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Learning of nature: The curious case of the naked mole rat

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ABSTRACT

Naked mole rats (NMRs) are the longest-living rodents known, living up to approximately 30 years and showing sustained good health. Nowadays, NMRs are considered excellent models for aging and, additionally, for cancer research, due to the evidence of a remarkable cancer resistance demonstrated through thousands of necropsies performed with very few cases that describe this pathology, which is believed to be a disease that unavoidably accompanies aging. Since some years ago, several studies have tried to explain the possible mechanisms underlying longevity and cancer resistance in NMRs through different perspectives and directions, creating new knowledge that subsequently could be used for cancer prevention and delaying aging in humans. Thus, the purpose of this review is to summarize the recent knowledge on naked mole rats with a particular emphasis on the molecular mechanisms associated with their longevity and cancer resistance.

1. Introduction

Naked mole rats (NMR, \textit{Heterocephalus glaber}; Rodentia, Bathyergidae) are rodents indigenous from hot and arid regions of Kenya, Somalia, and Ethiopia. They lead an exclusively subterranean existence, living in sealed burrows in large eusocial colonies of up to 300 individuals with strict division of labor, culminating in the existence, living in sealed burrows in large eusocial colonies of up to 300 individuals with strict division of labor, culminating in the existence, living in sealed burrows in large eusocial colonies of up to 300 individuals with strict division of labor, culminating in the presence of only one breeding female and 2–4 breeding males (Jarvis, 1981). NMRs are the longest-living rodents known, living up to, approximately, 30 years in captivity; 5.3 times longer than predicted on the basis of body size and nine times longer than similar-sized laboratory mice. Remarkably, they not only have an extraordinarily long lifespan, they also show sustained good health for at least 85% of their maximum longevity, and show no age-associated acceleration in mortality risk (Buffenstein et al., 2012). This is especially relevant for the development of cancer, which is believed to be an unavoidable accompanist of aging. Notably, of thousands of necropsies carried out in NMRs, only two cases have been reported describing the development of this pathology, an axillary adenocarcinoma possibly of mammary or salivary origin and a neuroendocrine carcinoma (Delaney et al., 2016). While mice are an excellent model for cancer development, progression and treatment, naked mole rats may be better for prevention, due to their remarkable cancer resistance (Deweerd, 2014). Thus, the purpose of this review is to summarize the recent knowledge on naked mole rats, with a particular emphasis on the molecular mechanisms associated with their longevity and cancer resistance.

2. Natural habitat

Longevity in NMRs have been suggested to be influenced by a favourable condition, which helps them to avoid predation and extreme climate conditions similar to those of flying vertebrates (bats and birds), but in this case it is attributed to their fossorial lifestyle (Healy et al., 2014). However, the adaptation to underground life has thrown-up several challenges for the NMR metabolism. The burrows are usually sealed and gas exchange through soil is limited, resulting in a hypoxic (10–15% oxygen) and hypercapnic (∼5% CO\textsubscript{2}) atmosphere. Additionally, the impact of high levels of ammonia or methane in nests and latrines could exacerbate respiration and acid–base balance (Buffenstein et al., 2012). In this way, NMRs have developed several specific features, including underdevelopment of the visual system (Buffenstein et al., 2013), skin insensitivity by a natural lack of sensory cations in neuropeptides substance P (Park et al., 2003) and calcitonin gene CALCA (Fang et al., 2014), together with the ability to tolerate a low body temperature (Goldman et al., 1999) and hypoxia conditions (Larson and Park, 2009).

On the other hand, the natural diet of wild NMRs, principally roots and tubers, in combination with various other plant constituents are a...
source rich in polyphenols, fibre and related compounds, possessing anti-microbial, anti-inflammatory, anti-oxidative, as well as anti-cancer activity, which may contribute to their exceptionally healthy lives (Buffenstein and Yahav, 1991; Debebe et al., 2016). Furthermore, NMRs naturally have a calorie and methionine restricted diet, two of the most powerful dietary regimens, resulting in longer lifespan (Brown-Borg and Buffenstein, 2016; Buffenstein, 2005), with a consequent low cystathionine β-synthase (CBS) activity and low sulphide (H₂S) levels (Dziegielewska et al., 2016), which in high levels have been suggested to promote angiogenesis and tumour growth, therefore contributing in this way to cancer resistance in this species (Hellmich et al., 2015).

3. Molecular mechanisms

3.1. Basal metabolism

Thyroid hormones play a major role in the metabolism of vertebrates. Buffenstein et al. (2001) reported low thyroxine (T4) and triiodothyronine (T3) levels in NMRs, suggesting a low metabolic rate that may help them, as adaptive strategy, to survive during periods of limited resources and reduce the need for oxygen in brain and other tissues. Additionally, the low body temperature of these animals (about 32°C) makes this low rate of metabolism possible (Goldman et al., 2011). However, similar to reports in reptiles, although NMRs are poikilothermic, they show an increase in thyroid activity in the cold (Buffenstein et al., 2001). Furthermore, NMRs do not exhibit age-related changes in basal metabolism, body composition, and bone mineral density (O’Connor et al., 2002). Therefore, these observations together with several theories linking metabolism, aging and lifespan, give clues for understanding, albeit in part, how NMRs, reducing their metabolism, decrease the inevitable side effects of metabolic processes.

3.2. Genome analysis

Most of the NMR genome (93%) showed synteny to human, mouse or rat genomes. The predicted NMR gene set included 22,561 genes, which is comparable to those of other mammals (22,389 in human, 23,317 in mouse, and 22,841 in rat). Genome sequencing revealed a relative reduction of SNPs due to CpG mutations, possibly because NMR genome exhibits a relatively low CpG density, mainly presenting CpG dinucleotides within CpG islands and thus, showing a lower methylation rate and contributing less to genetic variation (Kim et al., 2011). Additionally, MacRae et al. (2015a,b) found a slower nucleotide substitution rate in genome maintenance genes due to a greater decreasing in nonsynonymous nucleotide substitutions in relation with random genes, thus suggesting an evolutionarily more stable mechanism, which may be required for longevity and/or resistance to cancer in NMR. Furthermore, they reported evidence of gene expansion in two genes, CEBPγ and TINF2, suggesting a better protection against cellular stressors and specific control in telomerase activity and DNA damage signalling (MacRae et al., 2015b). Subsequently, comparing the expression of 130 genes involved in DNA repair activities between NMRs, humans and mice, they found a higher expression of these in the longer living species, human and NMR, than in mice, mainly for the base excision repair (BER) mechanism (MacRae et al., 2015a). Meanwhile, Keane et al. (2014) suggested the possibility of convergent evolution in the p53 proline-rich domain of NMRs, adding prolines and PXXP motifs for an enhanced DNA damage response (Keane et al., 2014).

3.3. Oxidative metabolism

NMRs provide equivocal support for oxidative stress theory of aging, which posits that the characteristic traits of aging are due to accrued damage by unchecked reactive oxygen species (ROS) generated during aerobic metabolism. Pérez et al. (2009) reported that young NMRs present more reversible cysteine oxidation products than young mice. However, NMRs sustain this level with aging, whereas, mice have an overall increase in irreversibly oxidized cysteine residues (Perez et al., 2009). Meanwhile, Fang et al. (2014) found that Cys272 and Ala230 of β-actin are converted to serine in the NMR (Fang et al., 2014). Normally, Cys272 is highly redox sensitive and may serve as a “redox sensor” (Lassing et al., 2007). These observations thus suggest that NMR β-actin is more resistant to oxidation despite its exposure to high ROS levels.

In spite of the data that support the susceptibility of NMRs for a more pro-oxidative cellular environment and damage accrual in all the biomolecules (Andziak et al., 2006), together with the near absence of cellular glutathione peroxidase (GPx) activity (Andziak et al., 2005), NMRs are able to maintain steady states of oxidative damage with no concomitant adverse effects. Additionally, because selenium is an important cofactor for different antioxidant enzymes, low concentration of selenium in different NMR tissues has been associated with lower levels of glutathione peroxidase activity, so selenium is able to perform a role in regulating antioxidant activity (Kasai et al., 2011).

Contrasting with the previous data, analysis of gene expression showed a strong overexpression of genes associated with the endoplasmic reticulum and mitochondrial matrix, reflecting a higher need for ROS protection and pointing towards a putative role of these organelles contributing with the antioxidant response and long lifespan (Yu et al., 2011).

On the other hand, hyaluronan, a glycosaminoglycan component of extracellular matrix and up-regulated in NMRs, presents antioxidant properties due to the creation of a mechanical barrier that limits ROS access to the cell surface and reduces DNA damage (Krasinski et al., 2009).

Lewis et al. (2015) found a positive correlation between NRF2 signalling activity and maximum lifespan. Normally, NMRs present a higher NRF2 levels and NRF2:ARE binding 50% higher compared with mice, given the pivotal role of NRF2 in cytoprotection, proteostasis and xenobiotic metabolism. NRF2 initiates the transcription of several hundred molecules that possess an antioxidant response element (ARE) such as antioxidants, molecular chaperones, and proteasome subunits. In addition, they showed that this close relationship in NMR is probably due to the higher expression of NRF2 and lower levels of its negative regulators, KEAP1 and βTrCP (Lewis et al., 2015).

3.4. Protein homeostasis

Good quality of proteins plays an important role in the maintenance of normal cellular homeostasis. Azpuru et al. (2013) found that NMR RNA does not display the typical banding pattern during denaturing gel electrophoresis; instead the NMR 28S ribosomal RNA appears split into two fragments of unequal sizes (Azpuru et al., 2013). Furthermore, they reported the place of “break” inside the D6 domain of 28S, which corresponds with a cystic GC- and simple repeat-rich intron in the Tala tucu-tuco (Ctenomys talarum), another vertebrate reported to produce shorter 28S RNA (Melen, 1999). This altered ribosomal structure is suspected to affect the overall translation fidelity of proteins based on their observations that NMR cells have substantially higher translation fidelity than mouse cells (Azpuru et al., 2013).

De Waal et al. (2013) while analyzing the activity of the enzymes TPI and PRDX1, enzymes that normally undergo carbonylation during aging, they observed that both present higher activity and lesser sensitivity to form higher-order non-reducible oligomers in NMRs compared to mice, thereby showing a protective cellular environment in NMR, which restores enzyme function (De Waal et al., 2013).

Furthermore, NMR proteome has been demonstrated to be more resistant to urea-induced unfolding and concomitant maintenance of functionality than observed in mice. On the other hand, the absence of age-related accumulation of ubiquitinated proteins and higher proteasome activity in NMRs reflects a rapid removal of oxidized and/or
misfolded proteins, and thereby better maintaining of protein homeostasis (Perez et al., 2009).

Rodriguez et al. (2014) found that elevated proteasome activity of NMRs and inhibition resistance are not due to intrinsic proteasome properties but, rather, to protective modulators of proteasome activity within the cytosolic milieu capable of protecting human, yeast, and mouse proteasomes. Therefore, they suggest the presence of a novel cytosolic factor maintaining a close relationship with the chaperones HSFl, HSP72, HSP40, and HSP25 that not only protects proteasome function against cell stressors but also enhances proteasome performance (Rodriguez et al., 2016, 2014). Additionally, NMRs have also shown higher activity in other pathways under stress induced condition to improve the proteostasis such as autophagy and elevated chaperone levels (Pride et al., 2015), thus suggesting that an improved proteostasis network may be necessary for the maintenance of longer health and life.

Autophagy has been demonstrated to be another important mechanism that plays a role in NMR cancer resistance due to higher levels of autophagy observed in them under basal conditions in adulthood and childhood (Zhao et al., 2014). Zhao et al. (2016) suggested that HIF-1α and p53 pathways are involved in the induction of autophagy in NMR skin fibroblasts, both being regulated by PI3K/Akt/mTOR signalling pathway, which is directed towards autophagy or apoptosis when active or inhibited, respectively (Zhao et al., 2016).

Notably, one of the most interesting over-expressed genes in the NMRs is the serum pan-protease inhibitor, alpha2-macroglobulin (A2M). A2M, as a result of a negative correlation observed in blood concentration between the birth and the old age, is suggested to play an important role in cancer and aging. Thieme et al. (2015) reported a higher concentration of A2M in NMR plasma, which presents lower proteolytic inhibitory capacity at a lower concentration, but higher overall inhibitory activity at a higher concentration than in humans. In spite of the high level of A2M, the concentration of LRPI, its receptor, was lower than in humans (Thieme et al., 2015), suggesting a better regulation in protease activity requiring less receptor-mediated clearance, thus improving the tissue homeostasis of these molecules and their products.

3.5. Stress resistance

Resistance to stressors is a common trait of long-living organisms. Salmon (2009) found that NMR-derived cells were indeed significantly more resistant than mouse cells to some forms of stress, i.e. cadmium, oxidative stress with paraquat, heat, and DNA-damaging agent methyl methanesulfonate. Surprisingly, they found that NMR cells were significantly more susceptible than mouse cells to the effects of H2O2, UV light and a mitochondrial electron-transport-chain inhibitor rotenone (Salmon et al., 2008). On the other hand, Lewis et al. (2012) observed that although NMR cells survive when they undergo a prolonged cell cycle arrest in response to genotoxins and DNA damage, the time it takes before they re-enter the cell cycle is more prolonged than that in mice cells, suggesting an overregulation in G1/S checkpoint to reduce the possibility of mutation event and where higher levels of p53 (50-fold higher than mice) take an important role (Lewis et al., 2012).

A relative resistance to low glucose concentrations was reported (Salmon et al., 2008). Interestingly, NMRs present an increased insulin sensitivity together with a modification in residue 22 of the insulin β-chain, changing an arginine for a glutamine (Edrey et al., 2011; Fang et al., 2014), whose mutation in humans is associated with reduced insulin processing, misfolding and diabetes (Liu et al., 2010). Fang et al. (2014) suggested a compensatory mechanism replacing insulin by IGF2 that mediate through fetal-like mode and modifying downstream signalling (Fang et al., 2014). IGF receptors are established contributors to the aging process, confirmed by the demonstration that decreased IGF signalling results in life-extending effects in a variety of species (Brohus et al., 2015).

3.6. Early contact inhibition

Seluanov et al. (2009) found that NMR presents a hypersensitive contact inhibition, mediated primarily by cell-cell contact, named to this phenomenon “early contact inhibition” (ECI). They described that ECI requires the activity of p53 and Rb pathways, and that inhibition is abolished only if both are depleted. Remarkably, the absence of either p53 or Rb activity caused an increase in the levels of apoptosis in NMRs cells, thus showing a second point safeguarding the ECI and avoiding the proliferation beyond of the confluence (Seluanov et al., 2009), which contrasted with the concerted necrotic mechanism in the blind mole rat (BMR) (Gorbunova et al., 2012). Additionally, they reported an increasing expression of p16 INK4a in ECI. However, when this mechanism is perturbed, naked mole-rat cells undergo, as humans and mice, contact inhibition associated with induction of p27KIP1. Therefore, these results indicate that naked mole-rat cells have two tiers of contact inhibition: ECI mediated by p16 INK4a and regular contact inhibition mediated by p27KIP1, thereby providing backup mechanisms that increase cancer resistance in vivo (Seluanov et al., 2009). In addition, Miyawaki et al. (2016) reported tumour resistance in NMR-induced pluripotent stem cells (NMR-iPSCs) based on ARF regulation and disruption of ERAS. Interestingly, NMR ARF suppression induces a phenotype similar to senescent cells, termed this phenomenon “ARF suppression-induced senescence” (ASIS) (Miyawaki et al., 2016).

Tian et al. (2013) culturing multiple lines of naked mole-rat fibroblasts noticed that the culture media became very viscous after a few days. They identified the viscous substance secreted as high-molecular-mass hyaluronan (HMM-HA). This HMM-HA has a molecular mass of 6–12 MDa, being bigger than hyaluronan in humans, mice and guinea pigs. Notably, they were able to identify the relationship between HMM-HA and ECI, connecting both across the CD44 receptor (main receptor for HA) and p16INK4a, repressing the mitogenic signalling. Interestingly, HMM-HA may be a secondary and fortuitous consequence of evolution because HMM-HA facilitates elasticity of the skin, which is a useful condition for adaptation to living underground in tight tunnels and confined spaces. Furthermore, comparing the expression of HAS1, HAS2 and HAS3, enzymes that synthetized HA found a higher expression of HAS2, whereas, HAS1 and HAS3 were similar in mouse, human and NMR cells. Collectively, these results carry on to identify the replacement of two asparagines that are 100% conserved among mammals with serines in the NMR HAS2, therefore modifying the cytoplasmic loop containing the enzyme’s active site. At the same time, the activity HA-degrading enzymes, HAases, was much lower than that in human, mouse or guinea pig cells. These experiments establish HMM-HA as a key component responsible for the elevated cancer resistance of the naked mole rat (Tian et al., 2013). Additionally, two years after, they detected a unique INK4 isoform in NMR composed of the first exon of p15INK4b and the second and third exons of p16INK4a. This transcript was named pALTPINK4a/b and expressed in several tissues, with the highest levels presented in lung and testes. Furthermore, they found that this new isoform has a tumour-suppressing function, similar to p15INK4b and p16INK4a, responding to hyaluronan-induced early contact inhibition and cell density, thus under stress conditions of UV-C irradiation, γ-irradiation or oncogene overexpression. However, pALTPINK4a/b showed an enhanced ability to mediate cell-cycle arrest, contributing to cancer resistance of this species (Tian et al., 2015).

3.7. Telomerase activity

As a normal cellular process, telomere length decreases with each cell division, so when telomere length reaches a critical limit, the cell undergoes senescence and/or apoptosis. Eukaryotes solve the “end-replication” problem by expressing a ribonucleoprotein complex named telomerase that replenishes these sequences each time a cell divides.
However, accumulation, localization and possibly other functions depend on cell types and species. Telomeres from all species contain an essential RNA component (TER), a unique reverse transcriptase protein (TERT) and various accessory proteins involved in assembly (Theimer and Feigon, 2006).

In NMRs, Kim et al. (2011) reported a positive selection from protein complex that alternates the lengthening of telomere pathway, TOP2A, along with TEP1 and TERF1. Furthermore, it is also of interest that TERT showed stable expression, regardless of age, thus pointing to altered telomerase function, which may be related to its evolution of extended lifespan and cancer resistance (Kim et al., 2011). Meanwhile, Liang et al. (2010) described resistance to experimental tumorigenesis in NMR cells that were induced by the expression of the oncogenic combination RAG12V and SV40 large T antigen. They observed a fast cellular crisis acting as a tumour suppressor mechanism that was surprisingly delayed adding human TERT in the combination, thus suggesting that although NMRs have an increasing telomerase activity, it is maintained under an overregulation (Liang et al., 2010). On the other hand, in a bioinformatics assay Evfratov et al. (2014) reported variations in the promoter region of TER gene and its structure, describing a unique regulatory element SOX17 site for NMRs, approximately 430 nucleotides upstream of the gene body, thus suggesting a better regulation in the expression of telomerase RNA. Furthermore, they found that a polymorphism G→A at position 421 leads the formation of a C–A non-canonical pair in the p8b stem that modify the CR7-p8b domain, thus improving the interaction between TCAB1 and TERT, making telomerase traffic more effective and therefore resulting in a more efficient telomere elongation (Evfratov et al., 2014).

On the other hand, a positive selection has been predicted for ANKD17, ABL1, FANCE, MSH3, FANCA, SLX4, BRCA1, XRCC5 and FANCL genes, where BRCA1, FANCE and FANCA work together responding to DNA damage and BRCA1 play a crucial role in telomere maintenance (Morgan et al., 2013).

4. Conclusion

In reality, it is very complicated to separate the mechanisms underlying longevity and cancer resistance because several ways are interconnected in contributing to both phenomena. However, we considered that Figs. 1 and 2 could be useful to explain, more easily, how NMRs have developed a natural model for delay aging and preventing cancer. It is important to note that all mechanisms involved in cancer resistance contribute to increased longevity, but not all mechanisms involved in increased longevity contribute to cancer resistance.

On the other hand, nowadays, there are a lot of information linking NMRs, aging and cancer with different perspectives and directions. However, it is necessary to focus on those that could be applied in our
daily lives, either through the development of drugs, food supplements or reduced exposure to substances, among many others, in order to delay the side effects of aging and improve the quality of life.

Conflict of interest
The authors declare no conflict of interest.

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References


