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The Naked Mole-Rat as a Model for Healthy Aging

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Abstract

Naked mole-rats (NMRs, *Heterocephalus glaber*) are the longest-lived rodents with a maximum life span exceeding 37 years. They exhibit a delayed aging phenotype and resistance to age-related functional decline/diseases. Specifically, they do not display increased mortality with age, maintain several physiological functions until nearly the end of their lifetime, and rarely develop cancer and Alzheimer's disease. NMRs live in a hypoxic environment in underground colonies in East Africa and are highly tolerant of hypoxia. These unique characteristics of NMRs have attracted considerable interest from zoological and biomedical researchers. This review summarizes previous studies of the ecology, hypoxia tolerance, longevity/delayed aging, and cancer resistance of NMRs and discusses possible mechanisms contributing to their healthy aging. In addition, we discuss current issues and future perspectives to fully elucidate the mechanisms underlying delayed aging and resistance to age-related diseases in NMRs.

1. INTRODUCTION

IGF: insulin-like growth factor

NMR: naked mole-rat

Living a long and healthy life is a wish of humankind. How are the life span and aging of animals, including humans, determined? To understand the mechanisms underlying animal aging and elucidate the key factors that may contribute to healthy aging of humans, many studies have been conducted using model animal species such as *Caenorhabditis elegans*, *Drosophila*, and laboratory mice. They revealed several evolutionarily conserved pathways involved in the regulation of aging and age-related diseases, such as the insulin-like growth factor (IGF) and mammalian target of rapamycin pathways (1, 2). The maximum life span of individuals and incidence of various age-related diseases, including cancer, vary widely among species (3, 4). Investigation of how species with longevity or resistance to aging-related diseases achieve such traits may lead to better understanding of the mechanisms and evolution of aging and facilitate the development of preventative and therapeutic strategies for aging and diseases in humans. Recent advances in omics technologies have made it possible to analyze molecular mechanisms even in nonmodel animal species. Genomic insight into the longevity of bowhead whales, the longest-lived mammals, and identification of potential molecular mechanisms underlying cancer resistance in African elephants and blind mole-rats are good examples (5–7). Furthermore, remarkable advances in genome editing technology, including the CRISPR-Cas9 system, may further promote research on these long-lived species.

The longest-lived rodent, the naked mole-rat (NMR, *Heterocephalus glaber*; **Figure 1**), is a nonmodel animal species that has received increasing attention in zoological and biomedical research

Figure 1

Adult naked mole-rats kept in an animal room.

in recent years. This small, furless rodent inhabits underground tunnels up to several kilometers long in arid savannas of Ethiopia, Kenya, and Somalia. Interestingly, colonies of NMRs have a eusocial structure similar to those of ants and bees, which is quite unusual among mammals, consisting of a single breeding queen, a few (usually one) breeding males, and dozens or hundreds of nonbreeding subordinates (workers) (8). However, it is debatable whether NMRs can truly be considered eusocial (9), according to Crespi & Yanega's (10) definition of eusociality, which is based on the existence of castes, groups of individuals whose behavior irreversibly differs prior to reproductive maturity. The underground, highly social lifestyle of NMRs prompted researchers to explore the regulatory mechanisms underlying their adaptation to subterranean environments, with lower O_2 and higher CO_2 levels than aboveground environments; their peculiar sociality; and their reproductive status. Furthermore, NMRs display other unusual features, including tolerance to low levels of oxygen (i.e., hypoxia) and high levels of carbon dioxide (i.e., hypercapnia), insensitivity to certain types of pain, exceptional longevity, and resistance to age-related diseases such as cancer. In mammals, the maximum life span is positively correlated with body mass; however, the documented maximum life span of NMRs exceeds 37 years, which is approximately fivefold longer than predicted based on their body mass (3, 11). Throughout their long life span, NMRs do not exhibit an increase in mortality risk, contrary to the Gompertz law of mortality, which states that mortality risk increases exponentially with age; are fertile; and show few age-related changes in cardiovascular function, body fat content, and bone mineral density (12–14). Moreover, NMRs are resistant to cancer and neurodegenerative diseases such as Alzheimer's disease (13, 15, 16). A growing number of studies have sought to identify the secrets of the longevity, delayed aging, and resistance to age-related disorders/diseases of NMRs. In addition to studies in NMRs of important factors known to be involved in aging and age-related diseases in model animals (e.g., oxidative stress, DNA repair, and translational fidelity), recent advances in genome, transcriptome, and metabolome analysis technologies have increased findings about NMR-specific regulatory mechanisms such as production of high-molecular-mass hyaluronan and peculiar regulation of cell senescence/death induction (15, 17–23).

The present review aims to summarize the current knowledge of factors associated with ecological adaptation and healthy aging of NMRs. We also explore the similarities and differences between the mechanisms in NMRs and other long-lived animals and discuss the limitations of current research and directions for future research.

2. UNIQUE SOCIALITY OF NAKED MOLE-RATS

The unique ecological characteristics of NMRs may be linked to their extraordinary longevity and delayed aging. As described above, NMRs live underground in arid regions and feed on underground tubers by burrowing (**Figure 2**). They form eusocial colonies containing an average of 75 individuals (up to 295) (8, 24). NMRs rely on their olfactory and auditory senses to communicate with colony members and can clearly distinguish between members and nonmembers through learning (25, 26). This group living is based on cooperative breeding, a breeding system in which nonparents take care of pups. Within the colony, the reproductive roles are clearly divided between breeders and nonbreeding subordinates. Subordinates, whose sexual maturation is suppressed (27, 28), perform various tasks for the whole colony, such as foraging, maintaining the burrow, and defending against intruders. Studies focusing on the division of labor among subordinates suggested that NMRs develop complex and dynamic working systems (29, 30). Cooperative pup rearing is supposedly underpinned by hormones, with estrogen levels in colony members increasing synchronously with that in the queen during gestation (31, 32). The reproductive capacity of subordinates is physiologically suppressed through physical contact with the

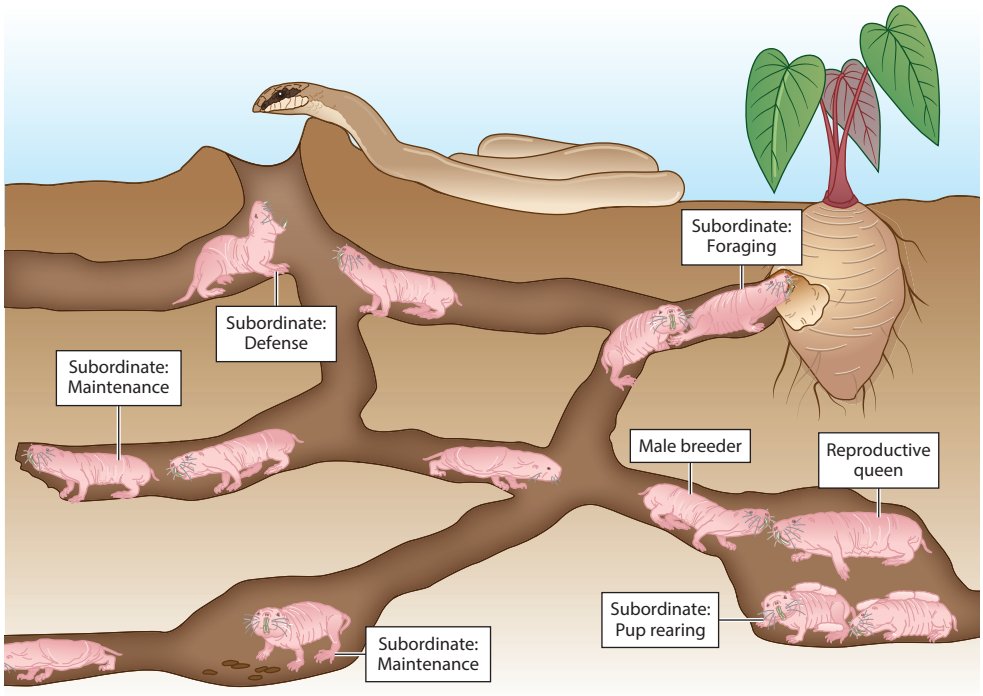

Figure 2

Diagram of an underground burrow of naked mole-rats. Naked mole-rats live in underground colonies in East Africa. They form eusocial colonies consisting of a reproductive queen, one to three male breeders, and subordinates whose sexual maturation is suppressed. Subordinates perform various tasks for the whole colony. Naked mole-rats' adaptation to the underground lifestyle and their sociality may contribute to their extraordinary longevity.

queen, and social isolation from the queen leads to sexual maturation of subordinates (33). The neuropeptide RFamide-related peptide-3 suppresses reproductive activity of subordinates (34). However, the behavior–neuron–endocrine system involved in suppressing sexual maturation is not fully understood (35).

Although NMRs are the longest-lived rodent (13), several social species of African mole-rats (AMRs), a phylogenetic group including NMRs, also have extended life spans (36–38). For instance, the maximum life span is approximately 20 years for Ansell's mole-rats (*Fukomys anselli*) (36), longer than 20 years for giant mole-rats (*Fukomys mechowii*) (39), and 20 years for Damaraland mole-rats (*Fukomys damarensis*) (38). What evolutionary and ecological factors common to social AMRs contribute to longevity? In general, energy investment in body maintenance and a low metabolic rate are the main proposed factors for longevity in animals (3, 39, 40). There is a trade-off between reproduction in early life and somatic maintenance in later life. Investment in somatic maintenance rather than early reproduction can be achieved in species with a low risk of extrinsic mortality that are expected to have sufficient breeding opportunities in later life (39, 40). Indeed, the relationship between extrinsic mortality risk and longevity is supported by interspecific comparative studies (41). The subterranean lifestyle of AMRs is thought to be adaptive due to the high predation pressure aboveground, and their subterranean habitat is characterized by physical shelter, low O_2 and high CO_2 levels, and relatively constant temperature and humidity (42).

AMR:
African mole-rat

Adaptation to such an underground environment may facilitate the evolution of energy conservation (43). In arid regions, the probability of finding food by burrowing alone is low due to the scarcity of tubers, and burrowing is energetically costly because soil is dry. Therefore, group living of AMRs in arid regions may have evolved to achieve efficient foraging through cooperative burrowing, resulting in a social shelter (24, 44). Thus, from an ecological perspective, a subterranean lifestyle in a cooperative group may contribute to longevity by reducing the risk of extrinsic mortality (42, 45–48). It is also believed that the cost of dispersal is substantially high in arid environments, which facilitates the development of a higher degree of sociality, such as cooperative breeding (24, 44). In addition, cooperative breeding leads to energy investment in somatic maintenance by inhibiting early reproduction of each member (48). The contribution of advanced sociality to longevity has been suggested in mammals and birds that display cooperative breeding (45, 49; see also 47, 50). For AMRs in arid regions, chronic food scarcity, in addition to the subterranean environment, may accelerate the evolution of energy saving (51). Furthermore, social AMRs habitually huddle in nest rooms to rest, facilitating thermoregulation (52). Thermoregulation through a stable subterranean environment and group living may underlie the heterothermic and hypothermic traits of AMRs (52–54), which may lead to effective energy saving (see the next section).

Despite having the smallest body size among AMRs, NMRs live longer than all other AMRs whose maximum life spans have been documented (13, 36–38, 55). Three features may contribute to the exceptional longevity of NMRs, which are attributed to the fact that NMRs inhabit particularly harsh areas that are hot and dry (24). The first is their extremely complex social system. In areas where food is scarcer, larger social groups may be necessary. Indeed, colony size and reproductive monopolization to the breeders are much greater with NMRs than with other social AMRs (24). The second is their high tendency to stay in stable habitats. Although the extent to which NMRs disperse is unclear, they may be less likely to disperse from their natal population than other AMRs given their unique tolerance for inbreeding (9). The reduced opportunity to leave a stable habitat may allow NMRs to enjoy more benefits of a subterranean lifestyle. The third is their unique furless phenotype. Loss of fur contributes to energy saving by promoting heterothermia and hypothermia. The NMR-specific furless phenotype might have evolved because of the need for rapid heat dissipation to ameliorate overheating associated with costly burrowing in their habitat (56), a stably hot environment, and the high population density due to more complex sociality. Therefore, we hypothesize that mutual synergistic effects of the subterranean lifestyle and social system, arising from the harsh habitat, may have contributed to the exceptional longevity of NMRs. Further comparative studies with other species are expected to improve our understanding of which ecological factors drive longevity.

3. MARKED HYPOXIA TOLERANCE OF NAKED MOLE-RATS

NMRs are one of the most hypoxia- and hypercapnia-resistant mammals. They can tolerate several hours to days of chronic hypoxia (3–8% O_2) and 18 min of anoxia (0% O_2) (57, 58). As described above, NMRs live in large groups in underground burrows where gas exchange is scarce, which probably contributes to their hypoxia and hypercapnia tolerance. Although a recent study of wild habitats reported that burrows are not constantly harsh environments, with average O_2 and CO_2 levels of 20.5% and 0.17%, respectively (59), some locations, such as the nest chamber where many individuals huddle together, may have low O_2 and high CO_2 levels. The physiological and molecular mechanisms relevant to hypoxia and hypercapnia tolerance in NMRs are outlined below (**Figure 3**).

How do NMRs respond to hypoxic conditions? When exposed to acute hypoxia, mammals attempt to balance oxygen supply and demand by increasing ventilation to compensate for reduced

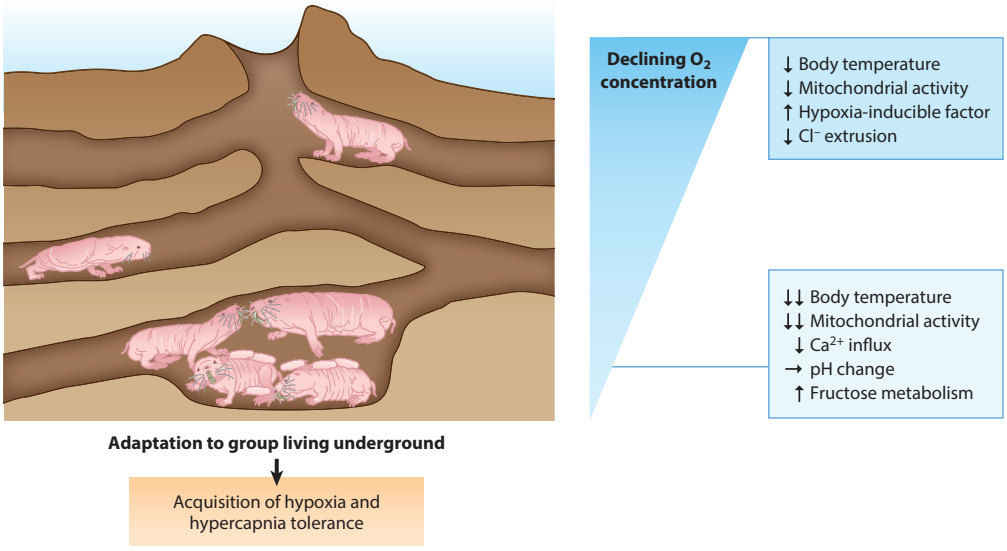

Figure 3

Hypoxia tolerance of naked mole-rats. Naked mole-rats are extremely tolerant of hypoxia and hypercapnia. These tolerances may have been acquired through adaptation to group living underground. The proposed mechanisms underlying hypoxia tolerance are shown.

oxygen levels and by decreasing body temperature and the metabolic rate to reduce oxygen consumption. In NMRs, the ventilatory rate does not increase with decreasing oxygen concentration, whereas the metabolic rate and body temperature fall by 60–85% and 2–4°C, respectively, from their basal state (57, 60, 61). To lower body temperature, mammals dissipate heat by modulating their behavior (e.g., moving to a cooler place and reducing huddling), changing circulatory functions (e.g., promoting heat loss by vasodilation), and reducing thermogenesis (e.g., suppressing heat production). In NMRs, suppression of thermogenesis rather than active heat dissipation seems to contribute significantly to metabolic and body temperature reductions during the hypoxic response (62, 63). Cheng et al. (64) reported recently that hypoxia decreases non-shivering thermogenesis in brown adipose tissue (BAT) of NMRs, accompanied by a decrease in protein expression of UCP1 and mitochondrial respiratory complexes. When NMRs are exposed to short-term or chronic hypoxia, mitochondrial respiration and activities of metabolic enzymes involved in glycolysis and the tricarboxylic acid cycle are also downregulated in tissues other than BAT, such as the brain and muscle (61, 65). Hence, functional suppression of mitochondria in several tissues, including thermogenic BAT, helps to reduce the metabolic rate and heat production to adapt to hypoxic conditions.

In general, hypoxia tolerance involves innate energy-saving mechanisms (e.g., low body temperature and low metabolic rate), in addition to suppression of energy production/consumption in response to hypoxia as described above. In fact, animals in hibernating or torpor-like states, which exhibit drastic decreases in body temperature and metabolism, are more tolerant of hypoxia than those in active states (66–68). NMRs are heterothermic mammals with a poor ability to maintain body temperature, which is at least partly due to their small body size and lack of fur. The body temperature of NMRs kept at 30°C is only 32–34°C (53, 69), and the basal metabolic rate of NMRs is considerably lower than that of other mammals of a comparable body size (70). When body temperature increases to 37°C by warming, tolerance to anoxia is shortened from tens of minutes to 6 min (58). As mentioned in the previous section, NMRs have evolved energy-saving

BAT: brown adipose tissue

systems through adaptation to the underground environment, which also likely contributes to healthy aging. Experimentally or genetically manipulated mice with reduced insulin/IGF signaling and a reduced body temperature often have a long life span and low cancer incidence (71). Zhao et al. (72) reported recently that low body temperature is a more significant contributor to longevity than the metabolic rate in mice and hamsters. In NMRs, body temperature is quite low, and insulin/IGF signaling may be diminished (73, 74). Furthermore, low mitochondrial activities even under normoxic conditions and reduced neuronal Cl^- extrusion due to a species-specific missense mutation in potassium–chloride cotransporter 2 facilitate energy saving in NMRs (75, 76). However, the mechanisms underlying energy saving, such as lowering the body temperature and metabolic rate, remain largely elusive.

TRF1: telomeric repeat binding factor 1

Under severe hypoxic and anoxic conditions, metabolic acidosis causes significant tissue damage. When the oxygen supply is decreased, energy production by anaerobic glycolysis increases to bridge the gap between aerobic energy production and the bioenergetic demand, and excessive accumulation of lactic acid as an end-product of glycolysis causes metabolic acidosis. To prevent metabolic acidosis, in anoxia-tolerant vertebrates, such as goldfish, crucian carp, and painted turtle, accumulated lactic acid is converted to ethanol, which diffuses across the gills into water, or is buffered by calcium and magnesium carbonates released from the shell (77, 78). In NMRs, gradual exposure to decreasing levels of O_2 (from 9% to 3%) did not significantly alter the blood or tissue pH, and there were no signs of metabolic acidosis (79). In addition, NMRs did not show a decrease in intraperitoneal pH after inhalation of 7.5% CO_2, which causes significant acidosis in mice (58). These observations suggest that NMRs have buffering abilities to ameliorate acidosis, although the underlying mechanism is unknown. Not only are NMRs less susceptible to acidosis, they are also resistant to pain and cell death caused by acid (80, 81). Gene mutations in the voltage-gated sodium channel Nav 1.7, which is implicated in insensitivity to acid-mediated pain, have also been found in cave-roosting little brown bats (*Myotis lucifugus*) and subterranean mammals other than NMRs (38), indicative of convergent evolution resulting in acquisition of hypoxia and hypercapnia tolerance. Furthermore, NMRs have hemoglobin with a higher oxygen affinity than mice (82) and therefore may be able to efficiently deliver oxygen to tissues even in a hypoxic environment, and anaerobic metabolism might not be strongly stimulated in NMRs. Changes in the succinate-to-fumarate ratio, an indicator of mitochondrial metabolic failure, after exposure to anoxia for 10 min are significantly smaller in NMRs than in mice (58).

In recent years, some molecular mechanisms underlying hypoxia tolerance of NMRs have been proposed. In general, the brain and heart consume large amounts of oxygen and are susceptible to damage upon oxygen deprivation. When brain slices or isolated hearts are exposed to low oxygen levels, functional depression of NMR tissues is lower than that of rat and mouse tissues (83). GLUT5, a highly selective fructose transporter expressed predominantly in the intestine and kidney in mice, is expressed abundantly in various tissues in NMRs, including the brain and heart. Furthermore, ketohexokinase (KHK), a fructose-metabolizing enzyme that can bypass feedback inhibition of glycolytic flux (84), is upregulated in the heart, brain, and liver of NMRs compared with mouse tissues (58). High expression of these genes enables the brain and heart of NMRs to use fructose as well as glucose as an energy source. This may contribute to the long-term maintenance of tissue function under anoxic conditions. NMR telomeric repeat binding factor 1 (TRF1) may contribute to the enhancement of the *KHK* expression and support glycolytic metabolism under hypoxic conditions (85). In general, neonatal mammals are more tolerant of hypoxia than adults (86). Expression of the *N*-methyl-D-aspartate (NMDA) receptor subunit GluN2D, which is normally highly expressed in the neonatal period, is retained in the adult stage in NMR brains (87). NMDA receptors containing the GluN2D subunit have lower channel activity than those containing other subunits upon exposure to hypoxia and attenuate

calcium influx into cells (88). Therefore, retention of GluN2D expression may prevent neuronal cell death associated with anoxic and hypoxic stress in NMRs. Interestingly, expression patterns of myosin heavy chain and troponin isoforms in adult NMR hearts are similar to those in neonatal mice (89). Regulation of this ectopic and heterochronic gene expression, which appears to play essential roles in hypoxia adaptation of NMRs, should be analyzed in the future. Transcriptional regulation by hypoxia-inducible factor (HIF) plays a central role in the adaptive response to hypoxic stress at the cellular level. Polymorphisms in genes involved in the HIF pathway are often identified as candidate factors for high-altitude adaptation in various species (90), whereas NMRs harbor mutations in HIF1α and von Hippel–Lindau, which is involved in degradation of HIF (20). Although there is no experimental evidence that these mutations stabilize HIF1α, there is a report that HIF1α is expressed at higher levels in tissues, such as the brain, liver, and kidney, of NMRs than in those of mice (91). Interestingly, blind mole-rats, which have strong hypoxia tolerance similar to NMRs, exhibit higher expression of HIF1α and more substantial increases in expression of target genes upon hypoxic stimulation in the kidney than rats (92). Thus, regulation of HIF expression and downstream signaling commonly controls hypoxia resistance.

Adaptation to low O_2 and/or high CO_2 levels may not only confer tolerance to adverse environments but also lead to an extended life span and ischemic tolerance in animals, as observed in humans and mice exposed to such environments (43, 93). Indeed, hypoxia-tolerant mammals, including NMRs, AMRs, blind mole-rats, and some bat species, tend to live longer than predicted based on their body mass. Hibernation, as well as hypoxia tolerance, may also be associated with longevity (94, 95). Exploration of the molecular mechanisms underlying adaptation to extreme conditions and energy saving may identify novel key factors for longevity and resistance to age-related diseases.

4. MARKED LONGEVITY AND AGING RESISTANCE OF NAKED MOLE-RATS

NMRs are the longest-lived rodent, with a maximum life span exceeding 37 years, and their mortality rate does not increase with age (12). In addition to their long life span, NMRs show minimal age-related declines in several physiological functions such as cardiac functions and reproductive capacity (14, 96). Moreover, NMRs have strong resistance to age-related diseases such as cancer and Alzheimer's disease (13, 15, 16). In this section, we discuss current understanding of the factors involved in the exceptional longevity and delayed aging of NMRs (**Figure 4**).

Genome sequencing analyses have identified several unique genomic features that may be associated with longevity and delayed aging of NMRs, such as positive selection of the *TERF1* gene encoding TRF1, which may help to protect telomeres (20, 97). Interestingly, breeders have longer life spans than subordinates in NMR colonies (12). A recent study showed that in NMRs, the methylation clocks (age-related changes in genomic DNA methylation) progress more slowly in queens than in subordinates, suggesting that queens possess unknown mechanisms to prevent age-associated changes in DNA methylation, which may contribute to further prolongation of the life span of queens compared with subordinates (98). Although some genomic regions have been suggested to be hypomethylated in breeding males, further analysis is required to determine if the methylation clock is also slow in these individuals.

DNA damage and somatic mutations are thought to be closely associated with the life span and aging of animals. RNA-sequencing analysis has shown that a group of genes involved in DNA repair pathways are highly expressed in NMR and human livers compared with mouse liver (18). Mutations and duplications of genes related to DNA repair have also been reported in other long-lived mammals such as bats (*Pteropus alecto* and *Myotis davidii*) and bowhead whales

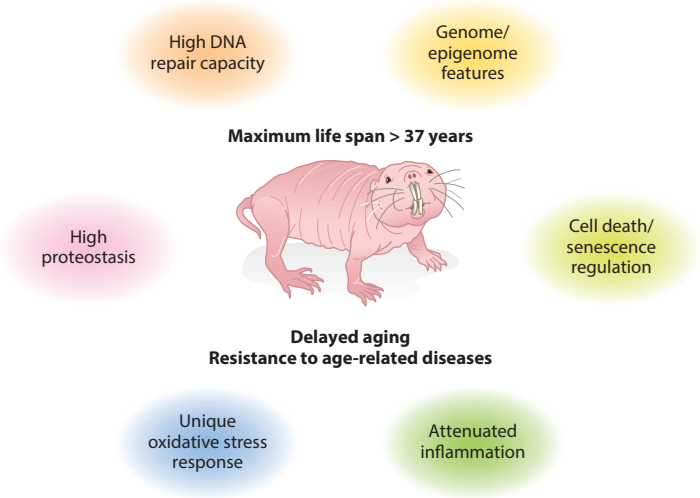

Figure 4

Potential mechanisms underlying longevity/delayed aging of the naked mole-rat. The naked mole-rat is the longest-lived rodent with extraordinary resistance to aging and aging-related diseases. Various mechanisms that help maintain genome stability, protein homeostasis, and tissue integrity are proposed to contribute to longevity and delayed aging in naked mole-rats.

(*Balaena mysticetus*) (5, 99). Tian et al. (100) reported that the DNA double-strand break (DSB) repair capacity of cultured fibroblasts correlates with the maximum life span of rodent species and that the activity of SIRT6, a DSB repair-promoting factor, is increased in long-lived rodent species, including NMRs. We recently isolated and cultured NMR neural stem/progenitor cells (NMR-NSPCs) from the subventricular zone of the neonatal brain and showed that they are more resistant to DNA damage than mouse NSPCs. When irradiated with the same dose of gamma radiation, NMR-NSPCs showed lower expression levels of gamma-H2AX, a DNA DSB marker; faster accumulation of 53BP1, which is involved in DNA repair at damaged sites; and less cell death than mouse NSPCs (101). These results suggest that NMR-NSPCs are more resistant to DNA damage than mouse NSPCs. Zhang et al. (102) reported recently that upon treatment with the DNA-damaging agent bleomycin, the mutation frequency is lower in fibroblasts from long-lived species such as humans and NMRs than in short-lived rodent species. Notably, a recent study that performed whole-genome sequencing of intestinal crypts from 16 mammalian species showed that the somatic mutation rate exhibited a strong inverse relationship with species life span (103). Therefore, a high DNA repair capacity may play an important role in NMR longevity and resistance to age-related diseases. Further studies are required to determine whether NMR cells have mechanisms to suppress DNA mutations or to efficiently eliminate mutated cells.

The oxidative stress theory holds that progressive and irreversible oxidative damage caused by reactive oxygen species (ROS) accumulates with age, and that the resulting cellular and tissue damage leads to functional decline of individuals. Puzzlingly, the response of NMR cells and tissues to oxidative stress is complex, and NMRs are both vulnerable and resistant to ROS (104). The levels of oxidative damage of lipids, DNA, and proteins are reportedly higher in NMRs than in mice (105). Glutathione peroxidase (GPx) activity, which is important for ROS detoxification, is very low in NMRs compared with mice, which is probably due to a mutation in GPx1 (106, 107). Furthermore, cultured NMR fibroblasts are much more vulnerable to hydrogen peroxide (H_2O_2) than mouse fibroblasts and are difficult to maintain in normoxic conditions (108). We showed

DSB: DNA double-strand break

NSPC: neural stem/progenitor cell

ROS: reactive oxygen species

recently that the vulnerability of NMR fibroblasts to H_2O_2 likely contributes to activation of cell death upon induction of cellular senescence, which can be considered "endogenous removal of senescent cells" (INK4a-RB-cell death described below) (23). However, there are several reports that NMR tissues and cells are resistant to ROS. Contrary to their response to H_2O_2, NMR cells are more resistant to paraquat, a pro-oxidant herbicide, than laboratory mice (108). Takasugi et al. (109) showed that treatment with high–molecular mass hyaluronan, which is secreted by NMR fibroblasts, suppresses *tert*-butyl hydroperoxide-induced cell cycle arrest and cell death in human fibroblasts by attenuating the p53 pathway. In addition, H_2O_2 consumption in mitochondria is higher in NMR tissues than in mouse tissues (17), and activity of hexokinase 2, which inhibits ROS production in mitochondria, remains high in older NMR individuals (110), suggesting that high antioxidant activity in mitochondria contributes to aging resistance. Nrf2-Keap1 signaling, which is involved in the response to oxidative stress, is also highly activated in NMR liver tissues (111). Thus, the responsiveness of NMRs to ROS may differ depending on the type of ROS and site of ROS generation. Further studies will elucidate novel mechanisms responsible for aging resistance of NMRs via appropriate management of oxidative stress.

iPSC: induced pluripotent stem cell

Loss of proteostasis is a hallmark of aging (112). Age-related changes in protein misfolding, ubiquitination, and proteasomal activity of tissues are markedly attenuated in NMRs; therefore, protein stability is considered to be high in these animals (113). Interestingly, proteasomal activity and resistance to proteasome inhibitors are higher in NMR livers than in those of mice, but the underlying mechanism is unknown. Heat shock proteins in the cytosol may contribute to this resistance (114). NMR fibroblasts display higher translational fidelity than mouse fibroblasts. NMRs have a unique cleavage site in 28S ribosomal RNA; however, its role in translational fidelity remains unclear (19). Thus, augmented proteostasis contributes to the longevity and aging resistance of NMRs; however, the underlying molecular mechanism is largely unknown.

In response to stresses such as excessive DNA damage, normal cells undergo cellular senescence (irreversible growth arrest) or apoptosis (115). Cellular senescence is an essential cancer suppression mechanism and has important roles in wound healing and development (116, 117). On the other hand, senescent cells secrete various factors that induce tissue inflammation, such as inflammatory cytokines and chemokines (senescence-associated secretory phenotype), whereby accumulated senescent cells in tissues promote individual aging and aging-related diseases such as cancer. Removal of senescent cells lengthens the healthy life span and ameliorates age-dependent disorders in mice (118). This has led to intense global competition in recent years to develop senolytic drugs that eliminate senescent cells in vivo (119). Seluanov et al. (120) reported that NMR fibroblasts exhibit continuous telomerase activity, proliferate indefinitely, and do not undergo replicative senescence. Meanwhile, Zhao et al. (121) reported that developmental senescence, oncogene-induced senescence, and DNA damage–induced senescence occur in NMRs. We generated NMR induced pluripotent stem cells (iPSCs) and found that during generation of iPSCs or activation of the proto-oncogene c-Myc, suppression of the tumor suppressor alternative reading frame (ARF) induces cellular senescence in NMR fibroblasts, which is not observed in mouse or human cells (ARF suppression-induced senescence) (22). Recently, Chee et al. (122) reported that β-catenin is highly expressed in NMR fibroblasts and that its knockdown causes cellular senescence. Thus, NMR fibroblasts undergo cellular senescence in response to various stimuli, at least in vitro. However, as described above, we found that when cellular senescence is induced in vitro, NMR fibroblasts progressively die via activation of the INK4a-RB pathway (INK4a-RB-cell death). The increase in cell death is due to activation of species-specific serotonin metabolism and resulting production of H_2O_2. INK4a-RB-cell death may be an endogenous senolytic program and prevent accumulation of senescent cells in NMRs (23; Y. Kawamura, K.

Oka, M. Takamori, Y. Sugiura, Y. Oiwa, et al., manuscript in revision). We recently revealed that NMRs have lost the ability to induce necroptosis, a type of programmed cell death that strongly induces inflammation (15) (see the next section for details). Thus, NMRs have unique cellular senescence/cell death regulatory mechanisms. Further studies are needed to determine how these characteristics contribute to their longevity and delayed aging.

We recently showed that NMR skin displays a unique, dampened inflammatory response upon exposure to various carcinogenic stimuli despite increased DNA damage and cell death (see the next section for details) (15). The unique regulation of cell death and cellular senescence, i.e., loss of the ability to induce necroptosis and INK4a-RB-cell death, may contribute to the attenuated tissue inflammatory response in NMRs. Inflammaging is a chronic, proinflammatory state that develops with aging (123). We speculate that the dampened inflammatory response upon tissue damage in NMRs may help to suppress inflammaging and thereby lead to their longevity and aging resistance. By performing single-cell RNA sequencing, Hilton et al. (124) showed that NMRs have a unique immune system characterized by a high myeloid/lymphoid cell ratio, including a novel lipopolysaccharide-responsive granulocyte cell subset, and a lack of natural killer cells. Similar differentiation bias has been observed in NMR hematopoietic stem cells (125). Emmrich et al. (126) showed that NMRs have an ectopic cervical thymus in addition to the canonical thoracic thymus and that thymic involution does not occur up to 11 years of age. Further studies are required to understand the unique NMR immune system and its contribution to delayed aging and cancer resistance.

In summary, high DNA repair activity, the unique response to oxidative stress, augmented proteostasis, unique regulation of cellular senescence and cell death, and the unique immune system leading to an attenuated inflammatory response may be involved in the longevity and delayed aging of NMRs. However, little is known about the molecular mechanisms and regulatory genes involved in these intriguing phenomena, and further studies are required.

5. MARKED CANCER RESISTANCE OF NAKED MOLE-RATS

NMRs are regarded to be highly cancer resistant because carcinogenesis was not observed in more than 2,000 necropsies of captive NMRs (13, 127). It should be noted that several recent reports described a few cases of carcinogenesis in NMRs kept in zoos; they reported one case each of an axillary adenocarcinoma, gastric neuroendocrine carcinoma, metastatic hepatocellular carcinoma, nephroblastoma, multicentric lymphosarcoma, cutaneous hemangioma, sacral chordoma, and presumptive esophageal adenocarcinoma (128–130). Detailed genomic sequencing analysis of normal and tumor tissues from these rare cases of spontaneous carcinogenesis in NMRs may provide insight into the novel mechanisms underlying their carcinogenesis resistance.

We showed recently that NMR individuals are highly resistant to chemical carcinogenesis induction (15). When treated with one of two types of carcinogens, 3-methylcholanthrene (3MC) (131) or 7,12-dimethylbenz[a]anthracene (DMBA)/12-O-tetradecanoylphorbol-13-acetate (TPA) (132), all laboratory mice (C57BL/6N) developed tumors within 24 or 40 weeks, respectively. By contrast, no tumors were observed for more than 2 years in NMRs. Buffenstein et al. also observed that NMRs did not develop any tumors after 6 months of DMBA/TPA administration (unpublished data of K.N. Lewis from the Buffenstein laboratory, cited in 133). Although further studies of the tumor incidence induced by other carcinogens in NMRs should be performed, it is evident that NMRs have an unusual resistance to chemical carcinogenesis induction at the individual level. However, understanding of the molecular basis of their carcinogenic resistance is still in its infancy. We summarize the findings to date below (**Figure 5**).

3MC: 3-methylcholanthrene

DMBA: 7,12-dimethylbenz[a]anthracene

TPA: 12-O-tetradecanoylphorbol-13-acetate

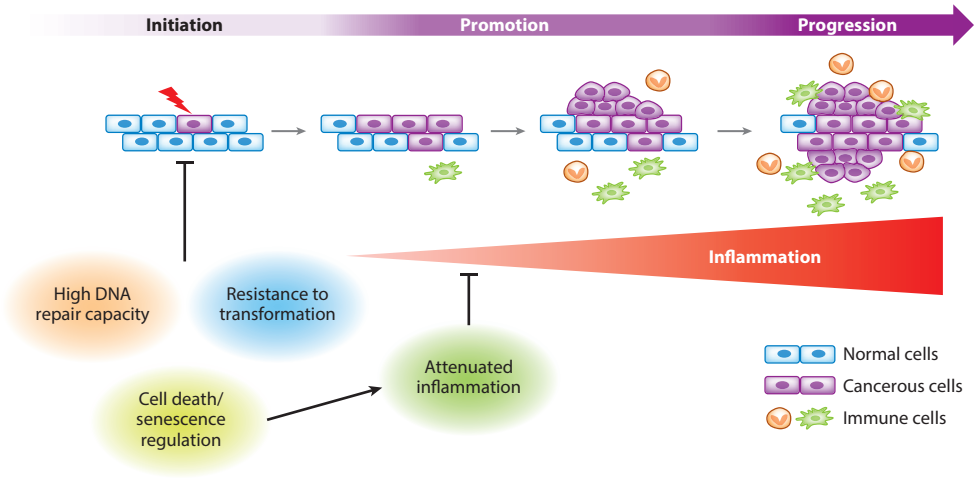

Figure 5

Potential mechanisms underlying carcinogenesis resistance of naked mole-rats. Naked mole-rats have unusual resistance to carcinogenesis. Cell-autonomous resistance mechanisms including high DNA repair capacity and high–molecular mass hyaluronan production may prevent the emergence of mutant cells. Unique cell death/senescence regulation systems also may attenuate carcinogenesis through inhibition of tumor-promoting inflammation.

In vivo carcinogenesis is a multistage process in which DNA damage initiates the generation of mutant cells, followed by promotion of mutant cell proliferation and changes in the tissue microenvironment surrounding mutant cells, leading to tumor progression (134, 135). In particular, chronic tissue inflammation is an important factor promoting tumor development that induces further genetic and epigenetic alterations of mutant cells (136–138). Histological analysis showed that DNA damage and cell death were increased in both NMR and mouse skin after treatment with 3MC or DMBA/TPA, indicating that carcinogenic insults damage tissues of both species. Notably, the number of inflammatory immune cells increased markedly after carcinogen administration in mouse skin, whereas the number of infiltrating immune cells was significantly lower in NMR skin (15). These results suggest that the tissue inflammatory response after carcinogenic insults, which is likely related to cancer promotion, is dampened in NMR tissues. We identified that the ability to induce necroptosis, a form of programmed necrosis, is lost in NMRs due to loss-of-function mutations in the receptor-interacting serine/threonine-protein kinase 3 (*RIPK3*) and mixed-lineage kinase domain-like protein genes, which are the master regulators of necroptosis (15). In general, necroptotic cells release large amounts of cellular components, which induce a strong tissue inflammatory response (139). Therefore, loss of the ability to induce necroptosis likely contributes to the attenuated inflammatory response and functions as a non-cell autonomous cancer resistance mechanism in NMRs. As described above, we also speculate that the weakened inflammatory response may contribute to the attenuation of inflammaging in NMRs. In addition, the high DNA repair activity and unique regulation of cellular senescence/death in NMRs described in the previous section may prevent the acquisition of oncogenic driver mutations in damaged cells and inhibit the accumulation and proliferation of mutant cells in tissues. Moreover, Seluanov et al. (140) reported $p16^{INK4a}$-mediated early contact inhibition of NMR fibroblasts, which may serve as a backup mechanism to restrict abnormal cell proliferation. However, whether this mechanism functions in vivo remains to be determined.

How do NMR cells behave when transduced with oncogenes? Two groups showed that NMR fibroblasts are resistant to experimental induction of transformation. They reported that introduction of constitutively active H-RAS (H-RASV12) and simian virus 40 Large T antigen (SV40 Large T), which is sufficient to transform mouse fibroblasts, did not transform NMR fibroblasts (21, 141). In addition to transduction of H-RASV12 and SV40 Large T, introduction of human telomerase reverse transcriptase (141) or suppression of NMR-specific high–molecular mass hyaluronan production (21) was required to transform NMR fibroblasts. On the other hand, Hadi et al. (142) recently showed that NMR fibroblasts are transformed upon introduction of only H-RASV12 and SV40 Large T. Deuker et al. (143) showed that p53 protein constitutively localized to nuclei of NMR fibroblasts and that, in contrast with p53 wild-type cells, expression of $KRAS^{G12V}$ reduced the proliferation potential of p53-knockout NMR fibroblasts under adherent culture conditions but allowed them to acquire anchorage-independent spheroid formation potential. We showed previously that suppression of the tumor suppressor *ARF* during reprogramming/oncogenic stress induces cellular senescence in NMR fibroblasts, which is not observed in mice and humans, and the generated NMR iPSCs exhibit upregulation of *ARF* expression and unique tumor resistance (22). Thus, the susceptibility of NMR cells to oncogenic transformation remains controversial. Further in vitro and in vivo studies are needed to determine whether NMR cells are resistant to induction of transformation upon oncogene transduction and how they behave when oncogenic driver mutations occur.

SV40 Large T: simian virus 40 Large T antigen

Are strategies for carcinogenesis resistance common among cancer-resistant animal species? Blind mole-rats, which are long-lived, cancer-resistant rodents phylogenetically distant from NMRs, show a very low incidence of carcinogenesis when treated with 3MC or DMBA/TPA (~9% or 0%, respectively) (144, 145). In contrast to in NMRs, DMBA/TPA treatment induces a robust inflammatory response and necrosis in blind mole-rat skin (144). Similarly, blind mole-rat fibroblasts undergo interferon-mediated necrotic death in response to hyperproliferation (146). This involves activation of the cGAS-STING pathway triggered by activation of retrotransposable elements (6). Activation of necrotic cell death and the immune response caused by hyperproliferation may contribute to cancer resistance in blind mole-rats by eliminating hyperplastic premalignant cells. Interestingly, the tissue response to carcinogens differs between NMRs and blind mole-rats, both of which are carcinogenesis-resistant, long-lived rodents. Elephants are large and have a huge number of cells; however, they have a low cancer mortality rate. The lack of a correlation between body size and cancer risk is known as Peto's paradox (147). The African elephant genome contains multiple copies of the *TP53* retrogene and an elephant-specific duplicated *LIF* gene (*LIF6*), which are implicated in enhancing apoptosis induction by activating the p53 signaling pathway in response to DNA damage (7, 148, 149). In addition, substitution rates are accelerated in genomic regions related to immune pathways in both African and Asian elephants (150). Thus, altered regulation of cell death and immune responses may be closely associated with cancer resistance in elephants. These results suggest that in cancer-resistant species, both common and species-specific mechanisms may protect against carcinogenesis. Intriguingly, the malignant cancer rates are lower in African than in Asian elephants (150). Comparative analyses of closely related species that are cancer-resistant but have different susceptibilities to cancer will lead to the identification of more important mechanisms for cancer suppression.

Considering their markedly low spontaneous carcinogenesis rate and strong resistance to chemical carcinogenesis induction, NMRs are likely exceptionally resistant to carcinogenesis. In addition to analysis of genes and cultured cells, detailed analysis of tissue responses upon induction of carcinogenesis is expected to elucidate novel molecular mechanisms related to the carcinogenesis resistance of NMRs.

6. CONCLUSION

In this review, the potential mechanisms underlying healthy aging of NMRs have been discussed from the perspective of ecology, hypoxia tolerance, aging, and cancer. Recent technological advances including multi-omics approaches have rapidly improved understanding of the unique characteristics of NMRs, which may involve numerous mechanisms. However, only a few molecular mechanisms have been experimentally demonstrated to contribute to resistance to aging and cancer in NMRs by genetic manipulation approaches, and further studies are needed. Aging and carcinogenesis in individuals are complex and multistep processes that involve various cell-to-cell and tissue-to-tissue interactions. Therefore, in vivo experimental approaches are essential for future research to reveal how aging and age-related diseases, including cancer, are prevented in long-lived species. Recently, we showed that NMRs lack the ability to induce necroptosis and that chemical carcinogenesis is delayed in mice in which necroptosis is inhibited by disruption of RIPK3 (15). Thus, it would be useful to generate genetically modified mice that mimic species-specific sequence/expression changes of a certain gene in NMRs and to determine whether these mice are resistant to aging or cancer. NMRs can be kept and bred in laboratories and are suitable for in vivo experiments. However, reverse genetics approaches cannot be applied, and there is no method to produce genetically modified NMRs. Due to the slow reproductive rate of NMRs (the gestation period is ~70 days) (151) and suppression of sexual maturation in subordinates, meaning only one female in a colony can breed, no developmental engineering method has been established. It is expected that future development of genetic modification technology for NMR individuals, in combination with rapidly developing technologies such as multi-omics, organoid formation, and in vivo imaging, will lead to a breakthrough and significantly improve understanding of the molecular mechanisms underlying healthy aging of NMRs. Further research on long-lived animals, including NMRs, using various new technologies developed in the future is expected to improve understanding of the evolutionary process of animal life span and aging and eventually develop new preventative and therapeutic strategies for age-related diseases including cancer in humans.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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