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Not just a cousin of the naked mole-rat: Damaraland mole-rats offer unique insights into biomedicine

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ABSTRACT

Evolutionary medicine has been a fast-growing field of biological research in the past decade. One of the strengths of evolutionary medicine is to use non-traditional model organisms which often exhibit unusual characteristics shaped by natural selection. Studying these unusual traits could provide valuable insight to understand biomedical questions, since natural selection likely discovers solutions to those complex biological problems. Because of many unusual traits, the naked mole-rat (NMR) has attracted attention from different research areas such as aging, cancer, and hypoxia- and hypercapnia-related disorders. However, such uniqueness of NMR physiology may sometimes make the translational study to human research difficult. Damaraland mole-rat (DMR) shares multiple characteristics in common with NMR, but shows higher degree of similarity with human in some aspects of their physiology. Research on DMR could therefore offer alternative insights and might bridge the gap between experimental findings from NMR to human biomedical research. In this review, we discuss studies of DMR as an extension of the current set of model organisms to help better understand different aspects of human biology and disease. We hope to encourage researchers to consider studying DMR together with NMR. By studying these two similar but evolutionarily distinct species, we can harvest the power of convergent evolution and avoid the potential biased conclusions based on life-history of a single species.

1. Introduction

Evolutionary medicine is a set of concepts and approaches first considered by Williams & Nesse (Williams and Nesse, 1991) about three decades ago. The essence of evolutionary medicine is to attempt to understand health and disease in the context of natural selection and to apply the principles and tools of evolutionary biology to help our understanding of human health and disease (Stearns, 2012). Using these concepts and approaches, evolutionary medicine has contributed to a better understanding of topics in human health including reproductive health (Ellison, 2003), mental illness (Keller and Nesse, 2006; Nesse, 1999, 2004), immune function and inflammation (Cooper and Herrin, 2010; Litman and Cooper, 2007; Straub, 2012), cancer (Greaves, 2010; Merlo et al., 2006), aging (Austad, 2009; Miller et al., 2011; Stearns et al., 2000), nutrition, and exercise (Chakravarthy and Booth, 2004; Eaton et al., 1999; Leonard, 2008).

One of the promises of evolutionary medicine is to use non-traditional model organisms, which could be more suitable for a particular health or disease question (Maher, 2009). Traditional animal models, which are simple, fast-growing, and tractable model systems (such as worms, flies, zebra fish, and laboratory mice and rats), allow the study of normal and pathological processes in a controlled and systematic way to investigate specific hypothesis or test the effects of drugs and genes that, can be turned on and off with genetic approaches (Magalhães, 2015; O'Connor et al., 2002). Research using model organisms has generated useful knowledge and significant breakthroughs regarding the mechanisms that contribute to different human diseases. However, these model organisms have been primarily chosen for convenience, rather than for specific features pertinent to human health and diseases (Buffenstein, 2005). This resulted in caveats which limit the information obtained from model organisms that could be translated to humans. For example, model organisms are typically short-lived and lack genetic diversity (Gasch et al., 2016). Using these model organisms in aging and/or oncology research could be insufficient because of the confounding biological difference between short-lived model organisms and long-lived human. As a result, more and more non-traditional model

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organisms have been employed to study specific human health and disease questions (Bolker, 2012). One such group of animals are the African mole-rats.

African mole-rats, are burrowing rodents of the family *Bathyergidae* and *Heterocephalidae* (Bennett and Faulkes, 2000). These mole-rat species represent a distinct evolution of subterranean lifestyles and have attracted the attention of biogerontologists due to their generally high longevity (Buffenstein, 2005; Buffenstein et al., 2021). Even though *Bathyergidae* and *Heterocephalidae* contain more than 20 species, our understanding of these mole-rats is heavily biased towards the naked mole-rat (NMR), *Heterocephalus glaber* (Faulkes et al., 2004). NMR have been intensively studied in a wide range of biomedical research because of their extreme characteristics such as long lifespan, cancer resistance, hypoxia- and hypercapnia-resistance, oxidative stress tolerance and high reproductive outputs for breeders (Buffenstein et al., 2021). Unfortunately, the unique physiology of naked mole-rats might make it difficult for translation into human biomedical research. For example, unlike other mammals including human, NMR is defined as heterothermic endotherms where their body temperature was the lowest in mammals (Buffenstein and Yahav, 1991). NMRs also exhibit extreme changes in the opioid system compared to other mammals (Park et al., 2003; Smith et al., 2015; St John Smith et al., 2012). Additionally, NMR have fewer C-fibers than laboratory rodents and other mole-rat species and do not produce the pain-relaying neuropeptides substance P and calcitonin gene-related peptide (Park et al., 2003; St John Smith et al., 2012). Moreover, mutations in Nav1.7 protein in NMR allow the organism to tolerate against acid or capsaicin in injested food and surrounding environments, which may offer advantages in food selection and survival in subterranean habitat (Smith et al., 2020). These unique features of NMR make it challenging to reconcile the discrepancy between the physiological and pathological responses in human and NMR. In this regard, other African mole-rat species, such as the Damaraland mole-rat (DMR; *Fukomys damarensis*; Fig. 1), who share many similar characteristics with NMR, could provide alternative explanations and might bridge NMR research to human biomedical research (Smith et al., 2015).

Just like NMR, DMR is a fully fossorial rodent living under eusocial structure (Faulkes and Bennett, 2016). It is important to note here that eusociality was evolved independently in NMR and DMR (Fig. 1). DMR reside in an arid habitat with unpredictable rainfall in southern Africa. Much of their distribution is characterized by red Kalahari arenosols, but they can also be found in a wide range of coarse sandy soils (Bennett and Jarvis, 2004; Faulkes and Bennett, 2016). DMR and NMR diverged ~26 million years ago (Fang et al., 2014) and exhibit similar unusual adaptations due to their comparable habitats and social structure (Fig. 1). The divergences between DMR and NMR could make DMR an alternative system to better understand the biology of some human diseases or conditions (Fang et al., 2014; Faulkes and Bennett, 2016; Smith et al., 2015). For instance, similar to humans, DMR is a homeothermic mammal that tightly controls its body temperature at a slightly lower temperature of 35 °C (Buffenstein and Yahav, 1991; Hislop and Buffenstein, 1994). Under hypoxia, DMR's responses resemble that of a non-fossorial species (including human), in that a ventilatory response was mounted and no metabolic depression was observed (Zhang and Pamenter, 2019a). Therefore, as a distant cousin of NMR, DMR exists as a system to investigate the adaptations to extreme environmental conditions while maintaining physiology similar to other mammalian species and humans. On the other hand, studying these two similar but also very different species together would allow us to harvest the power of convergent evolution to better understand the effects of natural selection and avoid the potential bias of conclusions based on life-history of a single species (Sackton and Clark, 2019). Studying DMR could also help us connect the knowledge between NMR and human physiology, and therefore enhancing the translatability of the scientific findings from NMR physiology and human biomedical research. The use of NMR in biomedical research has been intensively reviewed (Buffenstein et al., 2021), however, to the best of our knowledge, such a review is still lacking for DMR. In this review, we discussed the use of DMR as an extension of our current set of model organisms to help us better understand human biology, particularly, in aging, hypoxic/hypercapnic response as well as the neuroendocrine control of mammalian reproduction (Fig. 2).

1.1. Aging research – Insights on the rate of living theory, free radical theory of aging, DNA damage theory of aging and epigenetic oxidative redox shift theory of aging

Subterranean rodents have very long lifespans for their body weight, among which, fully fossorial DMR is one of the representatives (Edrey et al., 2011). The longest-lived DMR survived for more than 20 years in a laboratory setting (Dammann and Burda, 2007). DMR demonstrate a similar longevity quotient to that of humans and therefore present excellent models to study the biology of aging and age-related diseases (Dammann and Burda, 2007; Edrey et al., 2011).

Physiological research of DMR was pioneered on investigating their metabolic rates by Dr. Barry Lovegrove more than 30 years ago (Lovegrove, 1989; Lovegrove, 1986). These studies have documented that the resting metabolic rate was 43% lower than size-matched rodents, and 29% lower than other size-matched subterranean rodents. Together with other African mole-rat species, the fossorial rodents have low basal

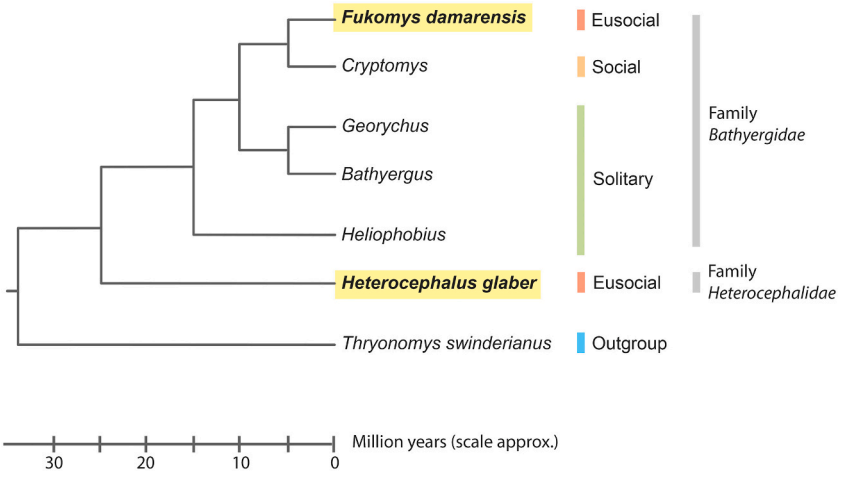

Fig. 1. Phylogeny for the *Bathyergidae* and the *Heterocephalidae*, together with a close outgroup (the cane rat *Thryonomys swinderianus*). Adapted from (Fang et al., 2014; Faulkes and Bennett, 2016; Ivy et al., 2020).

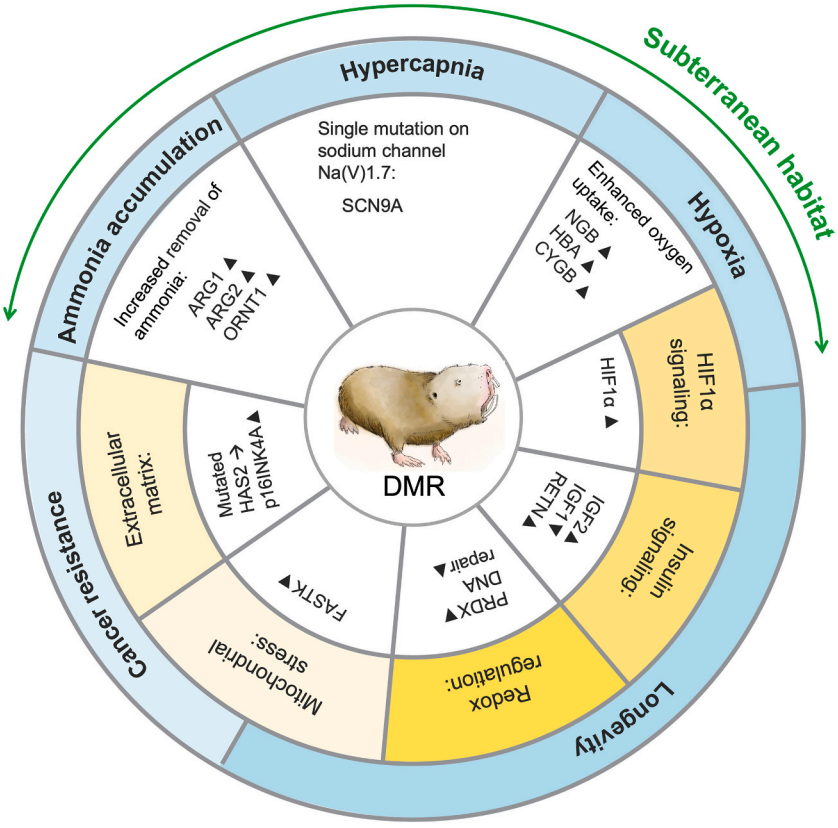

Fig. 2. Areas of interests in biomedical research in Damaraland mole-rat.

metabolic rates (BMR) compared to those expected for their size (McNab, 1966). These results support the Rate-of-Living theory which postulates that the slower an organism's metabolism, the longer its lifespan (Rubner, 1908). However, challenges to the Rate-of-Living theory indicated that BMR might not be a valid marker for total daily energy expenditure (DEE), where the ratio of BMR to total daily energy expenditure can vary between 1.6 and 8.0 among mammals (Speakman et al., 2002). Using doubly labelled water, DEE and sustained metabolic scope (the ratio of daily energy expenditure to basal metabolic rate) was measured, and metabolic scopes were lower for DMR (around 2) compared with other rodents (about 4.1) (Karasov, 1992; Scantlebury et al., 2006). More interesting, BMR does not vary among young, middle age, and old DMRs where BMR typically decreases with age for other laboratory rodents and humans (O'Connor et al., 2002; Yap et al., 2022). Besides lower but constant BMR at the organismal level, a recent study from our labs also demonstrated that DMR primary lung fibroblasts depended on glycolysis rather than oxidative phorsphorlation (OXPHOS) for ATP production in cells, and low basal proton conductance through mitochondria inner membrane in isolated lung mitochondria (Yap et al., 2022). These unique bioenergetic properties might result from adaptation to hypoxic environments since hypoxia-inducible factor 1 alpha subunit (HIF-1α) was upregulated (Yap et al., 2022). These metabolic adaptations and upregulated HIF-1α pathways, on the other hand, might contribute to their extreme longevity.

As inspired by the Free Radical Theory of Aging (Harman, 1972), the oxidative status of DMR have been studied to investigate the relationship between redox homeostasis and longevity (Labinskyy et al., 2006; Lambert et al., 2007). In a study conducted using isolated mitochondria, there was a lower capacity of superoxide and/or hydrogen peroxide production by mitochondrial Complex I during reverse electron transport in DMR compared to short-lived rodents (Lambert et al., 2007). The authors also reported a highly significant correlation between maximum lifespan and the capacity of mitochondrial Complex I H_2O_2 production during reverse electron transport in species of different lifespan (Lambert et al., 2007). These findings seem to echo the notion that aging is caused by accumulation of damage inflicted by reactive oxygen species (ROS). However, it is important to note that the capacity of ROS production measured from isolated mitochondria might not represent physiologically relevant mitochondrial ROS production in cells, tissues or *in vivo* (Zhang and Wong, 2021). In fact, low levels of ROS production in isolated mitochondria did not transfer to lower oxidative damage in tissues even when the antioxidant capacities were similar when compared to short-lived rodents (Lewis et al., 2013). Moreover, levels of antioxidant markers were similar between breeders and non-breeders, but both reduced (Schmidt et al., 2014) and constant (Jacobs et al., 2021) levels of oxidative damage makers had been shown for breeders in DMR. Interestingly, when experimentally increased cooperative contributions in breeding DMRs, oxidative stress was increased, without changing body condition (Mendonça et al., 2020). Consequently, it has been proposed that these long-lived mole-rats can tolerate high oxidative damage, maintaining somatic tissue function, without affecting their healthspan and lifespan (Lewis et al., 2013).

One of the mechanisms for tolerating high oxidative damage focused on resistance to DNA fragmentation and DNA repair pathways. In an *ex vivo* model of oxidative stress-induced vascular aging, epithelial and smooth muscle cells of DMR carotid artery were found to be resistant to H_2O_2-induced DNA fragmentation and caspase activation (Labinskyy et al., 2006). The study also demonstrated using NMRs, DMRs, guinea pigs and mice a negative correlation between the extent of oxidant-induced DNA fragmentation and maximal lifespan potential (Labinskyy et al., 2006). These findings suggest a more efficient DNA repair mechanism in DMR in response to oxidative stress compared to short-lived rodents. The notion of an improved DNA repair mechanism was supported by transcriptomic analysis of DMR (Fang et al., 2014). The transcriptome of DMR liver revealed differential expression and enrichment of several genes associated with oxidoreduction. Despite

downregulated expression (peroxiredoxins) and activity (glutathione peroxidases) of antioxidant enzymes, several genes associated with DNA damage repair and responses to stress also exhibited higher expression in DMR even during normoxia, suggesting improved DNA repair is an intrinsic mechanism of adaptation to an underground environment (Fang et al., 2014).

Besides DNA repair pathways, genomic and transcriptomic analyses also revealed an inactivation of Fas-activated serine/threonine kinase (FASTK) in the livers of DMR compared with short-lived rodents (Fang et al., 2014). FASTK serves as a regulator of Fas-mediated apoptosis and is located at the inner mitochondrial membrane (Jourdain et al., 2017). While FASTK has an important role in governing cell survival, its overexpression has been demonstrated to be involved in age-related pathological conditions such as tumorigenesis and immune-mediated inflammatory diseases (Simarro et al., 2010; Zhi et al., 2013). The role of FASTK in aging was corroborated by its effect on neuron elongation and regeneration. The ability of neurons to regenerate and their rate of elongation decrease with age whereas a statistically significant improvement of such was observed in FASTK null subjects (Loh et al., 2008). Therefore, the beneficial effect of FASTK knockdown on neuronal development may in part explain the outstanding longevity of DMR, particularly the integrity of neurological functions of DMR.

The longevity of DMR may also attribute to their distinct endocrine regulation of glucose homeostasis. DMR, like other mole-rats, harbor a divergent insulin β-chain sequence (Chan et al., 1984; Kramer and Buffenstein, 2004). Mutations in insulin β-chain are known to cause misfolded insulin and reduced insulin signaling in humans. The mutation Arg22Gln was found in DMR insulin and was reported to be associated with insulin misfolding (Liu et al., 2010). The alteration in insulin structure was also found to be associated with a decreased insulin growth factors 1 (IGF1)/growth hormone signaling (Fang et al., 2014). As an alternative mechanism, DMR expresses insulin growth factor 2 (IGF2) and insulin growth factor 2 binding protein and the autocrine/paracrine actions of IGF2 in the liver substitutes for insulin for glucose handling in DMR (Edrey et al., 2011; Fang et al., 2014). In humans, a reduced insulin level, an improved insulin sensitivity and an inhibition of the growth hormone/IGF1 axis are often considered to provide beneficial effects on longevity, as was the case, for example, in caloric restriction (Bartke et al., 2003; Janssen and Lamberts, 2004; Tatar et al., 2003; Yamaza et al., 2002). It is therefore hypothesized that the outstanding longevity of DMR, may in part be accredited to the divergence in insulin signaling.

1.2. Cancer resistance – the significant role of very high molecular mass hyaluronan in tumorigenesis

Consistent with other African mole-rats, the longevity of DMR also comes along with a rare incidence of cancers. This may partly be explained by the aforementioned inactivation of FASTK since its overexpression is strongly associated with tumor formation (Zhi et al., 2013). Another explanation for cancer resistance is related to the enzyme hyaluronan synthase 2 (Fang et al., 2014; Tian et al., 2013). Hyaluronan is an extracellular matrix polysaccharide that serves as an extracellular signal for early contact inhibition (Tian et al., 2013). A single amino acid substitution in a highly conserved region of hyaluronan synthase 2 in DMR leads to the synthesis of very high molecular mass hyaluronan which may confer cancer resistance by sensitizing DMR cells tocontact inhibition and triggering the downstream induction of the tumor suppressor P16INK4A (Fang et al., 2014; Tian et al., 2013). The discovery of anti-tumorigenesis activity of very high molecular mass hyaluronan has been a breakthrough in oncology research and opens up new avenues for anti-cancer therapies (Fisher, 2015; Gorbunova et al., 2020).

1.3. Hypoxia, hypercapnia, and ammonia metabolism – enhanced efficiencies of oxygen uptake, bicarbonate buffering and ammonia metabolism

Similar to NMR, DMR are also fully fossorial and live in densely populated, poorly ventilated underground burrows, within which they likely experience intermittent periods of hypoxia and hypercapnia (Faulkes and Bennett, 2016; Zhang and Pamenter, 2019a). Even though both species face similar hypoxic environments, the physiological responses to hypoxia are divergent. For NMR, hypoxia tolerance is achieved by depressions in metabolic rate, body temperature, and activity level with little change in ventilation. This hypoxia metabolic response is a hallmark of hypoxia adaptation and has been observed in most hypoxia-tolerance species. But DMR employed different strategy which resembles that of a non-fossorial species (including human). Upon exposure to acute and progressive hypoxia, DMR demonstrate a significant increase in ventilation and an absence of hypoxic metabolic response (Zhang and Pamenter, 2019a). The observation was consistent with most adult mammals of which the initial physiological response to low O_2 environments is a reflex increase in minute ventilation (Zhang and Pamenter, 2019a). This lack of hypoxic metabolic response but instead reliance on a robust hypoxic ventilation in DMR are similar to some other fossorial species, such as Middle East blind mole-rat (*Spalax ehrenbergi*). With chronic hypoxia, DMR demonstrated a further increase in ventilation upon ventilatory acclimatization to hypoxia compared to normoxia-acclimated animals (Zhang and Pamenter, 2019a). This observation indicates the occurrence of neuroplasticity within the ventilatory control circuits in DMR during prolonged exposure to hypoxia (Zhang and Pamenter, 2019a).

To better survive in a hypoxic environment, another important adaptation is an improved oxygen uptake to high oxygen-demanding tissues. The transcriptome and proteome of DMR revealed enhanced expression of proteins of the globin family (Fang et al., 2014). These proteins are responsible for the delivery and storage of oxygen in cells and tissues (Vinogradov and Moens, 2008). Under both normoxic and hypoxic conditions, there were higher protein expression of hemoglobin α and neuroglobin in the brains of DMR when compared to above-ground rodents (Fang et al., 2014). There was also a trend of elevated mRNA expression of cytoglobin, a member of the globin family that facilitates diffusion of oxygen through tissues, scavenges nitric oxide or reactive oxygen species to protect against oxidative stress in the brains of DMR (Fang et al., 2011, 2014; Hodges et al., 2008; Li et al., 2007; Reuss et al., 2016; Singh et al., 2014; Xu et al., 2006). These diverse physiological responses highlight the wide variety of adaptations that can mitigate systemic hypoxia. Importantly, the primary criterion of hypoxia tolerance is not the manifestation of physiological responses to a hypoxic stimuli, but rather the ability to successfully match metabolic demand to metabolic supply when oxygen is limited (Buck and Pamenter, 2006). Therefore, the true measure of hypoxia tolerance is the ability to tolerate prolonged and/or severe hypoxic exposure without detriment. Such concept provides a potential novel route for human studies in hypoxia condition, where increase hypoxia tolerance in organisms should target matching metabolic supply to metabolic demand regardless of physiological responses.

The underground burrows of DMRs present a gaseous environment range from normocapnic (0.1% CO_2) to hypercapnic (6% CO_2) conditions (Faulkes and Bennett, 2016; Zhang and Pamenter, 2019b). Surprisingly, unlike most burrow dwelling animals which exhibit delayed or blunted hypercapnic responses as an adaptation to reduce the energetic costs associated with hyperpnoea in hypercapnia (Barros et al., 2004; Boggs et al., 1984), the response of DMR to hypercapnia mirrors that of above-ground rodents. It was found that DMRs underwent hyperventilation along with the lack of change in metabolic rate during hypercapnia. The respiratory exchange ratio was also unchanged in hypercapnia suggesting DMRs do not adjust their metabolic fuel source in acute hypercapnia (Zhang and Pamenter, 2019b). Elevated CO_2 in the

body may result in respiratory and/or metabolic acidosis. When dissolved in blood, CO_2 dissociates into equilibrium between carbonic acid and bicarbonate, resulting in increased acidity (Branigan et al., 2018; Heinemann and Goldring, 1974). Dissolution of inhaled CO_2 in fluids of the upper respiratory tract and nasal mucosa also triggers painful, burning sensations that lead to behavioral avoidance (Brand et al., 2010). Despite the lack of metabolic adaptation, DMR were found to exhibit no sign of behavioral avoidance as manifested by no differences in movement velocity, distance travelled, spatial exploration, or body temperature at any level of environmental hypercapnia (Branigan et al., 2018). This ability of DMR is mediated by bicarbonate buffering at the level of the kidney or within the blood as acetazolamide, an inhibitor of carbonic anhydrase that potentiates whole animal acidosis, was able to sensitize DMR to hypercapnia-mediated behavioral avoidance (Branigan et al., 2018). Applications of such inhibitor on whole animal acidosis should be studied in other organisms including human.

The build up of CO_2 also evokes acid-induced pain. A negatively charged motif in the sodium channel $Na_v1.7$ protein (SCN9A), which is highly expressed in nociceptor neurons, prevents acid-induced pain signaling to the NMR brain (Smith et al., 2011). Such negatively charged motif is also present in the DMR, as well as other family *Bathyergidae* species (Fang et al., 2014; Smith et al., 2011). Interestingly, even though the negatively charge constellations [(−)(+)(−)] of the motif are the same between NMR and DMR, the exact mutation in $Na_v1.7$ protein are different, with Glu-Lys-Asp (EDK) in DMR compared to Glu-Lys-Glu (EDE) in NMR (Eigenbrod et al., 2019; Fang et al., 2014). Moreover, a closer look at the $Na_v1.7$ sequences revealed that domain III also carries two negative charges in NMR, but not in DMR (Eigenbrod et al., 2019). As a matter of fact, when screening through eight African rodent species, DMR showed robust pain behaviors to painful substances capsaicin, acid (HCl, pH 3.5), and allyl isothiocyanate, similar to laboratory rodents and humans; Whereas NMR showed no behavioral response to capsaicin and acid (Eigenbrod et al., 2019). Other than differences in $Na_v1.7$ protein, NMR also has fewer C-fibers (small unmyelinated axons associated with slow pain signaling) than other rodents, including the DMR. Both of these mechanisms have been exploited as targets for new pain therapies in human (Kingwell, 2019; Lynn, 1990). Consequently, these evidence serve as good examples that studies on DMR and other mole-rat species could bridge NMR research to human biomedical studies.

Living in densely populated underground burrows, the accumulation of ammonia which gives rise to the potent irritants, nitrogen and methane (Burda et al., 2007; LaVinka et al., 2009), presents a significant challenge for DMR survival. To cope with this, DMRs have evolved a divergent arginase 1, an enzyme that catalyzes the final step of the hepatic urea cycle, to enhance the efficiency of ammonia removal (Jenkinson et al., 1996). The single replacement of leucine/tyrosine residue by histidine at position 254 improves ammonia removal efficiency of arginase 1 by strengthening the assembly of arginase 1 homotrimer (Dowling et al., 2008; Fang et al., 2014; Lavulo et al., 2001; Sabio et al., 2001). Moreover, mitochondrial ornithine transporter (SLC25A15), another critical enzyme of the urea cycle, was also found to be expressed at high level in the livers of DMR (Fang et al., 2014). All together, these findings suggest enhanced ammonia detoxification of DMR as an adaptation to their natural habitat.

1.4. Neuroendocrine regulation – the control of reproduction in response to environmental changes

Being one of the two widely accepted eusocial mammals, the physiological and behavioral regulations of reproduction in DMR have been extensively studied. DMR exhibit a strong skew in lifetime reproductive success, with breeding restricted to a single female and one or two males (Clarke et al., 2001; Cooney and Bennett, 2000; Faulkes and Bennett, 2016). Non-breeding NMRs of both sexes are physiologically inhibited from reproducing, while in DMRs only the non-breeding females are physiologically suppressed (Bennett et al., 2018). Even under reproductive suppression, non-breeding members of the colony possess intact reproductive machineries and were shown to be able to switch from reproductively quiescent to reproductively active once the breeding female has died and a new, unrelated, individual becomes available or the fragmentation of the colony occurs (Faulkes et al., 1994; Molteno and Bennett, 2000; Snyman et al., 2006). For NMR, eusociality might be achieved by elevated circulating prolactin levels in non-breeders, but this is not the case for DMR (Bennett et al., 2018). In fact, the ovaries of female non-breeding DMRs have varying levels of follicular development, although they do not ovulate. Consequently, the reproductive suppression of non-breeding DMR is largely influenced by a self-restraint mechanism in order to minimize inbreeding and prevent incest until an unrelated male is present (Bennett, 1996; Clarke et al., 2001).

The underlying mechanisms for reproductive suppression of DMR might happen locally at tissue level. Inhibition of hypothalamo-pituitary-gonadal axis has been highlighted to be responsible for the neuroendocrine changes in DMR females (Bennett, 1996; Molteno and Bennett, 2000; Voigt et al., 2014; Voigt and Bennett, 2019; Voigt and Bennett, 2017). In DMR, the suppression of reproduction is achieved by the inhibition of ovulation, which is a consequence of a reduced level of gonadotropin-releasing hormone (GnRH)-induced luteinizing hormone from the pituitary (Bennett, 1996; Molteno and Bennett, 2000; Voigt et al., 2014). While the GnRH perikarya were found to be structurally and functionally intact in non-breeding DMR females, differential expression of GnRH was observed in different brain regions of non-breeding or breeding DMR females (Molteno and Bennett, 2000). Intriguingly, *in situ* hybridization revealed the elevated expression of GnRH mRNA along the rostral preoptic region of DMR breeding females compared to non-breeding females whereas the latter had increased GnRH mRNA level at the caudal level of the anterior hypothalamus (Voigt and Bennett, 2017). The delicate regulation of GnRH expression in specific neuron subpopulations is mediated by the region-specific expressions of neuropeptides such as dynorphin, neurokinin B, kisspeptin and RFamide-related peptide-3, and is considered as the key mechanism governing the switch between reproductive statuses (Voigt and Bennett, 2019).

1.5. Hearing – the importance of the precise arrangement/orientation of hair bundles in hearing

Subterranean rodent families show elevated auditory thresholds and restricted frequency ranges of hearing compared with other surface-dwelling rodents (Gerhardt et al., 2017; Heffner and Heffner, 1993; Heffner and Heffner, 1992; Okanoya et al., 2018; Pyott et al., 2020). DMRs hear within a narrow frequency range, between 125 cHz and 4 kHz, with the best hearing at around 1 kHz. Even at their best frequencies, audiograms indicate thresholds are elevated by 25 to 35 dB in DMR compared with above-ground rodents (Barone et al., 2019). Previous studies attempted to relate the elevated auditory threshold of DMR to the lack of pinnae which is known to be a feature that contributes to the poor sound localization (Heffner and Heffner, 1993). However, pinnae in rodents were shown to be responsible for acoustical gain at frequencies above 8 kHz, therefore the missing pinnae could not account for the elevated auditory thresholds at low frequency (Koka et al., 2011; Lauer et al., 2011). Furthermore, in NMR findings from microcomputed tomography also suggest that the gross dimensions of the middle and inner ear may not be sufficient to explain altered hearing (Mason et al., 2016). Based on well-curated hearing genotype-phenotype database, amino acid substitutions that matches pathogenic mutations in humans in the hair bundle link proteins were identified in DMR. These substitutions are consistent with abnormal hair bundle morphology observed by scanning electron microscopy and reduced cochlear amplification measured *in vivo* in DMR (Pyott et al., 2020). Missing and disorganized hair bundles from outer hair cells in the apical and apical/middle turns were observed in DMR (Pyott et al., 2020). Precise

arrangement and orientation of hair bundles across the sensory epithelium are critical for hearing as they allows efficient deflection of the OHC hair bundles, depolarization of the OHCs and prestin activation (McPherson, 2018). The impaired hearing ability of DMR is largely attributed to the disrupted hair bundles in the outer hair cells. The impaired hearing ability of DMR is largely attributed to the disrupted hair bundles in the outer hair cells, of which is consistent with many hearing pathologies in human. Currently, chinchillas, rabbits, paca, rats and mice of various genetic variations are the main animal models used to understand the biology of human auditory system (Chatterjee and Lufkin, 2011; Reis et al., 2017; Salvi et al., 2021). Identify gene mutations in DMR would help to understand physiological mechanisms associated with hearing, then further understand human hearing pathologies.

Recently, it has been shown that NMR has a colony-specific greeting (dialects) that is modulated by the "queen" of the colony and learned by other members of the colony in early life (Barker et al., 2021). Such vocal dialect facilitates recognition of colony members and thereby helps maintain colony cohesiveness (Buffenstein, 2021). DMR is another highly social, yet xenophobic, animals which can also make the birdlike chirps similar to NMR (Jacobs et al., 1998). It is still an open question on whether other eusocial mammals use similar strategies to maintain their highly organized social structure (Leedale et al., 2021).

1.6. Conclusion and future directions

Here we discussed some interesting aspects of the physiology of DMR as adaptations to their unique habitat and specific social structure, all of which provide valuable information and insights to the current biomedical research (Fig. 2). In the field of aging research, the use of NMR and to a lesser extent DMR has become popular due to the longevity of these mole-rat species. With extended human life expectancy, an unprecedented number of resources have been dedicated to aging research to promote health span by understanding the biology of age-related diseases. Based on the current use of DMR, several signaling pathways, as listed above, have been highlighted to be heavily involved in and may serve as therapeutic targets to slow down our aging process. This is also observed inoncology research in which the anti-tumorigenic molecule, very high molecular weight hyaluronan, was first identified to be naturally expressed in DMR. Furthermore, the effort to understand the reproductive skew of DMR females has broadened our knowledge of the delicate neuroendocrine regulation of reproduction in other mammals and human. This is useful information as female reproductive health has become an increasingly popular area of research.

However, challenges often result when scientists tried to validate these findings. Compared to laboratory rodents and othercommonly used model systems, the molecular biology and physiology of DMR are less defined and therefore the interpretation of results might not be as straightforward as it is for the more well-defined model systems. Fewer scientific tools and support are available for less defined systems as well. Reagents, such as antibodies for protein analysis or methodologies for functional assessments, may need to be re-validated and re-developed before conducting actual experiments. This makes the studies with DMR and NMR highly time- and resource-intensive. In this regard, this current review also intends to encourage researchers in evolutionary biology and biomedicine to allocate effort to the comprehensive characterizations of DMR and other non-traditional model organisms. Luckily, annotated genome for DMR is avaliable for use (Fang et al., 2014), which provides bases for other approaches such as qPCR, transcriptomic study and designing transfection plasmid and virus (Fang et al., 2014; Johnston et al., 2021). Protocols for establishing certain primary cells from DMR is also available to use (Johnston et al., 2021; Yap et al., 2022). Even though published transfection protocol specifically for DMR cells is still unavailable, however, methods used for NMR cells should apply for DMR cells as well (Seluanov et al., 2009). Expressing exogenous genes in primary DMR cells might be difficult because of mole-rats genome are relatively stable compared to other model organisms (Petruseva et al., 2017; Sahm et al., 2018). In short, establishing protocols for DMR might be difficult at first, but these lines of study will enable the subsequent development of a wealth of tools and resources to support further research with these organisms. Moreover, it should be of note that the eusocial structure of DMR has been reported to elicit considerable influence on multiple aspects of biology, these include but are not limited to the aging process (Healy, 2015; Lucas and Keller, 2020) and neurodevelopment (Anyan et al., 2011; Oosthuizen, 2020; Oosthuizen and Amrein, 2016). Therefore, there are potential caveats when trying to translate the findings from DMR to human and one should interpret these data with care.

Compared to NMR, DMR has attracted less attention from researchers even though these two species share many similar unusual traits together. As discussed throughout this review, the underlying physiological mechanisms for these unusual traits often differ between DMR and NMR. Comparative genomic study between NMR and DMR indicated both differences and similarities of their genomes (Fang et al., 2014; Lewis et al., 2016). The major differences between these two genomes lied in response to hypoxia, body temperature regulation, pain sensitivity, processing of rRNA (Fang et al., 2014). For example, neuroglobin is often elevated in the brains of subterranean rodents even during normoxia (Avivi et al., 2010). Higher neuroglobin expression was observed NMR brain, but not in the DMR brain. But DMR exhibited higher cytoglobin expression. This indicated species-specific expression of globins in response to hypoxia (Avivi et al., 2010; Fang et al., 2014). However, when comparing to above ground rodents, DMR and NMR genome showed lots of similarities. For example, because of the ammonia environment, arginase 1, which catalyzes the last step of the hepatic urea cycle, has changed in both the NMR and DMR: His254 replaces Leu/Tyr. This amino acid change was also detected in the subterranean coruro (*Spalacopus cyanus*) and the semi-subterranean degu (*Octodon degus*)(Fang et al., 2014). As a result, this mutation could be the adaptation for subterranean habitat both DMR and NMR occupied. Many areas of the "unique" underlying physiological mechanisms for different adaptations in NMR still remain untested for DMR. For some biomedical research topics, such as hypoxia adaptation, DMR could help bridge translational research to human health from NMR research. Consequently, results from studies on DMR are not only supplementary evidence to strengthen findings from NMR, but would also offer a unique perspective that will shed light on alternative physiological adaptations for similar evolutionary force. By studying these two similar but evolutionarily distinct species, we can avoid the potential bias of conclusions based on life-history of a single species. In addition to a holistic view of DMR, we also encourage researchers to consider the similarities and differences within the whole family Bathyergidae, or perhaps all subterranean rodents, particularly including the family *Spalacidae* (Fang et al., 2014). Understanding the broader phylogenetic context will be essential for understanding the evolution of these adaptations (Eigenbrod et al., 2019; Ivy et al., 2020; Smith et al., 2011; St John Smith et al., 2012).

Here, we reviewed a collection of studies with DMR and their potential influence on the development of current biomedical research. For complex biological questions, evolutionary approach is a powerful tool. The fundamental promise of an evolutionary approach is that natural selection is likely to discover solutions to complex biological problems, and by studying these solutions, we might discover mechanisms that differ from, and are superior to, those elucidated by the study of traditional model species. Though this is a powerful approach, we should always bear in mind that the findings of these studies may, to a certain extent, be confounded due to the lack of a comprehensive characterization of DMR and the discrepancies between DMR and human. More effort is warranted to broaden our understanding of DMR and to establish a good collection of experimental resources to support future research.

Author contributions

YZ was invited to complete the review. HSW, DAF and YZ wrote this commentary.

Declaration of Competing Interest

The authors declare no competing financial interests.

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