

# Evolution of cancer suppression as revealed by mammalian comparative genomics

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Cancer suppression is an important feature in the evolution of large and long-lived animals. While some tumor suppression pathways are conserved among all multicellular organisms, others mechanisms of cancer resistance are uniquely lineage specific. Comparative genomics has become a powerful tool to discover these unique and shared molecular adaptations in respect to cancer suppression. These findings may one day be translated to human patients through evolutionary medicine. Here, we will review theory and methods of comparative cancer genomics and highlight major findings of cancer suppression across mammals. Our current knowledge of cancer genomics suggests that more efficient DNA repair and higher sensitivity to DNA damage may be the key to tumor suppression in large or long-lived mammals.

## Addresses

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## Introduction

A major goal of most genomic research is to translate knowledge of life's genetic code into positive clinical outcomes for human diseases, including cancer. Cancer results from the somatic accumulation of mutations in cells [1], and a number of studies have shown mutations in common genes recurring in many different types of cancer [2]. Such genetic knowledge arms the biomedical fields with clues to the origins of cancer, how to better treat cancer, and how to prevent cancer. The rapid and recent growth in the field of cancer genomics has been

fueled by the falling cost of DNA sequencing. For instance, the human genome was sequenced in 2001 [3], and as of 2016 the UCSC Human Genome Browser has grown to now host 48 placental mammalian genomes alone [4] (see Table 1). The wealth of genomes from different species can contain valuable information about the link between genotypes and phenotypes on an evolutionary scale. Over evolutionary time, mutations that occur in the DNA sequences of genes may be subjected to natural selection, and beneficial mutations accumulate and contribute to species' adaptations at the phenotypic level. Thus, functional consequences of many mutations in cancer may also be predicted using multi-species genomic comparisons [5] (see Table 1 for current comparative genomics resources). In addition, it is likely that over evolutionary time natural selection has equipped many species with the means of tumor suppression in order to maintain cellular functioning and organismal fitness [6,7]. These adaptations may be encoded in the genomes of species where tumor suppression has evolved. Here, we highlight species whose biology evolved mechanisms of tumor suppression, and discuss comparative genomic efforts to understand the basis of this tumor suppression.

## Totally naked, nearly blind and (almost) cancer-free

The key to understanding cancer suppression mechanisms in nature is to target species in which cancer suppression is likely to have evolved. Obvious target species are those with low or negligible reported rates of cancer. While obtaining accurate estimates of cancer in wild and/or zoo populations remains challenging [8], some well-known examples exist. One such mammal is the African naked mole rat (*Heterocephalus glaber*), a mouse-sized rodent with a highly unique set of adaptations among mammals (Figure 1). Naked mole rats spend their entire lives underground and are eponymously hairless, have poor visual acuity, are eusocial in which a reproductive “queen” suppresses the reproduction of colony members, and can live up to 30 years. Despite decades of study of captive colonies and almost 400 necropsies, there have been no reported cases of spontaneous neoplasms in this species until very recently [9,10].

Despite being of similar size, naked mole rats have at least seven times the lifespan of a mouse, and exhibit negligible senescence during that time [11]. This suggests naked mole rats have evolved anti-aging defenses, which may be key to understanding cancer, a common

Table 1

## Current resources for studying cancer comparative genomics

Genomic databases	UCSC Human Genome Browser: visualize and browse genomes [4]	<a href="https://genome.ucsc.edu/">https://genome.ucsc.edu/</a>
	Ensembl: genomes and species tree [45]	<a href="https://ensembl.org/">https://ensembl.org/</a>
Multiple sequence alignment software	GenBank: open access sequence database [44]	<a href="https://www.ncbi.nlm.nih.gov/genbank/">https://www.ncbi.nlm.nih.gov/genbank/</a>
	MUSCLE: tool to align multiple sequences [52]	<a href="http://www.ebi.ac.uk/Tools/msa/muscle/">http://www.ebi.ac.uk/Tools/msa/muscle/</a>
	MAFFT: tool to align multiple genomic sequences [53]	<a href="http://mafft.cbrc.jp/alignment/software/">http://mafft.cbrc.jp/alignment/software/</a>
	PRANK: tool to align multiple genomic sequences [54]	<a href="http://www.ebi.ac.uk/goldman-srv/prank/">http://www.ebi.ac.uk/goldman-srv/prank/</a>
Phylogenetic information	TimeTree: database of divergence times [55]	<a href="http://www.timetree.org/">http://www.timetree.org/</a>
	Mammalian supertrees: evolutionary trees [39]	Fritz <i>et al.</i> [39]
Analysis tools	BLAST—basic local alignment search tool [46]	<a href="https://blast.ncbi.nlm.nih.gov/">https://blast.ncbi.nlm.nih.gov/</a>
	Codeml—test for positive selection on phylogenies using multiple sequence alignments [56]	<a href="http://abacus.gene.ucl.ac.uk/software/paml.html">http://abacus.gene.ucl.ac.uk/software/paml.html</a>
	PanTHERIA: contains trait data on extant and recently extinct mammals [57]	<a href="http://esapubs.org/Archive/ecol/E090/184/default.htm">http://esapubs.org/Archive/ecol/E090/184/default.htm</a>
Life history databases	Amniote: contains trait data on birds, mammals and reptiles [58]	<a href="http://www.esapubs.org/archive/ecol/E096/269/#data">http://www.esapubs.org/archive/ecol/E096/269/#data</a>
	AnAge: database of animal aging and longevity [40]	<a href="http://genomics.senescence.info/species/">http://genomics.senescence.info/species/</a>
Cancer gene databases	COSMIC: catalog of somatic mutations in cancer [59]	<a href="http://cancer.sanger.ac.uk/cosmic">http://cancer.sanger.ac.uk/cosmic</a>
	TSGene: tumor suppressor gene database [60]	<a href="https://bioinfo.uth.edu/TSGene/">https://bioinfo.uth.edu/TSGene/</a>

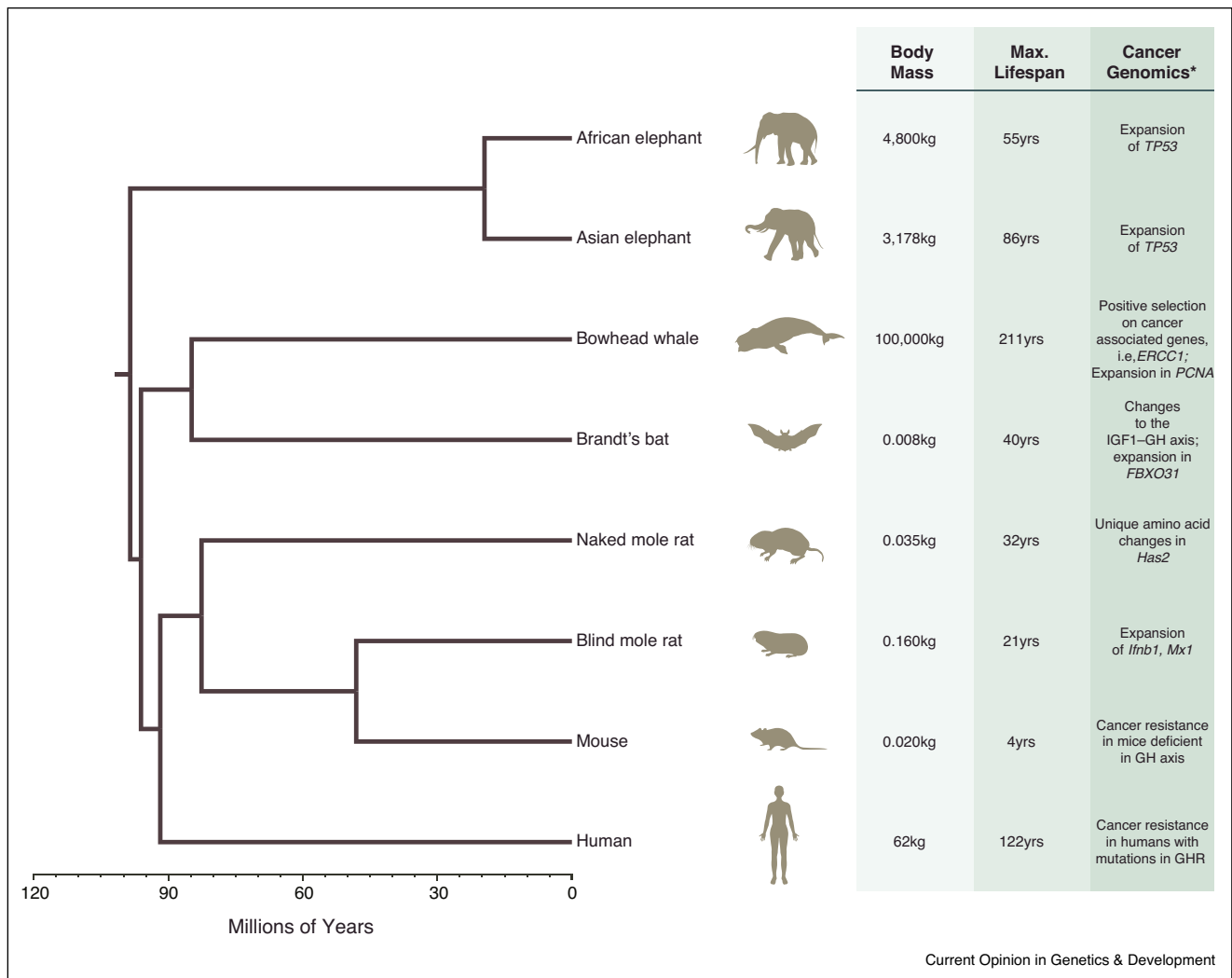
disease of aging. There has been careful genomic and functional work to demonstrate the cancer resistance mechanism in the naked mole rat. Early comparative cellular assays demonstrated that naked mole rat fibroblasts stop dividing at much lower densities than mouse fibroblasts, suggesting naked mole rat cells are extremely sensitive to early contact inhibition [12]. It was later found that hypersensitivity to contact inhibition in naked mole rats is made possible by the secretion of an unusually high-mass hyaluronan [13<sup>•</sup>], which accumulates abundantly in naked mole rat cells caused by decreased activity of hyaluronan-degrading enzymes.

The genome of the naked mole rat was sequenced in 2011 [14], and its analysis led to insights into the molecular mechanisms underlying the evolution of both longevity and cancer resistance. For instance, genes which have undergone positive selection (Box 1) along the naked mole rat lineage include *TEP1* (*telomerase associated protein 1*), which encodes a telomerase component, and *TERF1* (*telomeric repeat binding factor*) that, along with another positively selected gene *TOP2A* (*DNA topoisomerase II alpha*), contributes to the shelterin complex required to protect telomere length [15] and integrity [16]. Thus, comparative genomic analyses (highlighted in Table 1) support divergent telomerase activity (Box 2) in naked mole rats with respect to other mammals, and provides an additional functional hypothesis for increased longevity and cancer resistance in this species. The naked mole rat genome also revealed additional evidence for early contact inhibition. The p16<sup>Ink4a</sup> transcript required for early contact inhibition in the naked mole rat consists of three exons in both mouse and naked mole rat; however, there are premature stop codons in the naked mole rat's second exon and sequence similarity to mouse is low in the third exon, despite the apparent functional preservation of the

protein [14]. In addition, a unique genetic sequence in naked mole-rats for *hyaluronan synthase 2* (*HAS2*) was found, in contrast to the high degree of conservation at this locus across mammals [13<sup>•</sup>], supporting the hypothesis that the high-mass hyaluronan is involved in early contact inhibition. While the naked mole rat genome (<http://naked-mole-rat.org/>) may continue to provide insights into cancer resistance [17], a recent reanalysis found that the fragmentary nature of the assembly, including missing genes and imprecise annotations, require at least some caution with the interpretation of results [18]. As with all genomic assemblies, additional sequencing and annotation will continue to improve the quality of the genome, while experiments in the laboratory will be needed to confirm the functional significance of genomic changes.

Another cancer-resistant rodent is the blind mole rat (genus *Spalax*, Figure 1) [19], which is phylogenetically distant from the naked mole rat (Figure 1) and suggests alternative cancer suppression mechanisms evolved independently in these lineages. Surprisingly, the blind mole rat has a mutation in a highly conserved region of *TP53*, which is a well-known tumor suppressor gene (Box 2). This mutation is found frequently in human tumors [20]. Functional assays demonstrate blind mole rat fibroblasts secrete IFN- $\beta$  and can induce rapid cell death by necrosis [21]. Whole genome analysis of the blind mole rat found a duplication event in the gene *IFNB1* and evidence for positive selection in genes involved in necrosis and inflammation [22]. Both functional and genomic analyses of the blind mole rat suggests cell cycle control (via cell death) is the key to cancer resistance in this taxon. Ongoing work in both the blind and naked mole rats will help contribute to our understanding of the evolution of cancer resistance and perhaps provide insights that can one day help human patients.

Figure 1



Phylogeny of the taxa discussed in the context of cancer comparative genomics. Phylogenetic relationships and divergence times were obtained from an extant mammalian supertree [39]. Branch lengths represent million of years. Data on lifespan and body mass were collected from the AnAge database [40] and primary literature [23,26]. Column illustrated on the far right represents the current summary of cancer genomics across mammals. \*This summary reports the major findings and a fully comprehensive report can be found in the primary literature [13\*,22,25\*,26,28,30\*\*,41,42].

**Longevity, genome maintenance and cancer suppression**

Organisms with the ability to renew somatic tissue needed to evolve tumor suppressor mechanisms to regulate, control, and coordinate cellular proliferation, and thereby avoid the uncontrolled cell growth of cancer. Additionally, organisms with extended lifespans are at risk for higher accumulation of somatic mutations over the decades of cellular renewal (for longevity and body mass resources see Table 1). According to life history theory, in low extrinsic mortality conditions selection favors genes that result in a slow life history strategy. As such, genome maintenance systems are under selective pressure in longer-lived species and have the

potential to reduce this mutational accumulation [23]. Indeed, when examining proteins under accelerated evolution in long-lived mammalian lineages (n = 36), many proteins involved in DNA damage repair and response pathways, such as *DDB1* (damage-specific DNA-binding protein 1), *SMC1A* (structural maintenance of chromosomes 1A), and *CAPNS1* (calpain small subunit 1), show support for positive selection [24].

Another important way species can evolve redundant checks on neoplastic progression is the duplication of tumor suppressor genes (see cancer gene resources highlighted in Table 1). This is based on the assumption that gene duplication can be a source of functional

**Box 1 Comparative genomic methods.**

Comparative genomic methods primarily rely on a key assumption: homologous and functional genomic regions should be evolutionarily conserved across species. This is caused by purifying selection, which removes deleterious alleles that may reduce organismal fitness in populations. In contrast, non-functional genomic regions are thought to evolve via the process of neutral evolution, accumulating DNA substitutions as a linear function of time, without the variation-removing effects of selection. Therefore, we expect that between two species, functional homologous regions will be more similar to each other in terms of DNA sequence than non-functional ones, and that in non-functional regions the amount of sequence divergence will be correlated to the evolutionary distance between the two species.

Most genes owe their existence to gene duplication, a common mechanism in molecular evolution which can result from events such as recombination and retrotransposition [43]. When a gene duplicates, it creates a paralogous copy. A paralog may obtain a new function (neofunctionalization), or may maintain the original function with its parent copy (subfunctionalization). Gene copies across species derived from the same ancestral gene are orthologs. Orthologous genes often maintain similar functions across species, and the establishment of cross-species orthology is an important task for comparative genomics.

The most common method in comparative genomics is to ascertain sequence similarity to entries in databases such as GenBank [44] or Ensembl [45] using search algorithms such as BLAST [46] (Table 1). The coding regions of a gene are usually aligned to the ortholog from one or more species, and the sequence alignment can be searched for mutations of functional importance. Because of the degeneracy of the genetic code, synonymous substitutions are DNA mutations in a codon that will not change the amino acid sequence. Non-synonymous mutations are DNA substitutions that will change the amino acid sequence, and may alter protein structure and have a functional consequence. While the vast majority of non-synonymous mutations are deleterious, causing harm to the function of the gene, there are instances when the change is beneficial, and positive selection will favor the mutation. A common method of detecting positive selection using species comparisons measures the proportion of sites in a gene that contain either non-synonymous (dN) to synonymous (dS) substitutions, or dN/dS [47] (Table 1). Generally, when  $dN/dS < 1$  there are more synonymous mutations and purifying selection is inferred. When  $dN/dS > 1$  there are more non-synonymous mutations and therefore positive selection must have occurred. When  $dN/dS = 1$ , there are an equal number of synonymous and non-synonymous substitutions and the gene is undergoing neutral evolution. The latter usually occurs when paralogous gene copies that have lost their functionality are being studied.

divergence providing additional control of neoplastic progression (Box 1). A query of tumor suppressor genes across 36 mammalian genomes from Ensembl (Table 1) yielded 19 genes which exist as one copy in human but five or more paralogs in other species [25\*]. The tumor suppressor gene *FBXO31* had 63 copies in the microbat *Myotis lucifugus*, mirroring the 57 copies found in the Brandt's bat (*M. brandtii*) genome [26], and suggesting that the genomes of these small bats encode cancer suppression mechanisms caused by the heightened cancer risk associated with their longevity (Figure 1). Additionally, bats have a unique feature among mammals, as they are the only mammalian species capable of flying. Flying may reduce predation in this species, conferring to the bat's incredible lifespan, however flight increases the

**Box 2 Cancer suppression mechanisms.**

Tumor suppressor, *TP53*, is one of the most commonly mutated genes in human tumors. Nicknamed "guardian of the genome", *TP53* is activated in response to DNA damage and can halt the cell cycle. Depending on the type of DNA damage, the p53 protein can activate pathways involved in apoptosis, cell-cycle arrest or senescence. Individuals with the genetic disease Li-Fraumeni syndrome (LFS) have only one functional *TP53* allele, whereas healthy individuals have two alleles. Patients with LFS have more than a 90% lifetime risk of getting cancer [41]. Mice genetically altered to constitutively express a truncated form of *TP53* were shown to have enhanced resistance to tumors. Consequently, these mice also displayed early onset of traits associated with aging—highlighting the potential importance of *TP53* in regulating not only the lifespan of cells but of the organism as a whole [48]. Interestingly, mice carrying additional copies of *TP53* were shown to have an enhanced response to DNA damage without the undesirable effects of premature ageing [49]. The massive expansion of the *TP53* copy numbers during the evolution of the elephant lineage suggests that supernumerary copies of *TP53* regulate apoptosis and act as a tumor suppressor mechanism not only in artificial mouse experiments but in natural systems as well [30\*\*,31\*\*]. Interestingly, the cancer-resistant blind mole rat (genus *Spalax*) harbors a mutation in *TP53*, but likely caused by its uniquely hypoxic subterranean habitat. It may be that this rodent has evolved novel hypoxia-independent mechanisms that regulate cellular growth [22].

Another important cancer suppression mechanism is telomere length and telomerase activity, both of which regulate cellular growth. Telomeres cap the end of chromosomes and progressively shorten during cellular proliferation. Telomerase, a ribonucleoprotein enzyme, provides a cell with longer replicative capabilities by adding a telomere repeat sequence to the 3' end of the DNA. Telomerase activity is suppressed in somatic tissues of humans. Many tumors show reactivation of telomerase, which can then maintain the telomere length of the neoplastic cell. A comparative study examining 15 different members of the Rodentia clade found repressed telomerase activity co-evolved with body mass, not lifespan [50]. Small, long-lived rodents constitutively express telomerase [51], suggesting telomerase independent mechanisms of regulating cellular growth and cancer suppression.

metabolic demands of the species and can produce harmful by-products, such as reactive oxygen species (ROS). ROS damages DNA, thus genetic changes in bat evolution that would limit the accumulation of DNA damage would be advantageous in this taxa to avoid cancer. Accordingly, there is a concentration of positively selected genes that encode for proteins involved in DNA repair and DNA damage signaling (e.g., *ATM*, *TP53*, *RAD50*, *PRKDC* and *XRCC5*) and the innate immune system on the lineage leading to bats [27].

With an extraordinary lifespan of over 200 years, the bowhead whale is the longest living mammal. In 2015, the Bowhead Genome Resource sequenced the genome and two transcriptomes of *Balaena mysticetus* (<http://www.bowhead-whale.org>) [28] (Figure 1). Results from this sequencing effort found numerous aging and cancer-associated genes that were observed among 420 predicted orthologs with minke whale (*Balaenoptera acutorostrata*) with a  $dN/dS > 1$  (see Box 1), such as *ERCC1* (excision



*repair cross-complementing rodent repair deficiency, complementation group 1*), a gene which functions as part of the DNA repair pathway. In addition to genes under positive selection, analysis of the bowhead whale genome revealed duplications in genes important to cancer pathways, including a duplication of the gene *PCNA* (*proliferating cell nuclear antigen*) which is important in DNA repair, and a duplication in *LAMTOR1* (*late endosomal/lysosomal adaptor*) which is part of the mTORC1 activation pathway. mTORC1 is a nutrient and energy sensor of protein synthesis and thus an important player in cellular growth. Functional studies are now required to understand and validate the role of these specific genes in helping these long-lived species.

### Bigger and better

Larger and longer-lived mammals such as whales and elephants have lower cancer rates than would be expected given the number of cells and cell divisions that occur over an increased lifespan, a phenomenon termed Peto's Paradox [6,29<sup>\*</sup>]. While Peto's Paradox has been theoretically and mathematically explored [25<sup>\*</sup>,29<sup>\*</sup>], evidence of actual cancer incidence rates in mammals has just recently been published [30<sup>\*\*</sup>]. Empiric analysis of cancer incidence from necropsy data in 36 different mammalian species demonstrate no association between cancer incidence and body mass and/or lifespan. The largest living land mammal is the African savannah elephant (*Loxodonta africana*), which was shown to have much lower cancer rates than humans (<5% vs. 11–25%) despite being 100× larger [30<sup>\*\*</sup>]. This is consistent with a model that predicted elephants should have the highest level of cancer suppression across a panel of four non-human mammals (the second highest was naked mole rat) [29<sup>\*</sup>].

Comparative genomic efforts have also provided evidence of cancer suppression in elephants (Figure 1). For instance, 12 copies of *TP53* (Box 2) were revealed by *in silico* analysis of the Ensembl version of the African elephant genome [25<sup>\*</sup>]. Subsequent analyses included the cloning and sequencing of these copies as well as the mapping of high-throughput sequence reads to the genome assembly, yielding 20 copies of *TP53*, including 19 retrogenes [30<sup>\*\*</sup>,31<sup>\*\*</sup>]. Functional molecular analyses provided evidence that at least some of the *TP53* retrogenes are expressed and translated [30<sup>\*\*</sup>], and that the *TP53* retrogene proteins control the apoptotic response to DNA damage [30<sup>\*\*</sup>,31<sup>\*\*</sup>] by disrupting MDM2-related degradation of normal *TP53* signaling [31<sup>\*\*</sup>]. The expansion of *TP53* copy number and increased apoptotic response to DNA damage is thought to be correlated with body size evolution along the elephant branch of the mammalian phylogenetic tree (order Proboscidea), revealed through the mapping of high-throughput sequence reads derived from ancient DNA samples. For instance, there were between three and eight *TP53* retrogenes in the genome of the more primitive and

smaller extinct American mastodon (*Mammot americanum*) genome [31<sup>\*\*</sup>], approximately 14 in the extinct woolly mammoth (*Mammuthus primigenius*) and extinct Columbian mammoth (*Mammuthus columbi*) genomes [31<sup>\*\*</sup>], and between 12 and 20 *TP53* retrogenes in the extant Asian elephant (*Elephas maximus*) genome [30<sup>\*\*</sup>,31<sup>\*\*</sup>]. These complementary studies suggest a step-wise coevolution towards both larger body sizes and greater cancer suppression in proboscideans.

### The advantages of being small(er)

Selection works at the population level and while Peto's paradox may exist across organisms, evidence suggests it does not hold true at the species level. Smaller individuals within a population may have the advantage of cancer defense mechanisms selected at population level while lowering cancer risk (*e.g.*, fewer cells, lower levels of growth hormone). Indeed, there is some evidence to suggest taller humans are at a heightened risk for certain cancers [32], while smaller humans may be protected. One particular example of body size and cancer protection is the case of Laron syndrome in humans. Laron syndrome is caused by deletion or mutations of the growth hormone receptor (GHR) gene. The growth hormone (GH)/IGF1 axis is important in regulating body size and individuals with Laron syndrome are characterized by dwarfism [33]. Currently, only one non-lethal case of malignancy has been reported for individuals with mutations in the growth hormone receptor [34]. Additionally, experimentally induced GHR mutations have been associated with increased resistance to cancer in mouse models [35]. Further, the Brandt's bat genome discovered a deletion in a highly conserved region of GHR and multiple unique changes in the IGF1R gene. Gene expression data in the Brandt's bat showed similar patterns of expression to the GHR mutant mice [26]. These data suggest anti-cancer advantages to small body size, with emphasis on the control and decreased expression of the GHR/IGF axis. While we earlier discussed the potential advantages of studying lineages that have evolved large body size, efforts to study cancer resistance in lineages with evidence of a decrease in body size also may be advantageous.

### Conclusions and future directions

Evolution has, over millions of years, inherently explored cancer defense mechanisms with each new multicellular species. A comparative genomics approach to studying cancer can provide insight into both the common cancer suppression pathways of large and/or long-lived animals and highlight lineage- or clade -distinct adaptations. By identifying how certain species evolved mechanisms of cancer suppression, we can begin to identify which underlying molecular targets are potentially more exploitable for human cancer prevention and treatment.

Current sequencing efforts of cancer genomes has revealed that many kinds of cancers contain mutations

in DNA damage response pathways and genes, including the p53 pathway. Thus, it has become readily apparent that cancer is often caused by mutations that increase genomic instability. Many of the species discussed here contain molecular adaptations (via gene expansion or adaptive evolution) integral to the increased maintenance of genomic stability and control of cell proliferation. Our current understanding of cancer comparative genomics highlights four important mechanisms important for clinical intervention and developmental therapeutics: (1) contact inhibition, (2) telomere length and integrity, (3) more efficient DNA repair, and (4) a higher sensitivity to DNA damage.

Lastly, while this review focuses on comparative genomics of placental mammals, cancer is a problem that all multicellular organisms must solve [7<sup>\*</sup>]. However, we currently have a limited understanding on the rate of occurrence and types of cancers in other species, especially for amniote taxa outside of the mammalian orders [8]. Surveys of cancer reports from zoological data, field work, and primary literature can provide a comprehensive guide to which species – and genomes – to study. For instance, at the San Diego Zoo the incidence of cancer was found to be lower in birds than mammals (1.9% versus 2.8%, respectively) [8], suggesting the trove of recently sequenced avian genomes [36] may provide clues to cancer suppression. Also, the slow developmental rates and long lives of large reptiles suggest that they may hold further clues for cancer suppression [29<sup>\*</sup>], and the rapid development of genomic resources for reptiles [37] will provide ample opportunity to study genomic mechanisms of cancer suppression in ectothermic amniotes. In addition to studying cancer resistant wild taxa, we can also gain important insights into lineages that have a high prevalence for cancer, such as the domesticated dog [38]. Importantly, these future studies must be followed by functional experiments in the laboratory to truly understand evolution's mechanisms and strategies for cancer resistance. It is hoped that such studies may one day benefit human patients through the practice of evolutionary medicine. As our genomic technologies continue to improve, it will be necessary to expand analyses to other species and continue to discover both shared and novel adaptations to cancer defense mechanisms across the tree of life.

### Conflict of interest statement

JDS is Co-Founder and Shareholder of PEEL Therapeutics, Inc. and [ItRunsInMyFamily.com](http://ItRunsInMyFamily.com).

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have been highlighted as:

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paradox. They also analyze the African elephant genome and discover 20 copies of *TP53*, amplified via retrotransposition, which are transcriptionally active based on RT-PCR. Finally, they demonstrate that elephant cells undergo p53-mediated apoptosis much more readily than do human cells. Thus, this is the first published study to provide genomic and functional evidence of cancer resistance in elephants.

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