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Mini-Review

Prolonged longevity of hypopituitary dwarf mice

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Abstract

In two types of mutant dwarf mice, congenital deficiencies in pituitary function are associated with remarkably increased life expectancy. In this review, we will describe the key phenotypic characteristics of these animals, the evidence that they exhibit delayed aging, and the mechanisms that are suspected to account for their prolonged longevity.

1. Original description, mode of inheritance and key endocrine characteristics

In 1929, Snell reported the discovery of a recessive mutation that caused hereditary dwarfism in the mouse (Snell, 1929). This mutation was named dwarf, symbol *dw*, and subsequently became known as Snell dwarf. The affected animals, homozygous for this mutation (dw/dw), were of normal size at birth but grew slowly and reached only a fraction (approximately 1/3 by weight) of normal adult body size. Subsequent studies by many investigators provided evidence that the anterior pituitary of Snell dwarf mice is underdeveloped and devoid of cells producing the growth hormone (GH), prolactin (PRL) and thyroid stimulating hormone (TSH) (reviews in Grüneberg, 1952; Bartke, 1979a,b). By performing reciprocal transplants of pituitaries between normal and Snell dwarf mice, Carsner and Rennels (1960) succeeded in demonstrating that absence of GH secretion in the Snell dwarf mice was due to a primary pituitary defect rather than hypothalamic dysfunction. Studies in the laboratories of Karin (Theill and Karin, 1993) and Rosenfeld (Li et al., 1990) led to the identification of a homeotic gene, pituitary-1 (pit-1), which is

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responsible for differentiation of somatotrophs, lactotrophs and thyrotrophs (cells producing GH, PRL and TSH, respectively) during fetal development of the pituitary and to the demonstration that the Snell dwarfism is due to a mutation at the pit-1 locus. Failure to produce pit-1 protein in dw/dw mice is responsible for defects of endocrine function in these animals (Li et al., 1990).

In 1961, Schaible and Gowen reported discovery of another recessive mutant causing hereditary dwarfism in the mouse and named it Ames dwarf, symbol *df* (Schaible and Gowen, 1961). This mutation is located on a different chromosome than Snell dwarfism (chromosome 11 rather than 16), but apparently produces the same defects in pituitary development and thus an identical phenotype. Some differences between the Ames and Snell dwarf mice in the cytology of their pituitaries have been described (Gage et al., 1996), but both mutants appear to exhibit complete GH, PRL and TSH deficiency. In 1996, Sornson et al. reported that the Ames dwarfism is due to mutation of a gene that, in the course of the normal development of mouse pituitary, is expressed approximately one day earlier than pit-1 and is responsible for development of pit-1 positive cells. This gene was named Prophet of pit-1, Prop-1, and thus the Ames dwarfs are homozygous mutants at the Prop-1 locus (Sornson et al., 1996).

2. Breeding and husbandry of dwarf mice

Homozygous Snell (dw/dw) and Ames (df/df) dwarf mice are usually infertile (details below), but can be readily produced by mating heterozygous carriers of these genes. According to the Mendelian recessive inheritance, mating of two heterozygotes (e.g. df/+ × df/+) produces 25% of homozygous (df/df) animals among the progeny. The remaining animals are phenotypically normal but 2 out of 3 are heterozygous for the df gene. Female dwarfs (both dw/dw and df/df) are infertile due to PRL deficiency and the resulting failure of luteal function, and can reproduce only when PRL replacement is provided by means of daily injections or transplants of normal pituitaries under the kidney capsule (review in Bartke, 1979b, 2000). Most of the male dwarfs are also infertile, although, depending on their genetic background, an occasional male can sire a few litters. Fertility in male dwarfs can be induced by treatment with GH, PRL or thyroxine. When fertile dwarf males are mated with heterozygous females, half of the progeny is dwarf. All-dwarf litters can be produced by mating hormone-treated male and female dwarfs (reviewed in Bartke, 1979b, 2000). As was mentioned earlier in this review, dwarf mice appear normal at birth, but soon afterwards their growth rate begins to be reduced and between the ages of 10–14 days they can be distinguished from their normal siblings by smaller size, delayed eye opening and altered head shape.

The Snell and Ames dwarf mice do not require any special housing or husbandry conditions with the following exceptions: first, they may not thrive if weaned at the age of 21 days (the usual age of weaning normal mice) and thus should remain with the dam for a longer period. This is particularly helpful if the dam had mated during postpartum estrus and the dwarfs can remain with her for the second lactation. Second, these tiny animals appear to benefit from being group rather than single housed and it is

recommended to place them after weaning or with normal female animals or with other dwarfs.

3. Longevity of dwarf mice

As was mentioned at the onset of this chapter, the life span of the Ames and Snell dwarf mice is substantially longer than the life span of their phenotypically normal (homozygous +/+, or heterozygous +/df or +/dw) siblings. Prolonged survival of the Ames dwarf mice was reported by Brown-Borg and her colleagues in 1996 (Brown-Borg et al., 1996). The longevity of Snell dwarf mice was a subject of some early controversy (reviewed in Bartke, 2000), but increased life span of these animals was mentioned as early as 1972 (Silberberg, 1972) and was recently demonstrated conclusively (Miller, 1999; Flurkey and Harrison, personal communication). The mean age at death of dwarf mice exceeds the corresponding value in the normal controls by 40–65%, depending primarily on gender and genetic background (Brown-Borg et al., 1996; Miller, 1999). Both the average and the maximal life span are significantly greater in dwarfs than in normal mice, and the slopes of survival curves are parallel in dwarf and normal mice (Brown-Borg et al., 1996; Miller, 1999; Fig. 1). Therefore, it can be concluded that the extension of life span in dwarf mice is associated with, and most likely, due to delayed aging. Additional support for this conclusion is provided by results of longitudinal studies of various physiological and behavioral characteristics of these animals (Mattison et al., 2000; Kinney and Bartke, unpublished observations). It is of interest to point out that the magnitude of the extension of life in dwarf as compared to normal mice is at least as great as the maximal life extension that can be achieved in genetically normal animals by caloric restriction. Caloric restriction is the only well-documented method of delaying aging and prolonging life span in mammals and thus has become the "gold standard" of gerontological research.

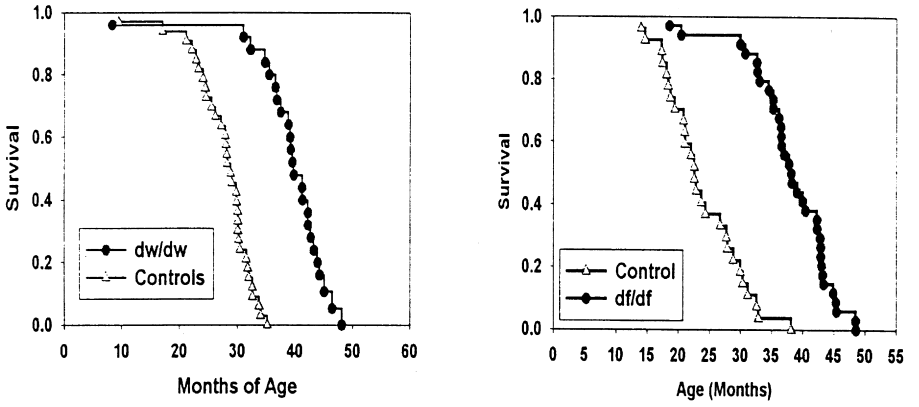

Fig. 1. Survival plots of dwarf mice. Left panel: Snell dwarf mice and normal controls (reproduced with permission from Miller, 1999); Right panel: Ames dwarf mice and normal controls (recalculated from data published by Brown-Borg et al., 1996). The graphs show proportion of animals remaining alive (survival) within the group calculated after death of individual animals.

4. Phenotypic characteristics

As can be expected from the multitude of important physiological actions of GH, PRL and thyroid hormones, combined deficiency of GH, PRL and TSH produces profound alterations of the phenotype. Phenotypic characteristics of dwarf mice that have been proposed or can be suspected to contributed to their prolonged longevity are listed below.

4.1. Anti-oxidant enzymes

Activity of catalase (CAT) are significantly elevated in the liver and kidney of Ames dwarf mice as compared to age- and sex-matched normal animals from the same strain (Bartke et al., 1998; Brown-Borg and Rakoczy, 2000). Moreover, hypothalamic activity of CAT and Cu/Zn superoxide dismutase (SOD) are elevated in Ames dwarf compared to normal mice (Hauck and Bartke, 2000). In as much as damage of cellular components by reactive oxygen species (ROS) are believed to be an important and perhaps a key mechanism of aging, increased activity of CAT and SOD, which are involved in removing ROS, stand out as a very likely mechanism of prolonged longevity of dwarf mice. In support of this possibility, there is some evidence for reduced oxidative damage in the Ames dwarf vs. normal mice (Bartke et al., 1998; Carlson et al., 1999; Brown-Borg and Rakoczy, 2000). To address the functional significance of enhanced activity of anti-oxidant enzymes in the Ames dwarf mice, we have examined their ability to survive exposure to Paraquat, a herbicide producing toxicity due to oxidative stress. After receiving a single intraperitoneal dose of 75 mg of Paraquat per kg body weight, Ames dwarfs survived significantly longer than normal mice (Bartke, Hauck and Wright, unpublished observations).

4.2. Body temperature and metabolic rate

Twenty-four hour telemetric recording of body core temperature (T_{co}) in the Ames dwarf and normal mice equipped with implantable transmitters revealed that T_{co} is markedly reduced in the dwarfs (Hunter et al., 1999). The difference between the dwarfs and normal mice averaged 1.5°C and persisted under conditions known to reduce or increase T_{co} (Hunter et al., 1999). Metabolic rate in Snell dwarf mice was measured and was found to be significantly reduced (Grüneberg, 1952). Reduction of T_{co} and metabolic rate in dwarf mice is consistent with primary TSH deficiency and the resulting hypothyroidism. Deficiency of GH could also contributes to these findings because GH exerts both anabolic and calorigenic effects. However, food consumption of the Ames dwarf mice, adjusted per gram of body weight, is increased rather than reduced (Mattison et al., 2000). This apparent discrepancy could be due to increased heat loss due to larger surface mass ratio of these tiny animals or to reduced efficiency of food utilization in dwarf vs. normal mice.

Although there is no clear or consistent relationship between metabolic rate and aging in homeothermic ("warm-blooded") animals, reduced metabolism of dwarf mice can contribute to their longevity by reducing production of ROS or by other mechanisms.

4.3. Regulation of plasma glucose levels

Plasma glucose levels are significantly lower in the Ames dwarf than in the normal mice (Borg et al., 1995). This was observed in animals with unrestricted access to food (Borg et al., 1995) and also after overnight fast (Turyn and Bartke, unpublished). Plasma insulin levels are reduced in fasted Ames dwarf mice (Turyn and Bartke, unpublished) and in ad libitium fed male dwarfs (Borg et al., 1995). Concomitant reduction in peripheral levels of glucose and insulin implied enhanced insulin sensitivity and, indeed, the suppressive effect of a single large dose of insulin on plasma glucose levels was significantly greater in dwarf than in normal mice (Mattison, Pazo and Bartke, unpublished). We believe that increased sensitivity of Ames dwarf mice to insulin is primarily due to deficiency of GH because animals with isolated GH resistance due to targeted disruption ("knock-out") of the GH receptor/GH binding protein gene are extremely insulin sensitive (Coschigano et al., 2000, and personal communication), while transgenic mice overexpressing GH are insulin resistant (Dominici et al., 1999).

Increased insulin sensitivity and reduced plasma glucose levels in Ames dwarf mice are probably not related to alterations in glucocorticoid or leptin signaling. Basal plasma corticosterone levels are not altered in females and increased in males (Borg et al., 1995), and we did not detect alterations in plasma leptin levels except for a reduction in middle-aged female dwarfs in comparison to age-matched normal females (Mattison and Bartke, unpublished).

Reduced plasma glucose levels in Ames dwarf mice are very likely to contribute to delayed aging and prolonged longevity of these animals. Nonenzymatic glycation of proteins is believed to represent an important mechanism of aging, and association of hyperglycemia with reduced life expectancy is very well documented.

4.4. Growth hormone deficiency

The most striking phenotypic characteristics of the Ames and Snell dwarf mice include suppression of postnatal growth and adult body size consequent to primary deficiency of GH and reduced peripheral levels of IGF-I (Holder et al., 1980; Chandrashekar and Bartke, 1993). We believe that absence of GH signaling in dwarf mice is a major, and most likely the key reason for extension of their life span. In support of this possibility, significant extension of life span was recently reported in mice with targeted disruption of the GH receptor/GH binding protein gene, which leads to GH resistance, suppression of plasma IGF-I levels, and approximately 50% reduction in adult body size (Coschigano et al., 2000).

In these GH receptor "knock-out" (GH-R-KO) mice, deficiency of GH signaling is the only primary genetic defect, prolactin levels are increased rather than reduced (Chandrashekar et al., 1999; and unpublished observations), both females and males are fertile, and hypothyroidism is mild (Hauck and Bartke, unpublished), and presumably secondary to GH/IGF-I deficiency. Thus, findings in GH-R-KO mice indicate that disruption of GH signaling is sufficient to delay aging (Kinney and Bartke, unpublished observations) and prolong life. In further support of the inverse relationship between GH signaling and life span, transgenic mice overexpressing GH live shorter than normal

mice and exhibit numerous symptoms of premature aging (reviewed in Bartke et al., 1998).

Mechanisms linking suppression of GH signaling and delayed aging remain to be elucidated. They are likely to include increased sensitivity of plasma glucose levels to insulin, which was discussed earlier in this chapter, reduced number of cell divisions (Winick and Grant, 1968), which presumably decreases the opportunities for ROS-induced damage and somatic mutations, and reduced risk of developing neoplastic lesions. The important role of IGF-I in tumorigenesis and stimulation of tumor growth is suggested by both epidemiological and in vitro studies. Small body size correlates with increased life expectancy in mice (review in Bartke et al., 1998), dogs (Patronek et al., 1997), and other species, apparently including the human (Samaras et al., 1999). One of the suggested mechanisms for this association is improved efficiency of the cardiovascular system and reduced cardiac work load in small individuals (Samaras et al., 1999). Thus, diminutive size of dwarf mice may confer longevity advantage in these animals.

5. Other potential mechanisms of delayed aging in hypopituitary dwarf mice

In addition to the mechanisms already mentioned, hypopthyroidism, hypoprolactinemia, delayed sexual maturation and hypogonadism may contribute to delayed aging of the Ames and Snell dwarf mice. However, prolonged longevity of GH-R-KO mice, which are mildly hyper- rather than hypoprolactinemic, fertile, and only mildly hypothyroid (details and references earlier in this chapter), suggests that the role of these phenotypic characteristics of dwarf mice is probably minor. We are currently using microarray technology to identify genes with altered levels of expression in the Ames dwarf as compared to normal mice.

6. Conclusions

The impressive extension of life span in the mouse by a loss-of-function mutation at a single locus provides novel models for the study of mechanisms of aging in mammals.

The results available to date suggest that alterations in hormonal signaling mediate genetic effects of the corresponding genes on longevity and thus are likely to be involved in genetic programming of aging and life span. Preliminary evidence of prolonged longevity in humans with hypopituitarism caused by a mutation at the Prop-1 locus, the same locus which is mutated in the Ames dwarf mice (Krzisnik et al., 1999), suggests that results obtained in hereditary dwarf mice may apply to man and other species.

The association of reduced growth, maturation, and fertility with prolonged longevity appears counterintuitive but is consistent with findings in calorically restricted animals and with data derived from animals selected for differences in body size (reviewed in Bartke et al., 1998; Bartke, 2000). It would appear that the normal rates of growth and sexual maturation, as well as attainment of normal stature and reproductive potential may incur significant “costs” in terms of aging and life expectancy. Consequently, suppression of growth and maturation by genetic mutations, gene knock-out, or caloric restriction can

prolong life, while acceleration of these processes by overexpression of GH and probably also by excessive nutrition can shorten life.

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