Review

Peto's Paradox: evolution's prescription for cancer prevention

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The evolution of multicellularity required the suppression of cancer. If every cell has some chance of becoming cancerous, large, long-lived organisms should have an increased risk of developing cancer compared with small, short-lived organisms. The lack of correlation between body size and cancer risk is known as Peto's paradox. Animals with 1000 times more cells than humans do not exhibit an increased cancer risk, suggesting that natural mechanisms can suppress cancer 1000 times more effectively than is done in human cells. Because cancer has proven difficult to cure, attention has turned to cancer prevention. In this review, similar to pharmaceutical companies mining natural products, we seek to understand how evolution has suppressed cancer to develop ultimately improved cancer prevention in humans.

The evolutionary theory of cancer

Cancer (see Glossary) is a consequence of multicellularity and a striking example of multilevel selection. The theory of cancer initiation and progression is deeply rooted in evolutionary and ecological concepts [1]. Cancer develops through somatic evolution, with genetic and epigenetic instability generating fitness variation among the cells in a body (Box 1). Selection at the level of organisms has led to the evolution of tumor suppressor mechanisms, such as cell cycle check points and apoptosis, which act as safeguards to prevent somatic mutations from propagating in the cell population. Nonetheless, cancer occurs at astonishingly high rates and can be responsible for 20–46% of total deaths in multicellular animals ranging from mollusks to mammals [2].

Peto's paradox

The challenge of suppressing somatic evolution dramatically increases with larger bodies and longer lifespans. Because cancer develops through the accumulation of mutations, each proliferating cell is at risk of malignant transformation, assuming all proliferating cells have similar probabilities of mutation. Therefore, if an organism has more cells (i.e. more chances of initiating a tumor), the probability of developing cancer should increase. Similarly, if an organism has an extended lifespan, its cells have more time to accumulate mutations. Because the probability of carcinogenesis is an increasing function of age [3], the lifetime risk of an organism for developing cancer should also scale with its lifespan. It is well known that larger organisms generally have longer lifespans [4], which exacerbates this problem.

There appears to be no correlation between body size, longevity and cancer across species and the absence of such a relationship is referred to as Peto's paradox [2,5]. Cancer rates across multicellular animals only vary by

Glossary

Angiogenesis: the process of growing new blood vessels. The size of a tumor is limited by the diffusion distance of oxygen and glucose before angiogenesis.
Angiogenic cell: a cell producing factors to induce angiogenesis.
Apoptosis: programmed cell death.
Cancer: a disease defined by the uncontrolled growth of abnormal cells that have the ability to invade other tissues or spread to a new part of the body.
Crypt: a well-like structure of epithelial cells. Stem cells remain at the base and, as cells differentiate, they move up the walls toward the top layer of the tissue. The surface of the intestine is made up of a sheet of crypts.
Haploinsufficient gene: a gene in a diploid organism that requires both alleles to be fully functional to exhibit a normal phenotype. A mutation in one allele will result in an abnormal phenotype.
Hras1: a proto-oncogene that encodes the Hras11 protein, which promotes cell growth and division. It is frequently mutated in cancers (GenBank Accession AY373386).
Late stage: cancer that has metastasized, requiring systemic therapy for treatment, rather than surgical excision.
Malignancy: synonymous to cancer.
Malignant transformation: the process through which normal cells become cancerous.
Metastasis: the spreading of cancerous cells from the initial tumor to a new location and/or tissue in the body.
p16 (CDKN2A): a tumor suppressor protein encoded by the cyclin-dependent kinase inhibitor 2A gene that regulates the cell cycle. In mouse, this is the product of *Cdkn2A* gene (GenBank Accession AF044335).
p53: a tumor suppressor protein that is involved in DNA repair, cell cycle regulation and apoptosis. In humans, this is the product of the *TP53* gene (GenBank Accession U94788) and the orthologous gene in mouse is *Trp53* (GenBank Accession AY044188).
p63: a tumor suppressor protein that is part of the p53 family and involved in cell differentiation. In humans, this is the product of the *TP63* gene (GenBank Accession BC039815).
p73: a tumor suppressor protein that is part of the p53 gene family and is involved in cell cycle regulation and apoptosis. In humans, this is the product of the *TP73* gene (GenBank Accession BC117251).
Proto-oncogene: a gene that increases the chance of progression to cancer when it is overexpressed or inappropriately activated by mutation.
Rb: a tumor suppression protein that inhibits cell cycle progression until the cell is ready to continue to the next phase of the cycle.
Simpson's paradox: the observation that a statistical trend within groups opposes the trend between groups [24]. For example, two variables could be positively correlated within a species, but an interspecific comparison would reveal a negative correlation across groups.
Tumor suppressor gene (TSG): a gene that increases the chance of progression to cancer when it is inactivated or deleted. These genes normally function to suppress the initiation of tumors, or to prevent and repair damage to the genome.

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Box 1. Somatic evolution and the development of cancer

Throughout the life of an organism, cells accumulate mutations caused by endogenous and exogenous damage, or errors in DNA synthesis, which are not properly repaired. In fact, somatic cells in tumors satisfy the three necessary and sufficient conditions for natural selection: (i) there must be variation within the population. A tumor is a heterogeneous population of cells with somatic genetic and epigenetic alterations; (ii) the variation must be heritable. Genetic and epigenetic alterations (mutations) are inherited by both daughter cells when a cell divides; (iii) there must be differential survival and reproduction (i.e. fitness) [79]. In some cases, the genetic and epigenetic mutations provide cells with survival and/or reproductive advantages over other cells.

The genetic and epigenetic changes in somatic cells can result in the six 'hallmarks of cancer', all of which provide a fitness advantage to somatic cells: (i) self sufficiency of growth signals; (ii) insensitivity to anti-growth signals; (iii) evasion of apoptosis; (iv) sustained angiogenesis; (v) limitless replicative potential (stabilization of telomeres); and (vi) the ability to invade tissue and metastasize [69]. The somatic evolution occurring within mutant cell populations can result in cancer [1,70]. Understanding this process through an evolutionary perspective is essential for knowing how a given treatment will affect the population dynamics and how one might be able to intervene to prevent the development of cancer all together.

approximately twofold, even though the difference of size among mammals alone can be on the order of a millionfold [2]. Natural selection interacts with the life history of a species and should suppress cancer through the expected period of fertility of an organism. Therefore, given the relative age of an organism, one would expect cancer rates to be similar across species. The question of Peto's paradox is how has natural selection changed the biology of large, long-lived organisms to achieve this scaling.

The exact functional relationship between body size and expected cancer risk is unclear; however, it is assumed to be an increasing function. In comparing laboratory rodents and humans, which differ in lifespan by a factor of 40 and in size by three orders of magnitude, approximately 30% of both rodents and humans will have cancer by the end of their life [6]. The general explanation for this is that large, long-lived animals are more resistant to carcinogenesis than are small, short-lived animals [5,7–9]; however, how they accomplish this resistance has yet to be established. Understanding this resistance could lead to new methods of cancer prevention in humans.

The need and potential for cancer prevention

Cancer has proven difficult to cure. Since former US President Richard Nixon declared the 'War on Cancer' almost 40 years ago, little progress has been made on reducing the lifetime risk of cancer and increasing survival rates for patients with late-stage diagnoses [10,11]. Most cancer research focuses on treatment rather than prevention, and this often leads to the recurrence of tumors that are resistant to therapy. With 10^9–10^{12} cells in a tumor and perhaps 10^5 mutations [12,13,14,15], it appears that, in many cases, therapy selects for a resistant clone [1]. Increasingly, attention is turning to cancer prevention so as to avoid this scenario entirely.

A proven strategy in drug development has been to seek natural products that have been honed by millions of years of evolution to generate the desired effect [16]. The evolution of large, multicellular organisms could hold the key to preventing cancer in humans. Peto's paradox suggests that large, long-lived animals, such as the blue whale (*Balaenoptera musculus*), have evolved mechanisms capable of suppressing cancer 1000 times better than those in humans. Research on how these large animals are suppressing cancer holds the promise of dramatic improvements in cancer prevention for humans.

Peto's paradox appears to be real

Cancer incidence records for wild and captive animals are not well documented for most species, making it difficult to compare incidence records of humans and other animals directly. However, it is still clear that cancer incidence does not scale with body size across species (Box 2). If blue whales developed 1000 times more cancer than did humans, they would probably die before they were able to reproduce and the species would quickly go extinct [17]. The mere existence of whales suggests that it is possible to suppress cancer many-fold better than is done in humans.

Cancer death rates vary approximately twofold across multicellular animals of drastically different sizes [2]. When wild mice (*Mus musculus*) are raised in protected laboratory conditions, 46% die of cancer [18]. Cancer is also responsible for approximately 20% of dog deaths [19], approximately 25% of human deaths in the USA [10] and 18% of beluga whale (*Delphinapterus leucas*) deaths [20]. Rare cases of cancer are discovered in blue whales, giving no evidence of elevated cancer risk in these species [20,21]. No matter the size or lifespan of the animal, cancer seems to account for approximately the same percentage of deaths.

Interestingly, within a species, size is associated with an increased cancer risk. In humans, 3–4 mm above the average leg length results in an 80% higher risk of nonsmoking-related cancers [22]. Also, children with bone cancers tend to be taller, and osteosarcomas occur in large dogs 200 times more frequently than in small- and medium-sized breeds [23]. There has probably not been enough time to evolve additional mechanisms to protect large dogs from this increased risk and counteract the extreme artificial selection for size. This suggests that animals that evolved to be larger as a species developed mechanisms to offset this increased cancer risk, whereas above-average individuals do not have additional defenses compared with smaller organisms within their species and, therefore, fall victim to cancer with greater probability. This divergent trend within versus between species is an example of Simpson's paradox [24].

Hypotheses to resolve Peto's paradox

Limited research efforts have been focused on resolving Peto's paradox. However, there are many hypotheses that might explain how organisms could overcome the burden of cancer despite an increased number of cells and extended lifespan. Some have been previously proposed [2,25,26,27,28,29] and others, to the best of our knowledge, are new in this review. Large bodies evolved independently along multiple lineages; therefore, one would not expect that all large, long-lived animals have evolved the same mechanism(s) to suppress cancer, unless the suppression stems from an innate characteristic common to all larger organisms. Differences in diet and carcinogenic exposures (including pathogens, which are

Box 2. All whales should have colorectal cancer by age 80

Calabrese and Shibata devised a simple mathematical equation to express the probability of a human developing colorectal cancer given their age [71]. Their equation produces results that closely match data from the Surveillance, Epidemiology and End Results (SEER) Program [72]. The probability of an individual developing colorectal cancer after a given number of cell divisions, which is proportional to age, is formulated in Equation I:

$$p = 1 - \{1 - [1 - (1 - u)^{d}]^{k}\}^{Nm} \quad \text{(I)}$$

where u is the mutation rate per gene per division, d is the number of stem cell divisions since birth, k is the number of rate limiting mutations required for cancer to occur, N is the number of effective stem cells per crypt and m is the number of crypts per colon [71].

The model also shows that the increased cancer risk observed in taller women in the SEER data set can be fit by simply increasing the parameter m to account for a larger colon [71]. Using the same rationale, we varied the parameter m from 1.5×10^3 to 1.5×10^{10} to see how the total number of stem cells in the colon changes the lifetime (90-year) risk of developing colorectal cancer (Figure I). We used the same values as Calabrese and Shibabta for all other parameters (Table I) [71]. If we use the blue whale as an example of an animal that is 1000 times the size of a human, where m could equal 1.5×10^{10} crypts, then this analysis reveals that more than 50% of blue whales would have colorectal cancer by age 50 and all would have colorectal cancer by age 80. The chance of an individual person developing colorectal cancer by age 90 is only approximately 2.5% according to this model and just over 5% as reported by the American Cancer Society [10]. It is implausible that 100% of blue whales actually get colorectal cancer by age 80. Although it is not known how often blue whales are developing colorectal cancer, they have been reported to occasionally have other cancers [20,21] and to live for more than 100 years [35].

This model suggests that there is something fundamentally different in the initiation and progression of cancer in large, long-lived animals, such as whales, compared with humans. Cancer rates for large, long-lived organisms could be made more similar to smaller animals by decreasing the mutation rate u; decreasing the rate of stem cell divisions, which would decrease d; increasing the number of rate limiting mutations (k) needed to get cancer; or decreasing the number of proliferating stem cells per crypt (N).

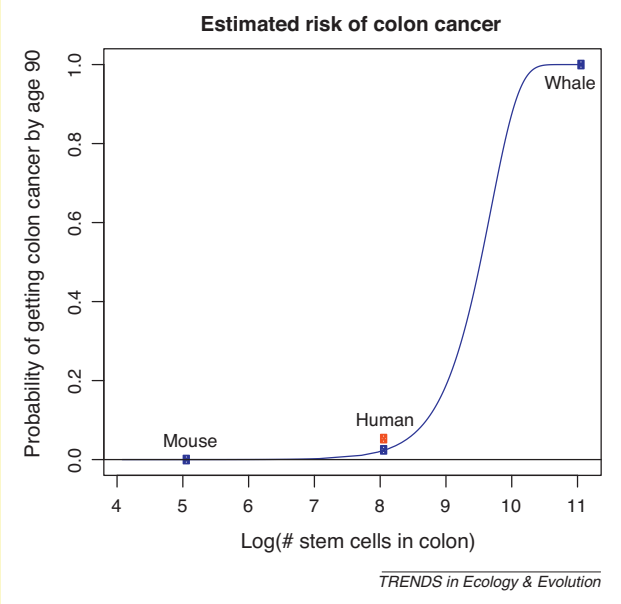

Figure I. Estimated probability of developing colorectal cancer by age 90 based on the number of cells in the colon. The probability of developing colorectal cancer at a certain age was calculated using Equation I [71]; parameter values are listed in Table I. This shows that, assuming all other parameters are equal, larger animals should have a greater lifetime risk of developing cancer compared with smaller organisms. Blue dots for mouse, human and whale indicate the estimated risk of colon cancer occurring within 90 years of life, given the approximate number of cells in a human colon, 1000 times fewer cells to represent the mouse and 1000 times more cells to represent the whale. The estimate for 1000 times smaller than a human (e.g. a mouse) is still barely above zero even after 90 years. In reality, a mouse only lives a maximum of 4 years [35], so they should never get colorectal cancer based on this equation. The red dot indicates the lifetime risk of colon cancer according to the American Cancer Society, which is approximately 5.3% for men and women averaged together [10].

Table I. Parameters used for the probability of developing colorectal cancer within 90 years

Parameter	Value*	Meaning
u	3×10^{-6}	Mutations per gene per cell division
d	8212.5	Divisions after 90 years, at a rate of one division every 4 days
k	6	Rate-limiting mutations needed to get cancer
N	8	Effective stem cells per crypt
m	$[1.5 \times 10^3–1.5 \times 10^{10}]$	Crypts in the colon

*All values taken from [71].

only associated with 15% of human cancers [30]) are unlikely explanations because there are many-fold differences in size between organisms with similar environments (e.g. dolphins and whales) and similar diets [e.g. elephants (*Loxodonta africana*) and mice are both herbivores]. Here, we present some possible mechanisms that might have evolved to obliterate the expected correlation between body size, lifespan and cancer risk.

Tumor suppression mechanisms that might vary across large, long-lived species

Lower somatic mutation rates

If large animals have lower somatic mutation rates per cell generation, then more cell divisions would need to occur for a cell to acquire the necessary mutations to become malignant compared with smaller animals. Mutation rate is a function of the error rate and the rate at which these errors are repaired. This could be achieved through several mechanisms, including better DNA damage detection and repair mechanisms. However, experimental data seem to suggest that mice and humans have comparable mutation rates [2], although better methods to measure somatic mutation rates *in vivo* are needed to explore this hypothesis.

Redundancy of tumor suppressor genes

Added redundancy of tumor suppressor genes (TSGs) could also suppress cancer in large animals [2,28] by requiring more mutations to occur to produce a malignant phenotype (Figure 1). Human cells require more mutations than do mouse cells to create immortalized cultures [31]. Both the

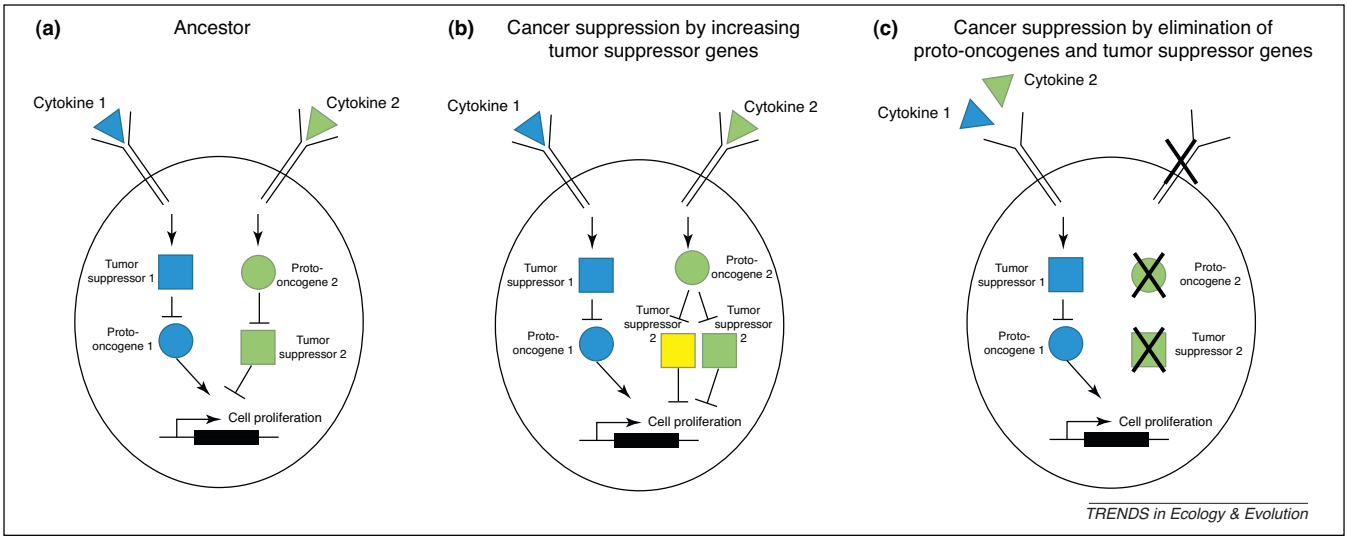

Figure 1. Alternative pathways to cancer hallmarks. **(a)** Assume that the ancestor of a large, long-lived organism has two pathways initiated by cytokines (triangles) such that, if either one is disrupted, the result is a hallmark of cancer. We illustrate this concept with cell proliferation; however, this could be replaced with any of the hallmarks. A large organism could decrease its risk of cancer by evolving redundant copies of TSGs (squares) **(b)** or by removing proto-oncogenes (circles) and TSGs to eliminate an entire pathway **(c)** so that there are fewer carcinogenic loci in the genome that are vulnerable to mutation. This option might be constrained by selective pressures on the remaining pathways to produce the adaptive phenotypes that had been encoded in the deleted pathway.

Rb and p53 pathways must be knocked out to immortalize human fibroblasts, whereas mouse cells require only the p53 pathway to be inactivated [25]. Mice genetically engineered to have extra copies of *Trp53* or *Cdkn2A* have increased tumor resistance [32,33]. Interestingly, the current build of the elephant genome (Ensembl release 59) has 12 orthologs of the human gene *TP53*, in addition to one copy of the genes that encode p73 and p63. The human genome only has one of each of these genes (*TP53*, *TP63* and *TP73*) [34]. If these all function as tumor suppressors, it might explain how elephants can have such large bodies and long lifespans (70 years in the wild) [35] but not succumb to cancer any more so than do smaller animals.

An opposing solution would be to eliminate some proto-oncogenes from the genomes of large, long-lived organisms. Having fewer proto-oncogenes decreases the chance of developing an oncogenic mutation and, therefore, decreases the overall probability of a cell becoming cancerous. This is supported by an experiment demonstrating that *Hras1*-null mutant mice develop significantly fewer papillomas than do wild-type mice [36]. If there were fewer pathways that could generate the phenotypes necessary for cancer, there would be fewer vulnerabilities in the genome and a reduced likelihood of cancer (Figure 1). Of course, proto-oncogenes are serving other functions, so eliminating them could be deleterious for other reasons.

Redundancy could also be in the form of expression. Many TSGs are tissue specific [37]. Cells of larger species could have evolved expression patterns such that, in any given cell, more TSGs are expressed compared with smaller, shorter-lived animals, even though there might be the same number of TSGs in the genome. This hypothesis would predict that large animals would have more ubiquitously expressed TSGs than do smaller species.

Lower selective advantage of mutant cells

A haploinsufficient gene in mice could be completely recessive in a larger animal, requiring mutations to occur on both alleles to gain a selective advantage over neighboring cells during carcinogenesis in the larger species [2]. This would decrease the possibility that mutations at this locus contribute to progression towards cancer. This has been observed in a tissue-specific manner. The tumor suppressor *Trp53* usually requires both alleles of the gene to be null to see a mutant phenotype; however, in some tissues, *Trp53* is haploinsufficient and losing only one allele produces a phenotype in mice [37].

Different tissue architecture

Changes in tissue architecture could influence the frequency of cancers by altering the way that cells are compartmentalized and/or the dynamics of the tissue [2]. Most tissues are comprised of small proliferative units; for example, the crypts of the intestines. It has been proposed that this hierarchical structure is a crucial cancer prevention mechanism [38]. Given that differentiating cells are evolutionary dead-ends, the effective population size of a somatic tissue probably depends mainly on the number and dynamics of stem cells (although a mutation that disrupts differentiation in a non-stem cell might also generate a carcinogenic cell lineage) [39]. Under a model of 'serial differentiation', it is possible to increase the number of cells and the amount of cell turnover without increasing the number or proliferative activity of somatic stem cells, simply by adding non-stem stages [40]. Altering the number of stem cells, the crypt density or the dynamics of differentiation and division could enhance the ability of the tissue to prevent malignant transformation.

More efficient immune system

Immune system efficiency against virus-associated cancers might account for some differences observed in cancer rates within humans [26], but this could also apply to non-viral cancers and we suggest that immune surveillance could explain differences in cancer resistance across species of all sizes. Tumors are initially immunogenic.

When mice are treated with carcinogens, tumorigenesis is delayed by immune system surveillance [41]. However, as the tumor coevolves with the immune system, tumor variants that go undetected are selected (termed 'immunoediting') [42]. 'Chronic antigenic stress' can result in exhaustion of the immune system, leading to ineffective surveillance, similar to observations of chronic viral infections [42]. Large, long-lived organisms might have better immune surveillance for neoplastic cells than do smaller organisms.

More sensitive or efficient apoptotic processes

The apoptotic propensity of cells might differ between large and small organisms. Cells from large bodies could be more sensitive to DNA damage or the activation of an oncogene and, thus, would be more apt to apoptose [26]. Support for this hypothesis comes from observations of human and mouse cell cultures. When human cells are irradiated, many die due to apoptosis triggered by DNA damage. A higher percentage of mouse cells survive and continue dividing regardless of the gross DNA damage inflicted by the radiation [43]. Apoptosis due to DNA damage eliminates the damaged cell from the population instead of repairing the DNA and possibly propagating remaining mutations in the tissue. However, there is likely to be a trade-off between apoptosis preventing cancer, but causing senescence due to depletion of the stem cell pool [44].

Increased sensitivity to contact inhibition

Selfish cellular proliferation can also be suppressed by signals from the microenvironment [26]. For example, cell contact inhibition has been noted to differ between human, mouse and naked mole-rat (*Heterocephalus glaber*) cells. In culture, naked mole-rat cells stop dividing at much lower densities than do human and mouse cells due to the early activation of the p16 pathway, which results in hypersensitivity to contact inhibition [45]. Although naked mole-rats and mice are small animals, the former live significantly longer than the latter (28 years [46] versus 4 years [47]). In all 250 necropsies of naked mole-rats that died in captivity, none had died of cancer [48]. Hypersensitive contact inhibition might have evolved to suppress cancer so that the naked mole-rat can live longer, although it has only been verified *in vitro* [45]. Signals for early cell senescence could be triggered in large, long-lived organisms to inhibit uncontrolled proliferation.

Shorter telomeres

Telomere length appears to be a fundamental check on the proliferative capacity of cells [49]. Telomeres shorten with every cell cycle and, when they become too short to protect the ends of the chromosomes, the cell senses those ends as DNA double-strand breaks, usually leading to apoptosis [50,51]. Even though stem cells express telomerase, which helps to rebuild telomeres, they generally do not express enough to prevent telomere shortening owing to proliferation [51]. We hypothesize that large, long-lived animals might have shorter telomeres (or erode them faster) than do smaller animals, limiting the number of times that their cells can divide and reducing opportunities to accumulate carcinogenic mutations.

Characteristics of all large organisms that might act as tumor suppression mechanisms

Fewer reactive oxygen species due to lower basal metabolic rate

A lower somatic mutation rate could also be a result of metabolism. Reactive oxygen species (ROS) are byproducts of metabolism and can cause DNA damage thought to contribute to aging and cancer [52,53,54]. The rate at which ROS are produced in a cell is a function of the basal metabolic rate (BMR) [55]. BMR per unit mass (mass-specific BMR) is proportional to (body mass)$^{-1/4}$ [56] and has been shown to correlate with the amount of oxidative damage [57]. Knocking out oxidative repair genes, and therefore allowing for DNA damage from ROS to persist, results in increased tumor susceptibility in a variety of tissues, suggesting that DNA damage caused by ROS has a causal role in tumor formation [58]. Large animals should produce fewer ROS due to their lower BMR and, consequently, have less endogenous damage to their DNA and an overall lower somatic mutation rate [29].

The average BMR of women is 10% lower than that of men after adjusting for body mass, composition, activity and age [29], and women consistently have lower rates of cancer [10]. Naked mole-rats, for which spontaneous cancer has yet to be reported [48], have a mass-specific BMR that is lower than expected given their size [35]. Caloric restriction inhibits cancers in animal models and one explanation for this is that the decrease in caloric intake reduces the metabolic rate, therefore producing fewer ROS and subjecting the DNA to less endogenous damage [59]. These observations could all be attributed to cells having less endogenous oxidative damage, which effectively results in a lower somatic mutation rate and a reduced cancer risk.

Formation of hypertumors

Nagy *et al.* have proposed an alternative hypothesis to resolve Peto's paradox [27]. Natural selection within a tumor might favor 'cheater' cells that take advantage of vasculature built by angiogenic cells. These 'cheaters' could grow and parasitize the primary tumor. This 'hypertumor' would reduce the overall fitness of the tumor and might even cause the tumor to regress. Nagy *et al.* argue that lethal tumors must be larger in larger animals, giving the hypertumor more time to evolve and force the parent tumor to become necrotic [27]. This model predicts that large animals would often carry macroscopic tumors that should be disproportionately more necrotic when compared with lethal tumors in smaller organisms [27], although this has yet to be verified experimentally. This hypothesis could be tested by serially passaging a cancer through mice, eventually generating enough cells that a hypertumor should evolve and the mean fitness of the tumor decrease.

Suggestions for the future

If the current understanding of cancer is correct, there must be something fundamentally different in large, long-lived organisms that enhances their suppression of carcinogenesis. These mechanisms have allowed for the evolution of large bodies and extended lifespans without increasing the burden of cancer. Most of the hypotheses that have been

Box 3. Outstanding questions for Peto's paradox

- What is the age-related incidence of cancer in most non-human (and non-experimental) animal species?
- Which of the many suggested mechanisms are valid explanations for the lack of correlation between body size, longevity and cancer incidence? Compared with humans, do larger, long-lived organisms have:
  - Lower somatic mutation rates?
  - More copies of TSGs?
  - Fewer proto-oncogenes?
  - Smaller selective advantages for somatic mutants?
  - Different tissue architecture (i.e. smaller proportion of stem cells or more quiescent stem cells)?
  - More efficient immune surveillance?
  - An apoptotic process that is highly sensitive to DNA damage?
  - Increased sensitivity to contact inhibition?
  - Shorter telomeres?
  - Less DNA damage due to fewer ROS?
- Is the presumed decrease in cancer incidence in lineages with lower than expected cancer incidence the result of several mechanisms that each contribute in a cumulative manner to decreasing the cancer risk of each cell, or rather of a single mechanism that has a drastic effect on a cell's probability of becoming malignant?
- Are such mechanisms shared among large, long-lived species or are they unique to each species?
- Does the cancer protection come from some innate characteristic of large organisms (i.e. low mass specific basal metabolic rate)?
- Can the cancer suppression mechanisms used by large, long-lived organisms be translated to humans as novel cancer preventive interventions?

proposed have not been directly tested, and most related questions remain open (Box 3).

Large bodies have evolved independently multiple times in the history of life, so each clade could have evolved different mechanism(s) to boost their tumor suppression abilities. An approach based on independent contrasts [60] of small and large species within each clade could prove fruitful for identifying cancer suppression mechanisms (Box 4).

Box 4. A phylogenetic approach to study Peto's Paradox

Large, long-lived organisms might have evolved to suppress cancer better than have small animals by duplicating TSGs [2,28] or eliminating some proto-oncogenes from the genome. A simple linear regression cannot be used to study whether a correlation exists between body size and the copy number of cancer-related genes because this assumes independence of each genome. In reality, the genomes have many traits in common owing to evolutionary decent from a common ancestor. An independent contrast model [73] should be used to partition the variance among species into comparisons that are independent of their evolutionary relationships. This can be done by studying multiple clades, each composed of closely related species that have large variance in body size.

Marine mammals belonging to the order Cetacea are an ideal clade for this study because they range in size from small dolphins, such as the Commerson's dolphin (~50 kg) [35] to the largest mammal on Earth, the blue whale (over 100 000 kg) [35]. The split between dolphins and whales occurred only 25–30 million years ago [74]. Unfortunately, the genomes of these animals are not currently available. Studies should focus on clades that include animals larger than humans, as opposed to looking at differences among various-sized rodents or between mouse and humans, because the goal is to find a way of preventing cancer that is superior to the endogenous tumor suppression mechanisms that occur in humans.

Unfortunately, the data needed to carry out these analyses is not currently available. Efforts should focus on sequencing genomes of large, long-lived species along with closely related small species, to determine whether TSGs duplicated, or oncogenes were deleted, during the evolution of a large body within a clade. Gene expression analy-

Box 5. Suggestions for future experiments

There are many experiments that could be done to test the hypotheses proposed to explain Peto's Paradox; however, they are limited by currently available information and assays as well as by the fact that large, long-lived animals cannot be easily genetically manipulated in a laboratory.

Lower somatic mutation rates
Mutation rates can be measured in elephant and whale cells *in vitro*; however, with better assays and longitudinal tissue sampling, the *in vivo* somatic mutation rate could be estimated [75].

More copies of TSGs or fewer proto-oncogenes
The copy number of cancer-associated genes can be studied using genomics to count the orthologs of known cancer genes using independent contrasts (Box 4). This is based on sequence information only, so functional studies would be necessary as follow-up.

Smaller selective advantages for somatic mutants
Fitness effects of mutations in cells of different animals could be estimated using *in vitro* cell competitions; however, this is not a realistic environment and might not reflect the true fitness caused by the mutation *in vivo*. Modern genetically engineered organisms could be used to measure the fitness of isolated mutations *in vivo* [76,77].

Different tissue architecture (smaller proportion of stem cells or more quiescent stem cells)
The mitotic index could be measured for crypts in intestinal tissue samples from elephants and whales. Given reliable stem cell markers, stem cells could also be counted.

More efficient immune surveillance
It might be possible to measure the immune response to mutant proteins that vary from the endogenous sequence by different degrees.

An apoptotic process highly sensitive to DNA damage
Cells from animals like elephants and whales can be irradiated *in vitro* to quantify how many cells apoptose as a function of the amount of DNA damage.

Increased sensitivity to contact inhibition
Cells can be grown *in vitro* to determine how the density of the cultures when the cells stop growing compares to the density of cultured cells from smaller organisms, as was done with the naked mole-rat [45].

Shorter telomeres
Telomere lengths can be measured and compared across species by using standard assays.

Less DNA damage owing to fewer ROS
New methods involving fluorgenic sensors for superoxide and hydroxyl radicals can detect ROS in cell cultures, tissues and *in vivo* [78].

ses of the same tissues might also reveal differential expression of cancer genes in large organisms. In addition, there are standard assays that could be used in comparative analyses to test many of the hypotheses for resolving Peto's paradox (Box 5), including measurements of DNA damage repair [61], telomere lengths [62], differentiation [63] and proliferation [64,65], apoptosis [66] and ROS [67].

Most cancer research is done on a small subset of organisms, which restricts understanding of cancer to the biology of those particular model systems. Furthermore, the qualities of model organisms that make them ideal to work with in laboratory conditions (short lifespan and small body) are the very things that make them poor models for cancer suppression [2]. The lack of functional data for non-model organisms is a major gap in the field. Function is often assumed from homology, which is not necessarily correct. For example, TSGs in *Drosophila* are largely non-overlapping with human tumor suppressors [68]. Studies that aim at a better understanding of the evolution of cancer suppression mechanisms will have to expand the variety of organisms that are studied in the laboratory setting.

There are not many large, long-lived organisms that have been fully sequenced, so testing Peto's paradox by doing comparative genomic analyses is difficult with current data. There is also the lack of robust epidemiological studies of cancer incidence in wildlife and captive populations. Captive populations will be useful for longitudinal studies and the predation-free environment will allow for better estimates of cancer rates. This will help researchers to better understand the nature of Peto's paradox.

Conclusion

There has been no observed correlation between body size, longevity and lifetime cancer risk [2]. Every additional cell and extra year of life should increase the probability of carcinogenesis. The fact that large, long-lived organisms are not over burdened by cancer suggests that they are more resistant to malignant transformation. Research focusing on what mechanisms have evolved to yield this cancer resistance will not only help explain Peto's paradox, but should also open new doors in the field of cancer prevention. Cancer treatments have not proven as effective as promised. If researchers can harness the cancer suppression mechanisms of large, long-lived organisms, then they could potentially eradicate cancer as a public health threat in humans. The initial step of a pharmaceutical company in developing a new class of drugs is to survey natural products to see whether evolution has already invented a solution to their problem. We are proposing that cancer prevention research capitalize on the same strategy. Humans have been invested in cancer research for decades while evolution has been tuning cancer suppression mechanisms for over a billion years. It is time to learn from the expert.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tree.2011.01.002.

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