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AGING AND CANCER IN TRANSGENIC AND MUTANT MICE

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TABLE OF CONTENTS

1. ABSTRACT

Mutant and genetically modified animal models, which are characterized by shortening or extension of the life span, give a unique possibility to evaluate the role of aging genes in mechanisms of carcinogenesis. Transgenic and null mutant ("knockout") animal models also offer an important opportunity to identify and study both carcinogens and chemopreventive agents. The analysis of the available data on transgenic and mutant mice has shown that only a few models represent examples of life span extension. Ames dwarf mutant mice, $p66^{-/-}$ knockout mice, alpha-MUPA and O^6-methylguanine-DNA methyltransferase (MGMT) transgenic mice live longer than wild-type strains. The incidence of spontaneous tumors in these mice was similar to those in controls, whereas the latent period of tumor development was increased. Practically all models of accelerated aging (excepting $p53^{+/m}$ mice) show the increased tumor incidence and shortening of tumor latency. These observations are in agreement with an earlier established positive correlation between tumor incidence and the rate of tumor incidence increase associated with aging and the aging rate in a population. Thus, genetically modified animals are a valuable tool in unraveling mechanisms underlying aging and cancer.

2. INTRODUCTION

The differences in longevity and pathology incidence, including cancer incidence among rodent strains, provide strong evidence of genetic influence on these parameters, although one can successfully argue that the differences between strains are also influenced by epigenetic mechanisms such as imprinting. There are existing genes that may affect the variation in life span within an inbred genetic background (1). These genes set up a response threshold and the degree of the integration of the regulatory network (2). During the last decade a number of genetic models with extended or reduced life spans have been generated. These models offer new approaches towards understanding the aging process. Spontaneous and induced genetic modifications, including homozygous null mutations ("knockout") and transgenic mammalian animals, have also been introduced in experimental gerontology (3-5). It is worth noting that, although the

effects of some genetic manipulations manifest themselves only at specific developmental periods of animal life, these genetic manipulations are in effect during embryonal development, as well as throughout adult life. Therefore, there are significant limitations in the interpretations of data generated by utilizing these genetic manipulations. Some of them have been discussed recently (2,4). On the one hand, the elimination of a specific activity or a specific pathway can lead to an erroneous conclusion regarding gene function because the compensatory mechanism may markedly alter the animal physiology. On the other hand, overexpression of a transgene may readily yield no effect on the life span or, for that matter, on any other aging parameter of the animal. Jazwinski (6) has noted that overexpression of a transgene will likely create interactions with other genes and with the environment, both external and internal.

A relationship between aging and carcinogenesis has been intensively discussed (7-17). The increased incidence of cancer as a function of aging has long been interpreted to suggest that multiple genetic changes are required for carcinogenesis to occur. Cancer cells differ from normal cells in many characteristics, including loss of differentiation, phenotypic changes, increased invasiveness, and decreased drug sensitivity (18-20). Although recent advances in molecular biology have helped to clarify the possible relationships between carcinogenesis and aging, it remains unclear whether genetic markers may be common to all cancer types or which markers may be associated with the increased age of cancer patients. Transgenic animal technology has resulted in a plethora of murine models for cancer research, providing insight into the complex carcinogenic events contributing to the loss of cell cycle control and tumor development. Transgenic and null mutant animal models also offer an important opportunity to identify and study both carcinogenic and chemopreventive agents (21,22). Genetically modified animal models, which are characterized by a shortening or extension of life spans, give a unique possibility to evaluate the role of aging genes involved in the mechanisms of carcinogenesis (23).

3. GENETICALLY MODIFIED MOUSE MODEL OF ACCELERATED AGING

3.1. Growth hormone (GH) transgene mice

In a number of experiments with transgenic animals expressing genes determining hyperproduction of human or animal GH, it was shown that these mice exhibit signs of premature aging and live only half as long as their wild-type siblings (24,25). Mice overexpressing GH exhibit increased indices of free radical production (26), significant reduction of catalase activity in the liver and kidney (27), signs of premature central nervous system aging (including reduced catecholamine turnover), increased astrogliosis, and impaired learning and memory (25,28,29). These animals reach sexual maturation earlier and cessation of reproduction sooner than wild-type controls (30,31). This effect in GH-transgenic mice is related to an accelerated degenerative process in the ovaries that is not present in the wild-type control (32). Most importantly, GH overexpressed mice have a high incidence of tumor development (33-35). It is worth noting that old (16 to 24 months of age) mice, transgenic with human growth hormone-releasing, hormone-developed pituitary adenomas, produced both GH and prolactin (36).

Age-related disturbances in the regulation of the insulin-like growth factors activity plays an important role in the development of some metabolic disorders and diseases, including cancer in the elderly (8,37,38). By using a construct in which the coding region of the mouse insulin-like growth factor-2 gene (Igf-2) was placed under the control of a keratin gene promoter, four transgenic lines were established, all of which displayed overgrowth of the skin, as judged by wrinkling (39). Transgene expression was high in the skin, in the gastrointestinal tract, and the uterus. Adult total body weight was slightly increased and there was no macroscopic evidence of tumor formation. However, the increase in cell proliferation was observed in the sites of Igf-2 expression.

3.2. Senescence accelerated mice (SAM)

The SAM strain was generated by selective inbreeding of AKR/J mice (40,41). There are several senescence-prone strains (SAMP), which live 12-15 months, and several senescence-resistant (SAMR) strains, which are normal controls for accelerated aging and have a life span of 24-30 months. It has been shown that SAMP mice develop normally until the age of 4 months and then they reveal signs of accelerated aging (such as loss of hair, skin ulceration, decrease of locomotor activity, deficiency in learning and memory, emotional disorders, abnormal circadian rhythms, brain atrophy, hearing impairment, cataracts, increased production of reactive oxidation specimens (ROS) and 8-hydroxyguanine levels in all organs (40-44)). The amount of Cu, Zn-SOD in the mitochondria fraction of the SAMP-1 was only half that of the SAMR-1 (45). The reproductive life span of SAMP was shorter than that of the SAMR and the reproductive senescence of the SAMP strain was more accelerated than that of the SAMR strain (46). It is worth noting that O^6-methylguanune-DNA methyltransferase, which repairs alkylated DNA, shows no difference in its activity in SAMP1 when compared with the SAMR1 strain (43).

The accelerated senescent-prone strain, SAMP-1, shows a striking increase in the frequency of chromosome aberrations from the age of 3 to 8 months, whereas the SAMR-1 strain shows only a slight increase of chromosome aberrations at the same age (47). Uryvaeva *et al*. (48) have shown an accelerated accumulation of micronuclear aberrations (with age) in the liver cells of SAMP mice as compared to the SAMR strain. The age-associated incidence of somatic *Hprt* mutations in splenic lymphocytes, as well as DNA damage (mainly DNA single strand breaks) in six organs, are also accelerated in SAMP1 mice as compared to the SAMR1 (49,50).

The incidence of spontaneous lymphomas is 17.5% in SAMP strains (from 0 % in SAMP11 and SAMP6 to 60.2% in SAMP7) and 13.7% in SAMR strains (from 2.7% in SAMR5 and 23.1% in SAMR4). The incidence of

other malignancies varies (from 0 to 4.8% in SAMP and from 3.8 to 4.1% in SAMR strains (40)). The levels of murine leukemia virus titres are found to be higher in the blood and spleen and much higher in the brain of SAMP-8 than in the same tissues of the SAMR1 strain (51). Sugimura *et al*. (52) revealed a high incidence of stromal hyperplasia, with fibrosis and inflammation in the dorsal lobe of the prostate gland, in SAMP mice. Atypical glandular epithelial cells and cribriform glandular deformities were observed in the dorsal and lateral lobes of the prostate gland of the SAMP strain.

3.3. Mutation in mouse klotho gene

Kuro-o *et al*. (53) established a novel mouse autosomal recessive mutant , *klotho*, that exhibits multiple phenotypes very similar to those observed in human aging, including a short life span (less than 100 days), decreased body weight, infertility, arteriosclerosis, skin and thymus atrophy, osteoporosis and emphysema. These mice are hypoglycemic and have decreased levels of insulin in the pancreas. Glucose tolerance and sensitivity to insulin are increased in *klotho* mice, compared to these parameters in wild-type mice (54,55). Uncoupling protein-1 gene expression of the brown adipose tissue and body temperature in *klotho* mice were lower than those in wild-type mice, suggesting that *klotho* mice have less energy expenditure than wild-type mice (54). The immunohistochemistry of the pituitary glands of *kl/kl* mice confirmed a decrease in the growth hormone, as well as the luteinizing hormone and follicle-stimulating hormone production. The gene has homology with the membrane sparring region and with the β-glucosidase enzymes. Recently the *β-klotho (βkl)* gene, which encodes a type I membrane protein, has been cloned (56). The treatment with human growth hormone did not increase the body weight in *klotho* mutant mice (57). Kuoro-o *et al*. (53) suggested that the *klotho* gene product may function as part of a signaling pathway, regulating aging *in vivo* and morbidity in age-related diseases.

The authors also concluded that *kl/kl* mice were not a model for mouse aging, but rather for human progeroid syndromes. There is no data on tumor pathology in these mice. The *klotho* mouse differs from SAM in several aspects: (a) the multiple aging-associated phenotypes in *kl/kl* mice are autosomal recessive and are not influenced by the genetic background, whereas the conditions of inheritance in SAM are more complex; (b) the multiple aging-associated phenotypes occur in *kl/kl* mice, whereas specific aging-associated phenotypes are typical for various SAM substrains; and (c) the aging-associated phenotypes in *kl/kl* mice manifest themselves much earlier than in SAM (53). It is worth noting that the *klotho* mouse is the first laboratory animal model with multiple phenotypes resembling human aging caused by a single gene mutation. The study, focused on osteopenia in *kl/kl* mice, has shown that a defect in the *klotho* gene expression causes the independent impairment of both osteoblast and osteoclast differentiation, leading to low cell turnover and osteopenia (58). Recently an association of human aging with a functional variant of *klotho* has been described (59,60).

3.4. DNA repair gene transgenic and knockout models

DNA repair plays an important role in genome stability. The DNA damage theory, being preceded by the somatic mutation theory of aging (8,61,62), assumes that aging in mammals is due to the accumulation of DNA damage in somatic cells. Taking into consideration the relationship among DNA damage, defective DNA repair, and carcinogenesis, it could be suggested that age-related changes, both in efficacy and the rate of DNA repair in an individual, might modify the susceptibility to exogenous or endogenous carcinogens (8). There are several types of DNA damage that occur in nature: spontaneous depurination and depyrimidination, cytosine deaminations, single-strand breaks, O^6-methylguanine, glucose and glucose-6-phosphate adducts, oxidative damage (thymine and thymidine glycols, hydroxymethyluracil, 8-hydroxideoxyguanosine, methyl adducts, cross-links and double-strand breaks (61). All of them could play a role in aging and carcinogenesis (8,11,61).

Xeroderma pigmentosum, characterized by a deficiency in the nucleotide excision repair and a greater than 1000-fold increased risk of skin cancer, represent a paradigm to understanding the role of unrepaired lesions in the development of cancer (63). Recently, two mouse models were generated with a defect in one of the nucleotide excision DNA repair genes (*XPD* and *CSB*), displaying distinctive symptoms of premature aging (64). Mice with a mutation in *XPD*, a gene encoding a DNA helicase that functions in both repair and transcription and that is mutated in the human disorder trichothiodystrophy (TTD) have been constructed (65). TTD mice were found to exhibit many symptoms of premature aging, including osteoporosis and kyphosis, osteosclerosis, early greying, cachexia, infertility, and reduced life span (average < 12 months, compared with > 2 years for wild-type littermates). TTD mice carrying an additional mutation in XPA that enhances the DNA repair defect, showed a greatly accelerated aging phenotype, which correlated with an increased cellular susceptibility to oxidative DNA damage. No data on tumor incidence in these mice has been reported yet.

These important findings raise many questions (66). At the molecular level, what are the lesions in DNA that ultimately cause the premature aging? The interaction of unrepaired damage and transcriptional deficiency results in premature aging in mice and humans, but are these same processes a major cause of aging in unaffected "normal" individuals? The model is very promising for studies in this direction.

The primary embryonic fibroblasts isolated from the xeroderma pigmentosum group G (*XP-G*) gene-deficient mice underwent premature senescence and exhibited the early onset of immortalization and accumulation of p53 (67). Xeroderma pigmentosum group A (*XPA*) gene-deficient mice have an almost complete deficiency in DNA nucleotide excision repair, and only 15% of the mice develop spontaneous tumors (hepatocellular adenomas) after 1.5 years (68). However, $XPA^{-/-}$ mice are very susceptible to ultraviolet B radiation and to different chemical carcinogens (68,69).

When crossed with mice carrying a mutation in the *Apc* tumor supressor (70), mice heterozygous for a targeted null mutation of *blm* gene, which encodes a recQ-like helicase and is the murine homolog of Bloom syndrome *BLM* gene, develop lymphoma earlier than wild-type littermates (in response to challenge with murine leukemia virus) and develop twice the number of intestinal tumors.

Failure of DNA repair and the fixation of DNA damage as mutation eventually lead to cellular transformation and carcinogenesis. In response to DNA damage, a nuclear enzyme, poly(ADP-ribose) polymerase (*Parp*), is activated, and poly(ADP-ribosyl) activates various nuclear proteins using NAD as a substrate. *Parp* is involved in the base-excision repair process and in DNA strand break repair (71,72), in the induction of cell death (73) and in the regulation of genomic stability linked with longevity (74). *Parp* knockout mice were established by disrupting *Parp* exon 1, 2 or 4 in the genetic background of 129Sv/C57BL6 or 129Sv/ICR mice (*Parp* $^{-/-}$) (75). It has been shown that these mice are very susceptible to the effects of alkylating agents and ionizing radiation. *Parp* $^{-/-}$ mice show severe myelosuppression (75). It is very important to investigate the survival and spontaneous tumor incidence in *Parp* $^{-/-}$ mice. However, these data have not yet been reported.

Ku80 is important for the repair of DNA double-strand breaks by the nonhomologous end-joining protein, Ku70. The Ku80-Ku70 heterodimer (Ku) binds to DNA ends, nicks, gaps, and hairpins. Ku80-mutant mice (*ku80*$^{-/-}$), when compared with wild-type littermates, prematurely exhibited age-specific changes, characteristic of senescence, that include osteopenia, atrophic skin and hair follicles, hepatocellular degeneration, hepatic hyperplastic foci, and age-specific mortality (76). The cancer and likely sepsis (suggested by reactive immune responses) were partly responsible for age-specific mortality in both cohorts. But diseases occurred earlier in *ku80*$^{-/-}$ mice. It is worth noting that the onset of age-related mortality in *ku80*$^{-/-}$ mice begins shortly after sexual maturity, possibly accounting for their reduced fecundity. It was observed that mouse cells deficient in Ku80 display a marked increase in chromosomal aberrations, including breakage, translocations and aneuploidy (77). Cancer incidence was reduced by 13-fold in *ku80*$^{-/-}$ mice in comparison to the control; however cancers were observed earlier in mutant mice (76). At the same time, knockout *ku70*$^{-/-}$ mice with the same genetic background (129Sv x C57BL/5) showed a reduction in life span and had a high incidence of $CD4^+$ $CD8^+$ T cell lymphomas at a mean age of 6 months (78,79), which suggests that one (or both) of these proteins works independently. It was shown that p53 monitors chromosome damage and either arrests the cell-cycle progression or triggers apoptosis in cells with unrepaired lesion (80). To determine whether p53 is involved in the growth arrest of *Ku80*$^{-/-}$ mice, double-mutant mice *Ku80*$^{-/-}$ *p53*$^{-/-}$ were generated (77). Although the mice developed normally, all of them died within 12 weeks after birth from disseminated B-cell lymphoma. In contrast, *p53-/-* mice are predisposed to thymic lymphomas (81), which develop at a slower rate than *Ku80*$^{-/-}$*p53*$^{-/-}$ pro-B-cell lymphomas, and *Ku80-/-* mice only occasionally develop T-cell lymphoma after seven months (76). It was concluded that Ku80 is a caretaker gene that maintains the integrity of the genome by a mechanism involving the suppression of chromosomal rearrangement (77).

3.5. Overexpression of Cu, Zn-SOD or catalase

Several studies have shown that aging cells and organisms accumulate increased levels of oxidant-damaged nuclear DNA (whereas superoxide dismutase, catalase, and some other enzymes and scavengers of free radicals protect a cell and an organism from oxidative stress (82,83)). The human Cu, Zn superoxide dismutase (*hSOD-1*) gene, catalyses the dismutation of O_2 to H_2O_2 and O_2. It is located in chromosome 21 in q22.1 and is overexpressed in Down's syndrome (DS) patients. These patients show various abnormalities (including mental retardation, congenital heart disease, immunological deficits, premature aging and increased cancer risk). Mutations in mitochondrial genes encoded by both mitochondrial DNA (mtDNA) and nuclear DNA have been implicated in a wide range of degenerative diseases (84).

In order to explore the potential role of *SOD-1* overexpression in DS, two lineages of transgenic mice for the *hSOD-1* gene have been generated and studied, at the ultrastructural level, to evaluate the effect of *hSOD-1* overexpression on the thymic microenvironment (85). Modification of the cellular architecture and morphology associated with a lipidic invasion, which are signs of a premature involution of the thymus, were observed in both lineages. A rupture of the filamentous network in the extracellular, and probably also in the intracellular matrix, was observed first. These results correlate the thymic alterations, visualized in light microscopy, on the thymus of DS patients and raise the question of the relationship between *SOD-1* overexpression and the different morphological alterations associated with the premature thymic involution observed in *SOD-1* transgenic mice. It was suggested that thymic and immunological impairments (present in DS patients) may be related to the *SOD-1* gene dosage effect. Overexpression of the human Cu, Zn-SOD gene was not beneficial to transgenic mice and caused increased lipid peroxidation in the brains of the animals (86). At the same time, in *hSOD-1* transgenic mice, age-related accumulation in the brainstem and the striatum of a marker of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine (8OHdG), and carbonyl oxidation products were significantly attenuated (compared to the wild-type control (87)). Twenty-four male mice transgenic with human Cu, Zn-SO, resulting in an overexpression of the cytosolic enzyme, were compared with 11 matched controls that were older than 19 months. There was no difference in longevity, locomotor activity, or dopamine uptake sites in the brain regions between the two groups (88). Five of the 24 transgenic mice died over the age of 19 months, together with five of the 11 controls (not statistically significant). It is very important to evaluate the rate of spontaneous tumor development in these mice.

Two types of transgenic mice were generated to evaluate the role of hydrogen peroxide in the formation of nuclear DNA damage. One set of lines overexpresses wild-type human catalase cDNA, which is localized to peroxisomes; whereas the other set overexpresses a human catalase construct that is targeted to the nucleus (89). Both types of transgenic animals had significant increases of catalase activities as compared to littermate controls. Despite enhanced activities of catalase, there were no changes in the levels of 8OHdG, a marker of oxidative damage to DNA. No data was reported on the survival rate and cancer incidence in these mice.

A specific DNA glycosylase, a product of the OGG1 gene, excises 8OHdG from DNA in eukaryotic cells. Homozygous *ogg1*$^{-/-}$ null mice were viable, but accumulated abnormal levels of 8OHdG in their genome (90). Despite this increase in potentially miscoding DNA lesions, OGG1-deficient mice exhibited only a moderately (but significantly) elevated spontaneous mutation rate in nonproliferating tissues, did not develop malignancies, and showed no marked pathological changes (90). It is important to note that the last two conclusions have been made because of results gained during a histopathological examination of two such animals sacrificed at 8 and 11 months of age (!) This evidence is totally insufficient for the evaluation of tumor incidence in mice, and the examination was carried out at a stage which was too early for the development of spontaneous tumors in the mouse strain 129 (8).

3.6. Mutant and transgenic models of the immunosenescence

During aging in mice and humans, a gradual decline in thymus integrity and function occurs (thymic involution) (91,92). To determine whether T cell reactivity or development affects thymic involution, the thymic phenotype in old (12 months) and young (2 months) mice (transgenic with rearranged α/β or β-2B4 T cell receptor (TCR) genes, mice made deficient for CD4 by gene targeting (CD4$^{-/-}$), mice made deficient for major histocompatibility complex (MHC)) class I (β2M$^{-/-}$) or class II genes (Aβ$^{-/-}$) have been compared (93)). The expected aging-related reduction in thymic weights were observed for all strains except those bearing disruption of both class I and class II MHC genes. Therefore, disruption of MHC class I and class II appeared to reverse or delay aging-related thymic atrophy at the age of 12 months. Immunohistochemical analysis of aging-associated alterations in thymic morphology revealed that TCR α/β transgenes, D4 disruption, and MHC class II disruption all reduced or eliminated these changes. All strains examined at 12 months showed alterations in the distribution of immature thymocyte populations (relative to young controls). These observations show that aging-associated thymic alterations can be separated and are therefore causally unrelated.

Mutant immunosuppressed NMRI mice (*nu/nu*), even when kept under germ-reduced conditions and fed with a germ-reduced diet, have an extremely short life span – the last mouse died at the age of 5 months (94,95). However in another background mouse strain (Swiss) the same mutations was followed by longer life span than in NMRI mice (96). Systematic observation of 1141 nude Swiss mice revealed 24 spontaneous tumors, 18 of lymphoreticular origin and 6 lung adenomas (96). Spontaneous tumors were seen at an average age of 9.1 months, and 22 of the tumors were seen only in that fraction of the group (324 mice) surviving for 5 months or more (6.8%).

Nevertheless, the incidence of spontaneous tumors in these nude mice was similar to the thymus-bearing background strain (96). The incidence and type of spontaneous tumors in athymic nude (*nu/nu*) mice, which were partially inbred (CBA/H) background, which were also carrying the viable yellow gene (*Avy*, derived from C57BL/6JAvy mice), and were comparable to those observed in the phenotypically normal nu/+ and +/+ control crosses carrying the *Avy* gene (97). The *Avy* gene increases the incidence of spontaneous tumors in most mouse strains. The effect of the nude gene heterozygocity on spontaneous AKR thymic lymphomagenesis was studied by comparing female littermates of AKR/Ms *nu*$^{-/+}$ and $^{+/+}$ (98). Overall incidences of thymic lymphomas were comparable in the two genotypes, but the mean latent period for lymphoma development was significantly shorter in (*nu/+)* mice (266 ± 11.6 days) than in the *(+/+)* mice (319 ± 7.9 days).

T-cell dysfunction and thymic involution are major immunologic abnormalities associated with aging (91,92). *Fas* (CD95) is a bifunctional molecule that is critical for apoptosis and stimulation during T-cell development. Using *fas*-transgenic mice, it was shown that T-cell senescence is associated with defective apoptosis and that the CD2-*fas* transgene allows for maintenance of the *Fas* apoptosis function and T-cell function in aged mice (99). In transgenic mice overexpressing the *bcl-2* gene in thymocytes, a resistance of immature thymocytes to apoptosis (mediated by corticosteroids and calcium ionophores) was observed (100). It was also shown that overexpression of *bcl-2* enabled a proportion of thymocytes and peripheral T-cells to escape the process of clonal deletion, which normally eliminates self-reactive T-cells during thymocyte maturation. These findings implicate the *Bcl-2* protein in regulating the life span of maturing thymocytes and in the antigen-selection process. The evaluation of a risk of spontaneous tumor development in *Bcl-2* transgenic mice is of critical interest.

Transgenic mice that contained constructs of the L-*myc* gene under the transcriptional control of the immunoglobulin heavy chain enhancer (E mu) developed thymic hyperplasia and were predisposed to T cell lymphomas and to highly malignant mesenchymal neoplasms that closely resemble human fibrous histiocytoma (101).

3.7. Transgenic and knockout models of age-related neurodegenerative diseases

Increased interest is emerging in the use of mouse models to assess the genetics of brain aging and age-related neurodegenerative disease (3). A mutant amyloid precursor protein (APP/RK), designed to interfere with processing by α-secretase, caused a severe phenotype in transgenic mice

(including behavioural abnormalities, (e.g., neophobia, aggression, hypersensitivity to kainic acid, and premature death) (102)). The major and consistent finding in these mice that died prematurely was extensive neurodegeneration and apoptosis (mainly in the hippocampus and cortex), accompanied by astrocytosis throughout the brain (103).

The formation of fibrillar deposits of amyloid-β protein in the brain is a pathological hallmark of Alzheimer's disease (AD). It was shown, however, that mice transgenic with the amyloid-β precursor protein that developed amyloid deposits in the brain do not show the degree of neuronal loss or *tau* phosphorylation found in AD (104). Shoji *et al*. (105) observed an age-related amyloid beta protein accumulation in transgenic mice which expressed a gene encoding 18 residues of signal peptide and 99 residues of the carboxyl-terminal fragment of the amyloid β precursor (under the control of the cytomegalovirus enhancer/chicken beta-actin promoter). The authors concluded that overproduction of amyloid β protein causes accumulation of the amyloid β fibrils, with accompanying cellular degeneration and macrophage activation, *in vivo*.

In another study it was reported that transgenic FVB/N mice overexpressing human or mouse Alzheimer amyloid precursor protein (APP695) died early and developed a CNS disorder that included neophobia and impaired spatial alteration, with diminished glucose utilization and astrogliosis mainly in the cerebrum (106,107). Age at the onset of neophobia and age at death decreased with increasing levels of brain APP. No extracellular amyloid was detected, indicating that some deleterious processes related to APP overexpression were dissociated from the formation of amyloid. It is worth noting that a similar clinical syndrome occurs spontaneously in 20% of wild-type mice when they reach mid- to late-adult age, suggesting that APP overexpression may accelerate naturally occurring age-related CNS disorders in FVBN mice (106). Aged Tg2576 transgenic mice overexpressing human βAPP695 have limited neuron loss and *tau* pathology, but also have frequent ubiquitin- and α-synuclein-positive, *tau*-negative neurites, resembling those seen in the Lewy body variant of Alzheimer's disease (108).

There is another model of Alzheimer's disease in transgenic mice harboring the human gene S-100β. This gene is a neurotrophic factor realised by astroglial cells and localised to chromosome 21 within the region that is considered obligate for Down's syndrome. S-100β is increased in the post mortem brains of both Down's syndrome and Alzheimer's disease. By 1 year of age, the transgenic animals have significant loss of dendrites compared to controls and the number of cells showing cell body staining was further increased. Behaviorally, younger transgenic animals could not perform learning tasks as well as controls (109). The authors suggest that the increased S-100β in the brain may lead to accelerated development, followed by increased aging.

It was shown that some cases of amyotrophic lateral sclerosis (a fatal disease in which spinal cord motor neurons degenerate resulting in progressive paralysis) are caused by mutations in the antioxidant enzyme Cu, Zn-SOD. Transgenic mice expressing amyotrophic lateral sclerosis-linked Cu, Zn-SOD mutation (SODMutM) exhibit a phenotype similar to that of human patients. The onset of the disease occurred in mice placed at 6 weeks of age (110). Dietary restriction failed to delay the onset of the disease or to shorten its duration. Overexpression of hepatocyte growth factor in the nervous system attenuated motoneuron death and axonal degeneration and prolonged the life span of transgenic mice overexpressing mutated Cu, Zn-SOD 1 (111).

It should be noted that data on the development of spontaneous tumors are practically absent in all the reviews included in this section of papers on genetically modified animal models of neurodegenerative diseases. However, this aspect is important and should also be studied in depth.

A murine model of ataxiatelangiectasia was created by disrupting the *Atm* locus via gene targeting (112-114). Homozygous *Atm* $^{-/-}$ mice displayed growth retardation, neurologic dysfunction, male and female infertility secondary to the absence of mature gametes, and defects in T lymphocyte maturation. The majority of animals developed malignant thymic lymphomas between 2 and 4 months of age. *Atm* 'knock-in' (*Atm*-Δ SRI) heterozygous mice harboring in-frame deletion show an increased susceptibility to developing tumors; however no tumors were observed in *Atm* knockout (*Atm* $^{+/-}$) heterozygous mice (115). *Atm*-Δ SRI homozygous mice developed thymic lymphomas and a variety of other tumors (sarcomas, adenomas, ovarian tumors, dermoid cysts, etc.), but live longer than *Atm* $^{-/-}$ mice.

3.8. p53 knockout mice

The cancer suppressor *p53* is a phosphoprotein barely detectable in the nucleus of normal cells. Upon cellular stress, particularly that induced by DNA damage, *p53* can arrest cell cycle progression (thus allowing DNA to be repaired) or it can lead to apoptosis. These functions are achieved, in part, by the transactivational properties of *p53*, which activates a series of genes involved in cell cycle regulation. In cancer cells bearing a mutant *p53*, this protein is no longer able to control cell proliferation, resulting in inefficient DNA repair and the emergence of genetically unstable cells. Downstream to *p53*, *p21* is responsible for growth arrest in G1, but other *p53* target genes are responsible for the G_2 cell-cycle arrest. The transcriptional activity of *p53* is progressively activated with the accumulation of cell doubling *in vitro* (116). Since senescence is characterized by a permanent cell-cycle block, significant emphasis has been placed on the *p53* targets that mediate cell-cycle arrest (117). At the same time it is worth noting that in some cancers (e.g., cervical carcinoma) the senescence signaling pathway may be *p53*-independent (118).

In response to genotoxic insult, *p53*-induced apoptosis results from overlapping downstream pathways that both suppress mutagenic (and survival) signaling and promote pro-apoptotic signaling. The frequency of

observed mutations in *p53* predicts that its inactivation is a requisite step in tumorigenesis (119). However, no significant differences were found in the mutation spectra and the mutation incidence in the liver, spleen, and brain between $p53^{-/-}$ and $p53^{+/+}$ mice with a lambda shuttle vector harboring the LacI gene (120,121). These findings suggest a need to reconsider the role of the *p53* gene as "guardian of the genome."

Transgenic mice with both alleles of the *p53* tumor suppressive gene product "knocked out" by gene targeting are susceptible to the early development of tumors, mainly lymphomas, malignant teratomas and hemangiosarcomas and are characterized by a reduced life span (122-127). Finch *et al*. (128) observed a reduction in the survival of the heterozygous *p53*+/- knockout mice as compared to the $p53^{+/+}$ wild-type mice.

A great deal of evidence shows that there is an age-related gradual decrease in thymus integrity and function (thymic involution) in humans and in animals (91,92). The development and aging of the immune system was accelerated in *p53*-deficient ($p53^{-/-}$) mice; the accumulation of memory T-cells was spontaneously accelerated; and a strong T-cell-dependent Ab response and Th2 cytokine expression (IL-4, IL-6, and IL-10) were induced by Ag stimulation in young *p53*-/- mice at the developmental stage (Ohkusu Tsukada *et al*., 1999). The authors showed that the high T-cell proliferative response in young mice rapidly progressed to a depressed proliferative response in adult mice. It was suggested that the loss of cell cycle regulation, DNA repair, and apoptosis by *p53* deficiency potentially leads to immunosenescence (with the accumulation of memory T-cells (129)). However, there are no other data on premature aging phenotype features in $p53^{-/-}$ mice. This aspect is under consideration in our current research on biomarkers of aging in $p53^{-/-}$ mice.

In order to examine whether a cooperation exists between inherited *p53* and Rb deficiency in carcinogenesis, crosses were made between *p53*- and Rb-deficient mice and these animals were monitored for subsequent tumor incidence (130). It was shown that $Rb^{+/-}$ or $p53^{-/-}$ developed pituitary adenomas or lymphomas and sarcomas, respectively; whereas mice deficient in both Rb and *p53* showed a faster rate of tumorigenesis and a wider array of tumors than animals deficient only in Rb or *p53*. It is worth noting that heterozygous *p53* knockout ($p53^{+/-}$) mice do not respond to many carcinogenic chemicals that show strain- or species-specific responses in conventional bioassays (131-133).

It was shown that mice functionally deficient in all isoforms of p73, which has high homology with the tumor suppressor *p53*, and in p63, a gene implicated in the maintenance of epithelial stem cells, exhibit profound defects (including hyppocampal dysgenesia, hydrocephalus, chronic infections and inflammation, as well as abnormalities in pheromone sensory pathways and a greatly reduced life span (134)). In contrast to *p53*-deficient mice, however, $p73^{-/-}$ mice showed no increased incidence of spontaneous tumors. Thus, after an autopsy of over 100 $p73^{-/-}$ mice ranging in ages from 2 to 15 months, the authors failed to observe an increased tumor incidence. However, due to the reduction in survival, it is impossible to conclude that the maximal age of the autopsied mice was sufficient for tumor development. The mean life span of the background mouse strain 129 is approximately 22-24 months and total incidence of spontaneous tumor reaches up to 21 % (135).

In a recent experiment by Tyner *et al*. (136) on mice that were genetically engineered (truncated *p53* in one allele ($p53^{+/m}$). This mutation confers phenotypes consistent with activated *p53* function causing markedly reduced longevity with extensive signs of premature aging (including osteoporosis, generalized organ atrophy, impair wound healing, and diminished stress tolerance). It was noted that $p53^{+/m}$ mice typically died with no obvious signs of disease, and the cause of death was often difficult to determine. These unexpected observations suggest that *p53* activation can cause premature aging in at least a subset of mammalian tissues, whereas wild-type p53 contributes to normal aging. The most impressive finding was the enhanced resistance of mutant ($p53^{+/m}$) mice to spontaneous tumors compared with wild-type ($p53^{+/+}$) mice. The authors noted that *p53* m-allele expression constructs enhance wild-type *p53* transactivation activity and can suppress cancer cell growth in the presence of wild-type *p53* mice. The enhanced tumor resistance in $p53^{+/m}$ mice dependent on wild-type *p53*, supports a model in which the m-allele product requires wild-type *p53* to promote tumor suppression. The authors claim that the association of early aging and tumor resistance in the $p53^{+/m}$ mice is consistent with the idea that senescence is a mechanism of tumor suppression (137,138).

The paradox that overactive *p53* suppresses cancer, but accelerates aging, can be explained by the fact that cancer results from the malfunctioning of *p53* in single cells, whereas aging involves a tissue-wide process (139). Cells with inactive *p53* ultimately shorten life span because cancer develops, whereas cell with abnormally high *p53* activity do not contribute to cancer, but instead undergo cell death or senescence. With time, these changes may compromise tissue physiology, shortening life span through aging. Thus, *p53* activity must be tightly controlled to balance a predisposition to cancer (too little *p53*) and premature aging (too much *p53*) (139). Another explanation of the $p53^{+/m}$ phenomena might arise from the concept of phenoptosis (programmed death of multicellular organism) (140). According to the hypothesis, aging represents a slow oxygen-reactive species-linked programmed death of organisms (eliminating individuals with damaged genomes and giving reproductive advantage to those who succeeded in a better preservation of their genomes from damage).

3.9. Regulation of cell-to-cell communication and knockout mouse models

In multicellular organisms, the role of gap junction intercellular communication in the regulation of cell proliferation, cell differentiation, and apoptosis is becoming increasingly recognized as one of the major

cellular functions through normal development to aging (141). The loss of cell-to-cell communication is one of the important characteristics of malignancy (141, 142). Connexins are subunits of gap junction channels, which mediate the direct transfer of ions, second messenger molecules, and other metabolites between contacting cells. *In vitro* studies with endothelial cells have shown that connexins play a role in the aging process (143). Deletion of different connexin genes from the mice results in various disorders (including cancer, heart malformation or conduction abnormality, cataracts, etc). (142). It was shown that $Cx^{-/-}$ mice develop a progressive demyelinating peripheral neuropathy, beginning at 3 months old, with a prevalence of motor fibers (144).

Modianova *et al*. (145) observed an age-related decrease of intercellular coherence strength in the lungs of strain A mouse, which is predisposed to spontaneous adenoma development, and in the livers of the CBA, C3H, and C3HA mice, which are predisposed to the development of hepatomas. Male and female one-year-old mice deficient for connexin-32 (Cx32) had 25-fold and 8-fold (respectively) more spontaneous liver tumors than wild-type mice (146). Transfection of connexin genes into tumor cells restores normal cell growth, supporting the idea that connexins form a family of tumor-suppressor genes (142).

3.10. Telomerase transfected and knockout mice

Telomeres are repetitive DNA sequences at the end of linear chromosomes. Each time a cell divides, telomeres shorten, which leads to an irreversible growth arrest state called replicative senescence. Telomere maintenance is thought to play a role in signaling cellular senescence. In most instances, cells become senescent before they can become cancer cells. However, almost all cancer cells are immortal, having overcome cellular senescence. Maintenance of telomere stability is required for cells to escape from replicative senescence and proliferate indefinitely. Telomerase, a cellular reverse transcriptase, is upregulated and reactivated in most human cancers and helps to stabilize telomere length by adding TTAGGG repeats onto the telomeres (147-150). However, the link between telomerase activity, telomere length, and the aging processes in an organism has not been established.

Expression of the catalytic component of human telomerase and human telomerase reverse transcriptase (hTERT) extends the life span of human fibroblasts, retinal pigment epithelial cells, large vessel and microvascular endothelial cells, and keratinocytes beyond senescence without causing neoplastic transformation (151-155).

However, it is worth noting that the ectopic expression of hTERT is not sufficient to immortalize normal human keratinocytes and mammary epithelial cells (156). Ectopic hTERT expression immortalized normal mesothelial cells and a premalignant, p16(INK4a)-negative keratinocyte line (155). Thus, telomere length stabilization alone is unable to permit keratinocytes to bypass senescence, but the subsequent slow, indefinitely continued growth permitted by telomerase expression permits immortalized variants to arise. Human mammary epithelial cells (HMEC), cultures of which normally stop dividing at 55-60 population doubling, after being infected with a hTERT retrovirus at the 40^{th} passage, were maintained until population doubling 250 (156). The increase in the expression of c-*myc* in HMEC-hTERT has been observed at the 107^{th} to 135^{th} population doublings (157). The authors concluded that, although telomerase activation extends the life span of HMECs, it is also associated with the overexpression of c-*myc* and therefore is not ultimately genoprotective. The extension of life span that is conferred by TERT causes c-*myc* activation and this immortalizes cells, in part by activating TERT expression. These findings indicate that the use of hTERT for expansion of normal human cells for therapeutic purposes must be approached with caution (158). Although it was demonstrated that hTERT-immortalized cells can retain normal growth and differentiation control mechanisms, it is possible that the loss of the p16-mediated growth arrest mechanism (loss of the pRB/$p16^{INK4a}$) and the unlimited replicative potential predispose such cells to further changes that may result in malignant transformation. It has been shown that expression of hTERT cooperates with the simian viruses 40 large T oncoprotein and oncogenic *ras* to transform human fibroblasts and kidney epithelial cells to tumorigenicity (159). These observations clearly provide evidence supporting recent proposals (160,161) that multiple "clocks" function to limit the proliferation capacity of human cells.

The telomerase knockout mice provide an opportunity to understand the effects associated with critical telomere shortening at the level of the organism (162). C57BL6 $mTR^{-/-}$ mutants have been generated that showed shorter telomeres than the original, mixed genetic background C57BL6/129Sv mice (163). These mice could be bred for only four generations and the survival of the late generation $mTR^{-/-}$ mice decreased dramatically with age as compared to their wild-type counterparts. Fifty percent of the 4^{th} generation of these mice died at only 5 months of age. This decreased viability with age in the late generation mice was coincident with telomere shortening, sterility, atrophy of the spleen, reduced proliferative capacity of B and T cells, abnormal hematology, and atrophy of the small intestine. The loss of telomere function in $mTR^{-/-}$ mice did not elicit a full spectrum of classical pathophysiological symptoms of aging (164,165); however, age-dependent telomere shortening and accompanying genetic instability were associated with a shortened life span, as well as with a reduced capacity to respond to stresses such as wound healing and hematopoietic ablation. It is interesting that an increased incidence of spontaneous malignancies (mainly lymphomas, teratocarcinomas) have been observed in $mTR^{-/-}$ mice (165). The appearance of these tumors is thought to be a consequence of chromosomal instability in these mice (166-168). Recently it was shown that late-generation $Terc^{-/-}$ mice, which have short telomeres and are telomerase-deficient, are resistant to the two-stage skin tumorigenesis (169). Early generations of telomerase-deficient $INK4A^{-/-}$ mice retain long telomeres and remain highly cancer prone, whereas the late generation of these mice have short dysfunctional

telomeres and are more cancer resistant (168). Moreover, these *in vivo* observations parallel those obtained from *in vitro*-based transformation assays (169). Experiments in the telomerase-deficient mice have shown that in the setting of a compromised *p53* pathway, telomere-based crisis can facilitate carcinogenesis by promoting chromosomal instability (170). Moreover, the unbalanced chromosomal rearrangements, caused by telomere compromise, closely resemble the regional losses of chromosomes in human carcinoma (15,170).

A recent study using telomerase-deficient mice has shown that differences in telomere length and regulation might impact dramatically on both the spectrum and cytogenetics of tumors during aging (127). It is important that the aging telomerase-knockout mice, heterozygous for mutant *p53*, exhibited a pronounced shift in their tumor spectrum to epithelial neoplasia (including mammary, colon, and skin carcinomas) (127). DePinho (15) suggests that these data are sufficient for understanding the role of telomere-induced genome instability in mechanisms of development of epithelial cancers in humans. Re-introducing telomerase in late generation telomerase-deficient mice, $Tert^{-/-}$, which have short telomeres and shows severe proliferative defects, restores telomerase activity followed by a rescue of chromosomal instability and premature aging (171).

To evaluate the possible risks of telomerase expression in adult somatic tissues, transgenic *K5-Tert* mice have been generated (150,172). These mice overexpress telomerase in stratified epithelia and have a greater wound-healing capability and increased incidence of spontaneous and chemically-induced tumors. The increased mortality and tumor incidence was more evident in a $p53^{+/-}$ background, indicating that telomerase activity cooperates with *p53* deficiency in carcinogenesis. The authors stressed that as an organism ages, high levels of telomerase activity in normal somatic tissues result in a decreased life span and an increased incidence of tumors (although telomerase is not a potent oncogene).

3.11. HER-2/neu transgenic mice

The human *HER-2/neu* protoncogene is a member of the epidermal growth factor receptor (EGFR) family to receptor tyrosine kinases (173). The *HER-2/neu* harboring transgenic mice revealed a high incidence of mammary carcinomas and died within four months after birth (174). In our laboratory it was shown that the mean life span of virgin female *HER-2/neu* transgenic mice (FVB background) was 311 ± 56 days and maximum life span was 431 days. Eighty percent of the mice developed mammary adenocarcinomas. Premature, age-related alterations in the estrus function were also observed in these mice (175). The survival of wild-type FVB/N female mice at 24-months of age was 62% and spontaneous tumor incidence was 66% (176). In these mice, lung adenomas, pituitary adenomas, ovarian tumors, lymphomas, histiocytic sarcomas, Harderian gland adenomas, and pheochromocytomas were observed; however no mammary adenocarcinomas have been observed. It is worth noting that reduced $p66^{Shc}$ expression may play a role in *HER-2/neu* -positive breast cancer development (177).

3.12. Circadian clock gene models

Functional roles for biological clocks have been demonstrated in organisms throughout phylogeny. The adaptive advantages of circadian organisation *per se* are largely a matter of conjecture (178). It is generally accepted, though without direct experimental evidence, that organisms derive primary benefits from the temporal organization of their physiology and behavior, as well as from the anticipation of daily changes in their environment and their own fluctuating physiological requirements (179,180). The loss of temporal organization with age (characterized by decreased circadian amplitude, loose internal synchronization, and poor response to external environmental time queues) is associated with poor health states and decreased longevity (181,182). It was shown that longevity in hamsters is decreased with a non-invasive disruption of rhythmicity and is increased in older animals given suprachiasmatic implants that restore higher amplitude rhythms (183). Chronic reversal of the external light/dark regimen (at weekly intervals) resulted in a significant decrease in the survival time in cardiomyopathic hamsters, with median life span being reduced by 11% (184). Disruption of normal circadian rhythmicity in an animal susceptible to early mortality due to cardiac disease results in a further decrease in longevity. The results substantiate the importance of the temporal organization of physiology and behaviour, provided by the circadian clock, to the health and longevity of an organism. The master circadian clock in mammals resides in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus. It was shown that mice deficient in the *mPer2*, one of eight circadian genes, are cancer prone and reveal a reduced survival rate (185). Thus, circadian organization is important in the control of both aging and cancer.

4. GENETICALLY MODIFIED MOUSE MODELS OF POSTPONED AGING

4.1. Ames dwarf mice

The Ames dwarf mouse, which typically has a longer life than other inbred mice, is one of the novel models in the investigation of aging (186,187). These mice are homozygous autosomal-recessive mutants (single point mutation in the Prophet gene) in a line of extreme non-agouti mice derived from a cross with descendants from an irradiation experiment. The dwarfs, which live from 50 to 64 % longer (males and females, respectively) than wild-type siblings (27,188), are one of the first mammalian examples of a single gene's ability to significantly extend individual life spans. The autosomal-recessive mutation results in the developmental failure of the pituitary to initiate synthesis and secretion of the growth hormone (GH) and the prolactin and thyroid stimulating hormone (TSH). These dwarf mice also have a low IGF-1 and blood insulin level, high sensitivity to insulin and decreased body temperature. Both male and female Ames dwarf mice are hypogonadal and infertile (186,187). Although they have normal antibody production in response to tetanus toxoid, Ames dwarf mice show signs of immunodeficiency, which is evident in the lymphocyte depletion in peripheral lymphoid tissue and in the involution of thymus, a decreased natural killer activity of splenic lymphocytes

(186,188,189). There is evidence that the tissues of the Ames dwarf mice have lower liver glutathione and ascorbate levels and a higher catalase activity (as compared to normal controls). They are also less vulnerable to oxidative damage (27,186,190,191). Spontaneous tumor incidence in aging dwarf and normal mice does not differ. However, dwarfs live significantly longer than normal mice; therefore, it is possible that either the tumors develop later in dwarfs or that the tumors grow more slowly (186,192).

4.2. Growth hormone receptor knockout mice

Homozygous growth hormone-receptor (GHR/BP) mice (knockout mice) were generated through gene targeting (193). Although $GHR^{-/-}$ mice showed severe postnatal growth retardation, proportionate dwarfism, decreased lengths of bones and bone mineral content, absence of the GHR and GH binding protein, significantly decreased serum insulin-like growth factor I, IGFBP-3, and elevated serum GH concentrations (193,194), they lived significantly longer than heterozygous *(+/-)* and wild-type mice (195,196).

4.3. Insulin/IGF-1 signaling pathway modifications and longevity in mice

Since the 1990s, intensive investigations in *C. elegans* and *D. melanogaster*, which have identified insulin signaling components (including daf-2, age-1 and daf-16 and their homologues (CHICO, InR *et al*.)) as the genes whose mutations lead to life span extension, have shed new light on molecular mechanisms underlying aging (197-202). It was demonstrated that FKHR, FKHRL1 and AFX, which are mammalian homologues of daf-16 forkhead transcription factor, function downstream of insulin signaling and akt/PKB under cellular conditions (203,204). However, it is an open question whether insulin signaling components, including forkhead transcriptional factors, play a critical role in aging and longevity in mammals, as well as in *C. elegans* and *D. melanogaster*. Daf-2 and InR are structural homologues of tyrosine kinase receptors in vertebrata. Such receptors 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 (205). At least three genes ($Pit1^{dw}$, $Prop1^{dw}$, *Ghr*) have been identified in knockout mice which leads to dwarfism (with reduced levels of IGF-1 and insulin) and to increased longevity (196,206). In Snell and Ames dwarf mice, sexual maturation is delayed, and male only few males are fertile, while females are invariably sterile (207). 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 challenge (207).

Reduction in both glucose and insulin levels and an increase in the sensitivity to insulin are a well-documented response to caloric restriction in rodents and monkeys (208,209). It was shown that improved sensitivity to insulin in calorie-restricted animals is specifically related to reducing visceral fat (210). It is worthy to note that $Ghr^{-/-}$ mice have a major increase in the level of insulin receptors (211), while Ames dwarf mice have a smaller increase in insulin receptors and substantially increased amount of insulin receptor substrates IRS-1 and IRS-2 (212).

In a bid to discover whether the IGF-1 receptor might control vertebrate longevity, Holzenberger *et al*. (213) inactivated the *Igf1r* gene by homologous recombination in mice. It was shown that $Igf1r^{+/-}$ mice live on average 26% longer than their wild-type littermates ($p<0.02$). It is worthy of note that long-lived mice do not develop dwarfism, and their energy metabolism was normal. Food intake, physical activity, fertility and reproduction were also unaffected in $Igf1r^{+/-}$. The spontaneous tumor incidence in the aging cohort of $Igf1r^{+/-}$ mice was similar to that in wild-type controls. It is very important that these $Igf1r^{+/-}$ mice, and mouse embryonic fibroblasts derived from them, were more resistant to oxidative stress than 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 regulated 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 (214). 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 were found to have an increase in mean life span (by 18%) with parallel increases in maximum life span. Extended longevity in FIRKO mice was associated with both a shift in the age at which the age-dependent increase in mortality risk becomes appreciable and a decreased rate of age-related mortality, especially after 36 months of age. In FIRKO mice, the resistance to obesity, despite normal food intake, suggests that metabolic rate is increased, rather than decreased (215). The authors believe that decreased fat mass could lead to a decrease in oxidative stress in FIRKO mice. Another possibility is that the increased longevity in these mice is the direct result of altered insulin signaling. It is worthy to note that a treatment with antidiabetic biguanide phenformin or buformin increases the susceptibility of tissues to insulin and increases the life span of mice and rats (8,216).

4.4. Knockout $p66^{shc}$ gene mice

An adaptor protein ($p66^{shc}$) becomes tyrosine phosphorylated (upon activation of growth factor receptors) and forms stable complexes with Grb2 (another adaptor protein for the *ras* exchange factor, SOS). However, it does not affect mitogen-activated protein kinase (MAPK) and does not inhibit *c-fos* promoter activation. $P66^{shc}$ is a splice variant of $p52^{shc}$/$p46^{shc}$, a cytoplasmatic signal transducer involved in the transmission of mitogenic signals from activated receptors to *Ras*. The Sch protein complexity increased during evolution (from one locus in Drosophila to at least three loci in mammals (197)). Genetic and biological evidence indicates that the mammalian Sch

isoforms regulate functions as diverse as growth ($p52/p46^{Sch}$), apoptosis ($p66^{Sch}$), and life-span ($p66^{Sch}$) (217). Targeted mutation of the mouse $p66^{shc}$ gene induces stress resistance to paraquat, which generates superoxide anions upon cellular intake and increases life span by 30% (218). The mean survival of homozygous $p66^{shc-/-}$ mice was 973 ± 37.3 days, whereas for wild-type mice it was 761 ± 19.0 days and for heterozygous $p66^{shc+/-}$ mice 815 ± 37.5 days. After 28 months, when all 14 wild-type animals had died, 3 of the 8 heterozygous (37%) and 11 of the 15 homozygous mice (73%) were still alive. No statistically significant differences were found in body weight and food consumption between the knockout and wild-type mice. There were no obvious abnormalities in the $p66^{shc-/-}$ mice. However, spontaneous tumorigenesis in these mice has not been adequately investigated.

A hypothesis was put forward in which $p66^{shc}$ is assumed to be involved in phenoptosis (i.e., programmed death of an organism, mediated by the oxygen-reactive, species-dependent massive apoptosis in an organ of vital importance (219)). The oxygen-reactive species are suggested to oxidize phosphatidyl serine in the inner leaflet of the cell plasma membrane, resulting in the appearance of this phospholipid in the outer membrane leaflet, an effect recognized by a special receptor and causing the $p66^{shc}$ phosphorylation as a serine residue. Serine-phosphorylated $p66^{shc}$ is proposed to block mitosis and initiate apoptosis. Large-scale apoptosis leads to phenoptosis and, hence, shortens the life span of the organism. A study on spontaneous tumor incidence, localization and type, as well as the susceptibility of $p66^{shc-/-}$ mice to carcinogens, would be very intriguing.

4.5. O^6-methylguanine-DNA methyltransferase (MGMT) transgenic mice

The DNA repair enzyme MGMT is a suicide acceptor protein that removes alkyl groups from the O^6 position of guanine alkylated by potent carcinogen nitroso compounds (220). The activity of this enzyme decreases with aging (8). Several transgenic mouse strains that overexpressed MGMT in the brain (150-fold increase) and liver (25-fold increase) were produced (221). In a pilot study it was shown that overexpression of MGMT in the liver reduced the frequency of spontaneous hepatocellular carcinomas in these mice compared to wild-type mice (221). The authors (C.A. Walter *et al*) have claimed that life span studies were initiated to determine whether an increased MGT activity affected rodent lifespan. They have suggested that these mice could demonstrate an increased life span and decreased spontaneous tumor incidence. However, the final results have not yet been published. Recently it has been reported that transgenic mice which overexpress the MGMT gene have fewer malignant tumors and survive longer, indicating that MGMT plays a protective role against malignant transformation (222). When overexpressed in the thymus, MGMT protects mice from N-nitrosomethylurea (NMU)-induced thymic lymphomas (223), whereas $MGMT^{-/-}$ knockout mice are more sensitive to the toxic effect of NMU and other alkylating agents (224).

4.6. Thioredoxin transgenic mice

Transgenic mice with an overexpression of human thioredoxin (TRX), a small redox-active protein, were produced to investigate the role of the protein in a variety of stresses (225). It was shown that bone marrow cells from TRX transgenic mice were more resistant to ultraviolet C-induced cytocide compared with those from wild-type C57BL/6 mice. It was reported that TRX transgenic mice have extended median and maximum life spans compared with wild-type mice - without apparent abnormality in them.

4.7. Urokinase plasminogen activator (α-MUPA) transgenic mice

α-MUPA is a line of transgenic mice that, compared with their wild-type counterparts, spontaneously eat less (approximately 20%) and live longer (also approximately 20%), thus resembling dietary-restricted mice (226). α-MUPA produced mRNA in the brain which encoded the extracellular protease urokinase plasminogen activator. These transgenic mice have significantly reduced food consumption, body weight and size, body temperature, and decreased plasma corticosterone at old age (compared to the wild strain (227)). α-MUPA mice also showed a high frequency of leg muscle tremor seen only in unstable body states (227). It is unfortunate that the authors did not study the incidence rate of spontaneous tumors, since caloric restriction inhibits spontaneous tumor development in a variety of mouse and rat strains (228,229).

5. CONCLUSION

Because DNA damage accumulates with aging (61) and plays a significant role in carcinogenesis (8), it would be reasonable to suggest that the risk of spontaneous tumor development should increase in long-living murine strains (compared to short-living strains). However, no significant positive correlation between life span and tumor incidence was found in the different strains of the inbred mice (8,135,230,231). The incidence of spontaneous tumors is determined by genetic background and sex, rather than by the duration of life span. It is well known that in some long-living and short-living mouse strains the incidence of spontaneous tumors is low; whereas in other mouse strains, characterised by different life spans, spontaneous tumor incidence is high (from 80 to as many as 100 % of cases) (8,135,232). It is worth noting that there is a close positive correlation between DNA repair of adducts of the carcinogen benzo(a)pyrene in different organs and the life span of C57BL/6 (long-living) and BALB/c (shorter living) strains (233).

Pour *et al*. (234) stressed that genetic factors were much more responsible for the variation in hamster spontaneous tumor incidence, localization, and histological type than for the life span of these animals in various populations. Similar results were obtained in the analysis of spontaneous tumor incidence in rats of different strains or stocks (231). Generally speaking, the available data show no positive correlation between spontaneous incidence and the life span of some strains or stocks of a species (8,11).

Table 1. Tumorigenesis in genetically modified mice with accelerated or postponed aging

Genetic modification, gene function	Effect on longevity	Effect on tumor development		References
		Incidence	Latency	
Accelerated aging				
HGH, bGH growth hormone overexpression	Decreases	Increases	Decreases	33,34
HGH-releasing factor overexpression	Decreases	Increases	Decreases	36
SAMP senescent accelerated mouse	Decreases < 14 mo.	No effect	Decreases	41
Klotho $kl^{-/-}$	< 100 days	No data	No data	53
$XPA^{-/-}$ DNA excision repair	Decreases	No effect	No data	68
$Parp^{-/-}$ base excision and DNA strand break repair	Decreases	Increased susceptibility to carcinogens		75
$Ku80^{-/-}$ DNA double-strand break repair	Decreases	Decreases	Decreases	76
$Ku70^{-/-}$ DNA double-strand break repair	Decreases	Decreases	Decreases	78
nu/nu athymic mice	Decreases	No effect	Decreases	96,98
$Atm^{-/-}$ ataxia telangiectasia	Decreases	Increases	Decreases	112
L-myc oncogene, DNA binding	Decreases	Increases	Decreases	101
$p53^{-/-}$ anti-oncogene, apoptosis	Decreases	Increases	Decreases	122
$p53^{+/m}$ anti-oncogene overexpression	Decreases	Decreases	Decreases	136
$Cx32^{-/-}$ connexin32; gap junction gene	Decreases	Increases	Decreases	146
$mTR^{-/-}$ telomerase	Decreases	Increases	Decreases	165
K5-Tert overexpression of telomerase	Decrease	Increases	Decreases	172
HER-2/neu oncogene, EGF receptor	Decreases	Increases	Decreases	175
mPer2 circadian clock gene	Decreases	Increases	Decreases	185
Postponed aging				
Ames dwarf mice	+ 50 – 64%	No effect	Increases	187
$Grh^{-/-}$ growth hormone receptor	Increases	No data	No data	196
Igf1r+/- IGF-1 receptor	+ 26%	No effect	No data	214
FIRKO fat specific insulin receptor	+ 18%	No data	No data	215
$p66^{shc-/-}$ adaptor protein	+ 30%	No effect	No data	218
MGTM DNA repair	Increases	Decreases	No data	221
TRX thioredoxin, redox-active protein	Increases	No effect	No data	225
α-MUPA urokinase plasminogen activator	Increases	No data	No data	227

However, in the latter case we are dealing with genetically different animals. Some of them are infected with oncornaviruses (e.g. murine mammary tumor virus, MuMTV, in C3H mice. Much more important is the correlation between the life span and spontaneous tumor incidence in different groups (populations) of animals of one strain or stock. Our data have shown that more "rectangular" patterns of survival curves are directly associated with an increased rate in the development of fatal tumors in rats of the same strain. By contrast, increased frailty in the animals at a younger age was followed by decreased mortality in older age, and correspondingly, by a decreased rate of fatal tumor development (8,11).

The analysis of the available data on transgenic and mutant mice has shown that only a few models represent examples of life-span extension. Ames dwarf mutant mice, $p66^{-/-}$ knock out mice, α-MUPA and MGMT transgenic mice live longer than wild-type strains. As usual, the incidence of spontaneous tumors in these mice was similar to those in controls, whereas the latent period of tumor development was increased. Practically all models of accelerated aging show increased tumor incidence and a shortening of tumor latency (Table 1). It is worth noting that this phenomenon has been observed both in mice that display a phenotype resembling the more natural aging process and in mice that show only some features of the normal aging process. Why is this the case? It is a fact that the aging processes predispose cells to accumulate mutations, some of which are necessary for initiation of tumorigenesis in target tissues (19,235).

Recent findings suggest that certain types of DNA damage and inappropriate mitogenic signals can also cause cells to acquire a senescent phenotype (10,14). Thus, the cells respond to a number of potentially oncogenic stimuli by adopting a senescent phenotype. These findings suggest that the senescence response is a failsafe mechanism that protects cells against malignant transformation. Despite the protection from cancer conveyed by cellular senescence and other mechanisms that suppress tumorigenesis, the development of cancer is almost inevitable as mammalian organisms age. It is certain that aging predisposes cells to the accumulation of mutations (62,236), several of which are necessary before malignant transformation occurs.

It was shown that there was an increase in tumor incidence, as well as an age-related accumulation of chromosome aberrations in the liver of the short-living mouse strain A, as compared to long-living C57L/6 mice (237). Short-living BDF1, SAMP6/Tan and A/J mice showed a significant age-related increase in spontaneous

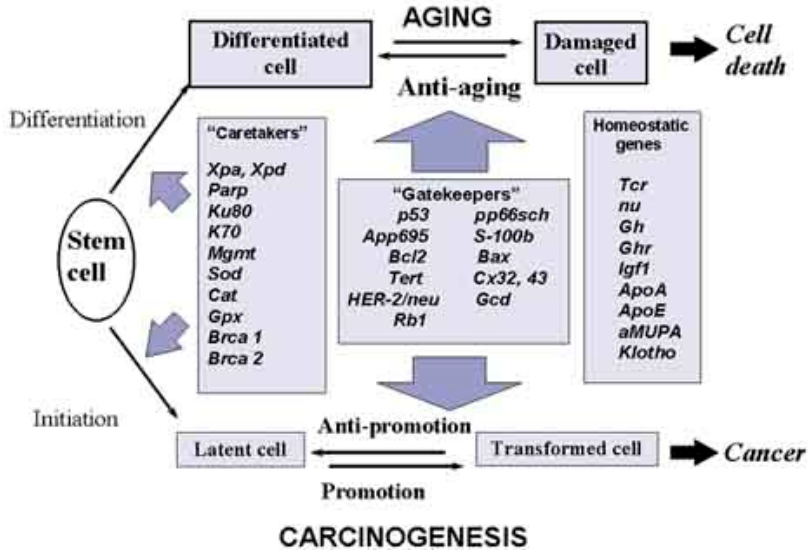

Figure 1. Cellular targets of gene effects on aging and carcinogenesis**.** Stem cells can be drived by differentiation finally to terminal differentiation and to cell death or to be subjected to initiation and promotion under the influence of endogenous or exogenous carcinogenic stimuli. Caretaker and gatekeepr genes control both processes determining a tissue homeostasis and normal function of cell, tissue and organism. Homeostatic genes control mainly growth and progression of transformed cells.

frequencies of micronucleated reticulocytes, whereas the long-living ddY, CD-1, B6C3F1, SAMR1, and MS/Ae did not show significant age-related differences in the mean frequencies of spontaneous micronuclei (238). Long-living mutant Ames dwarf mice and knockout $p66^{shc-/-}$ mice were less vulnerable to oxidative damage than wild-type controls (27,218); whereas the senescence-prone strain, SAMP, had increased production of ROS (41), DNA damage and somatic mutation, as compared to the senescence-resistant SAMR strain (50). MGMT-overexpressed mice are more resistant to alkylating agents (230,233); whereas $MGMT^{-/-}$ and $Parp^{-/-}$ mice, deficient in DNA repair, are more susceptible to the effects of alkylating chemicals and ionizing radiation (75,234). No significant differences were found in the mutation spectra and the mutation incidence between $p53^{-/-}$ and $p53^{+/+}$ LacI mice (120,121); however, the incidence of spontaneous tumors in $p53^{-/-}$ mice was increased, compared to the wild-type control (81,124,126). Gap junction-deficient mice ($Cx32^{-/-}$) have an extremely increased susceptibility to spontaneous and chemically induced carcinogenesis (146). Mice with a defect in the xeroderma pigmentosum group A (XPA) gene, have a complete deficiency in nucleotide excision repair and have a greater than 1000-fold higher risk of developing UV-induced skin cancer, as well as increased susceptibility of internal organs to mutagenesis and development of cancer after exposure to chemical carcinogens (68,69). However, the incidence of spontaneous tumors in these mice is relatively low – only 15% and, even then, these tumors develop only after the age of 18 months (68). It is very important to note that, with age, the rate of accumulation of somatic mutations significantly varies in the different tissues in mice (62,219-242).

Numerous benign or relatively well-controlled malignant tumors may also harbour many potentially oncogenic mutations, suggesting that the tissue microenvironment can suppress the expression of many malignant phenotypes (14,15). Cellular senescence has been proposed to contribute to the aging of an organism. With aging, senescent cells have recently been shown to accumulate in human tissues (14). It has been proposed that the accumulation of dysfunctional senescent cells disrupts the tissue microenvironment (14,243). Thus, the accumulation of mutations may synergize with the accumulation of senescent cells, leading to an increased risk of developing cancer, which is a hallmark of mammalian aging. However, in discussing the differences in human and mouse telomere biology, it has been suggested that, unlike human cells, mouse cells do not undergo replicative senescence (244,245).

According to the multistage model of carcinogenesis, the proportion of partially transformed cells that have progressed through some stages will increase with age (8,246). The evidence supporting age-related accumulation of "pre-malignant" cells in several tissues (skin, lymph node, thymus, spleen, liver, ovary, and mammary glands) has been summarized and discussed elsewhere (12,17).

Most cancer susceptibility genes were originally thought to directly control cell proliferation and death by acting as "gatekeepers". During the last few years it has become clear that genes which maintain the integrity of the genome (DNA repair genes) are "caretakers" and the disruption of these genes may be even more frequent causes of predisposition to cancer. Gatekeepers are genes that directly regulate (typically, inhibit) tumor growth. Inactivation of a given gatekeeper gene leads to a very specific tissue distribution of cancer. In contrast, inactivation of a caretaker gene leads to genetic

instabilities, which result in increased mutation of all genes, including gatekeepers (18). However, it seems that this classification is an oversimplification of the real situation. For example, defects of the DNA repair gene MSH2 result in a limited subset of colon cancer and do not affect other types of cancer in humans. At the same time, defects in *p53*- and *Rb*- pathways are present in 80-90% of tumors. Genes involved in metabolism, tissue growth pathways, and immune signaling genes (e.g. *GH, IGF-1, APO E, TCR,* etc.) also play an important role in tumor promotion and progression. These genes act as 'homeostatic' genes. Available data have shown that all types of genes are also involved in the control of aging (Figure 1). It is clear that both aging and carcinogenesis are complex multifactor processes that can have many causes. In this sense, new transgenic and knockout mouse models with prolonged or reduced longevity will be important instruments to evaluate the role of genes involved in aging in the mechanisms of carcinogenesis.

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