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Review

Mutant and genetically modified mice as models for studying the relationship between aging and carcinogenesis

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Abstract

Increased interest is emerging in using mouse models to assess the genetics of aging and age-related diseases, including cancer. However, only limited information is available regarding the relationship between aging and spontaneous tumor development in genetically modified mice. Analysis of various transgenic and knockout rodent models with either a shortened or an extended life span, provides a unique opportunity to evaluate interactions of genes involved in the aging process and carcinogenesis. There are only a few models which show life span extension. Ames dwarf mutant mice, $p66^{-/-}$ knockout mice, αMUPA and MGMT transgenic mice live longer than wild-type strains. The incidence of spontaneous tumors in these mutant mice was usually similar to those in controls, whereas the latent period of tumor development was increased. Practically all models of accelerated aging showed increased incidence and shorter latency of tumors. This phenomenon has been observed in animals which display a phenotype that more closely resembles natural aging, and in animals which manifest only some features of the normal aging process. 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 unravelling mechanisms underlying aging and cancer. Systemic evaluation of newly generated models

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should include onco-gerontological studies.

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1. 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 argue that the differences between strains are also influenced by epigenetic mechanisms such as imprinting. Genes may affect the variation in life span within an inbred genetic background (de Haan et al., 1998), possibly through a response threshold (Jazwinski, 1999a). 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. An overview of transgenic methods to increase the life span of fruit flies has recently been published (Tower, 2000). Spontaneous and induced genetic modifications, including homozygous null mutations ('knockout') and transgenic mammalian animals, have also been introduced in experimental gerontology (Gordon et al., 1993; Ingram and Jucker, 1999; Morgan et al., 1999; Guarente and Kenyon, 2000). It is worth noting that, although the effects of some genetic manipulations only manifest themselves 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 (Jazwinski, 1999a; Morgan et al., 1999). 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 on any other aging parameter of the animal for that matter. Jazwinski (1999b) 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 (Dix et al., 1980; Anisimov, 1983, 1987, 1998a,b; Miller, 1991; Campisi, 1997, 2000; Dix and Cohen, 1999; DePinho, 2000). The increased incidence of cancer as a function of aging has long been interpreted to suggest that multiple genetic changes are required for carcinogenesis. Cancer cells differ from normal cells in many characteristics, including loss of differentiation, phenotypic changes, increased invasiveness, and decreased drug sensitivity (Kinzler and Vogelstein, 1997; Lengauer et al., 1998; Hanahan and Weinberg, 2000). 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 carcinogen and chemopreventive agents (Alexander, 2000; Gulezian et al., 2000). 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.

2. Ames dwarf mice

Ames dwarf mice, which typically have a longer life than other inbred mice, is one of several novel models in the investigation of aging (Mattison, 2000; Bartke et al., 2001). 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 (Brown-Borg et al., 1996; Brown-Borg and Rakoczy, 2000), 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 (Mattison, 2000; Bartke et al., 2001). Ames dwarf mice show signs of immunodeficiency, which is evident in the lymphocyte depletion in peripheral lymphoid tissue and by the involution of thymus, a decreased natural killer activity of splenic lymphocytes, although they have normal antibody production in response to tetanus toxoid (Duquesnoy, 1972; Esquifino et al., 1991; Mattison, 2000). There is evidence that the tissues of the Ames dwarf mice have lower liver glutathione and ascorbate levels and a higher catalase activity compared to normal controls. They are also less vulnerable to oxidative damage (Brown-Borg et al., 1999; Mattison, 2000; Brown-Borg and Rakoczy, 2000). 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 the tumors develop later in dwarfs, or that the tumors grow more slowly (Mattison, 2000; Mattison et al., 2000).

3. Senescence accelerated mice (SAM)

The SAM strain was generated by selective inbreeding of AKR/J mice (Takeda et al., 1997; Takeda, 1999). 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 mice 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 in 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 (Takeda et al., 1997; Bulygina et al., 1999; Choi et al., 1999; Takeda, 1999; Yuneva et al., 2000). The amount of Cu, Zn-SOD in the mitochondrial fraction of the SAMP-1 was only half that of the SAMR-1 (Park et al., 1996). 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 (Miyamoto et al., 1995). 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 (Choi et al., 1999).

The accelerated senescent-prone strain, SAMP-1, shows a striking increase in the frequency of chromosome aberrations from the age of 3–8 months, whereas the SAMR-1 strain shows only a slight increase in chromosome aberrations at the same age (Nisitani et al., 1990). Uryvaeva et al. (1999) have shown an accelerated accumulation of micronuclear aberrations with age in the liver cells of SAMP mice 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 compared to the SAMR1 (Odagiri et al., 1998; Hosokawa et al., 2000).

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 (Takeda et al., 1997). The levels of murine leukaemia 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 (Meeker and Carp, 1997). Sugimura et al. (1994) 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 deformity were observed in the dorsal and lateral lobes of the prostate gland of the SAMP strain.

4. Mutation in the mouse *klotho* gene

Kuro-o et al. (1997) 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 hypoglycaemic 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 (Mori et al., 2000; Utsugi et al., 2000). 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 (Mori et al., 2000). 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 (Ito et al., 2000). Kuro-o et al. (1997) suggested that the *klotho* gene product may function as part of a signalling 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 are 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 (Kuro-o et al., 1997). 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 (Kawaguchi et al., 1999).

5. DNA repair gene transgenic and knockout models

DNA repair plays an important role in genome stability. The DNA damage theory assumes that aging in mammals is due to the accumulation of DNA damage in somatic cells, being preceded by the somatic mutation theory of aging (Anisimov, 1987; Bernstein and Bernstein, 1991; Vijg, 2000). Taking into consideration the relationship between 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 (Anisimov, 1987). 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 (Bernstein and Bernstein, 1991)). All of them could play a role in aging and carcinogenesis (Anisimov, 1987, 1998a; Bernstein and Bernstein, 1991).

Xeroderma pigmentosum, characterized by a deficiency in the nucleotide excision repair and an over 1000-fold increased risk of skin cancer, represent a paradigm to understand the role of unrepaired lesion in the development of cancer (Benhamou and Sarasin, 2000). 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 (de Boer et al., 1999). No data on tumor incidence in these mice have been reported yet.

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 (Harada et al., 1999). 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 (Van Steeg et al., 1998). However, $XPA^{-/-}$ mice are very susceptible to ultraviolet B radiation and to different chemical carcinogens (Van Steeg et al., 1998, 2000).

Failure of DNA repair and the fixation of DNA damage as mutation, eventually leads 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 (Ruscetti et al., 1998; Dantzer et al., 1999) and the induction of cell death (Berger et al., 1983). *Parp* knockout mice were established by disrupting *Parp* exon 1, 2 or 4 in the genetic background of 129Sv/C57BL6 or 129Sv/ICR mice ($Parp^{-/-}$) (Masutani et al., 2000). It has been shown that these mice are very susceptible to the effects of alkylating agents and ionizing radiation. $Parp^{-/-}$ mice show severe myelosuppression (Masutani et al., 2000). 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 (Vogel et al., 1999). 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 (Difilippantonio et al., 2000). Cancer incidence was reduced by 13-fold in $ku80^{-/-}$ mice in comparison to the control, however cancers were observed earlier in mutant mice (Vogel et al., 1999). 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 (Gu et al., 1997; Li et al., 1998) which suggests that one or both of these proteins work independently. It was shown that p53 monitors chromosome damage and either arrests the cell-cycle progression or triggers apoptosis in cells with unrepaired lesion (Levine, 1997). To determine

whether p53 is involved in the growth arrest of *Ku8*$^{-/-}$ mice, double-mutant mice *Ku80*$^{-/-}$*p53*$^{-/-}$ were generated (Difilippantonio et al., 2000). 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 (Jacks et al., 1997) which develop at a slower rate than *Ku80*$^{-/-}$*p53*$^{-/-}$ pro-B-cell lymphomas, and *Ku80*$^{-/-}$ mice only occasionally develop T-cell lymphoma after 7 months (Vogel et al., 1999). 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 (Difilippantonio et al., 2000).

The DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) is a suicide acceptor protein, which removes alkyl groups from the O^6 position of guanine alkylated by potent carcinogen nitroso compounds (Pegg, 2000). The activity of this enzyme decreases with aging (Anisimov, 1987). Several transgenic mouse strains that overexpressed MGMT in the brain (150-fold increase) and liver (25-fold increase) were produced (Walter et al., 1997). 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 the wild-type (Walter et al., 1997). The authors 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 still not 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 (Qin et al., 2000). When overexpressed in the thymus, MGMT protects mice from *N*-nitrosomethylurea (NMU)-induced thymic lymphomas (Allay et al., 1999), whereas MGMT$^{-/-}$ knockout mice are more sensitive to the toxic effect of NMU and other alkylating agents (Glassner et al., 1999).

6. Overexpression of Cu, Zn-SOD or catalase

Several studies have shown that aging cells and organisms accumulate 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 (Finkel and Holbrook, 2000). 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. 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 (Nabarra et al., 1997). Modification of the cellular

architecture and morphology associated with a lipidic invasion, 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 (Cebalos Picot, 1993). 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 (Cardozo Pelaez et al., 1998). Twenty-four male mice transgenic with human Cu, Zn-SO, resulting in an overexpression of the cytosolic enzyme, were compared with 11 matched controls over 19 months. There was no difference in longevity, locomotor activity or dopamine uptake sites in the brain regions between the two groups (Gallagher et al., 2000). 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 (Schriner et al., 2000). Both types of transgenic animals had significant increases of catalase activities 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 were 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 (Klungland et al., 1999). 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 (Klungland et al., 1999). It is important to note that the last two conclusions have been made on the background 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 (Anisimov, 1987).

7. Growth hormone (GH) transgenic and knockout mice

Homozygous growth hormone-receptor (GHR/BP) mice, knockout mice, were generated through gene targeting (Zhou et al., 1997). $GHR^{-/-}$ 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 (Zhou et al., 1997; Sjorgen et al., 2000), and lived significantly longer than heterozygous ($+/-$) and wild-type mice (Kopchik and Laron, 1999; Coschigano et al., 2000).

On the other hand, 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 wild-type siblings (Pendergrass et al., 1993; Steger et al., 1993). Mice overexpressing GH exhibit increased indices of free radical production (Rollo et al., 1996), significant reduction of catalase activity in the liver and kidney (Brown-Borg and Rakoczy, 2000), signs of premature central nervous system aging including reduced catecholamine turnover, increased astrogliosis, and impaired learning and memory (Steger et al., 1993; Miller et al., 1995; Meliska et al., 1997). These animals reach sexual maturation earlier and cessation of reproduction sooner than wild-type controls (Naar et al., 1991; Steger et al., 1994). This effect in GH-transgenic mice is related to accelerated degenerative processes in the ovaries, not present in the wild-type control (Mayerhofer et al., 1990). Most importantly, GH overexpressed mice have a high incidence of tumor development (Wolf et al., 1993; Bartke, 1998; Snibson et al., 1999). It is worth noting that old (16–24 months of age) mice transgenic with human growth hormone-releasing hormone developed pituitary adenomas, and produced both GH and prolactin (Asa et al., 1990).

Age-related disturbances in the regulation of the insulin-like growth factor II activity plays an important role in the development of some metabolic disorders and diseases, including cancer in the elderly (Anisimov, 1987; Dilman, 1994). By using a construct in which the coding region of the mouse insulin-like growth factor II 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 (Ward et al., 1994). Transgene expression was high in the skin, in the gastrointestinal tract and 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-II expression.

8. Mutant and transgenic models of immunosenescence

During aging in mice and humans, a gradual decline in thymus integrity and function occurs (thymic involution) (Remarque, 1998; Anisimov and Soloviev, 1999; Miller, 1999). 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 ($\beta 2M^{-/-}$) or class II genes ($A\beta^{-/-}$) have been compared (Lau and Spain, 2000). The expected aging-related reduction in thymic weights was 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 (Freisleben et al., 1994, 1997). Systematic observation of 1141 nude mice (Swiss background strain) that received human tumor grafts revealed 24 spontaneous tumors, 18 of lymphoreticular origin and six lung adenomas (Sharkey and Fogh, 1979). 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 (Sharkey and Fogh, 1979). The incidence and type of spontaneous tumors in athymic nude (*nu*/*nu*) mice partially inbred (CBA/H) background and which were also carrying the viable yellow gene (*Avy*, derived from C57BL/6JAvy mice), were comparable to those observed in the phenotypically normal nu/+ and +/+ control crosses carrying the *Avy* gene (Stutman, 1979). 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 $^{+/+}$ (Shisa et al., 1986). 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 (Remarque, 1998; Miller, 1999). *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 (Zhou et al., 1995). 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 (Siegel et al., 1992). 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 (Moroy et al., 1992).

9. 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 (Ingram and Jucker, 1999). 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 (Moechars et al., 1996). 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 (Moechars et al., 1999).

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 (Geula et al., 1998). Shoji et al. (2000) 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 (Hsiao et al., 1995; Chapman et al., 1999). 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 (Hsiao et al., 1995). Aged Tg2576 transgenic mice overexpressing human βAPP695 have limited neuron loss

and *tau* pathology, but frequent ubiquitin- and α-synuclein-positive, *tau*-negative neurites, resembling those seen in the Lewy body variant of Alzheimer's disease (Yang, F. et al., 2000).

There is another model of Alzheimer disease in transgenic mice harbouring the human gene S-100β. This gene is a neurotrophic factor realised by astroglial cells and localised to chromosome 21 within the region, which 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. Behaviourally, younger transgenic animals could not perform in learning tasks as well as controls (Whytaker Azmitia et al., 1997). 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 (Pedersen and Mattson, 1999). Dietary restriction failed to delay the onset of the disease or shorten its duration.

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.

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 (Miskin et al., 1999). 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 (Miskin and Masos, 1997). α-MUPA mice also showed a high frequency of leg muscle tremor seen only in unstable body states (Miskin et al., 1999). It is unfortunate that the authors did not study the occurrence of spontaneous tumor incidence, since caloric restriction inhibits spontaneous tumor development in a variety of mouse and rat strains (Weindruch and Walford, 1988; Anisimov, 2001).

In order to explore the role of *bcl-2* protooncogene, which protects various cell types from apoptotic cell death and which is expressed in the developing and adult nervous system, transgenic mice expressing *Bcl-2* under the control of the neuron-specific enolase promoter have been generated (Farlie et al., 1995). It was shown that these mice had an increased number of neurons; they learned faster, were more accurate in the Hebb–Williams maze and committed fewer errors (Coleman et al., 1999). It was also shown that the *Bcl-2* transgenic mice were faster than the wild-type mice, in particular the older mice.

A murine model of ataxia telangiectasia was created by disrupting the *Atm* locus via gene targeting (Barlow et al., 1996; Elson et al., 1996; Eilam et al., 1998). 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.

10. 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 activate 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 G_1, 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 (Bond et al., 1996). Since senescence is characterized by a permanent cell-cycle block, significant emphasis has been placed on the p53 targets that mediate cell-cycle arrest (Bringold and Serrano, 2000). At the same time it is worth noting that in some cancers (e.g. cervical carcinoma) the senescence signaling pathway may be p53-independent (Goodwin et al., 2000).

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 predict that its inactivation is a requisite step in tumorigenesis (Colman et al., 2000). 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 (Nishino et al., 1995; Buettner et al., 1997). 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 (Donehower et al., 1992, 1995; Hursting et al., 1995; Perkins et al., 1997; Atardi, and Jacks, 1999; Artandi et al., 2000). Finch et al. (1998) observed a reduction in the survival of the heterozygous $p53^{+/-}$ knockout mice 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 (Remarque, 1998; Miller, 1999). 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) was induced by Ag stimulation in young $p53^{-/-}$ mice at developmental stage (Ohkusu Tsukada et al., 1999). The authors showed that the high T-cell proliferative response in the young mice rapidly progressed to a depressed proliferative response in adult mice. It was suggested that the loss of regulation of the cell cycle, DNA repair, and apoptosis by p53 deficiency, potentially leads to immunosenescence with the accumulation of memory T cells (Ohkusu Tsukada et al., 1999). 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 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 (Harvey et al., 1995). 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 (Dass et al., 1999; Spadling et al., 2000; Sukata et al., 2000).

It was shown that mice functionally deficient in all isoforms of p73 which has high homology with the tumor suppressor p53, as well as with p63, a gene implicated in the maintenance of epithelial stem cells, exhibit profound defects including hypocampal dysgenesia, hydrocephalus, chronic infections and inflammation, as well as abnormalities in pheromone sensory pathways and a greatly reduced life span (Yang, A. et al., 2000). 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% (Storer, 1966).

11. 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 (Trosko et al., 2000). The lost of cell-to-cell communication is one of the important characteristics of the malignancy (Yamasaki et al., 1999; Trosko et al., 2000). 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 (Xie and Hu, 1994). Deletion of different connexin genes from the mice results in various disorders, including cancer, heart malformation or conduction abnormality, cataract, etc. (Yamasaki et al., 1999). It was shown that $Cx^{-/-}$ mice develop a progressive demyelinating peripheral neuropathy beginning at 3 months of age with a prevalence of motor fibers (Scherer et al., 1998).

Modjanova et al. (1983) observed an age-related decrease in intercellular coherence strength in the lungs of the mice strain A, predisposed to spontaneous adenoma development, and in the livers of the CBA, C3H and C3HA mice predisposed to the development of hepatomas. Male and female 1-year-old mice deficient for connexin-32 (Cx32) had 25-fold and eightfold more spontaneous liver tumors than wild-type mice, respectively (Temme et al., 1997). Transfection of connexin genes into tumor cells restores normal cell growth, supporting the idea that connexins form a family of tumor-suppressor genes (Yamasaki et al., 1999).

12. 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 mammalian (Luzi et al., 2000). 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 ($p66^{Sch}$) (Luzi et al., 2000). 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% (Migliaccio et al., 1999). 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 $p66^{shc\pm}$ mice 815 ± 37.5 days. After 28 months, when all 14 wild-type animals had died, three of the eight 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 reactive oxygen species-dependent massive apoptosis in an organ of vital importance (Skulachev, 2000). The reactive oxygen 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. The large-scale apoptosis leads to phenoptosis and, hence, shortens the life span of the organism. The study of spontaneous tumor incidence, localization and type, as well as susceptibility of the $p66^{shc-/-}$ mice to carcinogens, would be very intriguing.

13. Telomerase transfected and knockout mice

Telomeres are repetitive DNA sequences at the end of linear chromosomes. Each time a cell divides, telomeres shorten leading 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 (Cerni, 2000; Goyns and Lavery, 2000; Ishikawa, 2000). 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, 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 (Bodnar et al., 1998; Jiang et al., 1999; Morales et al., 1999; Yang et al., 1999; Dickson et al., 2000).

However, it is worth noting that ectopic expression of hTERT is not sufficient to immortalize normal human keratinocytes and mammary epithelial cells (Kiyono et al., 1998). Ectopic hTERT expression immortalized normal mesothelial cells and a premalignant, p16 (INK4a)-negative keratinocyte line (Dickson et al., 2000). Thus, telomere length stabilization alone is unable to permit keratinocytes to bypass senescence, but the subsequent slow, indefinite continued growth permitted by telomerase expression permits immortalized variants to arise. Human mammary epithelial cells (HMEC), cultures which normally stop dividing at 55–60 population doubling, being infected with a hTERT retrovirus at the 40th passage, were maintained until population doubling 250 (Wang et al., 1998). The increase in the expression of c-*myc* in HMEC-hTERT has been observed at 107th to 135th population doublings (Wang et al., 2000). 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 (Wang et al., 2000). 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 unlimited replicative potential predisposes such cells to further changes that may result in malignant transformation. It has been shown that expression of hTERT cooperates with the simian virus 40 large T oncoprotein and oncogenic *ras* to transform human fibroblasts and kidney epithelial cells to tumorigenicity (Hahn et al., 1999). These observations clearly provide evidence supporting recent proposals (Reddel, 1998; Wynford-Thomas, 1999) 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 (Kipling and Faragher, 1999). C57BL6 $mTR^{-/-}$ mutants have been generated, which showed shorter telomeres than the original mixed genetic background C57BL6/129Sv mice (Herrera et al., 1999). These mice could be bred for only four generations and the survival of the late generation $mTR^{-/-}$ mice decreased dramatically with age compared to their wild-type counterparts. Fifty percent of the 4th 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 (Lee et al., 1998; Rudolph et al., 1999). However, age-dependent telomere shortening and accompanying genetic instability were associated with a shortened life span, as well as 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 (Rudolph et al., 1999). The appearance of these tumors is thought to be a consequence of chromosomal instability in these mice (Blasco et al., 1997; Chin et al., 1999; Greenberg et al., 1999). Recently it was shown that late-generation $Terc^{-/-}$ mice, which have short telomere and are telomerase-deficient, are resistant to the two-stage skin tumorigenesis (Gonzalez-Suarez et al., 2000). Early generation 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 (Greenberg et al., 1999). Moreover, these in vivo observations parallel those obtained from in vitro-based transformation assays (Greenberg et al., 1999). 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 (Artandi and DePinho, 2000). Moreover, the unbalanced chromosomal rearrangements caused by telomere compromise, closely resemble the regional losses of chromosomes in human carcinoma (Artandi and DePinho, 2000; DePinho, 2000).

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 (Artandi et al., 2000). 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 (Artandi et al., 2000). DePinho (2000) suggests that these data are sufficient for understanding the role of telomere-induced genome instability in mechanisms of development of epithelial cancers in humans.

14. ErbB-2/neu transgenic mice

The human c-erbB-2/neu protooncogene is a member of the epidermal growth factor receptor (EGFR), family to receptor tyrosine kinases (Andrechek et al., 2000). The erbB-2/neu harboring transgenic mice revealed a high incidence of mammary carcinomas and died within 4 months after birth (Stocklin et al., 1993). In our laboratory it was shown that the mean life span of virgin female erbB-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 (Alimova, 2000). The survival of wild-type FVB/N female mice at 24 months of age was 62% and spontaneous tumor incidence was 66% (Mahler et al., 1996). 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 erbB-2-positive breast cancer development (Stevenson and Fracjelton, 1998).

15. Conclusion

Because DNA damage accumulates with aging (Bernstein and Bernstein, 1991) and plays a significant role in carcinogenesis (Anisimov, 1987), it would be reasonable to suggest that the risk of spontaneous tumor development should increase in long-living murine strains in comparison 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 (Storer, 1966; Smith et al., 1973; Anisimov, 1976, 1987). Table 1 shows that 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 up to 100% of cases) (Storer, 1966; Staats, 1980; Anisimov, 1987). It is worth noting that there is a close positive correlation between DNA repairs 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 (Boerrigter et al., 1995).

Pour et al. (1979) stressed that genetic factors were much more responsible for the variation in hamster spontaneous tumor incidence, localization, and histological type, than the length of 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 (Anisimov, 1976). Generally speaking, the available data show no positive correlation between spontaneous incidence and the life span of some strains or stocks of a species (Anisimov, 1987, 1998a). 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. In 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 (Anisimov, 1987, 1998a).

Taking into account the exponential character of the relationship between the incidence of the majority of tumors and age, we calculated the correlation coefficient between the parameters of the aging process and tumor development in 10

Table 1
Life span and spontaneous tumor incidence in 20 mouse strains (Storer, 1966; Anisimov, 1987)

Strain	Males		Females	
	Mean life span (weeks)	Tumor incidence (%)	Mean life span (weeks)	Tumor incidence (%)
C57BL/10	118	33	99	31
129	117	7	104	21
C3H	113	28	70	67
CBA	107	19	91	55
C3H.K.	104	44	–	–
DBA/2J	101	18	102	37
C57BR/odJ	100	25	99	34
129/J	97	12	93	20
C57BL/6J	96	7	99	14
RF/J	93	49	65	43
DBA/2	82	15	101	49
BALB/c	77	0	82	29
CBA/J	75	72	75	87
A/J	70	13	84	30
DBA/1J	62	2	107	22
ST/bJ	62	2	73	8
BDP/J	60	5	67	24
PL/J	55	0	64	71
C58/J	53	73	50	88
AKR/J	46	81	39	92

groups of intact rats, kept under identical conditions and which were used as control animals in our experiments (Anisimov, 1987). For each group, a life table was created, and the level of mortality (R), mean life span, and parameter α according to the Gompertz equation ($R = R_0 e^{\alpha t}$, where R_0 = mortality at time $(t) = 0$; α = constant) were calculated. The cumulative incidence for each group of animals was found by day 1000 of their life according to the actuarial method and by the equation: $Q = Q_0 e^{\kappa t}$, where Q = cumulative tumor incidence, t = time, $Q_0 = Q$ at $t = 0$, and κ = constant, the value of this constant (κ) was found. We observed a positive correlation between the population aging rate (evaluated as α) and tumor incidence Q ($r = 0.70$, $P < 0.05$) and the constant κ ($r = 0.77$, $P < 0.05$). It seems that the constant κ might be considered as an index of the age-related increase in tumor incidence in the population (tumor increase rate). Therefore, our data suggest a positive correlation between tumor incidence and tumor rate and the aging rate of a population (Anisimov, 1987). A similar conclusion has been made on the basis of the analysis of the effect of different geroprotectors on mortality curves in mice and rats (Anisimov, 1987, 1998a, 2001).

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, $p66^{-/-}$ knocked 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 (Tables 2–4). It is worth noting that this phenomenon has been observed both in mice which display phenotype resembling the more natural aging process, and in mice which show only some features of the normal aging process. Why is this the case? It is a fact that the aging processes predisposes cells to accumulate mutations, some of which are necessary for initiation of tumorigenesis in target tissues (Lengauer et al., 1998; Turker, 1998).

Recent findings suggest that certain types of DNA damage and inappropriate mitogenic signals can also cause cells to acquire a senescent phenotype (Campisi, 1997, 2000). 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 fail-safe 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 (Holliday, 2000; Vijg, 2000), 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, compared to long-living C57L/6 mice (Crowley and Curtis, 1963). Short-living BDF1, SAMP6/Tan and A/J mice showed a significant age-related increase in spontaneous 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 (Sato et

Table 2
Effect of mutations on longevity and tumor development in mice

Strain, mutation	Effect on signs of aging	Effect on longevity	Effect on tumor development		References
			Incidence	Latency	
Ames dwarf mice	Postponed aging	+50–64%	No effect	Increases	Mattison, 2000
SAMP (senescence-accelerated mouse)	Accelerates	Decreases	No effect	Decreases	Takeda et al., 1997
Klotho ($kl^{-/-}$)	Accelerates	Less than 100 days	No data		Kuro-o et al., 1997
nu/*nu* (athymic mice)	Immunosenescence	Decreases	No effect	Decreases	Sharkey and Fogh, 1979; Shisa et al., 1986

Table 3
Effect of gene overexpression on longevity and tumor development in transgenic mice

Gene, function	Effect on signs of aging	Effect on longevity	Effect on tumor development		References
			Incidence	Latency	
hGH, *bGH* (growth hormone)	Accelerates	Decreases	Increases	Decreases	Wolf et al., 1993; Bartke, 1998
hGH-releasing factor	Accelerates	Decreases	Increases	Decreases	Asa et al., 1990
MGMT (DNA repair)	No data	Increases	Decreases		Qin et al., 2000
hSOD-1 (Cu,Zn-superoxide dismutase)	Accelerates	Decreases	No data		Cebalos Picot, 1993
erbB-2/*neu* (oncogene, EGF receptor)	Accelerates	Decreases	Increases	Decreases	Alimova, 2000
L-*myc* (oncogene, DNA binding)	Accelerates	Decreases	Increases	Decreases	Moroy et al., 1992
α-MUPA (urokinase plasminogen activator)	Postponed aging	Increases	No data		Miskin et al., 1999

Table 4
Effect of gene knockouts on longevity and tumor development in mice

Gene, function	Effect on signs of aging	Effect on longevity	Effect on tumor development		References
			Incidence	Latency	
ghr$^{-/-}$ (growth hormone receptor)	Postponed aging	Increases	No data		Coschigano et al., 2000
XPA$^{-/-}$ (DNA nucleotide excision repair)	Accelerates	Decreases	No effect	Increases	Van Steeg et al., 1998
Parp$^{-/-}$ (base-excision and DNA strand break repair)	Accelerates	Decreases	Increased susceptibility to alkylating agent and ionizing radiation		Masutani et al., 2000
Ku80$^{-/-}$ (DNA double-strand break repair)	Accelerates	Decreases	Decreases	Decreases	Vogel et al., 1999
Ku70$^{-/-}$ (DNA double-strand break repair)	Accelerates	Decreases	Increases	Decreases	Gu et al., 1997
p66$^{shc-/-}$ (adaptor protein)	No effect	+30%	No effect		Migliaccio et al., 1999
Atm$^{-/-}$ (ataxia telangiectasia)	Accelerates	Decreases	Increases	Decreases	Barlow et al., 1996
p53$^{-/-}$ (anti-oncogene, apoptosis)	Accelerates	Decreases	Increases	Decreases	Donehower et al., 1992
Cx32$^{-/}$ (connexin32, gap junction gene)	Accelerates	Decreases	Increases	Decreases	Temme et al., 1997
mTR$^{-/-}$ (telomerase)	Accelerates	Decreases	Increases	Decreases	Rudolph et al., 1999

al., 1995). Long-living mutant Ames dwarf mice and knockout $p66^{shc-/-}$ mice were less vulnerable to oxidative damage than wild-type controls (Migliaccio et al., 1999; Brown-Borg and Rakoczy, 2000), whereas the senescence-prone strain, SAMP, had increased production of ROS (Takeda, 1999), DNA damage and somatic mutation compared to the senescence-resistant SAMR strain (Hosokawa et al., 2000). MGMT-overexpressed mice are more resistant to alkylating agents (Walter et al., 1997; Allay et al., 1999), whereas $MGMT^{-/-}$ and $Parp^{-/-}$ mice, deficient in DNA repair, are more susceptible to the effects of alkylating chemicals and ionizing radiation (Glassner et al., 1999; Masutani et al., 2000). No significant differences were found in the mutation spectra and the mutation incidence between $p53^{-/-}$ and $p53^{+/+}$ mice (Nishino et al., 1995; Buettner et al., 1997); however, the incidence of spontaneous tumors in $p53^{-/-}$ mice was increased compared to the wild-type control (Hursting et al., 1995; Jacks et al., 1997; Atardi and Jacks, 1999). Gap junction-deficient mice ($Cx32^{-/-}$) have an extremely increased susceptibility to spontaneous and chemically-induced carcinogenesis (Temme et al., 1997). Mice with a defect in the xeroderma pigmentosum group A (XPA) gene, have a complete deficiency in nucleotide excision repair and have a more 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 (Van Steeg et al., 1998, 2000). However, the incidence of spontaneous tumors in these mice is relatively low — only 15% and even then these tumors only develop after the age of 18 months (Van Steeg et al., 1998). It is very important to note that the rate of accumulation of somatic mutations with age significantly varies in the different tissues in mice (Dollé et al., 1997, 2000; Ono et al., 2000; Stuart et al., 2000; Vijg, 2000).

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 (Campisi, 2000; DePinho, 2000). Cellular senescence has been proposed to contribute to the aging of an organism. Senescent cells have recently been shown to accumulate in human tissues with aging (Campisi, 2000). It has been proposed that the accumulation of dysfunctional senescent cells disrupts tissue microenvironment (Rinehart and Torti, 1997; Campisi, 2000). 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 (Wright and Shay, 2000).

According to the multistage model of carcinogenesis, the proportion of partially transformed cells that have progressed through some stages will increase with age (Vainio et al., 1992; Anisimov, 1998a). The evidence supporting age-related accumulation of 'premalignant' cells in several tissues (skin, lymph node, thymus, spleen, liver, ovary, mammary gland) has been summarized and discussed elsewhere (Anisimov, 1998b).

Most cancer susceptibility genes were originally thought to directly control cell proliferation and death, 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 may even be 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 (Kinzler and Vogelstein, 1997). 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 (Fig. 1). It is clear that both aging and carcinogenesis are a complex multifactor process that can have many causes. In this sense, new transgenic and knockout mouse models with prolonged or reduced longevity will be an important instrument to evaluate the role of genes involved in aging in the mechanisms of carcinogenesis.

It has to be stressed that the number of animals used in the studies on the effect of either overexpression or suppression of individual genes on longevity, is usually too small to draw an ultimate conclusion concerning the relationship between the aging rate and tumorigenesis in genetically modified and wild-type animals. Typically, only a few biomarkers of aging have been evaluated in the reviewed studies. Sometimes it is also difficult to distinguish a true acceleration of normal aging from a progeroid syndrome. In a number of studies, the information on the cause of animal death, as well as on tumor incidence, is omitted. A program for the adequate testing of biological interventions to promote healthy aging has recently been discussed (Warner et al., 2000). There are standardized protocols for long-term and short-term assays for the evaluation of cancer risk from chemicals and other interventions in experimental rodents (Montesano et al., 1986). Gerontological studies have now been included in the protocols of systemic evaluation of newly generated mouse strains. Researchers will frequently wait up to long periods for cancer to develop in the life span of mice; however, little attention is paid to the aging process of these mice. It appears that researchers need to introduce approaches that involve both the study of cancer and the aging.

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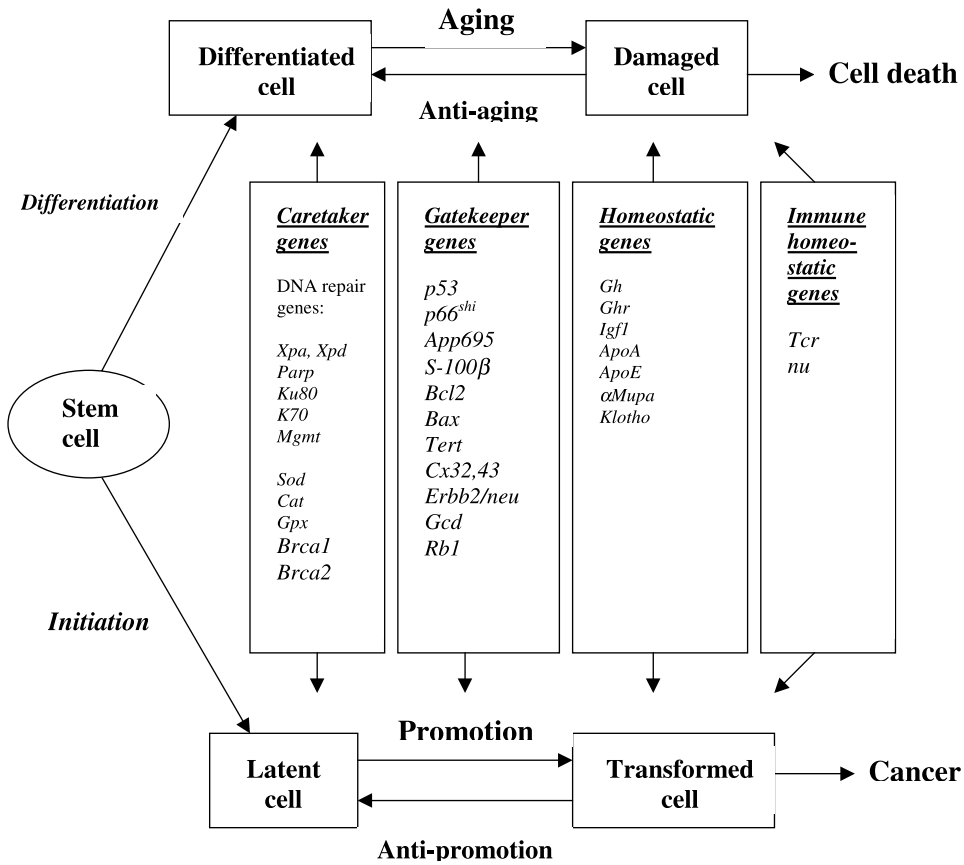

Fig. 1. Cellular targets of gene effects on aging and carcinogenesis.

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