DISEASE MECHANISMS

Comparative genetics of longevity and cancer: insights from long-lived rodents

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Abstract | Mammals have evolved a remarkable diversity of ageing rates. Within the single order of Rodentia, maximum lifespans range from 4 years in mice to 32 years in naked mole rats. Cancer rates also differ substantially between cancer-prone mice and almost cancer-proof naked mole rats and blind mole rats. Recent progress in rodent comparative biology, together with the emergence of whole-genome sequence information, has opened opportunities for the discovery of genetic factors that control longevity and cancer susceptibility.

Maximum lifespans
The maximum documented lifespans achieved by representatives of various species. They are typically documented in a captive environment protected from predators.

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Mammalian species show remarkably diverse maximum lifespans, which range from 2 years of a short-tailed shrew to 211 years of a bowhead whale[1]. This diversity can be exploited to understand the genetic mechanisms of ageing and the diseases associated with ageing — most notably, cancer. Historically, comparative studies of species have provided substantial advances in biology, the best example of which is Darwin's discovery of natural selection as the driving force that underlies species diversity. However, the focus in biological research subsequently shifted to the study of a few model species, which yielded important discoveries in cell and molecular biology but lacked the species breadth of previous work. Now, with the new molecular tools and genomic approaches available for studying the cellular and physiological mechanisms that control ageing, a comparative approach can be used at a vastly more sophisticated level. Indeed, in recent years there has been a renewed interest in using interspecies comparisons and unconventional long-lived animal models for ageing studies[2–4]. A big step towards understanding the molecular basis of human ageing and its interaction with disease would be achieved if we could discover the mechanisms responsible for the >100-fold differences in lifespan between mammalian species.

In this Review, we focus on the genetic factors that underlie the greatly diverse ageing rates and cancer risks among rodents. Given the fairly close genetic make-up and yet profoundly different lifespans of rodents, they proved especially suitable for identifying key longevity assurance systems, including tumour suppressor mechanisms such as telomere maintenance and global genome maintenance. We review recent progress in understanding how the longest-lived rodents — the blind mole rat and the naked mole rat — achieve cancer resistance and long lifespans. Finally, we discuss recent advances in whole-genome sequencing of very long-lived mammals and how this can increase our understanding of the genetics of human longevity, as well as the resistance to cancer and other age-related diseases.

Rodents as models for comparative studies

Rodents are the most prevalent mammals on earth, and ~40% of mammalian species are rodents. They are an ideal group of species for comparative studies of ageing. These animals are phylogenetically related, but their lifespans are extremely diverse and range from 3–4 years in mice and rats, to >20 years in blind mole rats, beavers, porcupines and squirrels, and >30 years in naked mole rats (FIG. 1a). This nearly tenfold variability in lifespan among rodent species is greater than that observed in other mammalian orders and is hypothesized to be connected to the variability among rodent species in extrinsic mortality caused by predation (BOX 1). The longest-lived rodents — naked mole rats, blind mole rats, beavers, porcupines and squirrels — all belong to different phylogenetic groups, which indicates that slow ageing has independently evolved at least four times in rodents[2] (FIG. 1a). Moreover, a tenfold range in lifespans far exceeds differences in lifespans observed within a species and is certainly much greater than anything that has been achieved in extending lifespan using genetic,

pharmacological or dietary interventions in mice or rats. Indeed, dietary restriction, which is the best-documented intervention to extend lifespan, only results in a maximum increase of ~40%[5].

Rodents also differ markedly in body mass. The smallest rodents, such as mice, have an average body mass of 20–30 g, whereas the largest rodent capybara weighs 55 kg. This diversity is useful for comparative studies of ageing, as many ageing-related traits show a dependence on body mass[6–8]. Importantly, rodents include species with all possible combinations of lifespans and body mass; that is, they include species with large body mass and average lifespans (capybaras), large body mass and long lifespans (beavers and porcupines), small body mass and short lifespans (mice), and small body mass and long lifespans (naked mole rats).

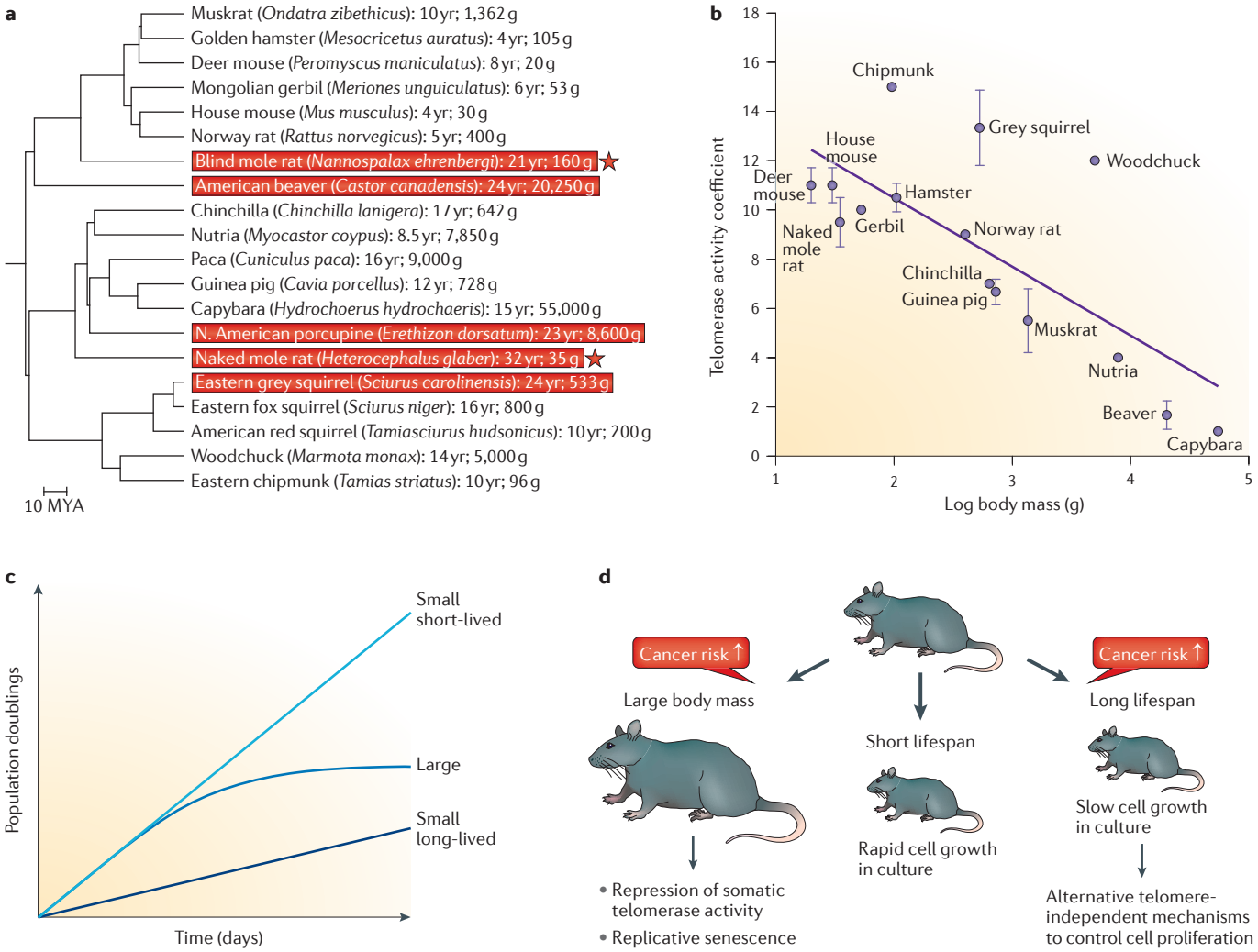

Figure 1 | **Evolution of tumour suppressor mechanisms.** **a** | Slow ageing and resistance to cancer have evolved multiple times in rodents. Maximum lifespan in years (yr) and body mass in grams of each of these species are shown[1,81]. Red shading highlights slow-ageing species with maximum lifespans >20 years. Stars indicate species for which cancer resistance had been documented. **b** | Correlation of telomerase activity with body mass is shown. A strong negative correlation is observed between telomerase activity in somatic tissues and body mass. Telomerase is repressed in somatic tissues of large rodents. Error bars represent one standard deviation. **c** | Cell proliferation patterns are shown for primary fibroblasts isolated from species with different body mass and maximum lifespans. Small body mass and short lifespans correlate with rapid cell proliferation *in vitro* and the absence of replicative senescence. Large body mass (>10 kg) correlates with rapid cell proliferation *in vitro* followed by replicative senescence due to telomere shortening. Finally, cells of small but long-lived (maximum lifespan >10 years) animals tend to proliferate very slowly but do not enter replicative senescence. **d** | The model summarizes evolution of tumour suppressor strategies depending on lifespans and body mass. When species evolve large body mass, the cancer risk is increased owing to increased number of cells. To mitigate this risk, large body mass co-evolves with repression of telomerase activity and with replicative senescence. Small short-lived species require fewer tumour suppressors. Finally, evolution of longer lifespans in small species is associated with telomere-independent tumour suppressor mechanisms that stringently control cell proliferation, and the cells of these species are characterized by very slow proliferation rates *in vitro*. MYA, million years ago; N., north. Part **b** reproduced from Springer *Age (Dordr.)*, **30**, 2008, 111–119, Rodents for comparative aging studies: from mice to beavers., Gorbunova, V., Bozzella, M. J. & Seluanov, A., figure 2a. With kind permission from Springer Science and Business Media. Part **d** reproduced with permission from REF. 15 © 2008 Seluanov, A. *et al. Aging Cell* © Blackwell Publishing Ltd/Anatomical Society of Great Britain and Ireland 2008.

Box 1 | **How do lifespans evolve?**

Evolutionary theory of ageing was developed by Haldane[74], Williams[75] and Medawar[76], who concluded that the force of natural selection declines with age. In the natural world, animals die from predation and accidents; thus, genes that confer fitness and longevity beyond the expected lifespan of a given species based on extrinsic risks would be largely ignored by natural selection. In other words, there is little benefit for a species to invest in maintaining physical fitness and fecundity beyond the time that the individuals of that species are expected to survive based on extrinsic pressures. Hence, one can predict that the differences in ageing rates between species are driven by differences in extrinsic mortality as a result of predation, starvation or accidents. Species that are subject to high extrinsic mortality gain no advantage from investing resources into somatic maintenance and longevity, and instead benefit from reproducing prolifically while they are still alive. By contrast, in species that are shielded from predators by specific adaptations, molecular mechanisms evolve to allow them to live longer. Experimental evidence for this theory was provided by classic experiments in *Drosophila melanogaster*, in which long-lived flies were artificially selected by only allowing older flies to reproduce[77–79] such that the force of natural selection no longer decreased with age. Additionally, studies of opossums showed that the animals living on a predator-free island reproduced later and aged slower than animals of the same species on the more hazardous mainland[80]. The correlation between lifespan and environmental vulnerability holds true even for a large collection of >500 mammalian species[8,19]. The evolutionary theory helps to understand the diversity of lifespans in the rodent clade (FIG. 1a). For example, the quills of a porcupine make it inaccessible to predators, which coincides with a long maximum lifespan of 24 years. The sturdy lodges and imposing body size of the beaver, which lives for >24 years, protect the animal from predators. Moreover, the subterranean lifestyle of blind mole rats and naked mole rats shields them from the majority of predators. In all of these cases, a low level of extrinsic mortality is likely to have promoted the evolutionary emergence of pro-longevity and tumour suppressor mechanisms that could be uncovered by in-depth comparative genetic analyses of such species.

The ageing process in rodents, similar to that in humans, is associated with an increased incidence of disease. This is especially relevant for cancer, which is a typical disease of the aged. Short-lived rodents such as mice and rats are prone to cancer, and cancer incidence in some strains can be as high as 95%[9,10]. However, the two longest-lived rodent species — the naked mole rat and the blind mole rat — are remarkably cancer resistant, and no cases of spontaneous cancer have been reported in either species after multiyear observations of large animal colonies[11–13]. This disparity in cancer rates between species that are so closely related phylogenetically offers an inimitable opportunity to understand how cancer resistance can be achieved in these related species and possibly in mammals in general.

The independent evolution of long-lived, cancer-resistant rodent species provides a unique opportunity to study what are possibly multiple mechanisms that lead to a long life without cancer. Long-lived rodents are especially interesting owing to their relationship to the best-studied ageing models — mice and rats. However, in contrast to many long-lived laboratory mouse mutants that cannot compete in the wild[11–13], natural long-lived species are well adapted to their respective environments and offer an opportunity to identify longevity mechanisms that can be modulated without undesirable effects on fitness.

Telomerase
A ribonucleoprotein enzyme that elongates telomeres by synthesizing the telomeric repeat sequence using an RNA templete.

Cross-species biological comparisons

Before the emergence of genomic data for the longest-lived rodents, important biological insights related to longevity and tumour suppressor mechanisms were obtained through cross-species comparisons.

Telomere maintenance and replicative senescence. Permanent arrest of cell proliferation induced by progressive telomere shortening — replicative senescence — is one of the key anticancer mechanisms in humans[14]. Telomerase activity is repressed in the somatic tissues of humans but is constitutively active in mice. The prevailing view had been that long-lived species evolved repression of telomerase activity to enable replicative senescence. This hypothesis was tested using a comparative analysis of 15 rodent species with diverse lifespans. Surprisingly, it was found that telomerase activity did not co-evolve with lifespan but instead co-evolved with body mass[7] (FIG. 1b). During evolution, larger animals (body mass >10 kg) tended to evolve repressed telomerase activity in somatic cells to mitigate increased cancer risk conferred by the large number of cells. Consistent with repressed telomerase activity, fibroblasts from large rodents undergo replicative senescence when cultured *in vitro*[15] (FIG. 1c). These findings were confirmed using a wider range of mammalian species[16]. These studies experimentally showed the importance of body mass as a risk factor for cancer. If cancer results from a random mutation in a cell, then cancer risk must be proportional to the number of cells. However, larger animals do not have higher cancer incidence, and this concept is known as Peto's paradox[17]. The answer to Peto's paradox is that larger animals must evolve additional anticancer mechanisms, one of which is replicative senescence. If the increase in size from 30 g in a mouse to 55 kg in a capybara commands additional tumour suppressor mechanisms, then one can only speculate on the number of novel tumour suppressor mechanisms that are awaiting discovery in animals with even greater body mass, such as elephants and whales.

Mechanisms controlling cell proliferation. Analogous to the evolution of replicative senescence as a tumour suppressor mechanism to counteract the heightened cancer risk from increased body mass, tumour suppressor mechanisms would also be expected to evolve to counteract the increased cancer risk in organisms with longer lifespans. Interestingly, despite the absence of replicative senescence, small rodents with short and long lifespans show a striking difference: cells from small shorter-lived species show a rapid rate of proliferation *in vitro*, whereas cells from small longer-lived species (the maximum lifespan of which is >10 years) proliferate much more slowly *in vitro*[15] (FIG. 1c). Fibroblasts divide infrequently *in vivo*, whereas *in vitro* cells are induced to overproliferate by growth factors that are present in the serum in the culture medium. Therefore, *in vitro* growth could be a surrogate test for susceptibility to malignant transformation. The slow growth of fibroblasts from long-lived species *in vitro* is likely to reflect more stringent cell cycle control mechanisms that restrict overproliferation of cells. This suggests that cells of small long-lived rodents, which lack replicative senescence, have evolved alternative tumour suppressor

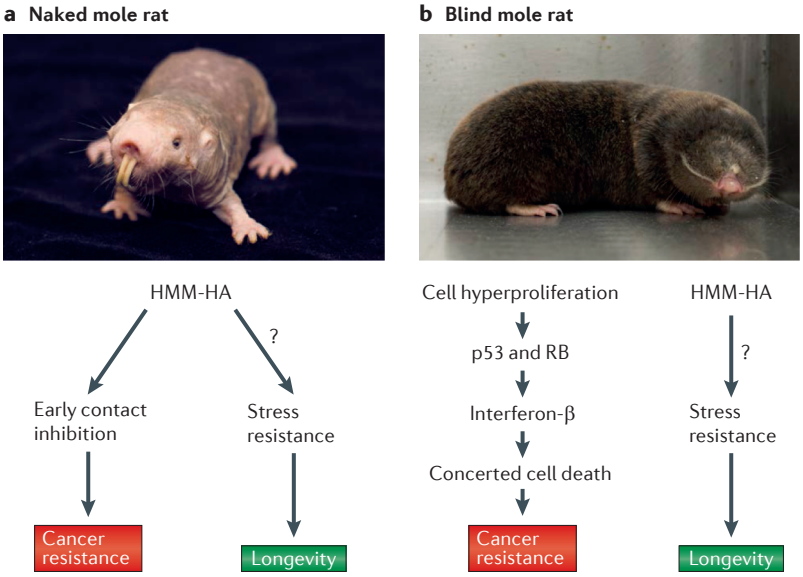

Figure 2 | **Two mole rat species independently evolved longevity and resistance to cancer. a** | The naked mole rat is the longest-lived rodent that is almost cancer-proof[11,41]. Cancer resistance in the naked mole rat is mediated by high-molecular-mass hyaluronan (HMM-HA), which results in early contact inhibition (that is, hypersensitivity of naked mole rat cells to contact inhibition). HMM-HA may also contribute to longevity by increasing stress resistance as a result of the antioxidant and cytoprotective properties of hyaluronan. **b** | The blind mole rat is one of the longest-lived rodents that is also resistant to cancer. Cancer resistance in the blind mole rat is mediated by an interferon-mediated necrotic cell death mechanism. Blind mole rat cells produce HMM-HA but, in contrast to naked mole rat cells, do not show early contact inhibition. Antioxidant properties of HMM-HA in the blind mole rat can increase stress resistance and contribute to longevity in this species. RB, retinoblastoma protein.

mechanisms that slow cell growth *in vitro* and that might prevent proliferation of pre-malignant cells *in vivo*.

Body mass and lifespan shape tumour suppressor mechanisms. The analysis of diverse rodent species suggests a set of rules for how increased cancer risk conferred by large body mass or long lifespan drives the evolution of tumour suppressor mechanisms (FIG. 1d). Both body mass and lifespan contribute to the evolution of tumour suppressor mechanisms but in different ways. Body mass greater than ~10 kg co-evolves with replicative senescence, whereas lifespans longer than ~10 years are associated with the evolution of more stringent cell cycle control mechanisms that increase the sensitivity of cells to growth conditions and manifest slow cell proliferation in culture (FIG. 1d). Replicative senescence, despite being a potent anticancer mechanism, also contributes to ageing of tissues through accumulation of senescent cells[18]. Thus, small long-lived species are free of the pro-ageing effects of replicative senescence while being protected from cancer through alternative mechanisms. Remarkably, members of the Sciuridae family — such as the grey squirrel, woodchuck and chipmunk — have particularly high telomerase activity that may be beneficial for wound healing and a robust immune response, which are processes that require rapid cell proliferation[7,15]. Studies of such small long-lived species open new avenues of research into novel tumour suppressor mechanisms.

It is important to emphasize that the rules discussed above relate to interspecies comparisons, in which body mass refers to an average body mass for the species. The correlations between longevity and cancer resistance between and within species show opposing trends. Larger species tend to live longer than smaller species — a trait shaped by millions of years of evolution[8,19]. For example, the longest-lived mammals besides humans are elephants and whales. However, larger individuals within a given species are often shorter lived. This is particularly striking when comparing large and small dog breeds, with smaller breeds being longer lived and less susceptible to cancer than larger breeds[20–22]. Similarly, dwarf mice with mutations in genes involved in the insulin-like growth factor 1 (IGF1)–growth hormone (GH) axis are longer-lived and resistant to cancer[23], and patients with Laron syndrome characterized by GH deficiency are protected from cancer[24].

Lifespan and genome stability. Genome maintenance capacity has long been implicated in the evolution of species-specific maximum lifespans[25]. For example, cells from short-lived species, such as mice and rats, are less capable of repairing DNA damage induced by ultraviolet light[26] or repairing DNA double-strand breaks[27] than cells from long-lived humans, possibly owing to the lower expression levels of the DNA repair factors poly(ADP-ribose) polymerase 1 (PARP1) and DNA-dependent protein kinase (DNA-PK, which consists of the catalytic PRKDC subunit, X-ray repair cross-complementing protein 5 (XRCC5) and XRCC6)[28,29]. In spite of these deficiencies, mouse or rat cells have survival characteristics after treatment with DNA damaging agents that are similar to those of human cells[30]. This raises the possibility that cells from short-lived species have a reduced stringency of DNA damage control at the cost of increased mutations[31]. Finally, a mouse cell in culture has a much higher probability of undergoing karyotypic changes and of becoming neoplastically transformed than a human cell[32], which also indicates a superior genome maintenance system in longer-lived species. Spontaneous immortalization never occurs in primary populations of human cells[33].

Naked mole rats and blind mole rats

The naked mole rat (*Heterocephalus glaber*) and the blind mole rat (*Spalax ehrenbergi*) represent two striking examples of animals that live in protected environments and that show extreme longevity and resistance to cancer[12,34–36]. The naked mole rat is a eusocial rodent that lives in large colonies in Africa[37], whereas the blind mole rat is a solitary animal found in the Middle East[38]. The two species are phylogenetically distant from each other: the naked mole rat is closer to guinea pigs, and the blind mole rat is closer to mice and rats (FIG. 1a). Strikingly, however, the protective environments of these two mole rat species allowed them to evolve independent mechanisms of exceptional longevity and cancer resistance[11–13,39,40] (BOX 1; FIG. 2).

Hyaluronan mediates tumour resistance in the naked mole rat. Cancer resistance in the naked mole rat is mediated by the extreme sensitivity of their cells to contact inhibition[41]. Normal animal cells arrest their proliferation when they come into contact with each other, which is a powerful anticancer mechanism. This growth arrest is associated with the activation of the *Ink4* locus in naked mole rats[41].

The trigger for the contact inhibition of naked mole rat cells is a polysaccharide, hyaluronan[42]. It is an abundant molecule in the body and a major non-protein component of the extracellular matrix that is termed 'extracellular goo' (REFS. 43,44). Hyaluronan is produced by the hyaluronan synthases HAS1, HAS2 and HAS3, which differ in the sizes of molecules produced[45]. The widely distributed form of hyaluronan in normal tissue has a high molecular mass, which forms a highly viscous network. At the sites of inflammation, injury and tumours, hyaluronan may be present in a low-molecular-mass form. Both high- and low-molecular-mass forms of hyaluronan bind to CD44 but trigger distinct biological outcomes[46]. High-molecular-mass hyaluronan binding to CD44 arrests the cell cycle by repressing mitogenic signalling[47]. By contrast, low-molecular-mass hyaluronan binding to CD44 promotes cell cycle progression[48]. In addition, high-molecular-mass hyaluronan has anti-inflammatory properties, whereas low-molecular-mass hyaluronan promotes proliferation and inflammation[46]. Thus, high-molecular-mass hyaluronan has antitumor activity, and the low-molecular-mass form may promote tumorigenesis. Naked mole rat tissues contain hyaluronan of extremely high molecular mass, which is five times longer than the hyaluronan of mice or humans. Naked mole rat cells cannot be malignantly transformed by a combination of oncoproteins that cause mouse cells to form tumours[49]. However, when the *Has2* gene responsible for hyaluronan synthesis was knocked down or when the hyaluronoglucosaminidase 2 (*Hyal2*) gene that is responsible for breaking down hyaluronan was overexpressed, naked mole rat cells readily formed tumours[42], which indicates that high-molecular-mass hyaluronan is a key to the cancer resistance of this species. Two mechanisms contribute to high hyaluronan levels in the naked mole rat. The *Has2* gene in the naked mole rat has a unique sequence, which may confer an enhanced activity to the enzyme, and the rate of hyaluronan turnover is very slow in naked mole rat tissues. It is anticipated that finding ways to manipulate hyaluronan degradation or to alter the hyaluronan synthase activity may lead to new therapies to treat or prevent cancer in humans.

Accurate protein synthesis in the naked mole rat. Another peculiar feature of the molecular biology of naked mole rats is that its 28S ribosomal RNA is cleaved into two fragments[40]. Such 28S rRNA cleavage has been described for only one other species of vertebrates[50]. This unusual structural feature of the naked mole rat ribosome is associated with higher fidelity of translation than that of the mouse. Thus, naked mole rat cells produce fewer aberrant proteins, which supports the hypothesis that a more stable proteome in the naked mole rat contributes to its longevity[40].

Interferon mediates cancer resistance in the blind mole rat. Anticancer mechanisms in the blind mole rat evolved independently and have taken a different path than in the naked mole rat. Remarkably, as an adaptation to hypoxic conditions underground, blind mole rats have evolved an amino acid change in p53 (which is encoded by the *Tp53* gene) that corresponds to a mutation frequently found in human tumours[51]. In blind mole rats, p53 lost the ability to induce transcription of apoptotic peptidase activating factor 1 (*Apaf1*) and increased the transcriptional induction of *Mdm2* (which encodes a negative regulator of p53)[52]. Thus, p53 in the blind mole rat cannot induce apoptosis, which makes the tumour resistance of these animals even more surprising. Unlike naked mole rats, blind mole rat cells do not show early contact inhibition but instead use a 'scorched earth' strategy to kill pre-cancerous cells and their neighbours. Pre-malignant blind mole rat cells secrete interferon-β1 that mediates massive necrosis of the surrounding cells[13]. An independent study confirmed that blind mole rat cells secrete a soluble factor that inhibits proliferation of cancerous cells and triggers their death[39]. Activation of a similar interferon-mediated cell death mechanism has been observed in mice with defective p53, in which cell death is driven by the activation of transcription of repetitive elements and non-coding RNAs[53].

In addition, the blind mole rat was shown to express an alternatively spliced form of heparanase that acts as a dominant negative form to repress heparan sulphate degradation in the extracellular matrix[54]. This heparanase splice variant has antitumor activity[54]. The mechanism leading to a more stable extracellular matrix in the blind mole rat resembles the cancer resistance mechanism of the naked mole rat (that is, the production of high-molecular-mass hyaluronan and the slow hyaluronan turnover).

Hyaluronan evolved in long-lived subterranean rodents. Interestingly, blind mole rat cells, similarly to naked mole rat cells, produce high-molecular-mass hyaluronan but do not show hypersensitivity to contact inhibition. Besides regulating cell proliferation, hyaluronan is also a potent antioxidant. It is possible that hyaluronan contributes to longevity of the mole rats by ameliorating oxidative stress. For example, the heart of naked mole rats contains very high levels of hyaluronan[42], which could delay the onset of heart disease. Why did these two species evolve high-molecular-mass hyaluronan? We hypothesize that hyaluronan production was initially upregulated as an adaptation for subterranean life to provide elastic skin needed for the life in underground tunnels. Later, this trait might have been co-opted to provide cancer resistance and longevity. In conclusion, the studies of naked mole rats and blind mole rats represent a successful example of how moving research efforts to non-canonical long-lived rodent models can lead to discoveries that may benefit human health.

Pseudogenized genes
Genes that have lost their functional gene products, for example, through accumulation of frameshifts or stop codons. They also arise when a gene is processed by a retrotransposon such that a portion of the mRNA transcript of a gene is reverse transcribed back to DNA and inserted into chromosomal DNA.

Synteny
Shared genomic organization between related species. It is usually seen as a shared relative order of genes or other functional elements on a portion of a chromosome.

Comparative genomics of ageing and cancer

Genomic and genetic approaches provide opportunities for unbiased discovery of genes, pathways and molecular mechanisms behind the exceptional longevity and cancer resistance observed in some rodents. Particularly appealing is the fact that these traits evolved independently, for example, in naked mole rats, blind mole rats and possibly some other species. Comparative genomics of rodents also benefits from the fact that these animals are among the best represented by completely sequenced genomes. Owing to their small body size (compared to most other mammalian orders), great diversity, abundance and availability of tissues, rodents have also been subject to many 'omic' approaches — most notably, transcriptome analyses. Finally, as mice and rats have short lifespans and high susceptibilities to cancer, rodents offer a direct experimental system to examine the predictions that arise from comparative genomic studies.

Strategies for comparative genomics. Analyses of rodent genomes and their associated biology, such as unique and common traits of the examined species, may proceed at several levels and use diverse approaches[55,56] (FIG. 3). Genome alignments, such as the University of California Santa Cruz (UCSC) genome browser, that include both closely related and distant species are helpful for comparative studies, as they provide both genome-wide and gene-centric views. One approach in comparative genomics is to search for patterns of lost genes and pseudogenized genes (that is, pseudogenes), which may provide information on the processes and systems that are no longer needed by the species of interest. For example, occupying the subterranean niche, naked mole rats lost many genes that are involved in visual function. It is also useful to analyse the lost genes and pseudogenes with regard to the pathways and systems in which they have biological roles. Analyses of synteny often accompany such searches to ensure that the identified pseudogenes correspond to functional orthologues in other species.

Another approach is to analyse positive selection, unique amino acid replacements or accelerated evolution[57]. Applied across whole mammalian genomes, these searches identify a multitude of genes (and sites within genes), and hence require robust filtering methods to remove false positives, such as genes with repeats. Further prioritization of the list of candidate genes and regions may be guided by pathway enrichment that underlies biology or other considerations.

A third approach is to integrate these analyses with omic data sets (for example, gene expression profiles, proteomics or metabolite profiles) and to contrast the species or lineages with broader groups of organisms, or to examine the consequences of system perturbations or interventions[58]. In this case, prioritization of molecular targets for further studies may be improved when several methods point to a particular gene, pathway or mode of regulation. The omic studies may also be appropriately designed to improve their impact. For example, studies on ageing and longevity may examine the omic data across lifespans or under conditions that affect lifespans.

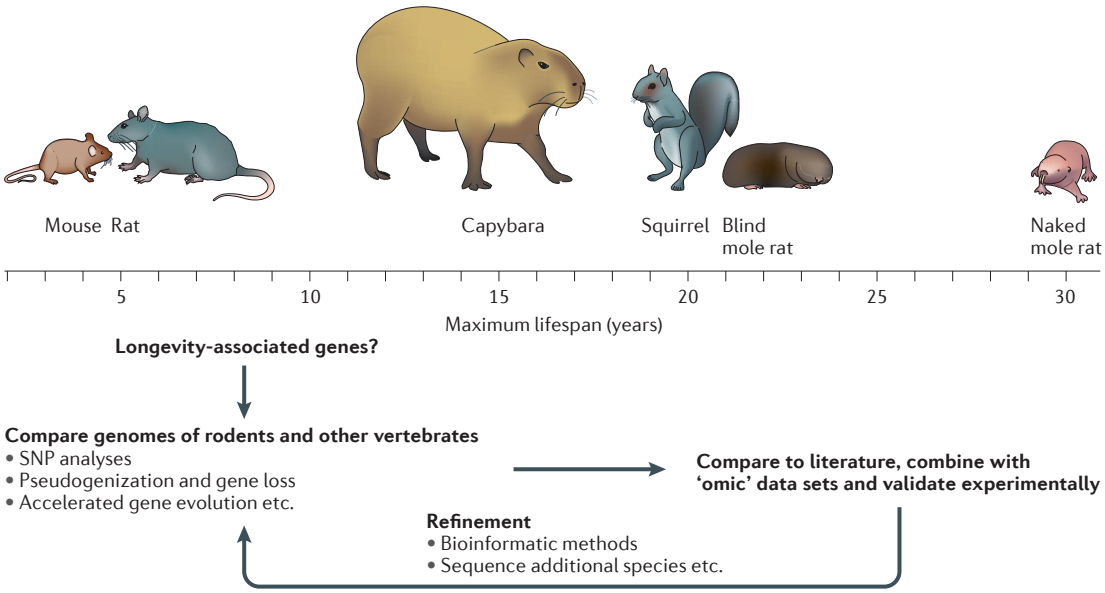

Figure 3 | **Comparative genomics of ageing.** Strategies for comparative genomic analyses of rodents are shown. Such analyses begin with the genomes of organisms with widely different lifespans and focus on genetic adaptations of long-lived species, such as the naked mole rat and the blind mole rat. These may lead to the identification of functionally relevant genes that contribute to the examined traits. For example, these approaches may uncover lineage-specific genetic changes that are associated with longevity and cancer resistance. In addition, the use of 'omic' approaches may support analyses across rodents, thereby characterizing common strategies used by these organisms to regulate species lifespan and cancer susceptibility. SNP, single-nucleotide polymorphism.

Box 2 | **Comparative genomics reveals longevity adaptations of the Brandt's bat**

The genome of an exceptionally long-lived mammal, the Brandt's bat, has recently been completed[64]. This mammal of 4–8 g can live for >40 years. Comparative genomic analyses revealed unique changes in the transmembrane domains of growth hormone (GH) receptor and insulin-like growth factor 1 (IGF1) receptor, which might have contributed to the evolution of long lifespan in the Brandt's bat and other microbats, and perhaps to the evolution of their small body mass. Interestingly, these genes are well known for their role in regulation of longevity. The IGF1–GH axis has been implicated in the extended lifespan of various dwarf mice and in the decreased incidence of age-related diseases in humans with Laron syndrome, which is characterized by GH receptor dysfunction. Of note is that this adaptation to longevity is distinct from those found in naked mole rats and blind mole rats.

Beyond studies on evolution of lifespan, microbats have recently been the focus of comparative genomic approaches that target other unique traits, such as echolocation, and these studies have identified the genes involved[64,65]. Another study used this approach to uncover genes involved in DNA repair and genome maintenance in microbats[68], whereas a study that examined placental mammals identified altered telomere maintenance genes in microbats[44].

Finally, another approach is to focus the searches on genes with known connections to the biological processes of interest[59]. For example, known ageing-related genes and cancer driver genes could be examined in the genomes of interest when one focuses on longevity and cancer. In such focused studies, it is easier to analyse the associated regulatory regions (such as promoters, enhancers, allosteric sites and untranslated regions), as well as gene variants. There is currently no universally accepted strategy to uncover genomic features that underlie biological functions, but methods are rapidly advancing, and there are already numerous available methods and approaches.

Genomics of the naked mole rat. The completed genome of the naked mole rat[55] represents the first case of an animal genome sequenced with the explicit purpose of explaining the exceptional longevity and cancer resistance. Comparative genomic analyses within vertebrates or mammals (including several rodents) revealed various genes and processes that may contribute to the unique traits of the naked mole rat. For example, these animals evolved a shortened version of the tumour suppressor $p16^{INK4A}$, which was found to contribute to early contact inhibition of naked mole rat fibroblasts[41]. The modified $p16^{INK4A}$ structure could make the cell cycle machinery more sensitive to hyaluronan-mediated contact inhibition, which is controlled by $p16^{INK4A}$. Another important protein that uniquely changed in the naked mole rat is the thermogenesis regulator mitochondrial brown fat uncoupling protein 1 (UCP1), which is altered at a conserved site that is regulated by fatty acids and nucleotides. Being poikilothermic, naked mole rats are unique among mammals in their inability to maintain a stable body temperature, and the detection of UCP1 as an altered protein is consistent with this phenotype[55]. However, additional studies are needed to determine how this change affects UCP1 activity and/or regulation. Pseudogenes of the naked mole rat are highly enriched for visual perception function, which is consistent with the strictly subterranean life of these animals[55]. Naked mole rats also pseudogenized both melatonin receptors and altered two proteins involved in telomere maintenance[55]. Although a causal role of these changes in longevity is unknown, the findings offer direct molecular targets for subsequent experimental analyses.

Genomics of the blind mole rat. A recent analysis of the whole-genome sequence of the blind mole rat showed that *Ifnb1* (which encodes interferon-β1) underwent a duplication event compared with the genomes of mice, rats and naked mole rats[60]. Other genes that underwent expansion in the blind mole rat include the *Mx1* genes from the interferon signalling pathway and several genes involved in regulation of cell death and inflammation: *Nfkb*, *Tnfrsf1a*, *Birc3*, *Fem1b* and *Aifm1*. Furthermore, three genes involved in necrosis and inflammation (*Tnfrsf1a*, *Tnfsf15* and *Nfkb1*) show evidence of positive Darwinian selection[60]. Together with the earlier work on the mutant form of p53 in this species (see above), these studies suggest that blind mole rats evolved a 'weak' p53 possibly as an adaptation to hypoxia. To compensate for the insufficient function of p53, blind mole rats then evolved a very efficient cancer resistance mechanism that relies on increased immunoinflammatory response through gene amplification in the interferon-β1 pathway.

Independent adaptations to subterranean life. The recently completed genome of the blind mole rat[60] allows comparisons between the two mole rat species, which independently evolved adaptations to subterranean life, long lifespans and resistance to cancer. The analysis revealed that clock circadian regulator (*Clock*) genes responsible for adaptation to darkness showed convergent evolution between these species[60]. *Scn9a* (also known as *Nav1.7*, which encodes a proton-gated nociceptor sodium channel) also shows convergent evolution, which may represent an adaptation to a high carbon dioxide environment by blocking pain induced by tissue acidosis[61,62]. However, no obvious common patterns of evolution of genes involved in cancer and ageing were identified. This suggests that these species evolved different mechanisms to achieve longevity and resistance to cancer, or that a more sophisticated data analysis involving a greater number of species is needed to obtain this insight.

Comparative genomics of rodents and other mammals. High-quality genome sequences are available for numerous short-lived mice and rats, and several additional rodent genomes have been sequenced for the species with short and intermediate lifespans[55,63]. There is no doubt that the availability of rodent genomes will

increase in coming years. For example, it would be useful to obtain genome sequences of the Damaraland mole rat, which is closely related to the naked mole rat. With these genomic resources, the longevity trait should be amenable to the analyses of convergent evolution of mammalian traits, as was recently shown by the studies of echolocation in bats and dolphins[64,65], adaptation to aquatic life in whales[66], horse evolution[67] and the comparative genomics across 29 mammals[63].

One hypothesis that can be more rigorously tested by genomic studies is whether species-specific enhanced longevity is related to superior genome maintenance efficacy, as suggested by the studies discussed above. Given their broad range of lifespans, rodent species offer a unique opportunity in this case. Indeed, with complete genome sequences available for many rodent species, it is possible to comparatively analyse genome maintenance genes in relation to lifespan. Beyond rodents, a recent analysis of two bat genomes showed that the DNA repair and DNA damage signalling genes *ATM*, *TP53*, *RAD50*, *PRKDC* and *XRCC5* are under selection in bats, which suggests that genome maintenance systems are under selective pressure in longer-lived species[68]. It would be interesting to determine whether such repair genes have unique signatures in long-lived species. Both germline and somatic mutation frequency can now be analysed directly using whole-genome sequencing of parent–offspring trios[69] and single somatic cells[70]. The identification of genes and pathways that are responsible for more efficient genome maintenance in long-lived species may help to develop strategies to increase genome stability in humans.

An example of the application of comparative genomics for revealing the molecular basis of mammalian traits is the evolution of echolocation in mammals[64,65]. Similar comparative genomic approaches can be applied to longevity and cancer resistance. In addition, these approaches can be extended to the analyses of gene expression profiles[71], proteomics, ribosome profiles[72] and metabolite profiles across the group of mammals. In this case, in contrast to species- or lineage-specific

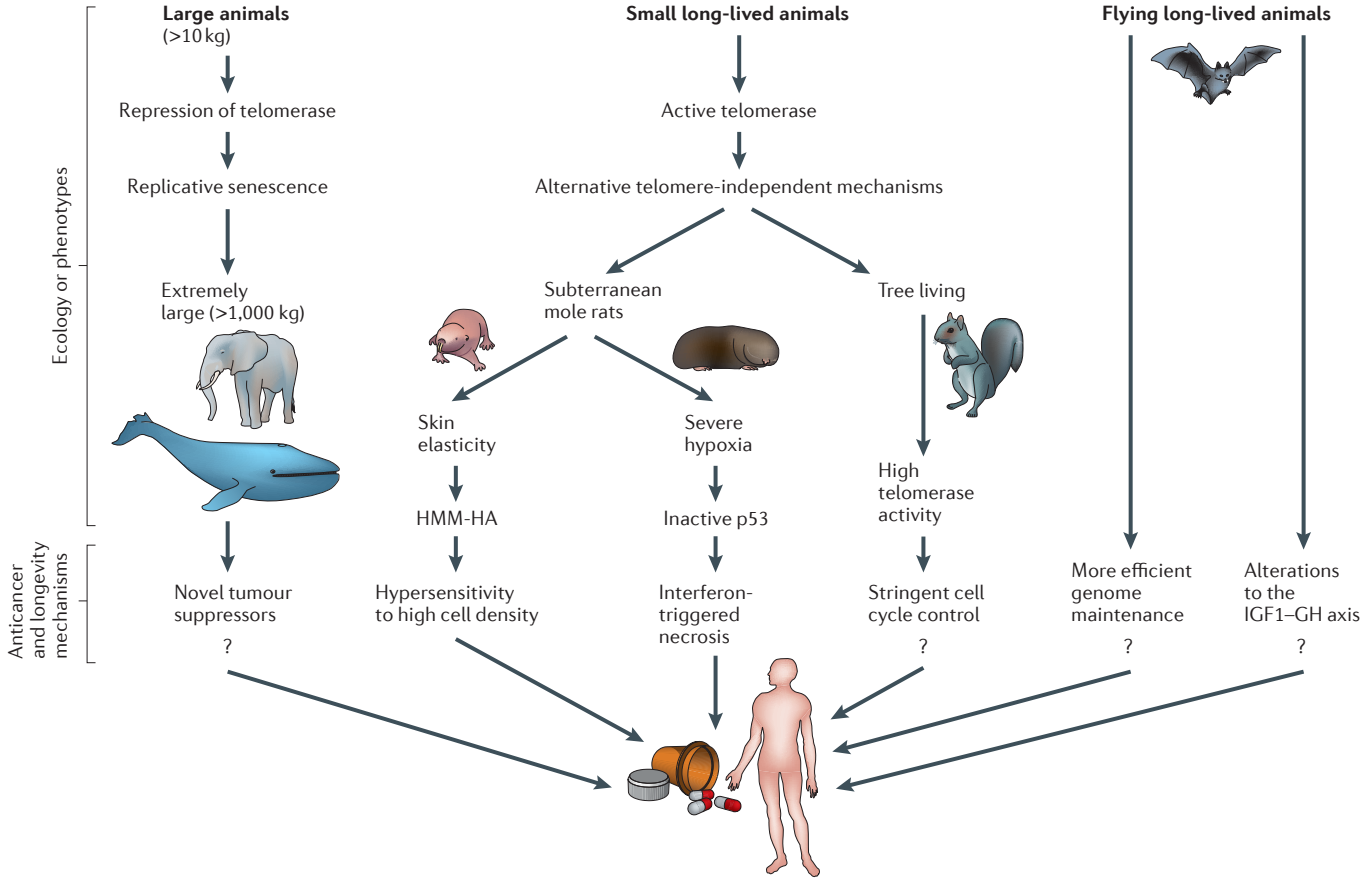

Figure 4 | **Lineage-specific mechanisms of longevity and cancer resistance that evolved in species with diverse ecology could be adapted to benefit human health.** The upper panel depicts three groups of species with an ecology or a phenotype that is associated with the evolution of longevity and anticancer adaptations, which are shown in the lower panel. Large body size (>10 kg) is associated with the evolution of replicative senescence (left panel). The giant mammals such as elephants and whales are hypothesized to evolve novel tumour suppressor mechanisms that are absent in smaller species, including humans. Small long-lived species are characterized by diverse anticancer adaptations, such as high-molecular-mass hyaluronan (HMM-HA), interferon-triggered necrosis and stringent cell cycle control (middle panel). Long-lived bats possibly evolved more efficient DNA damage repair systems, as well as alterations in the insulin-like growth factor 1 (IGF1)–growth hormone (GH) axis (right panel). Question marks indicate adaptations for which exact molecular mechanisms are still unknown. These longevity and anticancer mechanisms hold promise to benefit human health.

adaptations highlighted above using the examples of the naked mole rat, the blind mole rat and the Brandt's bat (BOX 2), longevity or cancer resistance traits across multiple clades can be characterized. These studies may be especially informative about the coordinated changes in the levels and activity of cellular components, such as transcripts, proteins and metabolites. These components can be further assessed at the level of pathways and networks, and integrated into cellular models of extended longevity. Overall, future studies may reveal both common adaptations that contribute to the longevity trait in many species- and lineage-specific adaptations, as exemplified by UCP1 in naked mole rats, interferon in blind mole rats and the IGF1–GH axis in microbats (BOX 2).

Conclusions

Increased insights into the molecular mechanisms at the interface of ageing and age-related disease, such as cancer, are crucial for making further progress in improving human health. In this Review, we show that comparative studies of rodents are highly informative in this respect and have already led to a new understanding of how cancer can be suppressed in an animal. The paradigm that emerges from these initial studies suggests that species ecology contributed to the evolution of lifespan and body mass, which have collectively driven the evolution of distinct tumour suppressor strategies in different species, such as the contact inhibition mediated by high-molecular-mass hyaluronan in the naked mole rat and the interferon-mediated cell death mechanism in the blind mole rat (FIG. 4).

Interestingly, other species within the rodent clade are likely to harbour additional novel tumour suppressor mechanisms. For example, the grey squirrel is a long-lived, diurnal rodent that lives above ground. In tissue culture, grey squirrel cells do not secrete high-molecular-mass hyaluronan but instead show extremely high telomerase activity and slow proliferation[15], which indicates the presence of a cell cycle control mechanism that has yet to be discovered. Such a mechanism, which prevents telomerase-positive cells from undergoing malignant transformation, can potentially be exploited to prevent cancer formation in cells with naturally active telomerase, such as human stem cells and the germ line. Another example of species with unique adaptations is animals with extremely high body mass. As the studies of rodents have shown, body mass above ~10 kg is associated with telomerase repression in somatic cells and with replicative senescence. It is plausible that species with even greater body mass, such as elephants and large whales, have unique anticancer adaptations that have yet to be discovered. As unique species ecology determines the evolutionary path for longevity and anticancer adaptations, humans would not be expected to possess all of these mechanisms and could benefit from them. Thus, the study of such species-specific adaptations and the subsequent application of these strategies in humans open new avenues for cancer prevention and lifespan extension. One successful example of this strategy is the invention of antibiotics, in which an antibacterial mechanism that evolved in fungi is used to benefit humans. This may be a powerful alternative to the studies focusing on conserved mechanisms of longevity that operate across rodents and beyond. For example, high-molecular-mass hyaluronan can be directly administered to humans, and pharmacological inhibitors of HYAL enzymes (which are responsible for hyaluronan degradation) could be developed to increase the levels and molecular mass of endogenous hyaluronan.

Recent advances in DNA sequencing technologies have enabled comparative genomics by sequencing whole genomes of organisms, and these studies indicate that multiple DNA damage and repair genes are under selection in long-lived species[73]. The understanding of how more efficient genome maintenance is achieved may open avenues for improving DNA repair and genome stability in humans. Comparative genomics also offers an unbiased view on other adaptations of long-lived animals and their unique genetic strategies. The first three genomes of exceptionally long-lived mammals — the naked mole rat, the blind mole rat and the Brandt's bat (BOX 2) — revealed different lineage-specific mechanisms that are used by these animals in lifespan control. The availability of additional genomes of long-lived animals will undoubtedly offer additional insights into the genomics of longevity and cancer resistance.

In conclusion, it is important to diversify the range of standard model organisms and to include various long-lived and cancer-resistant species. This is crucial because the short-lived and cancer-prone mice and rats may be missing the mechanisms for longevity and cancer resistance. Future studies that are focused on comparative genomics and in-depth molecular studies of long-lived rodent species hold promise to drive the fields of ageing and cancer forward.

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