorbol-13-acetate (TPA), a well-known skin tumor promoter, causes free radical-mediated DNA alterations, such as sister chromatid exchanges and expression of proviruses and retroviruses²².

Discovery of oncogenes and of their function has provided new insight 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)²³.

To overcome the obvious limitations of two (three)-stage model, a multistage model of carcinogenesis has been conceived, in which the number of stages is not limited, the stages are envisioned as a continuum, and the influence of factors other than specific carcinogens may be properly accounted for in Figure 2²⁴. The principles of this model are as follows. First, neoplastic transformation involves the transition of target cells through multiple stages, the number of which varies for different neoplasms (with

Figure 2. Integral scheme of carcinogenesis



a minimum of one intermediate stage). Secondly, passage from one stage to another is a stochastic event, the rate of which depends on the dose of a carcinogen that affects the cell. Finally, all cells at any stage of carcinogenesis may enter the next stage independently of each other.

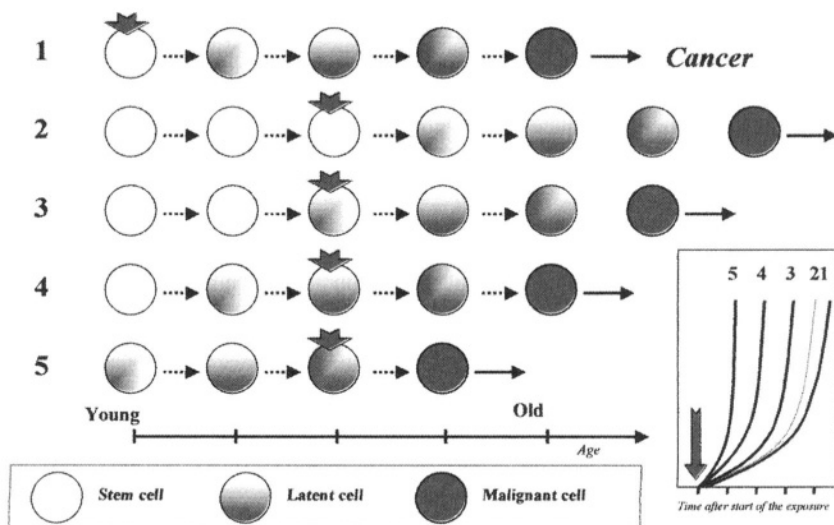
According to this model, the tumor develops only if at least one cell goes through all the necessary stages, and the clonal growth of this cell causes clinical cancer, as a critical volume of neoplastic cells accumulates. In this model, the exact origin of the various stages is ignored and the changes in cell function during the process of carcinogenesis are not assessed. The grade of malignancy is considered to increase with every stage. Various carcinogenic agents (exogenous as well as endogenous) may modulate the process. In addition, some agents act at early stages of carcinogenesis and others at later stages²⁴. Epidemiological data, analyzed within the framework of a multi-stage model, have helped to estimate the contribution of various factors to the development of cancer. These factors include the time from the start of carcinogenic exposure, and the age of onset of exposure.

It is worthy to note that in every tissue the number of events occurring in the stem cell before its complete transformation is variable and depends on many factors, in particular the rate of aging of the target tissue and its regulatory system(s)^{6,14}. This model is consistent with the analysis of age-related distribution of tumor incidence in different sites in humans and laboratory animals^{1,3}.

Important differences between early and late-stage carcinogens should be highlighted, to illustrate potential interactions of aging and carcinogenesis. Exposure to early stage carcinogens requires a latent period for the development of cancer. During the latent period the transformed cell goes through the subsequent carcinogenic stages. Clearly, elimination of early-stage carcinogens from the environment will not result in immediate cessation in the incidence of cancer. Carcinogens acting at late stages of carcinogenesis cause the tumor incidence rate to rise after a relatively short period of time. The increased rate of tumor incidence will be reversed almost immediately on cessation of exposure²⁴.

This risk of cancer after exposure to a carcinogen may be calculated as: $I = (\text{age})^{k-1} - (t)^{k-1}$ where I is the risk of cancer, t is the time from initial exposure to the carcinogen, and k is the number of stages that the target cells have undergone before the exposure to the carcinogen. This formula is based on the assumption that with aging there is a progressive accumulation of partially transformed cells primed to the effect of late-stage carcinogens (Figure 3). Age is considered as a variable because older cells may already present in advanced carcinogenic stages, are primed to the effects of environmental carcinogens and consequently may develop cancer more rapidly and at higher rate when exposed to these substances. A number

Figure 3. The multistage carcinogenesis induced by single exposure to a carcinogenic agent at different ages.



of factors, including genetic predisposition, oxidative stress, and previous exposure to carcinogens may be responsible of the molecular changes that prime aging cells to environmental carcinogens.

2. EFFECT OF AGING ON THE SUSCEPTIBILITY TO CARCINOGENESIS IN VIVO

Animal experiments seem to confirm that there are age related differences in sensitivity to carcinogen in some tissues. Thus, with age, susceptibility to carcinogens in murine mammary gland, small intestine and colon, thyroid, ovarian follicular epithelium decreases, in subcutaneous tissue, cervix uteri and vagina increases and in others (lung, hemopoietic tissues) it remains stable (Table 1). For details see references 1,5-6). Age-related differences in cancer susceptibility have been observed after exposure to the same carcinogens in experimental systems. For example, in female rats exposed to N-nitrosomethylurea (NMU) in doses 10, 20 or 50 mg/kg at the age of 3 month developed mammary carcinomas, tumors of the kidney, ovaries and colon. In contrast to young animals, the rats exposed to the same doses of the carcinogen at the age of 15 months showed a higher frequency of tumors of the corpus and cervix uteri, and a lower frequency of mammary and intestinal adenocarcinomas and tumors of the ovary and kidney ²⁵. Comparison of the results with the data on DNA alkylation, synthesis and O⁶-methylguanine repair obtained on the same model suggests a critical role of age-related proliferative activity changes occurring in the target tissues in the mechanism of age in modifying the effect on carcinogenesis. Obviously, there are no common patterns of age related changes in DNA synthesis and repair or in proliferative activity of different tissues with age ^{1,5,6}.

There are several possible reasons for this wide variation in experimental results. These include factors related to the experimental model and factors related to the tumor-host. Model-related factors involve the characteristics of different carcinogens (direct or indirect action, chemical structure, mechanism of action), route of administration, exposure duration, presence of local and systemic activity, and time of observation. Host-related factors involve animal species, strain, sex, and age. The effective dose of an indirect carcinogen, requiring metabolic activation, may vary significantly in old and young animals, because the activity of the enzymes necessary for carcinogen activation in the liver and/or target tissue(s) may change with age ^{5,26,27}. Critical factors that determine the susceptibility of a tissue to carcinogenesis include DNA synthesis and proliferative activity of that tissue at the time of carcinogen exposure, and the efficacy of repair of damaged DNA. The

Table 1. Effect of aging on the susceptibility of laboratory rodents to carcinogenesis (Anisimov, 1987; 1998)

Target tissue	Carcinogenic agent	Effect of aging
Skin	DMBA, MCA, BP, TC, UV, β -irradiation	↑
	Fast neutrons, electrone-irradiation	↓
Subcutaneous soft tissues	BP, DMBA, MCA, NMU, polyurethane sponge, Moloney virus	↑
Bone	^{224}Ra , ^{227}Th , ^{239}Pu , radionuclides	=
Vascular vessel	DENA, DMH	=
	Vinylchloride	↑
Hematopoietic tissue	X-rays, γ -irradiation, estrogens	↓
	NMU, pristan	↑=
Mammary gland	DMBA, MCA, NMU, AAF, X-rays	↓
	Estrogens	↑
Uterus	DMH, NMU	↑
Vagina	DMBA	=
Ovary	X-rays, Biskind's operation	↓
Testis	Fast neutrons	↑
Thyroid gland	Fast neutrons, X-rays	↓
Lungs	DENA, DBA, urethane	↓
	NMU, fast neutrons, X-rays	↑
Pleura	Asbestos	↑
Liver	AAF, AFB ₁ , DMNA, DENA,	↓
	Phenobarbital, CCl ₄	↑
Pancreas	NMU	↑
Esophagus	DENA	↓
Forestonach	DENA	↓
Stomach	MNNG	↓
Small intestine	MAMNA	↓
Colon	DMH, MAMNA	↓ (rat)
	DMH, NMU	↑ (mouse)
Kidney	AAF, NMU, DMNA	↓
Bladder	DMBA, BHBNA	↑

Abbreviations: AAF- 2-acetylaminofluorene; AFB₁ – aflatoxin B₁; BHBNA – N-butyl-N-(4-hydroxybutyl)nitrosamine; BP–benzo(a)pyrene; DBA – 1,2,5,6-dibenzanthracene; DENA– N-diethylnitrosamine; DMNA – N-dimethylnitrosamine; DMH- 1,2-dimethylhydrazine; MAMNA – N-methyl-(acethoxymethyl)nitrosamine; MCA – 20-methylcholanthrene; MNNG – N-methyl-N'-nitro-N-nitrosoguanidine; NMU – N-nitrosomethylurea; TC – tobacco smoke condensate; UV- ultra violet irradiation; CCl₄ – carbon tetrachloride; X-rays - Roentgen irradiation.

↑ - **increase** in incidence of tumors or decrease in tumor latency; ↓ - **decrease** in incidence of tumors or increase in tumor latency; = no effect.

available data concerning age related changes of these parameters have been discussed elsewhere^{1-3,23,28}. Obviously, there are no common patterns of age related changes in DNA synthesis and repair or in proliferative activity of different tissues with age.

The homeostatic regulation of cell numbers in normal tissues reflects a precise balance between cell proliferation and cell death. Programmed cell death (apoptosis) provides a protective mechanism from cancer, by removing senescent, DNA damaged, or diseased cells that could potentially interfere with normal function or lead to neoplastic transformation^{23, 29}. Apoptosis plays a substantial role in many other aspects of aging and cancer, including control of the life span of most members of transformed cells, and the rate of growth of tumors³⁰. p53 mediated apoptosis was suggested as a safeguard mechanism to prevent cell proliferation induced by oncogene activation³¹.

3. AGING AND SUSCEPTIBILITY TO CARCINOGENESIS IN VITRO

Some *in vitro* observations support the suggestion on accumulation in tissues of premalignant cells. Thus, transformed by 24-hours exposure to DMBA, foci in murine bladder epithelium have appeared earlier (on the 40th to the 60th day) and more often (25%) in explants of old (28-30 months) donors in comparison with 100 days and 0.9% in cultures received from five to seven month old mice. A spontaneous transformation of bladder epithelium occurred only in the explants received from old donors³². The aging of the tissue donor was associated with increased susceptibility of primary cultures of rat fibroblasts to transformation induced by SV-40³³. However rat embryonal fibroblasts were much susceptible to *v-scr* transformation than when they were isolated from an adult rat³⁴. Nettesheim et al.³⁵ reported that the sensitivity of trachea epithelium explants of old animals to chemical carcinogens was lower in comparison to explants from young animals.

Susceptibility to transformation varies during the different stages of proliferative senescence depending on the carcinogen. Thus, young cells are more susceptible to transformation by chemical carcinogens and by low-dose ionizing radiation, susceptibility to ultraviolet radiation is identical throughout the life span of human fibroblasts, whereas susceptibility to a tumor promoter is identical through the cell life span with exception of the final stage, and susceptibility to SV40 is highest during the final stage^{36,37}.

Thus, experiments both *in vivo* and *in vitro* provide evidence that the age related factors limiting the susceptibility to carcinogens are tissue specific^{1,6}. This conclusion may explain, at least in part, both age related

changes in susceptibility to carcinogenesis in target tissues, and organ and tissue variability in age distribution of spontaneous tumor incidence. This conclusion generates a critical question: does the aging accompanied by the accumulation of premalignant lesions in target tissues?

4. EFFECT OF AGING ON THE SUSCEPTIBILITY TO TUMOR PROMOTERS IN VIVO

There is evidence of age-related accumulation of cells that are in the late stage of multi-stage process of carcinogenesis. Numerous experiments support this model. Thus, single skin application with 7,13-dimethylbenz[*a*]anthracene (DMBA) in mice aged 8 and 48 weeks at doses ranging from 10 to 300 μg caused increased skin papilloma incidence in older mice³⁸. Also, the average diameter of the tumors was larger in the older animals. Of particular interest are the experiments using skin transplants. TPA failed to induce tumors in the skin of 2-month-old mice grafted to animals of different ages, but caused the same tumor incidence in the skin of 1-year-old donors irrespective of the recipient's age^{39,40}. These results indicate that the age of the target tissue, more than the age of the host, determines susceptibility to late-stage agents. Delaying wounding 16 weeks after initiation with a carcinogen led to a more pronounced skin tumor response compared with delay of only 6 weeks in young mice⁴¹. Delaying promotion has also been reported to lead to an increased tumor response with the promoters chrysarobin⁴² or mezerein⁴³. These findings are in agreement with data on age-related decrease in cellular DNA repair capacity in skin^{44,45} and increasing *p53* mutation frequency with advancing age in human normal skin⁴⁶ and in basal-cell skin carcinomas^{47,48}. Post-ultraviolet DNA repair capacity was found to undergo an age-related decline to which corresponded age-related increase in post-ultraviolet mutability in cultured primary skin fibroblasts from normal donors from the first to the tenth decade of life⁴⁴. It was suggested that there was the age-related increase in the number of telomerase positive basal cells in the skin⁴⁹. However in some studies the papilloma response either decreased with age or was the same as the response in younger mice⁵⁰⁻⁵².

In Tg.AC transgenic (*v-Ha-ras*) mice, skin tumor incidence and multiplicity were strongly age-dependent, increasing with increasing age of the animal when first treated with TPA, or exposed to wounding, or UV-light⁵³. The authors suggest that natural developmental changes in keratinocytes are co-opted by the molecular mechanisms that regulate the induction of transgene expression, thus stimulating tumor formation in older Tg.AC mice.

Age-related accumulation of cells in advanced carcinogenic stages may also be inferred by other types of experiments. The mouse model of

hepatocarcinogenesis is very convenient for this purpose because of the availability of strains of animals with different susceptibility to hepatic carcinogenesis. In the liver of highly susceptible mice, the concentration of hepatocytes in advanced stages of carcinogenesis was increased early in life before the exposure to experimental carcinogens⁵⁴. In the liver of F344 rats the number of spontaneous proliferative foci is proportional to the animal age^{55,56}. The incidence of proliferative foci and hepatic tumors induced by phenobarbital, carbon tetrachloride or peroxisome proliferators in rodents is also a function of age⁵⁵⁻⁵⁷.

Another pertinent model involves induction of lymphomas in mice receiving transplants of splenic, thymic and lymphoid cells from syngeneic donors⁴⁰. The incidence of neoplasms was related to the age of the donor, but not to the age of the recipient. Geschickter⁵⁸ observed mammary tumor development in estrogen-treated one and 20 month-old rats with a latency period of 9.5 and 3.0 months, respectively. The data on age-related susceptibility to tumor promoters are given in Table 2.

Table 2. Effect of aging on susceptibility of different tissues to tumor promoters in rodents (Anisimov, 1998)

Target tissue	Species	Treatment, agent	Age groups, months	Effect of aging	References
Skin	Mouse	TPA*	4 and 14	↑	39
Liver	Mouse	Phenobarbital	1,5 and 12	↑	55
	Rat	Phenobarbital	1 and 26	↑	56
		Partial hepatectomy + phenobarbital	5 and 18	↑	154)
		CCl ₄	1-6 and 12	↑	155
		Clofibrat, nafenopin	3 and 18	↑	57
		Clofibrat, Wy-14643	2,5 and 23	↑	156
Mammary gland	Rat	Estradiol	1 and 20	↑	58
Ovary	Rat	Biskinds' operation**	3 and 14	↑	157

*- 12-O-tetradecanoylphorbol-13-acetate

** Transplantation of the ovary into the spleen after ovariectomy.

Single intravenous injection of NMU at doses of 10, 20 or 50 mg/kg was administered to female rats aged 3 or 15 months²⁵. The NMU carcinogenic dose dependence in different age groups was considered in the context of a multi-stage model. It was calculated that the number of events necessary for complete malignant transformation in 15-month-old rats under the influence of NMU was lower than in three month-old. In this experiment as well as in another sets of experiments in rats and in mice it was shown that tumors developed earlier in older than in younger animals after exposure to the same doses of NMU^{14,59-62}. The combined incidences of severe endometrial hyperplasia and adenocarcinomas tended to increase with the increase in intervals between a start of promoting estradiol treatment after N-nitrosoethylurea initiation in mice⁶².

5. EFFECT OF AGING ON TRANSPLANTABLE TUMOR GROWTH

An important question related to the integrated carcinogenic model (Figure 2) concerns age-related changes in tissue microenvironment as these changes may both favor or oppose carcinogenesis in different circumstances. Should aging alter the environment in which tumor develops, the growth rate of transplantable tumors may vary with the age of the tumor recipient⁶³. These experiments bypass the effect of age on carcinogenesis itself and explore the role of age-related changes in the organism on the growth and progression of transformed cells. Evaluation criteria for such experiments should include: (a) tumor transplantability, (b) rate of tumor growth, and (c) survival time of tumor bearing animals. The natural history of spontaneous tumors in humans (the rate of tumor doubling, metastasizing potential) and on the survival of cancer patients newly diagnosed at different ages provide 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 (Table 3)^{1,6}. In general, an "age effect" may be recognized both in experimental and in human malignancies.

Tissue origin (histogenesis) and immunogenicity of tumor are the principal factors determining age-related differences in tumor growth. There is increasing evidence that age-related changes in tumor microenvironment might play also a significant role. In our experiments, lung-affine cells of rat rhabdomyosarcoma RA-2 were intravenously inoculated into rats of different ages⁶⁴. It was observed that the number of lung tumor colonies was highest in one month-old 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 (IGF-1) activity in the lung.

Table 3. Effect of aging on growth of subcutaneously transplanted tumors in rodents (Anisimov, 1987, 2003). ↑ - Increase in transplantability and/or the rate of growth and/or decrease in survival time; ↓ - Opposite effects; = No effect.

Tumor	Species	Age at the time of tumor transplantation, months	Effect of aging
Epidermoid carcinoma H.Ep.#3	Mouse	4-8 and 20-23	↑
Squamous-cell cervical carcinoma SCC	Mouse	3 and 12	=
		3 and 18	↑
Melanoma B16	Mouse	3 and 12	↓
		3 and 22	=
		3 and 24	↓
Mammary carcinoma: Spontaneous	Mouse	3,5 and 16,5	↓
		10-11 and 21-22	↓
Ehrlich ascite carcinoma		3 and 16,5-18	↑
EMT6		3-4 and 20-28	↑
MAT-21		2 and 4- 5	↓
A-755		3 and 18	↑
64pT		3 and 24	↓
Walker-256	Крыса	2 and 24	↓
Lewis lung carcinoma	Mouse	3 and 18	=
		2 and 24	↑
		3 and 33	↓
Lung carcinoma-1	Mouse	3-8 and 18-23	↑
Hepatoma-22a	Mouse	3 and 14-16	↑
Novikoff's hepatoma	Крыса	4,5 and 27,5	↓
Teratocarcinoma OTT 6050	Mouse	2 and 16	↓
Methylchlanthrene sarcoma	Mouse	6 and 22	↑
		2-3 and 10-21	↑
	Крыса	1-10 and 12-15 8-20 and 29-32	↓ ↑
Fibrosarcoma 1023	Mouse	2 and 4-5	↑
Fibrosarcoma 1591	Mouse	2-6 and 10	↑
Sarcoma 180	Mouse	3 and 18	↑
Osteogenic sarcoma	Mouse	2-3 and 10-17	=
Uterine sarcoma	Mouse	3 and 12	=
Fibrosarcoma	Крыса	4 and 12	↓
Ascitic fibrosarcoma	Крыса	3-4 and 16-18	↑
Mastocytoma P815	Mouse	3 and 25	↑
		3-12 and 20-32	↑
Reticulocell tumor, type A	Mouse	8 and 11-17	↓
Leukemia L1210	Mouse	3 and 11	=
Hemocytoblastoma La	Mouse	3 and 18	=
Myeloma LCP-1	Mouse	2-3 and 19-20	↓

In another experiment, RA-2 cells from a 3-month-old donor were inoculated into 2-3 or 21-23-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” host⁶⁰. The results obtained suggest the critical role of host and donor microenvironment in lung colony forming potential of RA-2 cells.

McCullough *et al.*⁶⁵ 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 represents an important determinant in the age-related tumorigenic potential of transformed cells.

Krtolika and Campisi⁶⁶ have shown that senescent stromal fibroblasts can stimulate the hyperproliferation and malignant progression of preneoplastic and neoplastic cells in culture. They also tested the ability of senescent fibroblasts to stimulate epithelial cell growth in vivo by inoculation of preneoplastic epithelial cells with presenescent or senescent human fibroblasts into nude mice⁶⁷. None of the tumors when injected alone. Both preneoplastic mouse mammary epithelial cells and preneoplastic human keratinocytes did not form tumors in the presence of presenescent fibroblasts but formed large lethal tumors in the presence of senescent fibroblasts. In the case of human breast cancer cells, senescent fibroblasts markedly stimulated the rate of tumor growth⁶⁷.

6. MECHANISMS OF INTERACTION OF AGING AND CARCINOGENESIS

Cancer is a common denomination given to a number of different diseases. Common features to all cancers include^{23,68}

- potential immortality of cancer cells due to avoiding apoptosis
- ability to invade surrounding tissues due to reduced sensitivity to signals from neighboring cells aimed to offset proliferation
- cell de-differentiation with re-appearance of some embryonal proteins (e.g. α -fetoprotein) in cytoplasm
- growth signals autonomy, which allows cancer cells to proliferate in absence of outside signals due to only inner growth signals
- release of growth factors and promotion of angiogenesis in tissue, which favor tumor growth and metastasis
- increase in metabolism and number of mitochondria in cancer cells

Gene mutations, as well as changes in regulation of gene expression, which can produce these typical features, were suggested to be key genetic events leading to cancer development^{23,68,69}. Down regulation of apoptosis gene, *p53*, as well as upregulation of *myc* and *ras* genes, which may favor excessive proliferation, could be examples of such events^{69,70}.

Both carcinogenesis and aging are associated with genomic alterations, which may act synergistically in causing cancer^{23,68-71}. In particular, three age-related changes in DNA metabolism may favor cell transformation and cancer growth. These changes are genetic instability, DNA hypomethylation, and formation of DNA adducts.

Genetic instability involves activation of genes that are normally suppressed, such as the cellular proto-oncogenes, and/or inactivation of some tumor suppression genes (*p53*, *Rb*, etc.)^{23,31}. DNA hypomethylation is characteristic of aging, as well as of transformed cells. Hypomethylation, a potential mechanism of oncogene activation, may result in spontaneous deamination of cytosine and consequent base transition, i.e., substitution of the pair thymine:adenine. Accumulation of inappropriate base pairs may cause cell transformation by activation of cellular proto-oncogenes²³. Age-related abnormalities of DNA metabolism may be, to some extent, tissue- and gene-specific. For example, hypomethylation of the *c-myc* proto-oncogene has been found in the hepatocytes, but not in the neurons of old mice^{72,73}. Within the same cell, different DNA segments express different degrees of age-related hypomethylation. The uneven distribution of hypomethylation may underlie selective overexpression of proto-oncogenes by senescent cells. For example, the transcription of *c-myc* is progressively increased in the liver but not in the brain of rats between the ages of 4-22 months, whereas the transcription of *c-sis* and *c-src* does not appear to be age-related in any tissues^{72,73}. The different extent of DNA abnormalities among aging tissues may account in part for the different susceptibility of these tissues to carcinogens^{74,75}.

The damage caused by endogenous oxygen radicals has been proposed as a major contributor to both aging and cancer⁷⁶⁻⁷⁸. Endogenous oxidative damage to lipids and proteins increases with age^{77,78}. It was shown that oxygen free radicals may induce active mutations of the human *c-Ha-ras* proto-oncogene⁷⁸. The level of one oxidized nucleoside, 8-hydroxy-2'-deoxyguanosine (oh8dG) in the DNA increased with age in liver, kidney, and intestine but remained unchanged within brain and testes of rats, whereas the urinary excretion of the nucleoside decreased with age of rats⁷⁹. A variety of cellular defense systems are involved in protecting cellular macromolecules against devastating action of oxygen-based radicals. These systems include antioxidant enzymes (Cu,Zn- superoxide dismutase (SOD), manganese-containing SOD, catalase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase), some vitamins

(α -tocopherol, ascorbic acid), uric acid and the pineal indole hormone melatonin⁸⁰⁻⁸³.

There is evidence of an age-related accumulation of spontaneous mutations in somatic and germ cells⁷¹. Accumulation with age of some spontaneous mutations or mutations evoked by endogenous mutagens can induce genome instability and, hence, increase the sensitivity to carcinogens and/or tumor promoters. It has been shown that clonally expanded mtDNA mutations accumulate with age in normal human tissues as well as in human tumors^{84,85}. The finding that deleted mtDNA accumulated in human muscle tissue as well as evidence for partially duplicated mtDNA in aged human tissues⁸⁵ suggests the important role of clonal expansion of mutant mtDNA in the age-related increase of systemic oxidative stress in the whole organism⁸⁶. A significant trend toward increasing *p53* mutations frequency with advancing age was found in some normal and malignant tissues^{46,47}. Simpson⁹ suggested that the aging human body accumulates enough mutations to account for multistep carcinogenesis by selection of preexisting mutations. The evidence showed that both genetics of the selected cellular clone and the epigenetics of the selective environment contribute to tumor development⁸⁷.

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. Dolle et al.⁸⁸ using a *lacZ* plasmid transgenic mouse model, determined spectra of spontaneous point mutations in different organs in young and old mice. While similar at a young age, the mutation spectra among these organs were significantly different in old age. The authors stressed that the replicative history *per se* is not the underlying causal factor of age-related organ-specific differences in mutations spectra. Rather, differences in organ function, possibly with association with replicative history, may explain the divergence in mutation spectra during aging. In turn, this may explain both age-related increase in spontaneous tumor incidence and age-related changes in susceptibility to carcinogens in various organs.

Multistage carcinogenesis is accompanied by disturbances in tissue homeostasis and perturbations in nervous, hormonal, and metabolic factors that may favor tumor growth and lessen natural antitumor defenses. The development of these changes depends on the susceptibility of various systems to a carcinogen and on the dose of the carcinogen. Changes in the microenvironment may condition key carcinogenic events and determine the duration of each carcinogenic stage, and sometimes they may even reverse the process of carcinogenesis. These microenvironmental changes influence the proliferation rate of transformed cells together, the total duration of carcinogenesis and, consequently, the latent period of tumor development (Figure 2).

Crosstalk between mesenchyme and epithelium has been described as a known driver of differentiation and development^{89,90}. It was shown that changes in stromal behavior can promote epithelial transformation^{66,89}.

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. 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. Table 4 summarizes the data available in 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. Carcinogens could be supposed to initiate 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 hand.

7. THE ROLE OF THE INSULIN/IGF-1 SIGNALING PATHWAY IN AGING AND CANCER

The potential link between aging and insulin/IGF-1 signaling has attracted substantial attention during last years, on the basis of evidence including age-related increase in incidence of insulin resistance and type 2 diabetes in accelerated aging syndromes and life span extension by caloric restriction in rodents. Concomitant reduction in plasma insulin and plasma glucose levels, which implies increased sensitivity to insulin, emerged as a hallmark of increased longevity^{91,92}. Hyperglycemia is an important aging factor involved in generation of advanced glycosylation endproducts (AGEs)^{93,94}. There are evidence that hyperinsulinemia favors accumulation of oxidized protein by reducing its degradation as well as facilitates protein oxidation by increasing steady-state level of oxidative stress⁹⁵. Untreated diabetics with elevated glucose levels suffer many manifestations of accelerated aging, such as impaired wound healing, obesity, cataracts, vascular and microvascular damage⁸. It was shown that centenarians have a preserved glucose tolerance and sensitivity to insulin as well as lower degree of oxidative stress as compared to aged persons⁹⁶. It is worthy to note that hyperinsulinemia is an important factor not only in aging but also in the development of cancer^{8,97,98}.

The intensive investigations in *C. elegans* since 1990's, which have identified insulin signaling components including *daf-2*, *age-1* and *daf-16* as the genes whose mutations lead to life span extension shed new light on

Table 4. Similarity of changes developing in an organism during natural aging and carcinogenesis (Anisimov, 1997, 2003, with modifications)

Parameters	Aging	Carcinogenesis
<i>Molecular level</i>		
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
<i>Cell/tissue level</i>		
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
<i>Systemic/ organism level</i>		
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
<i>Population level</i>		
Cancer incidence	Exponential pattern	Exponential pattern
Progeria	Acceleration	Increases
Exposure to 5-bromodeoxyguanine	Acceleration	Increases
Exposure to ionizing radiation	Acceleration	Increases
Treatment with geroprotectors	Postponement	Decreases or latency increases
Rate at the oldest age	Decreases in mortality	Decrease in incidence

* Related to clonally proliferating malignant cells

molecular mechanisms underlying aging^{91,92,99}. In *D. melanogaster*, the mutation of genes operating in the signal transduction from insulin receptor to transcription factor *daf-16* (*age-1*, *daf-2*, *CHICO*, *InR* и др.) are strongly associated with longevity^{99,100}. It was demonstrated that FKHR, FKHL1 and AFX, which are mammalian homologues of *daf-16* forkhead transcription factor, function downstream of insulin signaling and akt/PKB under cellular conditions^{101,102}.

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). 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}*, *Ghr*) whose knockout leads to dwarfism have been identified. The expression of these genes is associated with reduced levels of IGF-1 and insulin and increased longevity^{103,104}. In Snell and Ames dwarf mice, sexual maturation is delayed, and only 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 acute challenge⁹¹.

Recently, strong support for the role of 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 was provided by Hsieh et al^{105,106}. It was shown that in the Snell dwarf mice, GH deficiency would lead to reduced insulin secretion and alterations in insulin signaling via *InRβ*, IRS-1 or IRS-2 and P13K affects genes involved in the control of longevity. The authors 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 the sensitivity to insulin are a well-documented response to caloric restriction in rodents and monkey^{107,108}. It is worthy to note that *Ghr^{-/-}* mice have a major increase in the level of insulin receptors¹⁰⁹, while Ames dwarf mice have a smaller increase in insulin receptor and substantially increased amount of insulin receptor substrates IRS-1 and IRS-2¹¹⁰. The development of tumors in Ames dwarf mice was postponed and the incidence was reduced as compared to the control¹⁰⁸.

The crucial event of the effect of caloric restriction is low levels of insulin and IGF-1 and also the increase of insulin sensitivity in rodents¹¹¹ as well as in monkeys¹¹². Many characteristics of these long-lived mutants and GH-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 al.¹¹³ inactivated the *Igf1r* gene by homologous recombination in mice. It was shown that *Igf1r*^{-/-} mice died early in life, whereas heterozygous *Igf1r*^{+/-} mice live on average 26% longer than wild-type littermates. These mice did not develop dwarfism; their energy metabolism was normal. Food intake, physical activity, fertility and reproduction were also unaffected in *Igf1r*^{+/-} mice. These mice and 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 *Shc*, both main substrates of IGF-1 receptor, showed decreased tyrosine phosphorylation. *p66*^{Shc} mediated cellular responses to oxidative stress. Two main pathways – the extracellular-signal related kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K)-Akt pathway – were downregulated in *Igf1r*^{+/-} mice.

The extension of longevity was observed in fat-specific insulin receptor knockout (FIRKO) mice^{114,115}. These animals have reduced fat mass and were protected against age-related obesity and its subsequent metabolic abnormalities including deterioration in glucose tolerance, although their food intake was normal. Both male and female FIRKO mice had increased mean life span (by 18%) with parallel increases in maximum life span. Extended longevity in FIRKO mice was associated with a higher age threshold beyond which age-dependent increase in mortality risk became appreciable and a decreased age-adjusted mortality rate, especially after 36 months of age. In FIRKO mice, the resistance to obesity, despite normal food intake, suggested that metabolic rate is increased, rather than decreased¹¹⁵. The authors believe that decreased fat mass could lead to a decrease in oxidative stress. Another possibility is that the increased longevity in these mice is the direct result of altered insulin signaling.

Shimokawa et al.¹¹⁶ designed 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 an intermediate phenotype in plasma IGF-1, food intake, and body weight between *tg/tg* and control (*-/-*) rats. The life span of *tg/tg* rats was 5 to 10% shorter than *-/-* rats. In contrast, the life span of *tg/-* rats was 7 to 10% longer than *-/-* rats. It was found that tumors caused earlier death in *tg/tg* rats; in contrast, *tg/-* rats had reduced nonneoplastic 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 the GH-IGF-1 axis function needs for longevity in mammals.

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 mutation located downstream to *daf-16* in insulin signal transduction system is associated with longevity¹¹⁷. It is worth noting that centenarians display lower degree of resistance to insulin and lower degree of oxidative stress as compared with elderly persons before 90 years⁹⁶. The authors suggested that centenarians may have been selected for appropriate insulin regulation as well as for the appropriate regulation of tyrosine hydroxylase (TH) gene, whose product is rate limiting in the synthesis of catecholamines, stress-response mediators. It was shown that catecholamines may increase free radical production through induction of the metabolic rate and auto-oxidation in diabetic animals¹¹⁸. Recent study on aging parameters of young (up to 39 years) and old (over 70 years) individuals having similar IGF-1 serum levels provides evidence of important role of this peptide for life potential¹¹⁹. Roth et al.¹²⁰ analyzed data from the Baltimore Longitudinal Study of Aging and reported that survival was greater in men who maintained lower insulin level.

Several years ago, it was suggested to use biguanide antidiabetics as a potential anti-aging treatment⁸. The antidiabetic drugs, phenformin (1-phenylethylbiguanide), buformin (1-butylbiguanide hydrochloride) and metformin (N,N-dimethylbiguanide) were observed to 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^{8,121,122}.

Buformin supplemented at the concentration of 0.1 mg/ml to nutrient medium during the larvae stage and over the life span of *C. elegans* increased the mean life span of the worms by 23.4% and the maximum life span by 26.1% as compared to the controls¹²³. The treatment with phenformin or buformin slightly decreased the body weight of rats, in comparison with the control slow down the age-related switching-off of the reproductive function in female rats prolonged the mean life span of female C3H/Sn mice and LIO rats^{1,6,124-128}. Recently it was found that metformin significantly increases the life span of rats (G.S. Roth, personal communication).

Several other effects of treatment with antidiabetic biguanides related to reproduction and aging, are known from earlier studies. For example, it decreased hypothalamic threshold of the sensitivity to feedback inhibition by estrogens¹²⁵⁻¹²⁸, which is one of the most important mechanisms regulating age-related decline and switch-off of the reproductive function¹²⁵⁻¹³⁰. Treatment with metformin may improve menstrual regularity,

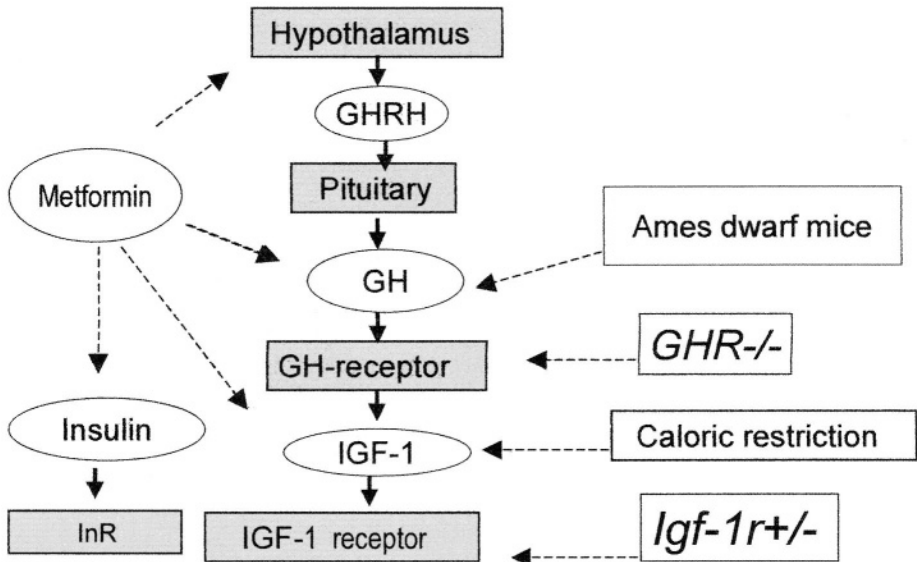
leading to spontaneous ovulation, and enhance the induction of ovulation with clomiphene citrate in women with polycystic ovary syndrome ¹³¹. The treatment with phenformin also decreased hypothalamic threshold sensitivity to feedback regulation by glucocorticoids and by metabolic stimuli (glucose and insulin) ⁸. It was recently shown that elements involved in the insulin/IGF-1 signaling pathway are regulated at the expression and/or functional level in the central nervous system. This regulation may play a role in the brain's insulin resistance ¹³², in the control of ovarian follicular development and ovulation ¹⁰², and brain's control of life span ^{111,133}. Antidiabetic biguanides also alleviated age-related metabolic immunodepression ⁸. These mechanisms can be involved in geroprotective effect of biguanides. Treatment with chromium picolinate which elevated the insulin sensitivity in several tissues, including hypothalamus, significantly increased the mean life span and decreased the development of age-related pathology in rats ¹³⁴. We hypothesized that antidiabetic biguanides and possibly chromium picolinate regulate tyrosine hydroxylase and insulin/IGF-1 signaling pathway genes both associated with longevity ^{99,135}. It was shown that the polymorphism at TH-INS locus affects non-insulin dependent type 2 diabetes ¹³⁶, and is associated with hypothalamic obesity ¹³⁷, polycystosis ovary syndrome ¹³⁸, hypertriglyceridemia and atherosclerosis ¹³⁹.

The anticarcinogenic effect of antidiabetic biguanides has been demonstrated in several models of spontaneous and induced carcinogenesis. The treatment with phenformin normalized the tolerance to glucose and serum insulin and IGF-1 level in rats exposed to intravenous injections of N-nitrosomethylurea (NMU) and inhibited mammary carcinogenesis in these animals ^{124, 140}. Treatment of rats with 1,2-dimethylhydrazine (DMH) caused the decrease in the level of biogenic amines, particularly of in the hypothalamus, the decrease of glucose tolerance and the increase of the blood level of insulin and triglycerides. Administration of phenformin restored immunological indices and inhibited DMH-induced colon carcinogenesis ^{140, 141}. The colon 38 adenocarcinoma growth was significantly inhibited in liver-specific IGF-1-deficient mice, whereas injections with recombinant human IGF-1 displayed sufficiently promoted the tumor growth and metastasing ¹⁴².

A decrease of glucose utilization was found in the 3-month-old female progeny of rats exposed to NMU on the 21st day of pregnancy ^{124,140}. 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 ¹⁴³⁻¹⁴⁴. In high fat-fed hamsters, the 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 the carcinogen and metformin¹⁴⁵.

Figure 4. Proposed effects of metformin, calorie restriction and genetic modifications on insulin/IGF-1 signaling pathway in control of aging. The broken arrows on the figure show the targets for the genetic modifications, calorie restriction and for metformin in IGF-axis.



Thus, 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 tumors¹²⁵⁻¹²⁷.

The comparative study of 10-years results of metabolic rehabilitation (included fat and carbohydrate dietary restrictions and treatment with biguanides) of cancer patients had suggested increase in the survival of breast and colorectal cancer patients, increase in the length of cancer-free period, decrease in the incidence of metastasis as compared with control patients¹²².

Although it is known that free radicals are produced during metabolic reactions, it is largely unknown which factor(s) modulate their production *in vivo*. It has been suggested that hyperinsulinemia may have increase free radicals and therefore promote aging, independent of glycemia^{8,94,96}. Plasma

levels of lipid hydroperoxides are higher, and antioxidant vitamins are lower in individuals who are resistant to insulin-stimulated glucose disposal but otherwise glucose tolerant, nonobese, and normotensive^{93,95}. There is substantial evidence supporting the hypothesis that selective resistance to insulin-stimulated (muscle) glucose disposal consequent hyperinsulinemia triggers a variety of metabolic effects, likely resulting in accelerated oxidative stress and aging^{8,93,95}.

The anti-diabetics biguanides inhibit fatty acid oxidation, inhibit gluconeogenesis in the liver, increase the availability of insulin receptors, inhibit monoamine oxidase¹²¹, increase sensitivity of hypothalamo-pituitary complex to negative feedback inhibition, reduce excretion of glucocorticoid metabolites and dehydroepiandrosterone-sulfate⁸. These drugs have been proposed for the prevention of the age-related increase of cancer and atherosclerosis, and for retardation of the aging process⁸. 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, 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 hyperlipoproteinemia⁸. There are data on antioxidative effect of biguanides^{133,146} and its neuroprotective activity¹⁴⁷. It was shown that biguanides inhibits complex I of the respiratory chain in mitochondria that leads to an activation of physiological intracellular inhibition of mitochondrial respiration¹⁴⁸. Biguanides stimulate a protein kinase cascade inhibiting an expression of transcription factor SREBP-1. An interaction 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 FFA¹⁴⁹. It was shown also that in vivo biguanides inhibits an appetite^{150,151} and serum levels of leptin and IGF-1¹⁵². It was suggested that biguanides regulate energy balance of the organism at the fat tissue level¹⁵³. In general, results of bioguanides effects seem very similar to those of calorie restriction.

Table 5. Comparison of characteristics of rodents subjected to normal aging, caloric restriction, genetic modifications or treatment with antidiabetic biguanides.

Parameters	Aging	Calorie restriction	Dwarf mice	<i>GH R^{-/-}</i>	<i>Igf1r^{+/-}</i>	FIRKO	Biguanides
Life span extension	↓	+40-50%	+50%	+46 %	+33%	+18%	+20%
Tolerance to glucose	↓	↑	↓	↓	↑↓ ^a	= or ↑	↑
Sensitivity to insulin	↓	↑	↑	↑	↑	↑ in fat	↑
Serum level: Insulin	↑	↓	↓	↓	=	↓	↓
	↓	↓	Absent	↑	ND	↓	↓
GH	↓	↓	↓	↓	↓	↓	↓
IGF-1							
Body size	↑	↓	↓	↓	↓	↓	↓
Body fat content	↑	↓	↑	ND	↑↓	↓	↓
Reproductive function	↓	↓ ^b	↓ ^b	↓ ^b	= ^b	ND	↑
Thyroid function	↓	↓	↓	↓	=	ND	↑
Serum corticosterone	↑	↑	=	=	ND	ND	↓
Immune function	↓	↓	= or ↓	ND	ND	ND	↑
Resistance to oxidative stress	↓	↑	↓	↓	↑	↑	↑
Tumor incidence	↑	↓	= or ↓	=	=	ND	↓

Note: ↓ - decrease; ↑ - increase; = no effect; ND – no available data.

^a The tolerance to glucose is increased in females but decreased in male.

^b Reproductive function in relation to normal aging mice.

8. CONCLUSION

The incidence of cancer increases with age in humans and in laboratory animals alike, but patterns of age-related distribution of tumors is different for different tissues and different tumors. Aging may increase or decrease the susceptibility of individual tissues to early carcinogens and usually facilitates promotion and progression of carcinogenesis. Aging may predispose to cancer by 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 effect of late-stage carcinogens is found both in aged animals and elderly humans, as predicted by the multistage model of carcinogenesis. Studies in mammals have led to the suggestion that hyperglycemia and hyperinsulinemia are important factors both in aging and in the development of cancer. Insulin/insulin-like growth factor 1 (IGF-1) signaling molecules that have been linked to longevity include DAF-2 and InR and their homologues in mammals, and inactivation of the corresponding genes followed by the increase in life span in nematodes, fruit flies and mice. It is possible that the life-prolonging effects of caloric restriction are due to decreasing IGF-1 levels. A search of pharmacological modulators of insulin/IGF-1 signaling pathway mimetic effects of life span extending mutations or caloric restriction could be a perspective direction in regulation of longevity. Some old and new observations suggest that antidiabetic biguanides could be promising candidates for both the life span extension and the prevention of cancer.

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