

Review

Fighting with Aging: The Secret for Keeping Health and Longevity of Naked Mole Rats

Wenjing Yang^{1#}, Yifan Hu^{2,3#}, Shufang Cui^{1*}

¹Laboratory Animal Science Department, Basic Medical School, Naval Medical University, Shanghai, China.

²Changhai Hospital, Naval Medical University, Shanghai, China

³No. 904 Hospital of the PLA Joint Logistics Support Force, Wuxi, China

[Received November 17, 2023; Revised January 6, 2024; Accepted January 9, 2024]

ABSTRACT: Health and longevity are the dreams of mankind and the main field of the medical community. The naked mole rat (NMR) is a unique murine animal with extremely long lives (exceeding 38 years), revealing little signs of aging such as reproductive decline, neural degenerative diseases, and cancer. They provide us with valuable perspectives on preventing age-related diseases. This review systematically summarized the characters of different systems of naked mole rats in aging resistance, and furtherly exploited the mechanisms for aging resistance form genome, telomeres, protein recycling, metabolism, and oxidative stress attitudes. As a species with a high similarity with human beings, it cannot be ruled out that after reasonable validity, safety and ethical evaluation, the dominant genes of naked mole rats will be developed in medical transformation, to realize the dream of human health and longevity.

Key words: naked mole rat, aging, ROS, genome, metabolism

Longevity and health should be a major goal of all the people. The occurrence of chronic diseases is often positively correlated with aging [1], such as cancer, cardiovascular disease, and neurodegeneration [2-4]. Many studies have been performed in model animals such as *Caenorhabditis elegans* and lab mice, such as the cGas/Sting pathways and mTOR pathways [5, 6]. These animals are excellent in Drug evaluation and mechanism research, but show limitations in providing novel perspectives to fight age-related diseases from evolutionary ways. The naked mole rat (NMR) is a unique murine animal with extremely long lives (exceeding 38 years) [6], revealing little signs of aging such as reproductive decline [7], Alzheimer's disease [8], and cancer [9], et al. Therefore, it is imperative to find effective gene, environmental, and drug interventions to delay aging, improve functional loss, and reduce the

incidence of late-life disease. Advantages in omics and genetic editing by CRISPR/Cas9 system will enable the transmission of advantageous genes across species. This study will systematically summarize the physiological characteristics of naked mole rats, and exploit the mechanisms for keeping healthy and longevity, to provide a useful valuable message for all human beings' dreams of health and longevity.

1. Characteristics of long-lived naked mole rats

The Naked mole rat, who lives in a resource-poor, humid, and harsh subterranean environment, is just one of more than 50 kinds of subterranean rodent species worldwide [10]. The life quotient of naked mole rats is (the ratio of maximum observed lifespan to weight-predicted lifespan) greater than 4, similar to human beings [11]. They are

*Correspondence should be addressed to: Prof. Shufang Cui, Laboratory Animal Science Department, Basic Medical School, Naval Medical University, Shanghai, China. Email: youngstar_sf@163.com. #These authors contributed equally to this work.

Copyright: © 2024 Yang W. et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

eusocial animals, like bats, social bees, wasps, and ants. They are resistant to cancer [12, 13] and hypoxia [14, 15]. Besides, there remains a large area to draw a systematic picture of their evolutionary routes.

2. Physiological performances of aging resistance in naked mole rats

2.1 Hematopoietic and immune system characters in aging resistance

Immune senescence related to aging, is characterized by changes in immune components, such as loss of adaptive immune diversity [16], susceptibility to infection in older people, autoimmunity, and cancer. But Naked mole rats show delayed immune senescence [17]. Naked mole rat hematopoietic systems consist of a large pool of quiescent hematopoietic stem cells (HSCs). Transcriptomic data show molecules in cell protection pathways but not inflammatory markers are upregulated during aging in naked mole rats, which is quite different from human beings and lab mice. The inherent myeloid bias in the bone marrow of naked mole rats does not predispose hematopoietic stem cells and progenitor cells to reduced lymphocyte differentiation, thus naked mole rats do not reveal an age-related reduction in early T-cell progenitors [18]. So, there is no thymic involution in Naked mole rats until 11 years old [17]. The high levels of Aire and FOXP1 also ensure thymic function at neonatal levels [17]. Erythropoiesis in the spleen sustains into adulthood, which is also crucial for the long-term survival of naked mole rats [19]. Due to an increase in myeloid cells and a decrease in lymphoid lineages in spleens, Naked mole rats exhibit a lower level of adaptive immunity [19], but a high level of innate immunity [20], which may be energy-efficient in immune surveillance to defend heterologous infection. The Langerhans cells that settle in the skin (derived from bone marrow and spleen) are conserved and show no signs of aging in Naked mole rats [21], which may also play critical roles in protecting them from various infections. Interestingly, the number of skin stem cells and the epidermal gene levels showed surprising stability during aging [21], indicating the excellent renewal and healing power in naked mole rats [22]. Functional skin healing experiments have shown that wound closure rates in young and old animals are strictly equivalent to 33, exhibiting a very strong anti-aging phenotype. RNAseq analysis revealed that the transcription levels of several longevity-related genes (IGFBP3, IGF2BP3, Ing2) and tumor suppressor genes (BTG2, CDKN1A, CDKN2C, Dnmt3a, HIC1, SOCS3, SFRP1, SFRP5, THBS1, TSC1, ZFP36) were significantly increased in senescent NMR skin compared with aged human or mouse skin [22].

2.2 Forever young cardiovascular systems of naked mole rats

The naked mole rat hearts show little changes in aging hearts. There was no age-related change in NMR left ventricular function either at rest or under exercise stress. ECG analysis did not find any arrhythmias in all ages of naked mole rats, while it significantly increased in aged mice. The E/A ratio of only female naked mole rats (used to evaluate ventricular diastolic function) decreased significantly with age. Interestingly, a decrease in diastolic function in female naked mole rats does not lead to cardiovascular disease (CVD) and morbidity or mortality [23]. In particular, QRS, an important aging sign of heart function in lab mice, showed no incensement in both young and aged naked mole rats [23]. Morphologically, cardiomyocyte cross-sectional area left ventricular size, and left ventricular wall thickness in naked mole rats over their life expectancy [24], which are great differences between humans and lab mice [25]. Although cardiomyocyte hypertrophy has been found in naked mole rats, it does not develop into heart disease [23, 24, 26, 27]. In the study of cardiac fibrin profile analysis, naked mole rats mainly depend on β -MHC, which is economical in ATP consumption compared with α -MHC [26].

Age-related arteriosclerosis, aortic blood pressure, and pulse wave velocities have not been found changed with aging in naked mole rats [24]. Though high levels of oxidative have been detected in arteries, there were quite a few lesions in vascular or apoptosis in vascular endothelial cells [28, 29]. Apoptotic cells were significantly increased (about 250-300%) in the arteries of aged rats (2 years old), 5-6-fold (about 50%) of those in aged naked mole rats (12 years old). This suggests that blood vessels in naked mole rats tolerate high levels of oxidative stress to maintain young vessel stabilities [28].

2.3 Aging resistance in naked mole rat central nervous system

The hypoxia tolerance of naked mole rats plays an important role in aging resistance. There are three ways to help naked mole rats switch between normoxia and hypoxia rapidly. First of all, it has been found a unique anaerobic glycolysis pathway that rapidly energizes nerve cells in naked mole rats during hypoxia [15]. Secondly, there are sustained greater proportion of the GluN2D subunit till adulthood compared with mice, which play critical roles in delaying calcium entry into neurons when hypoxia exposure [30]. Thirdly, reducing the energy expenditure of nerve cells. Though much more neural stem/progenitor cells (NS/PCs) in naked mole rats proliferated more slowly and stayed in the G0/G1 phase.

But after stimulus such as γ irradiation, NMR-NS/PCs initiate inner mechanisms rapidly against DNA damage and keep cells away from dying [31]. Finally, there is postnatal neuromorphogenesis and spatial synaptic refinement in partial brain regions of naked mole rats, including the hippocampus and olfactory, helping naked mole rats adapt to extreme hypoxia and avoid neurodegenerative processes [32]. Interestingly, although Alzheimer's disease (AD)-associated proteins (A β and phosphorylated tau) are extremely high in naked mole rat brains, neither A β plaques, neurofibrillary tangle, nor neuronal loss occurs. This provides new insights into the mechanisms of AD [33, 34]. Maybe the low aggregation rate of A β ₁₋₄₂ in naked mole rats raises the threshold for neuronal tolerance toxicity [35]. Other proteins in the naked mole-rat brain are also of interest. It has been shown that NRG-1 prevents the impairment of hippocampal function by endogenous and exogenous neurotoxins [36]. Proteomic studies of the naked mole rat brain identified nine proteins associated with neurite growth and neurotransmission: Cofilin-1, dihydropyrimidinase-related protein 2 (isoform 2), aka collapsin response mediator protein 2, spectrin alpha chain (isoform 3), Septin-7, Syntaxin-binding protein 1, synapsin-2 isoform IIB, and dynamin1 (isoform 3 and 4). These proteins increase significantly with age and play critical roles in maintaining the neuroplasticity of naked mole rats during aging [37].

2.4 Unique musculoskeletal system

Although there were no significant differences between naked mole rats and other Girls in terms of bone composition, mechanical properties, growth patterns, and basic structure, the cortical bones of the naked mole rats are completely circumferential and layered, lacking a mineralized cartilage island. Furthermore, the naked mole rat skeletons reveal no evidence of remodeling at any age [38]. The skeletal homeostasis, bone mineral structure, and mechanical properties are stabilized in aged naked mole rats, but not in aged mice and human beings [25]. High levels of high molecular weight hyaluronic acid (HMWHA) in naked mole rats may be responsible for the resistance to trauma and common degenerative diseases such as osteoarthritis [39].

Healthy skeletal muscle tissue persists in naked mole rats for decades. Muscle fiber integrity and mitochondrial ultrastructure were well maintained in aged naked mole rats, revealing few signs of age-related muscle atrophy or mitochondrial dysfunction. Mitochondrial complex IV expression and activity remained stable, but complex I expression was significantly reduced in naked mole rats. Mitochondrial DNA copy number increased significantly in skeletal muscle fibers of naked mole rats with age [40],

which may be beneficial for maintaining normal movements of muscles. Interestingly, mitochondrial DNA rearrangements only exist in senescent skeletal muscle tissue in naked mole rats, but not in human beings and other rodents. These changes may be responsible for the physical function of naked mole rats to remain healthy throughout their lives.

2.5 Sustained female reproduction ability

Although reproduction ensures the survival of the entire species, it reduces an individual's lifespan [41]. This theory is not true in the queen of naked mole rats. The Queen of naked mole rats, which breed throughout their lifespans, does not suffer from the energy costs of reproduction. Maybe extremely high levels of RRAGB and TMEM8C are contributed to the longevity and sustained reproduction ability [41, 42]. As eusocial animals, the queen has absolute power to dispatch energy and ensure the survival of the community, which is an important way for this species to gain a foothold on Earth. This is the result of a eusocial lifestyle with the most arranged energy at the expense of the reproductive capacity of the workers.

On the other hand, the reproductive senescence of female naked mole rats is also manifested in expanded ovarian reserves [43]. In human beings and mice, a limited ovarian reserve is established from birth [44], and reproductive capacity gradually declines as the reduction of ovarian reserve with age [45, 46]. The fertility of the mice was maintained for a maximum of 9 months [47]. The naked mole-rats ovaries remained largely germ cell at 90 days after birth and showed a 10-fold greater ovarian reserve by 6 months than lab mice [48]. This is one of the main reasons for the queen in the colony to restore her reproductive ability until the end of her life [48].

3. Mechanisms of aging resistance in naked mole rats

This evolutionary tinkering, and the consequent alteration or recycling of various components, promotes integrative adaptation through a process of natural selection [49]. Large differences in the maximum lifespan potential of species [MLSP] must ultimately be encoded by genes. However, if a specific lifespan program exists, a genetic response mutant can identify such a program and help human beings achieve immortality.

Large amounts of genomic and transcriptomic data from naked mole rats have been processed in recent studies [13, 15, 50]. About 88% of the naked mole rat genes are orthologous to those of human [50].

In comparison with mammals, there are 750 acquired and 320 lost genes found in naked mole rats [50], and about 66 unique additional genes have been found in

naked mole rats [50]. Naked mole rats strive to maintain genetic stability, and on the other hand, make genetic changes at minimal cost to adapt to extreme environments, making their evolutionary differential genes of great value to human beings.

3.1 Roles of the genome in aging resistance

3.1.1 Gene evolutions

Gene evolutions make naked mole rats inhabit the environment. Interestingly, naked mole rats are genetically much similar to human beings than mice [51]. Interspecies differences in mutation rates are generally considered to indicate different evolutionary pressures. Compared with mice and guinea pigs, the incidence of synonymous and non-synonymous differences in naked mole rats decreased significantly [51].

Activities of splicing factor are higher in naked mole rats from youth to old [52], which may contribute to better molecular stress responses and avoidance of senescence [52]. Comparative analysis revealed that ADAMTS9, the known inhibitor of the mTOR pathway, performing a driver of senescence, is extremely high in bats naked mole rats at all ages [53]. The convergent evolution of ADAMTS9 may therefore be responsible for the extraordinary longevity and anti-tumor activity of bats and naked mole rats. Elevated levels of p16 are associated with the early contact inhibition in dermis fibroblasts of naked mole rats [54], due to the unique splicing pattern of pALT^{INK4a/b} in the Ink4 transposon of naked mole rats, thus affecting cell cycle progression and tumorigenesis [55, 56].

There was no significant differences in copy number between the naked mole rat genome and that of human oncogenes and tumor suppressor gene [55]. Moreover, expression levels of many enzymes targeting DNA repair are higher in the liver, brain, and testis than in mice [55, 56], including enzymes involved in tumor suppression (e.g. TP53), base excision repair, mismatch repair, and non-homologous end ligation [56].

3.1.2 Maintenance of Genome Stability

DNA damages affect most aspects of the aging phenotype [57]. There was not only lower mutation frequency but also stronger DNA repair in naked mole rats, such as higher levels of Nrf2 activity in naked mole rats than mice [58]. RNA-seq data on the brains, livers, and kidneys of newborn, young (4-year-old), and old (20-year-old) naked mole rats showed that few genes in naked mole rats show differential expression between 4 to 20 years old, especially in the brain. For example, genes associated with macromolecular degradation, such as GSTA1,

DERL1, and GNS, are not upregulated with age [59]. Genes encoding mitochondrial proteins (NDUFB11, ATP5G3, and UQCRCQ) [59] are not downregulated, which is consistent with the stable maintenance of mitochondrial function during aging. There are also higher copy numbers of CEBPG (a DNA repair regulator), Tinf2 (a protector of telomere integrity), and α -2 macroglobulin (A2M) [60] in the naked mole-rat liver, which play an important role in supporting gene stability.

Moreover, naked mole rat fibroblasts are resistant to radiation-induced apoptosis. It takes a much higher dose of γ -ray irradiation to trigger naked mole rat cell senescence [61]. Gene expression analysis of senescence-related changes showed that senescence-related secretory phenotype genes in naked mole rat cells were similar to mice, including induction of SASP, IFN, TNF, inhibition of protein translation, and cell cycle. The insufficient protection mechanisms in DNA metabolism, transcription, and translation [61], make mice susceptible to induced senescence.

3.1.3 Gene expression and regulation

Interestingly, naked mole rats reveal senescence in epigenetic levels. Lnc RNAs in naked mole rats share many similarities with other vertebrate species, such as tissue specificity and low expression. However, the shortest Lnc RNA has only been found in naked mole rats, compared to other long-lived species such as Bowhead whales and Brandt's bat [62]. Besides, CpG subpopulations underwent similar methylation changes during aging in naked mole rats compared with human beings [63].

3.2 Ambiguous roles of telomeres in aging resistance

In most eukaryotes, telomere shortening is thought to be critical in senescence. When they reach a critical length the cell enters a state of senescence, which is termed the 'senescence/senescence replication theory'. However, long telomeres and high telomerase activity are tumor-promoting factors. The largest comparative study of telomeres and telomerase involving more than 60 mammalian species has found that smaller, shorter-lived species tend to have longer telomeres and higher levels of telomerase [64]. In summary, the evolution of macrosomia and longevities appears to be closely related to the evolution of short telomeres and telomerase inhibition, possibly to suppress tumors.

Telomeres of naked mole rats are similar in length to those of human beings, and much smaller (1/3 to 1/2) than those of mice and rats [64]. Interestingly, the relative telomere length (RTL) in rat tissues (kidney, lung, and muscle) decreases with age but shows a slightly

significant elongation in naked mole rats with age oppositely [65, 66]. This phenomenon may be caused by low telomerase activity in naked mole rats, which is demonstrated by the tight binding of TRF1 to telomeres in naked mole rats, leading to lower cancer incidence and longevity [67]. Besides, TERT showed stable expression at all ages, also consistent with the role of the telomerase complex highlighted by positive selection on TEP1 and TERF1 [50]. Currently, the role of telomeres in aging remains unclear, and naked mole rats may provide novel attitudes exploring relations between telomeres and aging.

3.3 Protein recycling and aging resistance

The cellular proteome performs highly diverse functions to sustain life [68]. It has involved a complex network of protein quality control (PQC) pathways in cells, including degradation pathways and numerous chaperones and co-chaperones, to assess protein quality, and by refolding or eliminating proteins through degradation pathways to initiate appropriate responses to mitigate damage. But this quality control system will decline as cell senescence, leading to a series of diseases, including neuro-degeneration and so on.

In general, the lifespan is negatively correlated with the turnover of high-abundance proteins. Compared with mice, cells from naked mole rats exhibit slower protein turnover and a robust protein homeostasis system [69], which reduces the risk of many unnecessary mutations. The unique 28S rRNA in naked mole rats guarantees high fidelity and stability in protein synthesis [70]. It is shown that proteolytic degradation mechanisms and maintenance of protein quality play a key role in the lifespan of rodents [71]. An efficient ubiquitin-proteasome system (UPS) in naked mole rats enables the rapid degrading of misfolded proteins [72]. During senescence, higher proteasome activities and autophagic rate have been found in naked mole rat organs than those of mice [69]. High levels of the key chaperones HSP72, HSP40, and HSP25 in naked mole rats protect proteasome function from cellular stressors [73]. There was a significant positive correlation between MLSP, HSP25, HSF1, proteasome activity, and autophagy-related protein 12 (ATG12). High basal autophagic activity in naked mole rat cells mediated by ATG5 contributes to the inhibition of p53/Rb-induced apoptosis and increases their lifespan [74].

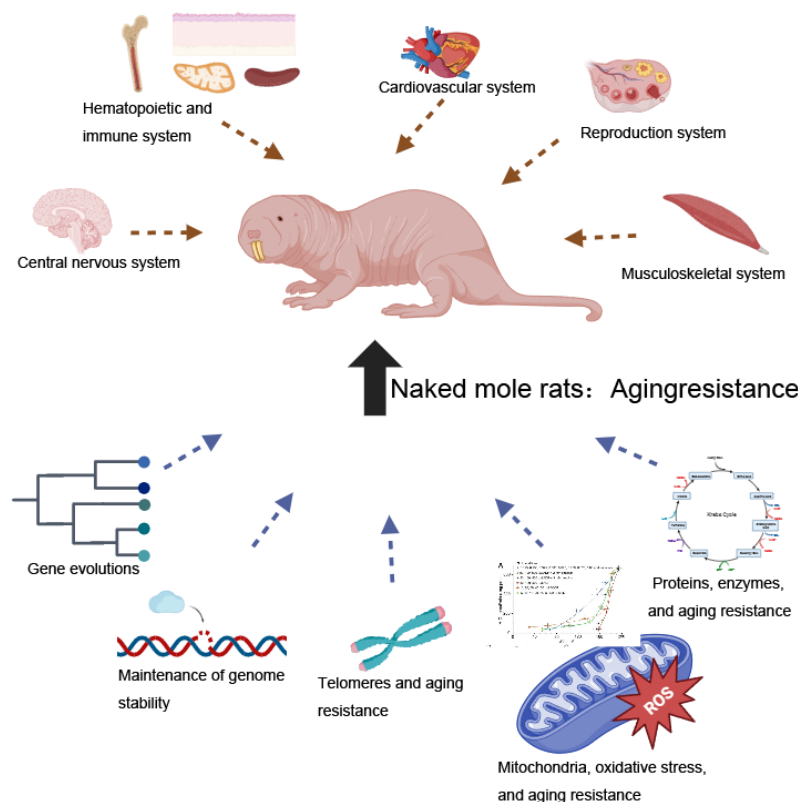


Figure 1. Characters and mechanisms of aging resistance in naked mole rats. Various organisms of naked mole rats show aging resistance. The underlying mechanisms may be due to gene evolutions, maintenance of genome stability, telomeres, ros, and protein recycling mechanisms.

3.4 Metabolism and aging resistance

A moderate reduction of O₂ concentration may be beneficial to longevity [50, 75]. The overall metabolic rate was maintained at a lower level throughout the whole life span in naked mole rats [76]. The stable levels of HIF-1 α ensured the stability of naked mole mouse proteins at different oxygen concentrations, increasing the efficiency of oxygen consumption. The attenuation of the HIF-1 α function is related to senescence of mice [77]. Besides, the use of fructose to produce ATP under special conditions is also a relatively energy-saving method [15]. Interestingly, the gut microbiota of naked mole rats is capable of using soil sulfate as a terminal electron acceptor to maintain anaerobic oxidative metabolism [78]. Amino acid metabolism is more dominant than fatty acid metabolism in naked mole rats [79, 80]. In terms of protein metabolism, both PK levels and PKM levels in naked mole rats increased with aging, accompanied by a decreased phosphorylation state. High PKM activity is responsible for raising the efficiencies of cellular function in aging.

3.5 Controversial theories on oxidative stress and aging

Oxidative stress theory predicts that the rate of oxidative damage generation and accumulation in long-lived species should be lower than in short-lived species, and these differences should be evident even when comparing younger, healthy individuals [81]. Multiple comparative studies have shown that mitochondrial ROS (mtROS) production rates and oxidative damage to mitochondrial DNA are inversely associated with maximal lifespan, especially in long-lived animals [82]. But the long-lived naked mole rat is an exception. The horizontal comparison showed that the level of oxidative stress damage accumulated in the naked mole rats was much higher than that of mice of comparable age, but the longitudinal comparison showed that the elderly naked mole rats reveal similar high levels of oxidative stress damage as the young naked mole rats [83]. This is a challenge to the classical theory of oxidative stress aging and may force a re-evaluation of the interrelationship between oxidative stress damage and aging (Fig 1.).

4. Conclusions

Health and longevity are also the dreams of mankind and the main field of the medical community. For a long time, evolution, long-lived Naked mole rats have developed an efficient way of using energy, including their true social lifestyle, as well as the way the organism metabolizes and distributes energy. Adjustments at the genome and protein

levels allow the species to exist for generations. With the update of gene editing, gene therapy, and cell therapy technology, some researchers have verified the function of the dominant genes of naked mole rats on other species and effectively improved some pathological states of other species [9]. But quite a lot of discoveries are demonstrated by omics, without experimental verification, which may be a restriction in medical transplantation (Table 1). But, as a species with high similarities with human beings, it cannot be ruled out that after reasonable validity, safety, and ethical evaluation, the dominant genes of naked mole rats will be developed in medical transformation, to realize the dream of human health and longevity.

Table 1. Advantages and limitations in applications of naked mole rats.

Advantages in applications	Limitations in applications
Unique genes/proteins in aging resistance: pALT ^{INK4a/b} [54], HMWHA [9], 28S rRNA [70]	Roles of telomeres in aging resistance [50, 64-67]
Common genes with different expression levels: ADAMTS9 [53], Nrf2 [58], GSTA1 [59], DERL1 [59], GNS [59], CEBPG [60], Tinf2 (a protector of telomere integrity) [60], A2M (α -2 macroglobulin) [60]	Oxidative stress and aging [82, 83]
Epigenetics: Lnc RNAs [62], CpG subpopulations [63]	
Protein recycling and aging resistance: HSP72 [73], HSP40 [73], HSP25 [73], ATG5 [74]	
Metabolism: stable HIF-1 α [77], PK and PKM levels [79, 80]	

Acknowledgments

This work was supported by State Key Research and Development Program of China (2021YFF0702400), the National Nature Science Foundation of China (No. 82271915, No. 31700923), Natural Science Foundation of China of Shanghai (23ZR1477700), Science and Technology Innovation Program of Shanghai, China (Nos. 22140900300, 20140900100), PLA Special Issue of Animal Projects (No. SYDW [2020]-12), 2020 Shanghai Military-Civil Integration Development Special Project(2020-jmrh1-kj9), National Sci-Tech Support Plan (No. 2015BAI09B02). We thank all the group members for providing help in this study.

Conflict of Interest statement

The authors declare no conflicts in this manuscript.

Author Contributions

This review has been conceived and designed by WJ Y and SF C. WJ Y and YF H are responsible for collecting literature. This manuscript is written by WJ Y and YF H, and revised by WJ Y and SF C.

References

- [1] Crimmins EM (2015). Lifespan and Healthspan: Past, Present, and Promise. *Gerontologist*, 55:901-911.
- [2] Niccoli T, Partridge L (2012). Ageing as a risk factor for disease. *Curr Biol*, 22:R741-752.
- [3] Jagger C, Gillies C, Moscone F, Cambois E, Van Oyen H, Nusselder W, et al. (2008). Inequalities in healthy life years in the 25 countries of the European Union in 2005: a cross-national meta-regression analysis. *Lancet*, 372:2124-2131.
- [4] Stenholm S, Head J, Aalto V, Kivimäki M, Kawachi I, Zins M, et al. (2017). Body mass index as a predictor of healthy and disease-free life expectancy between ages 50 and 75: a multicohort study. *Int J Obes (Lond)*, 41:769-775.
- [5] Liu H, Zhang H, Wu X, Ma D, Wu J, Wang L, et al. (2018). Nuclear cGAS suppresses DNA repair and promotes tumorigenesis. *Nature*, 563:131-136.
- [6] Oka K, Yamakawa M, Kawamura Y, Kutsukake N, Miura K (2023). The Naked Mole-Rat as a Model for Healthy Aging. *Annu Rev Anim Biosci*, 11:207-226.
- [7] Briño-Enríquez MA, Faykoo-Martinez M, Gobin M, Grenier JK, McGrath A, Prado AM, et al. (2023). Postnatal oogenesis leads to an exceptionally large ovarian reserve in naked mole-rats. *Nat Commun*, 14:670.
- [8] Brown-Borg HM, Buffenstein R (2017). Cutting back on the essentials: Can manipulating intake of specific amino acids modulate health and lifespan? *Ageing Res Rev*, 39:87-95.
- [9] Zhang Z, Tian X, Lu JY, Boit K, Abulaeva J, Zakusilo FT, et al. (2023). Increased hyaluronan by naked mole-rat Has2 improves healthspan in mice. *Nature*, 621:196-205.
- [10] Lewis KN, Soifer I, Melamud E, Roy M, McIsaac RS, Hibbs M, et al. (2016). Unraveling the message: insights into comparative genomics of the naked mole-rat. *Mamm Genome*, 27:259-278.
- [11] Buffenstein R (2005). The naked mole-rat: a new long-living model for human aging research. *J Gerontol A Biol Sci Med Sci*, 60:1369-1377.
- [12] Delaney MA, Ward JM, Walsh TF, Chinnadurai SK, Kerns K, Kinsel MJ, et al. (2016). Initial Case Reports of Cancer in Naked Mole-rats (*Heterocephalus glaber*). *Vet Pathol*, 53:691-696.
- [13] Piersigilli A, Meyerholz DK (2016). The "Naked Truth": Naked Mole-Rats Do Get Cancer. *Vet Pathol*, 53:519-520.
- [14] Larson J, Park TJ (2009). Extreme hypoxia tolerance of naked mole-rat brain. *Neuroreport*, 20:1634-1637.
- [15] Park TJ, Reznick J, Peterson BL, Blass G, Omerbašić D, Bennett NC, et al. (2017). Fructose-driven glycolysis supports anoxia resistance in the naked mole-rat. *Science*, 356:307-311.
- [16] Lee KA, Flores RR, Jang IH, Saathoff A, Robbins PD (2022). Immune Senescence, Immunosenescence and Aging. *Front Aging*, 3:900028.
- [17] Emmrich S, Tolibzoda Zakusilo F, Trapp A, Zhou X, Zhang Q, Irving EM, et al. (2021). Ectopic cervical thymic and no thymic involution until midlife in naked mole rats. *Aging Cell*, 20:e13477.
- [18] Emmrich S, Trapp A, Tolibzoda Zakusilo F, Straight ME, Ying AK, Tyshkovskiy A, et al. (2022). Characterization of naked mole-rat hematopoiesis reveals unique stem and progenitor cell patterns and neotenic traits. *Embo j*, 41:e109694.
- [19] Bégay V, Cirovic B, Barker AJ, Klopffleisch R, Hart DW, Bennett NC, et al. (2022). Immune competence and spleen size scale with colony status in the naked mole-rat. *Open Biol*, 12:210292.
- [20] Hilton HG, Rubinstein ND, Janki P, Ireland AT, Bernstein N, Fong NL, et al. (2019). Single-cell transcriptomics of the naked mole-rat reveals unexpected features of mammalian immunity. *PLoS Biol*, 17:e3000528.
- [21] Savina A, Jaffredo T, Saldmann F, Faulkes CG, Moguelet P, Leroy C, et al. (2022). Single-cell transcriptomics reveals age-resistant maintenance of cell identities, stem cell compartments and differentiation trajectories in long-lived naked mole-rats skin. *Aging (Albany NY)*, 14:3728-3756.
- [22] Fatima I, Chen G, Botchkareva NV, Sharov AA, Thornton D, Wilkinson HN, et al. (2022). Skin Aging in Long-Lived Naked Mole-Rats Is Accompanied by Increased Expression of Longevity-Associated and Tumor Suppressor Genes. *J Invest Dermatol*.
- [23] Grimes KM, Lindsey ML, Gelfond JA, Buffenstein R (2012). Getting to the heart of the matter: age-related changes in diastolic heart function in the longest-lived rodent, the naked mole rat. *J Gerontol A Biol Sci Med Sci*, 67:384-394.
- [24] Grimes KM, Reddy AK, Lindsey ML, Buffenstein R (2014). And the beat goes on: maintained cardiovascular function during aging in the longest-lived rodent, the naked mole-rat. *Am J Physiol Heart Circ Physiol*, 307:H284-291.
- [25] Can E, Smith M, Boukens BJ, Coronel R, Buffenstein R, Riegler J (2022). Naked mole-rats maintain cardiac function and body composition well into their fourth decade of life. *Geroscience*, 44:731-746.
- [26] Grimes KM, Barefield DY, Kumar M, McNamara JW, Weintraub ST, de Tombe PP, et al. (2017). The naked mole-rat exhibits an unusual cardiac myofilament protein profile providing new insights into heart function of this naturally subterranean rodent. *Pflugers Arch*, 469:1603-1613.
- [27] Grimes KM, Voorhees A, Chiao YA, Han HC, Lindsey ML, Buffenstein R (2014). Cardiac function of the naked mole-rat: ecophysiological responses to working underground. *Am J Physiol Heart Circ Physiol*, 306:H730-737.

- [28] Csiszar A, Labinskyy N, Orosz Z, Xiangmin Z, Buffenstein R, Ungvari Z (2007). Vascular aging in the longest-living rodent, the naked mole rat. *Am J Physiol Heart Circ Physiol*, 293:H919-927.
- [29] Labinskyy N, Csiszar A, Orosz Z, Smith K, Rivera A, Buffenstein R, et al. (2006). Comparison of endothelial function, O₂-* and H₂O₂ production, and vascular oxidative stress resistance between the longest-living rodent, the naked mole rat, and mice. *Am J Physiol Heart Circ Physiol*, 291:H2698-2704.
- [30] Peterson BL, Park TJ, Larson J (2012). Adult naked mole-rat brain retains the NMDA receptor subunit GluN2D associated with hypoxia tolerance in neonatal mammals. *Neurosci Lett*, 506:342-345.
- [31] Yamamura Y, Kawamura Y, Oiwa Y, Oka K, Onishi N, Saya H, et al. (2021). Isolation and characterization of neural stem/progenitor cells in the subventricular zone of the naked mole-rat brain. *Inflamm Regen*, 41:31.
- [32] Penz OK, Fuzik J, Kurek AB, Romanov R, Larson J, Park TJ, et al. (2015). Protracted brain development in a rodent model of extreme longevity. *Sci Rep*, 5:11592.
- [33] Ward JM, Cartoceti AN, Delaney MA (2021). Brain Lesions in Aging Zoo-Housed Naked Mole-Rats (*Heterocephalus glaber*). *Vet Pathol*, 58:142-146.
- [34] O'Connor PJ, Tomporowski PD, Dishman RK (2015). Age Moderates the Association of Aerobic Exercise with Initial Learning of an Online Task Requiring Cognitive Control. *J Int Neuropsychol Soc*, 21:802-815.
- [35] Edrey YH, Medina DX, Gaczynska M, Osmulski PA, Oddo S, Caccamo A, et al. (2013). Amyloid beta and the longest-lived rodent: the naked mole-rat as a model for natural protection from Alzheimer's disease. *Neurobiol Aging*, 34:2352-2360.
- [36] Edrey YH, Casper D, Huchon D, Mele J, Gelfond JA, Kristan DM, et al. (2012). Sustained high levels of neuregulin-1 in the longest-lived rodents; a key determinant of rodent longevity. *Aging Cell*, 11:213-222.
- [37] Triplett JC, Swomley AM, Kirk J, Grimes KM, Lewis KN, Orr ME, et al. (2016). Reaching Out to Send a Message: Proteins Associated with Neurite Outgrowth and Neurotransmission are Altered with Age in the Long-Lived Naked Mole-Rat. *Neurochem Res*, 41:1625-1634.
- [38] Carmeli-Ligati S, Shipov A, Dumont M, Holtze S, Hildebrandt T, Shahar R (2019). The structure, composition and mechanical properties of the skeleton of the naked mole-rat (*Heterocephalus glaber*). *Bone*, 128:115035.
- [39] Taguchi T, Kotelsky A, Takasugi M, Chang M, Ke Z, Betancourt M, et al. (2020). Naked mole-rats are extremely resistant to post-traumatic osteoarthritis. *Aging Cell*, 19:e13255.
- [40] Stoll EA, Karapavlovic N, Rosa H, Woodmass M, Rygiel K, White K, et al. (2016). Naked mole-rats maintain healthy skeletal muscle and Complex IV mitochondrial enzyme function into old age. *Aging (Albany NY)*, 8:3468-3485.
- [41] Bens M, Szafranski K, Holtze S, Sahm A, Groth M, Kestler HA, et al. (2018). Naked mole-rat transcriptome signatures of socially suppressed sexual maturation and links of reproduction to aging. *BMC Biol*, 16:77.
- [42] Buffenstein R, Jarvis JU (2002). The naked mole rat--a new record for the oldest living rodent. *Sci Aging Knowledge Environ*, 2002:pe7.
- [43] Grive KJ, Freiman RN (2015). The developmental origins of the mammalian ovarian reserve. *Development*, 142:2554-2563.
- [44] Hertig AT, Adams EC (1967). Studies on the human oocyte and its follicle. I. Ultrastructural and histochemical observations on the primordial follicle stage. *J Cell Biol*, 34:647-675.
- [45] Wallace WH, Kelsey TW (2010). Human ovarian reserve from conception to the menopause. *PLoS One*, 5:e8772.
- [46] Findlay JK, Hutt KJ, Hickey M, Anderson RA (2015). What is the "ovarian reserve"? *Fertil Steril*, 103:628-630.
- [47] Ensrud KE, Ewing SK, Taylor BC, Fink HA, Stone KL, Cauley JA, et al. (2007). Frailty and risk of falls, fracture, and mortality in older women: the study of osteoporotic fractures. *J Gerontol A Biol Sci Med Sci*, 62:744-751.
- [48] Place NJ, Prado AM, Faykoo-Martinez M, Briño-Enriquez MA, Albertini DF, Holmes MM (2021). Germ cell nests in adult ovaries and an unusually large ovarian reserve in the naked mole-rat. *Reproduction*, 161:89-98.
- [49] Jacob F (1977). Evolution and tinkering. *Science*, 196:1161-1166.
- [50] Kim EB, Fang X, Fushan AA, Huang Z, Lobanov AV, Han L, et al. (2011). Genome sequencing reveals insights into physiology and longevity of the naked mole rat. *Nature*, 479:223-227.
- [51] Pamenter ME, Dzal YA, Thompson WA, Milsom WK (2019). Do naked mole rats accumulate a metabolic acidosis or an oxygen debt in severe hypoxia? *J Exp Biol*, 222.
- [52] Lee BP, Smith M, Buffenstein R, Harries LW (2020). Negligible senescence in naked mole rats may be a consequence of well-maintained splicing regulation. *Geroscience*, 42:633-651.
- [53] Lambert MJ, Portfors CV (2017). Adaptive sequence convergence of the tumor suppressor ADAMTS9 between small-bodied mammals displaying exceptional longevity. *Aging (Albany NY)*, 9:573-582.
- [54] Liang S, Mele J, Wu Y, Buffenstein R, Hornsby PJ (2010). Resistance to experimental tumorigenesis in cells of a long-lived mammal, the naked mole-rat (*Heterocephalus glaber*). *Aging Cell*, 9:626-635.
- [55] Hulbert AJ, Pamplona R, Buffenstein R, Buttemer WA (2007). Life and death: metabolic rate, membrane composition, and life span of animals. *Physiol Rev*, 87:1175-1213.
- [56] Kim EB, Fang X, Fushan AA, Huang Z, Lobanov AV (2011). Genome sequencing reveals insights into physiology and longevity of the naked mole rat. *Nature* 479:223-227.

- [57] Schumacher B, Pothof J, Vijg J, Hoeijmakers JHJ (2021). The central role of DNA damage in the ageing process. *Nature*, 592:695-703.
- [58] Lewis KN, Wason E, Edrey YH, Kristan DM, Nevo E, Buffenstein R (2015). Regulation of Nrf2 signaling and longevity in naturally long-lived rodents. *Proc Natl Acad Sci U S A*, 112:3722-3727.
- [59] Sahm A, Bens M, Szafranski K, Holtze S, Groth M, Görlach M, et al. (2018). Long-lived rodents reveal signatures of positive selection in genes associated with lifespan. *PLoS Genet*, 14:e1007272.
- [60] MacRae SL, Zhang Q, Lemetre C, Seim I, Calder RB, Hoeijmakers J, et al. (2015). Comparative analysis of genome maintenance genes in naked mole rat, mouse, and human. *Aging Cell*, 14:288-291.
- [61] Zhao Y, Tyshkovskiy A, Muñoz-Espín D, Tian X, Serrano M, de Magalhaes JP, et al. (2018). Naked mole rats can undergo developmental, oncogene-induced and DNA damage-induced cellular senescence. *Proc Natl Acad Sci U S A*, 115:1801-1806.
- [62] Jiang JJ, Kong QP (2020). Comparative analysis of long noncoding RNAs in long-lived mammals provides insights into natural cancer-resistance. *RNA Biol*, 17:1657-1665.
- [63] Kerepesi C, Meer MV, Abulaeva J, Amoroso VG, Lee SG, Zhang B, et al. (2022). Epigenetic aging of the demographically non-aging naked mole-rat. *Nat Commun*, 13:355.
- [64] Gomes NM, Ryder OA, Houck ML, Charter SJ, Walker W, Forsyth NR, et al. (2011). Comparative biology of mammalian telomeres: hypotheses on ancestral states and the roles of telomeres in longevity determination. *Aging Cell*, 10:761-768.
- [65] Leonida SRL, Bennett NC, Leitch AR, Faulkes CG (2020). Patterns of telomere length with age in African mole-rats: New insights from quantitative fluorescence in situ hybridisation (qFISH). *PeerJ*, 8:e10498.
- [66] Adwan Shekhidem H, Sharvit L, Leman E, Manov I, Roichman A, Holtze S, et al. (2019). Telomeres and Longevity: A Cause or an Effect? *Int J Mol Sci*, 20.
- [67] Augereau A, Mariotti M, Pousse M, Filipponi D, Libert F, Beck B, et al. (2021). Naked mole rat TRF1 safeguards glycolytic capacity and telomere replication under low oxygen. *Sci Adv*, 7.
- [68] Chiti F, Dobson CM (2017). Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade. *Annu Rev Biochem*, 86:27-68.
- [69] Pride H, Yu Z, Sunchu B, Mochnick J, Coles A, Zhang Y, et al. (2015). Long-lived species have improved proteostasis compared to phylogenetically-related shorter-lived species. *Biochem Biophys Res Commun*, 457:669-675.
- [70] Azpurua J, Ke Z, Chen IX, Zhang Q, Ermolenko DN, Zhang ZD, et al. (2013). Naked mole-rat has increased translational fidelity compared with the mouse, as well as a unique 28S ribosomal RNA cleavage. *Proc Natl Acad Sci U S A*, 110:17350-17355.
- [71] Rodriguez KA, Valentine JM, Kramer DA, Gelfond JA, Kristan DM, Nevo E, et al. (2016). Determinants of rodent longevity in the chaperone-protein degradation network. *Cell Stress Chaperones*, 21:453-466.
- [72] Swovick K, Firsanov D, Welle KA, Hryhorenko JR, Wise JP, Sr., George C, et al. (2021). Interspecies Differences in Proteome Turnover Kinetics Are Correlated With Life Spans and Energetic Demands. *Mol Cell Proteomics*, 20:100041.
- [73] Rodriguez KA, Osmulski PA, Pierce A, Weintraub ST, Gaczynska M, Buffenstein R (2014). A cytosolic protein factor from the naked mole-rat activates proteasomes of other species and protects these from inhibition. *Biochim Biophys Acta*, 1842:2060-2072.
- [74] Kim J, Chee WY, Yabuta N, Kajiwarra K, Nada S, Okada M (2020). Atg5-mediated autophagy controls apoptosis/anoikis via p53/Rb pathway in naked mole-rat fibroblasts. *Biochem Biophys Res Commun*, 528:146-153.
- [75] Kim D, Langmead B, Salzberg SL (2015). HISAT: a fast spliced aligner with low memory requirements. *Nat Methods*, 12:357-360.
- [76] O'Connor TP, Lee A, Jarvis JU, Buffenstein R (2002). Prolonged longevity in naked mole-rats: age-related changes in metabolism, body composition and gastrointestinal function. *Comp Biochem Physiol A Mol Integr Physiol*, 133:835-842.
- [77] Frenkel-Denkberg G, Gershon D, Levy AP (1999). The function of hypoxia-inducible factor 1 (HIF-1) is impaired in senescent mice. *FEBS Letters*, 462:341-344.
- [78] Debebe T, Biagi E, Soverini M, Holtze S, Hildebrandt TB, Birkemeyer C, et al. (2017). Unraveling the gut microbiome of the long-lived naked mole-rat. *Sci Rep*, 7:9590.
- [79] Viltard M, Durand S, Pérez-Lanzón M, Aprahamian F, Lefevre D, Leroy C, et al. (2019). The metabolomic signature of extreme longevity: naked mole rats versus mice. *Aging (Albany NY)*, 11:4783-4800.
- [80] Chee WY, Kurahashi Y, Kim J, Miura K, Okuzaki D, Ishitani T, et al. (2021). β -catenin-promoted cholesterol metabolism protects against cellular senescence in naked mole-rat cells. *Commun Biol*, 4:357.
- [81] Andziak B, O'Connor TP, Buffenstein R (2005). Antioxidants do not explain the disparate longevity between mice and the longest-living rodent, the naked mole-rat. *Mech Ageing Dev*, 126:1206-1212.
- [82] Barja G, Cadenas S, Rojas C, Pérez-Campo R, López-Torres M (1994). Low mitochondrial free radical production per unit O₂ consumption can explain the simultaneous presence of high longevity and high aerobic metabolic rate in birds. *Free Radic Res*, 21:317-327.
- [83] Andziak B, O'Connor TP, Qi W, DeWaal EM, Pierce A, Chaudhuri AR, et al. (2006). High oxidative damage levels in the longest-living rodent, the naked mole-rat. *Aging Cell*, 5:463-471.