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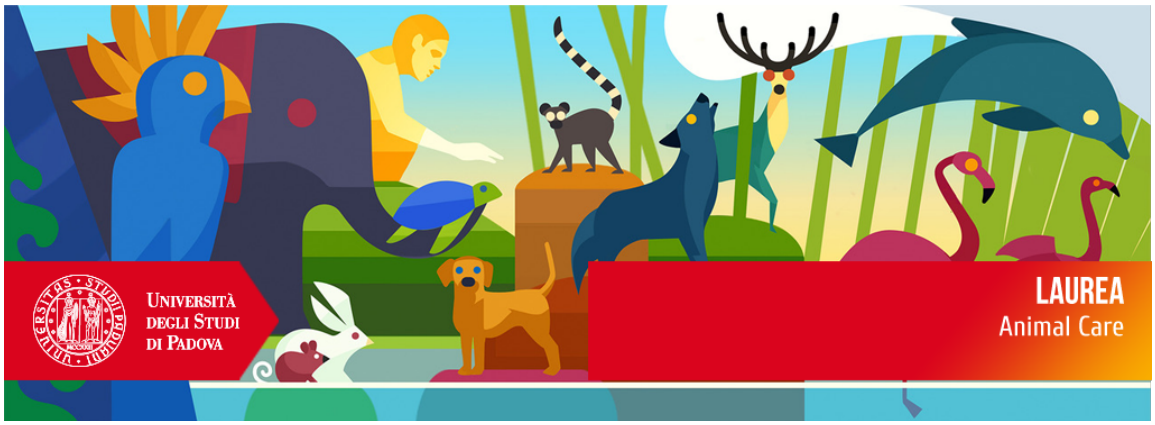

Overview of Cancer Resistance Mechanisms Observed in Mammals

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ABSTRACT

Cancer is a global health challenge, affecting various species across the mammalian kingdom and it is a complex and ubiquitous disease characterized by uncontrolled cell growth with the potentiality to disrupt the physiological integrity of an organism.

It is important to understand the mechanisms underlying cancer resistance in different mammals for elucidating the evolution of cancer defenses.

The investigation begins with an examination of Peto's paradox, a phenomenon that challenges conventional theories of cancer development concerning body size and lifespan. Despite the expected correlation between increased cell count and heightened cancer risk, some long-lived mammals, such as elephants and whales, exhibit a paradoxically low incidence of cancer. This observation has prompted inquiries into their unique anti-cancer defenses.

Continuing, exploring the intricate cancer resistance mechanisms observed in long-lived bats, naked mole rats, and blind mole rats. These species have evolved a repertoire of strategies, including telomerase expression, genomic adaptations, and specific protein interactions, to mitigate the risk of tumorigenesis.

Elephants, renowned for their remarkable cancer resistance, emerge as a focal point of this investigation. Their defenses includes an abundance of TP53 gene copies, a heightened DNA damage response, and an intriguing susceptibility to hyperplasia-induced cell death.

Additionally, investigating into the captivating variation in cancer susceptibility between Asian and African elephants. While both species share some resistance mechanisms, the Asian elephant's heightened susceptibility to cancer challenges.

In conclusion, understanding these adaptations and mechanisms of cancer resistance in these species not only enriches the understanding of cancer biology but also offers potential avenues for advancing cancer research and treatment in humans.

1. INTRODUCTION

1.1 Definition and development of cancer

Cancer in animals, much like in humans, presents a complex and challenges landscape. As it is a pervasive and insidious disease that has been steadily on the rise over the years. This concerning and continuous growth in cancer incidence demands a concrete effort from the scientific and medical communities to understand its causes, mechanisms and effective interventions.

In 2021, the National Cancer Institute defined cancer as a disease characterized by the uncontrolled growth and spread of some of the body's cells to other parts of the body. Under normal circumstances, when the body requires new cells to replace old or damaged ones, healthy cells go through a meticulously regulated process known as cell division *(National Cancer Institute, 2021).* However, there are instances when this orderly process falters, leading to the proliferation of abnormal or damaged cells at inappropriate times.

The animal body inherently possesses mechanisms designed to eliminate cells containing DNA damage before they can progress towards the development of cancer. Yet, with the passage of time and the natural aging process, the efficiency of these protective mechanisms tends to wane, diminishing the body's capacity to efficiently remove such compromised cells.

This decline in the body's defense mechanisms can be attributed to genetic alterations responsible for governing cellular functions, especially those related to cell growth and division. These genetic modifications can be prompted by exposure to detrimental environmental factors, such as the harmful ultraviolet rays from the sun, or they may be inherited from one's parents *(National Cancer Institute, 2021).* As a result of these genetic changes, cells can undergo a disruptive transformation, ultimately culminating in the formation of tumors.

The process by which cancer develops is called carcinogenesis, which is not a single event but rather a multistage process of accumulation of genetic and epigenetic mutation in a mitotic cell that occur over time *(Armitage P., et al., 1954).*

The probability of cancer emerging is intricately linked to the frequency of cell divisions occurring within a specific timeframe, since each cell division carries a probability of experiencing a cancer-causing somatic mutation. In essence, the higher the rate of cell division, the greater the potential for the accumulation of genetic and epigenetic mutations, thereby elevating the susceptibility to cancer *(Caulin AF., et al., 2015).*

For this reason, long-lived and large bodied organisms should encounter a higher risk of cancer during their lifetime simply related to the fact that their bodies contain an higher number of cells and so they will undergo more cell divisions *(Abegglen LM., et al., 2015).*

1.2 Peto's Paradox

In 1975, scientist Richard Peto made a noteworthy discovery: when analyzing cancer incidence at the species level, it became apparent that there was no apparent correlation between an organism's cell count, lifespan or body size with the probability to develop cancer *(Peto R., et al., 1975).*

For example, the incidence of cancer in humans is much higher than the incidence of cancer in whales *(Nagy JD., et al., 2007)*, despite whales have an high number of cells compare to humans. If the probability of carcinogenesis were constant across cells, one would expect whales to have a higher incidence of cancer than humans *(Nunney L., 2013).*

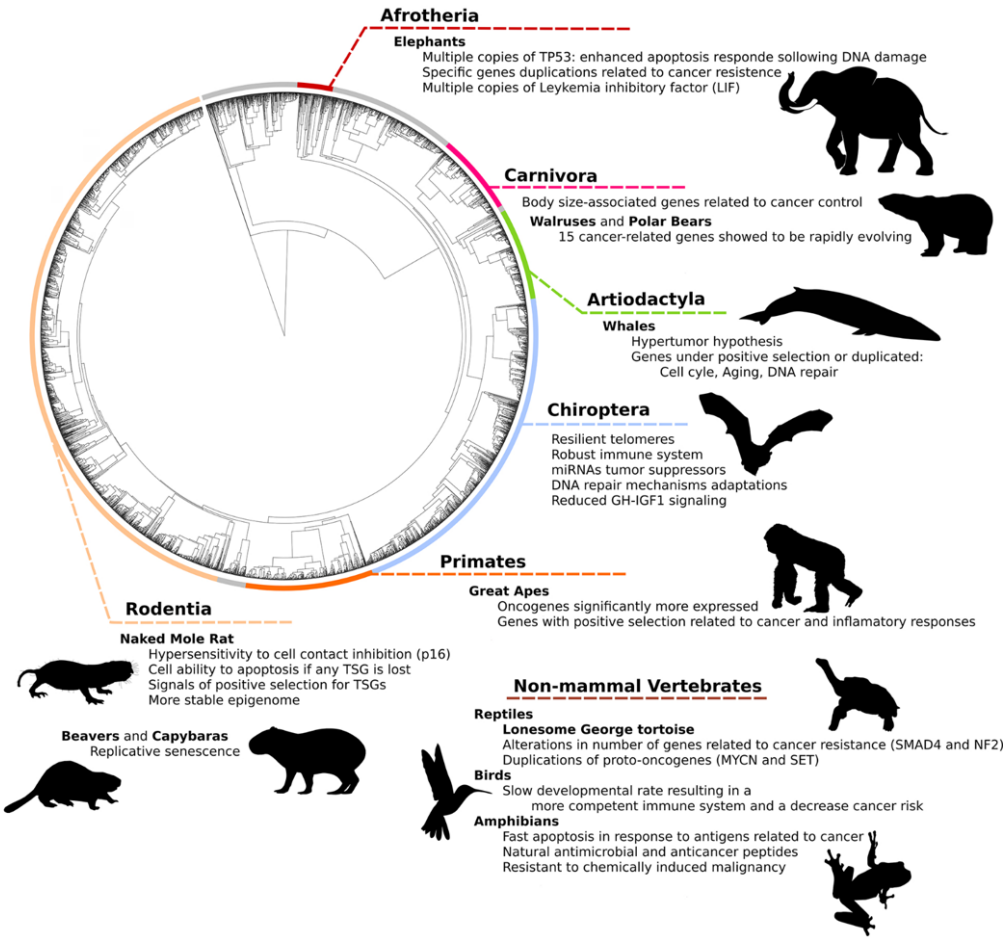

Figure 1. Summary of the main genetic mechanisms related to cancer resistance in vertebrates (Nery MF, et al., 2022).

To explain this paradox, researchers have proposed several theories. Given that the mechanisms of cancer suppression can intensify in individuals who face a higher cancer risk, one of the primary explanations is that larger animals with extended lifespans have developed stronger mechanisms for suppressing cancer.

A key factor contributing to this phenomenon is the metabolic distinctions between larger and smaller species. Metabolic rate refers to the mechanism at which an organism utilizes energy and executes various biochemical processes within its body. For instance, elephants or whales have notably slower metabolic rates in contrast to smaller animals like mice.

As a result, the occurrence of mutagenic agents, especially chemically reactive molecules such as reactive oxygen species (ROS) and free radicals, capable of inducing harm to DNA and other cellular constituents when overly abundant, tends to be less frequent in larger animals. *(Sies H., et al., 2020).*

Metabolic rate affects also the rapidity of cell division and telomere shortening.

As cells divide, a portion of the telomere is lost, and over time, this can lead to the telomeres becoming critically short. When telomeres become too short, it can have various biological consequences, including cellular aging and, in some cases, contributing to age-related diseases.

This cellular aging process can manifest in several ways, including replicative senescence, cellular differentiation, or apoptosis. These mechanisms act as natural safeguards to impede the development of tumors. However, cancer cells have evolved strategies to circumvent these built-in safeguards. One such strategy involves the upregulation of an enzyme called telomerase, a characteristic feature of stem cells and cancer cells. Telomerase can elongate telomeres, allowing these cells to continue dividing without encountering the usual limitations imposed by telomere shortening.

Alternatively, cancer cells can activate a distinct mechanism known as 'alternative lengthening of telomeres (ALT)'. ALT relies on a common DNA repair process called homologous recombination *(Roake CM., et al., 2016).* In this process, a damaged DNA strand is repaired by using a healthy and identical strand from a related DNA molecule as a template. ALT essentially employs this DNA repair mechanism to extend telomeres, promoting unhindered cell division. An alternative pathway that some animals employ to curtail uncontrolled cell proliferation involves inducing replicative senescence. In this state, cells cease dividing and lose their ability to function properly. Replicative senescence plays a vital role as a late-stage barrier to impede tumor progression *(Seluanov A., et al., 2008).*

Another hypothesis proposes the existence of what scientists have termed "hypertumors." This concept suggests that bigger tumors evolved in larger animals, exhibit slower growth rates and become susceptible to what are referred to as "cheater" cells *(Nagy JD., et al., 2007).*

These "cheater" cells are a subpopulation of cells within a tumor that have developed certain traits or behaviors that allow them to exploit the tumor's microenvironment and resources for their own advantage; in this case they exploit the tumor's angiogenic properties, which are mechanisms that promote the growth of new blood vessels to supply the tumor with nutrients and oxygen. By doing so, these "cheater" cells divert essential resources away from the main tumor mass, compromising its overall fitness.
The presence of "hypertumors" may potentially reduce the overall lethality of cancer within the bodies of larger animals.

Another possible solution of Peto's Paradox is that larger animals may have evolved an enhanced immunocompetence, which means they have a more effective immune system capable of detecting and combating neoplastic cells, or cells that are undergoing abnormal and potentially cancerous changes *(Tollis M., et al., 2017).*

2. DISCUSSION OF THE TOPICS

2.1 Development of cancer resistance's mechanisms in mammals

Natural selection assumes a pivotal role in individuals who has long lifespan and larger size, as it actively contributes to the development of anti-cancer defenses.
When individuals within populations face the selective pressure of cancer risk, the population must undergo adaptive evolution to develop mechanisms for suppressing cancer. Failure in doing this would result in fitness costs and potentially even extinction *(Roche B., et al., 2012).*
Hence, within this context, the evolutionary process has gave upon certain individuals the ability to defer the onset of cancer risk until after their reproductive years resulting in a higher frequency of cancer in older animals *(Seluanov A., et al., 2008),* as a result, cancer tends to afflict older individuals who are no longer subject to the forces of natural selection.
While some of these mechanisms are shared and maintain across species, such as the repression of telomerase activity in mammals with body mass greater that 5-10 kg, others are distinct and have been influenced by the specific lifestyle and ecological characteristics of each species *(Seluanov A., et al., 2008).*
These skills may involve decrease in the copy number of oncogenes, an increase in the copy number of tumor suppressor genes, a reduced retroviral activity and load, narrow metabolic rates leading to decreased free radical production, increased immune surveillance and selection for 'cheater' tumors

that parasitize the growth of other tumors, among many others *(Nunney L., 1999; Leroi et al., 2003; Caulin, et al., 2011; Katzourakis et al., 2014).*

An example is the mutations in the growth hormone receptor (GHR) or deficiencies in growth hormone (GH) signaling, such as those observed in individuals with Laron-type dwarfism (characterized by short-stature), which seems to be linked to heightened cancer resistance in both humans and mice *(Ikeno Y., et al., 2009; David A., et al., 2011; Guevara-Aguirre J., et al., 2011).* Therefore, diminishing GH-IGF1 signaling could potentially play a role in the enhanced cancer resistance observed in long-lived bats.

The exact mechanisms through which reduced GH-IGF1 signaling confers cancer resistance are not fully understood and are still an active area of research. However, it is believed that the modulation of this signaling pathway may affect various cellular processes involved in cancer development and progression, such as cell proliferation, apoptosis, and DNA repair.

2.1.1 Long-lived Bats

Research has revealed that long-lived bats *(Myotis),* known for their remarkably extended lifespans, possess unique regulatory mechanisms that provide resistance against tumorigenesis and promote cellular damage repair. These mechanisms, combined with their ability to alleviate oxidative stress, significantly contribute to their exceptional longevity *(Brook CE., et al., 2015; Seim I., et al., 2013; Zhang G., et al., 2013).*

One facet of these mechanisms arises from the identification of an unusually high abundance of genes undergoing positive selection within bat genomes *(Zhang G., et al., 2013).*

Specifically, scientists have identified 21 telomere maintenance genes with distinctive expression patterns, featuring 14 associated with DNA repair and five linked to alternative telomere-lengthening mechanisms.

DNA repair mechanisms involve proteins like ataxia-telangiectasia mutated (ATM) and senataxin (SETX) *(Foley NM., et al., 2018),* which are crucial for maintaining genomic stability and responding effectively to DNA damage.

ATM is used for detecting and repairing DNA double-strand breaks, which are one of the most severe types of DNA damage. When double-strand breaks, ATM is activated, initiating a cascade of events that lead to DNA repair, ensuring that the two broken ends are correctly reconnected. If double-strand breaks aren't repaired properly, they can lead to genomic instability, which is a hallmark of cancer development.

SETX, instead, is involved in a variety of DNA repair processes. One of its crucial roles is in resolving DNA-RNA hybrids, which can form when the DNA double helix interacts abnormally with RNA molecules. Senataxin helps prevent these hybrids from interfering with normal DNA processes and causing damage. Disruptions in this process can lead to genomic instability and potentially contribute to cancer *(Foley NM., et al., 2018).*
While these proteins are not unique to bats and are found in various organisms, their specific function and regulation within bat species may be optimized to provide them with a unique advantage in terms of cancer resistance and longevity.

Another aspect of these mechanisms lies in their remarkable adaptations in mitochondrial function, effectively managing reactive oxygen species (ROS) that typically induce apoptosis when excessively high. This adaptation involves the formation of autophagosomes, cellular structures that eliminate damaged mitochondria, promoting cell survival and preventing apoptosis *(Brook CE., et al., 2015).*
Moreover, these organelles appear to possess multifaceted functions, potentially playing a role in tumor resistance and pathogen control, as observed in the blood miRNAomes (microRNA profiles) and transcriptomes (gene expression patterns) of Myotis myotis, commonly known as the mouse-eared bat.

Another significant observation in bats involves the upregulation of three specific miRNAs out of four, specifically miR-101-3p, miR-16-5p, and miR-143-3p. Upregulated miRNAs are microRNAs expressed at higher levels or exhibiting increased activity in bats compared to other organisms. The importance of this upregulation lies in the fact that these miRNAs have been recognized as tumor suppressors, actively demonstrating anti-cancer properties.

2.1.2 Naked Mole Rats

The naked mole rat *(Heterocephalus glaber)* is classified as long-lived mammals where replicative senescence is absent, meaning their cells can divide and replicate without undergoing the typical limitations seen in other mammals, and rely on early-acting tumor suppressor mechanisms that prevent excessive cell growth *(Buffenstein R., et al., 2010)*.

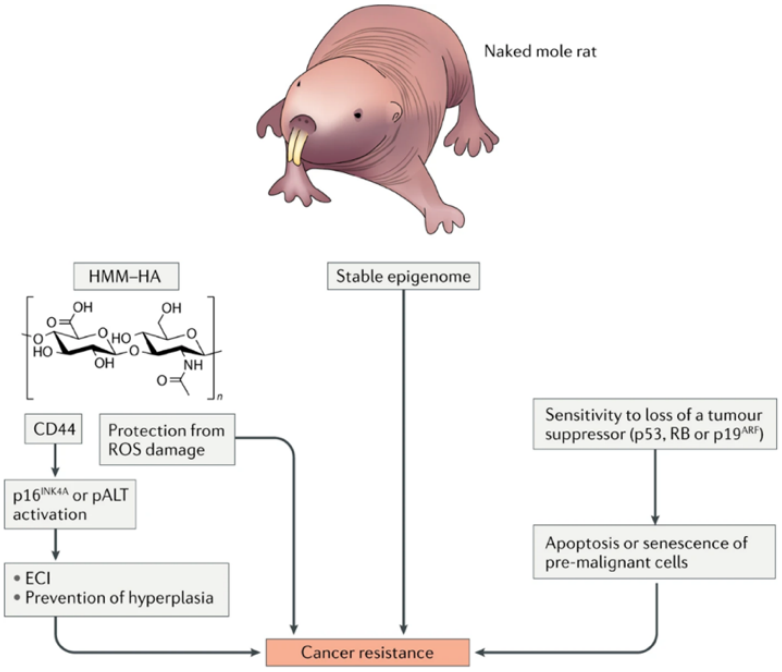

Figure 2. Naked mole rat cells possess several distinctive features that contribute to their cancer resistance. These include the production of HMM-HA, ECI through CD44 receptors, a strong extracellular matrix, antioxidant properties, epigenetic stability, and the ability to detect and respond to the loss of tumor suppressor genes. (Seluanov, A, et al., 2018)

The main mechanism of the naked mole rat cells regard the extracellular contact inhibition (ECI), this means that cells stop their proliferation upon coming into contact with neighboring cells, effectively signaling a cessation in growth to prevent tissue overcrowding.

ECI occurs through high molecular mass hyaluronan (HMM-HA), a linear glycosaminoglycan and a primary non-protein constituent of the extracellular matrix (ECM) *(Tian X., et al., 2013),* which possess greater molecular size, an increased number of repeating units within their structure and exhibit anti-proliferative, anti-inflammatory, and anti-metastatic properties *(Toole B., 2004)*.

Its secretion and concentration can be modulated in response to various factors, including the need to regulate cell proliferation and tissue integrity.

This signaling pathway is mediated by the CD44 receptors, present in the cell membrane, that are activated by the interaction with HMM-HA. This binding helps to stabilize and reinforce the extracellular matrix, enhancing the structural integrity of tissues and inhibiting uncontrolled cell growth and movement.

CD44 activation can also trigger intracellular signaling pathways, including the activation of gene expression at the Cdkn2a-Cdkn2b locus, which is critical for maintaining proper control over the cell cycle and preventing the development of cancer *(Kim EB., et al., 2001; Tian X., et al., 2015).*
Another pathway pass through the activation of the p16INK4, a tumor suppressor protein that plays a crucial role in controlling the cell cycle. When cells make contact with one another, p16INK4A acts as a "stop signal" to halt cell division, reinforcing the effects of CD44-HMM-HA interactions. This differs from the typical response observed in other species, such as humans and mice *(Seluanov A., et al., 2009)*, which prefer to follow the p27 pathway.
The p27 is a cyclin-dependent kinase inhibitor, which role is to block the activity of Cyclin-dependent kinases (CDKs), avoiding the progression of the cell cycle.

An additional mechanism regards the ability of naked mole rat to recognize when important tumor suppressor genes, Tp53 (p53) and Rb1 (Retinoblastoma 1), are either absent or malfunctioning. These genes normally act as guardians of the cell cycle, ensuring that cells do not divide uncontrollably. *(Rangarajan A., et al., 2004).*
One response to the absence of these tumor suppressors is the activation of a process called apoptosis which is controlled and programmed cell death mechanism.
In this case, the cells that lack functional Tp53 or Rb1 are "flagged" for destruction. This self-destructive process eliminates potentially cancerous cells from the body, preventing them from dividing uncontrollably and forming tumors.
The second response is for the affected cells to enter a state of permanent growth arrest (senescence) *(Miyawaki S., et al., 2016).*

Naked mole rats have also developed mechanisms to enhance proteolysis, which helps remove damaged or unwanted proteins from cells. This process is facilitated through autophagy, which is a cellular process where cells engulf and digest damaged organelles and proteins. It acts as a form of cellular "clean-up" through the proteasome, a cellular structure responsible for breaking down proteins that are tagged for degradation as damaged or unnecessary *(Azpurua J., et al., 2013; Zhao S., et al., 2014; Rodriguez KA., et al., 2014; Lewis KN., et al., 2015)*. By doing so, they contribute to the suppression of cancer development by reducing the accumulation of mutations and maintaining controlled cellular proliferation and genomic stability.

The presence of these mechanisms in naked mole rats implies that they have developed distinctive abilities to finely regulate pluripotency, possibly as an evolutionary response to their remarkable cancer resistance.

These unique capabilities become particularly relevant in the context of reprogramming experiments. Scientists perform reprogramming experiments to understand how cells can be manipulated to revert to a pluripotent state, where they possess the remarkable capacity to differentiate into a wide array of distinct cell types.

One way to confirm their pluripotency is by assessing their ability to form teratomas, which contain cells from all three germ layers (endoderm, mesoderm, and ectoderm) *(Miyawaki S,, et al., 2016; Tan L., et al., 2017; Lee S., et al., 2017).*

While induced pluripotent stem cells (iPSCs) themselves are not cancerous, the reprogramming process can activate or mimic cellular pathways and characteristics that are shared with cancer cells *(Folmes CD, et al., 2001; Ben-David U, et al., 2011; Suva ML, et al., 2016)*, such as the activation of proto-oncogenes, or due to these epigenetic alterations there can be a disruption of normal gene regulation, potentially leading to the activation of oncogenes or the silencing of tumor suppressor genes. Therefore, there is a concern that iPSCs may have an increased propensity to develop cancerous properties if not properly controlled during their generation or use in research and therapies.

It was observed that naked mole rat cells possess a remarkable resistance to reprogramming *(Miyawaki S., et al., 2016; Tan .L, et al., 2017; Lee S., et al., 2017)*, even if researchers successfully reprogram naked mole rat cells into iPSCs, these cells display a notably low efficiency in forming teratomas.

The fact that naked mole rat cells can be transformed into pluripotent cells without displaying tumor characteristics is indeed an important discovery.

This research is crucial for regenerative medicine and understanding diseases like cancer.

Finally, the naked mole rat has developed a form of glycolysis that is primarily driven by the metabolism of fructose, instead of relying solely on glucose.

This has many advantages: first fructose can be metabolized through glycolysis in a way that requires less oxygen compared to glucose, allowing them to thrive in their subterranean habitats (*Park TJ., et al., 2017; Liu H., et al., 2010)*; secondly fructose metabolism produces less lactic acid (produced when glucose is metabolized under low-oxygen conditions), reducing the risk of cellular damage.

It is noteworthy that the metabolic pathway used by naked mole rat cells to generate energy, under oxygen-deprived conditions, shares similarities with the way by which cancer cells generate energy.

This observation raises the possibility that naked mole rat cells might have an increased susceptibility to cancer, however, this potential vulnerability is effectively mitigated by a multitude of previously mentioned tumor-suppressive adaptations.

2.1.3 Blind Mole Rats

In contrast to mice and rats, the blind mole rat (*Spalax ehrenbergi* superspecies) boasts remarkable longevity, living up to 21 years, and displays notable cancer resistance *(Edrey YH., et al., 2012; Gorbunova V., et al., 2012).* Indeed, there have been no documented instances of spontaneous tumor formation in blind mole rats *(Ashur-Fabian O., et al., 2004).*

These rodents have the ability to produce the enzyme telomerase in their non-reproductive (somatic) cells, this can prevent or delay cellular aging (replicative senescence) that typically occurs when telomeres become too short. This mechanism acts as an anti-cancer defense because it helps cells maintain their normal function and prevents them from becoming cancerous due to age-related cellular changes *(Gorbunova V., et al., 2012).*

Moreover, in low-oxygen environments, cells often undergo apoptosis, as a protective mechanism to eliminate cells that are damaged or at risk of becoming cancerous due to oxygen deprivation.

In order to thrive in such conditions and evade programmed cell death (apoptosis), blind mole rats have undergone targeted genetic alterations within a gene known as TP53 *(Shams I., et al., 2004).* This includes specific changes, like replacing the amino acid arginine with lysine in the DNA-binding domain of the TP53 protein. This gene encodes a protein known as TP53, which plays a crucial role in regulating cell growth and preventing cancer.

Furthermore, attempts to induce tumors using carcinogens in vivo revealed a strong initial necrotic response without subsequent tumor formation *(Manov I., et al., 2013)*. The necrotic response coincides with increased interferon-β (IFNβ) expression. IFNβ, produced by cells in response to various triggers, plays a pivotal role in the body's immune defense against viruses, enhancing the activity of certain immune cells, such as natural killer (NK) cells and macrophages, which are responsible for identifying and eliminating infected or abnormal cells, or inducing apoptosis.

This adaptation is due to the blind mole rat’s exceptional sensitivity to hyperplasia, characterized by uncontrolled cell growth. In response, these cells trigger an interferon-based reaction, with a significant release of IFNβ into the surrounding medium *(Gorbunova V., et al., 2012)* and culminating in the elimination of hyperplastic cells through a process called 'concerted cell death' (CCD), a combination of necrotic and apoptotic pathways (refer to **Figure 3**).

Genomic analysis of the blind mole rat has uncovered multiple copies of genes related to the interferon (IFN) pathway. This suggests that the evolution of IFN-mediated CCD might be a

compensatory mechanism for the blind mole rat, since it helps address the challenge of reduced pro-apoptotic (cell death-inducing) function in the p53 protein (in the blind mole rat, the p53 protein appears to have a reduced ability to induce apoptosis compared to other animals, like humans), which is crucial for eliminating damaged or cancerous cells. These duplicated IFN pathway genes potentially enhance the blind mole rat's immune response, aiding in the removal of cells that should undergo apoptosis but are less efficient due to limitations in the p53 protein *(Gorbunova V., et al., 2012).*

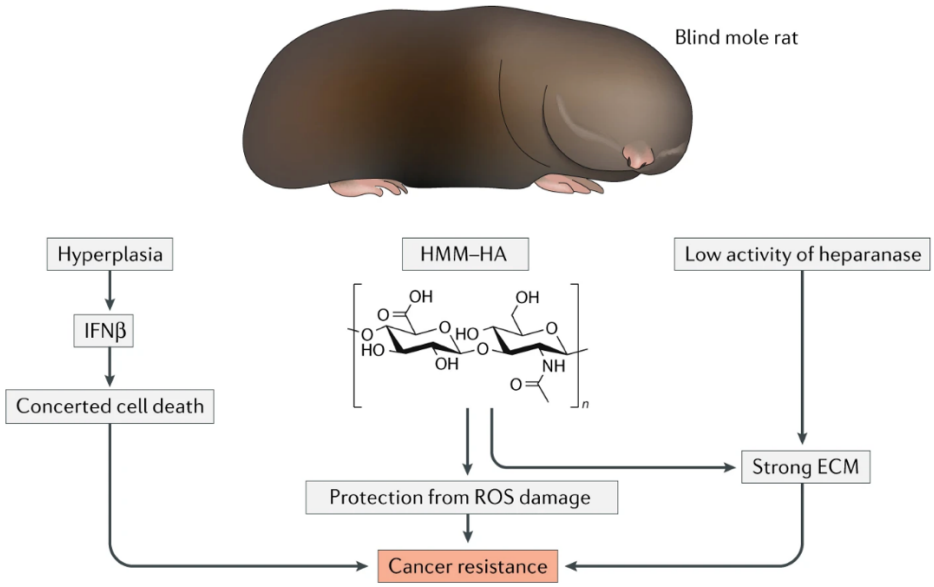

Figure 3.They release interferon-β (IFNβ), triggering a process called concerted cell death (CCD), which combines necrotic and apoptotic pathways to effectively eliminate pre-malignant hyperplastic cells. These rats also produce significant amounts of high molecular mass hyaluronan (HMM-HA), similar to naked mole rats, but they lack early contact inhibition. HMM-HA appears to protect their cells from damage caused by reactive oxygen species (ROS), potentially contributing to their cancer resistance.
Additionally, blind mole rats express a dominant-negative splice variant of heparanase, which, along with HMM-HA, reinforces the extracellular matrix (ECM). This reinforcement inhibits tumor growth and metastasis, further enhancing their cancer resistance. (Seluanov, A., et al., 2018)

In addition to the interferon-mediated mechanism, blind mole rat cells produce HMM-HA *(Tian X., et al., 2013)*, providing protection against reactive oxygen species (ROS). Notably, unlike the naked mole rat, these cells lack Extracellular Contact Inhibition (ECI*) (Manov I., et al., 2013)* it implies that cells can continue to grow and divide even when in contact with other cells.

Conclusively, blind mole rats possess a unique version of the heparanase gene, generated through a process known as splicing, which involves the removal of non-coding regions (introns) from a pre-messenger RNA (pre-mRNA) molecule and the connection of the coding regions (exons).
The specific variant of the heparanase gene found in blind mole rats acts as a "dominant negative", exerting a suppressive or inhibitory effect. In this context, it impedes the degradation of the Extracellular Matrix (ECM), which constitutes a complex network of proteins and carbohydrates offering structural and biochemical support to cells. Consequently, the blind mole rat's heparanase variant disrupts the usual breakdown or degradation of the ECM, potentially influencing processes such as tissue growth and repair *(Nasser NJ., et al., 2009)*. Along with abundant HMM-HA expression, this potentially results in a more organized ECM structure that restricts tumor growth and spread *(Tian X., et al., 2013).*

2.1.4 Whales

It is intriguing to note that during the sequencing and analysis of genomes from various whale species *(Cetacea),* including the bowhead whale *(Balaena mysticetus)* which boasts an exceptional lifespan, no duplications of the TP53 gene were found *(Yim HS., et al., 2014; Foote AD., et al., 2015; Foote AD., et al., 2016).* Whales have developed distinct anti-cancer adaptations that are not observed in other species *(Tacutu R,, et al., 2013).*

Through comparative analysis of the genomic and transcriptomic data of bowhead whales, researchers have detected genes undergoing positive selection that are associated with cancer and aging *(Seim I., et al., 2014; Keane M., et al., 2015)*, specifically the duplication of the proliferating cell nuclear antigen (PCNA) *(Kean, M.. et al., 2015).* PCNA plays a vital role in DNA replication and repair, critical for maintaining genomic stability. Alterations in its copy number can impact DNA maintenance and potentially influence an organism's susceptibility to cancer and its aging processes.

Notable examples of genes that have undergone positive selection also include excision repair cross-complementation group 1 (ERCC1), responsible for encoding a DNA repair protein, and uncoupling protein 1 (UCP1), which codes for a mitochondrial protein found in brown adipose tissue *(Keane M., et al., 2015).*
Considering that both ERCC1 and PCNA are integral to DNA repair processes, it's plausible that these proteins offer protection against cancer by reducing mutation rates. Consequently, whales may not require additional copies of TP53, as their cells do not accumulate cancer-causing mutations and do not progress to a pre-neoplastic state.

Moreover, researchers have pinpointed specific changes in gene expression exclusive to bowhead whales *(Balaena mysticetus),* particularly involving genes associated with insulin signaling. This discovery holds significance as alterations in genes related to insulin signaling can exert considerable influence over a wide array of biological processes, including metabolism and cellular growth, since insulin is a hormone pivotal in regulating blood sugar levels and managing energy storage and utilization *(Seim I., et al., 2014).*

3. ELEPHANTS CASE STUDY

3.1 Elephants cancer resistance mechanisms

In light of Peto's paradox, it becomes evident that larger-bodied and longer-lived animals might have evolved extra safeguards to suppress tumors, compensating for their higher cell counts. Moreover, many large animals live longer lives, which means they need additional defenses against cancer throughout their extended lifespans.

The heightened sensitivity of elephant cells to genotoxic stress serves as a potential anticancer mechanism, as it enables the elimination of damaged cells more effectively, preventing their progression into precancerous stages. However, this increased cell death may potentially deplete the pools of stem and progenitor cells within the tissues.
To counterbalance this potential consequence, elephants may have developed additional adaptations or mechanisms to ensure the preservation of their stem cell populations and the maintenance of healthy tissues *(Abegglen LM., et al., 2015; Sulak, M., et al., 2016).*

Moreover, elephant blood cells display an impressive susceptibility to DNA damage induced by ionizing radiation. Rather than opting for DNA lesion repair, compromised elephant cells seem to have evolved a proactive strategy—inducing apoptosis. This proactive approach effectively thwarts the development of early-stage tumors.

Furthermore elephants possess multiple copies of the leukemia inhibitory factor (LIF) *(Vazquez JM., et al., 2018)*, with a standout transcript, LIF6, exhibiting heightened expression in response to DNA damage activated by p53. This upregulated expression significantly contributes to an enhanced DNA damage response within elephants. The LIF6 gene, housing a p53-responsive element, is speculated to have transformed from a pseudogene into a functional target gene of p53 *(Vazquez JM., et al.,*

2018). Collectively, these gene duplications equip elephants with additional tumor suppressors, substantially reducing the risk of cancer. The heightened apoptosis triggered by DNA damage plays a pivotal role in maintaining genome integrity without incurring undesirable cell loss.

In conclusion, the most significant mechanism concerns the TP53 gene.
In a groundbreaking discovery, two independent research teams *(Abegglen LM., et al., 2015; Sulak, M., et al., 2016)* simultaneously identified 19 additional copies of the TP53 gene within the elephant genome (see **Figure 5**). Notably, all of these additional TP53 copies are categorized as pseudogenes, which are essentially genes rendered non-functional, losing their capacity to produce proteins or functional RNA molecules. Pseudogenes typically emerge during evolution when mutations or structural alterations, often involving deletion patterns, accumulate in a previously functional gene, rendering it inactive.
Among these 19 TP53 pseudogenes, the most intriguing discovery was that two of them demonstrated active translation in elephant fibroblasts, a category of connective tissue cells distributed throughout the body, encompassing the skin, tendons, and various organs. TP53, the parent gene from which these pseudogenes originated, plays a crucial role in regulating cell growth and preventing tumor formation. Mutations in TP53 are frequently linked to cancer.
The translation of these specific pseudogenes in elephant fibroblast cells suggests they may still possess functional capabilities. They retain the ability to contribute to the production of some proteins *(Sulak M., et al., 2016).*
However, it's essential to emphasize that these additional TP53 copies lack critical components such as DNA-binding domains, preventing them from effectively interacting with DNA to control gene expression. Furthermore, they lack the nuclear localization signal, which is a sequence within the TP53 protein necessary for its movement into the cell's nucleus, where many crucial cellular processes take place. As a result, these extra TP53 copies cannot function as transcription factors.

What adds an intriguing layer to this discovery is the observation that specific mutations within the TP53 gene exhibit a unique behavior of being transcribed from adjacent transposable elements. These transposable elements, essentially mobile DNA fragments embedded within the genome, have the remarkable ability to relocate themselves within the genetic structure over an extended period of time.

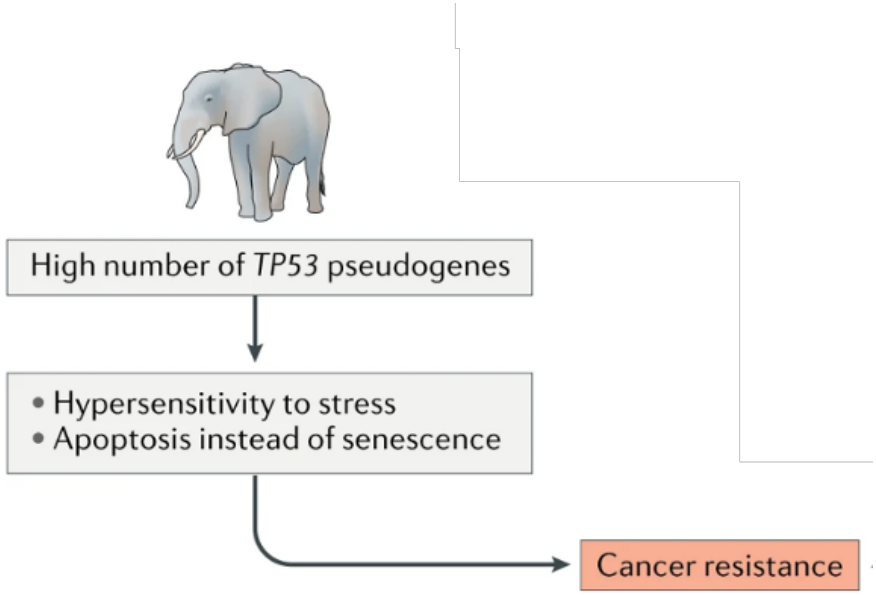

Figure 5. In terms of cell count, elephants possess a greater number of cells within their bodies, which statistically would suggest a higher likelihood of developing cancer. However, the actual occurrence of cancer does not correlate with the body mass of a species. This is due to the fact that elephants have evolved supplementary mechanisms to suppress tumor growth. They have developed multiple copies of the TP53 gene known as pseudogenes, which are connected to an amplified apoptotic response (Seluanov, A., et al., 2018).

Elephant cells exhibit an enhanced p53-dependent DNA damage response, leading to increased apoptosis (cell death) when compared to smaller members of their biological family, such as armadillos, hyraxes, and aardvarks *(Sulak M,. et al., 2016).*

P53 is often referred to as the "custodian of the genetic code" because it plays a pivotal role in overseeing the cell cycle and functioning as a guardian against the development of tumors *(Simmons H., et al., 2023).*

While the exact mechanism through which these novel TP53 gene variants exert their effects remains not fully understood, one hypothesis suggests that the protein products, generated by these TP53 gene variants, may contribute to stabilizing the wild-type p53 protein. This stabilization could enhance the normal p53 protein's stability, making it more resistant to changes or degradation. Such stability might reinforce the cell's ability to prevent cancer development or respond to DNA damage (*Abegglen LM, et al., 2015; Sulak M., et al., 2016).*

3.2 Evolution of *TP53*

TP53 duplicates identified in elephants have displayed signs of positive selection, providing additional evidence of their functional significance *(Abegglen LM, et al., 2015; Sulak M., et al., 2016).* A study conducted by Lynch's team discovered over a dozen instances of TP53 gene copies in two extinct mammoth species. In contrast, the same gene appeared only once in elephants' contemporary relatives. It is hypothesized that the surplus gene copies emerged during the evolutionary expansion of the elephant lineage due to increased size.

Roughly 55-60 million years ago, the retrogenes of TP53 were believed to have emerged in the common ancestor of manatees (*Trichechus*) and elephants. Following this, around 45 million years ago, a proliferation of TP53 began in a shared ancestor of Asian and African elephants, persisting throughout the evolutionary history of elephantids.

The research conducted by *Marc Tollis, Lisa M. Abegglen et al.,* shed light on an intriguing aspect of elephant genetics. They discover that the genome assembly of the African bush elephant contains approximately 19 instances of TP53, while the genome assembly of the Asian elephant harbors between 9 to 11 copies of TP53, as indicated by the two species' respective assemblies.

Approximately 21 to 24 variations in TP53 copy numbers were identified within the genomes of forest elephants. Woolly mammoths, on the other hand, displayed an estimated range of 19 to 28 *TP53* copies in their genomes. The genome of the straight-tusked elephant contained approximately 22 to 25 TP53 copies *(Sulak M., et al., 2016).*

This abundance of TP53 copies within the genomes of different elephant species suggests a significant evolutionary adaptation.

It implies that elephants, as large, long-lived mammals, may have developed this surplus of TP53 copies as a means to enhance their ability to prevent and repair DNA damage effectively.

3.3 Different susceptibility in developing cancer between Asian and African Elephants

Nowadays are present three elephant species, the African bush elephant (*Loxodonta africana*), the African forest elephant (*L. cyclotis*), and the Asian elephant (*Elephas maximus*) *(Shoshani J., 1998).* Asian elephants, known for their impressive longevity of nearly 80 years, and African elephants, which typically live around 65 years, have evolved distinctive strategies for survival *(Moss C. J., 2001; Chapman S. N., et al., 2019).* The variations in susceptibility to diseases between these species hold immediate and critical implications for the conservation of elephants *(Greenwald R., et al., 2009).*

Researches, in order to determine the occurrence of abnormal tissue growth and malignancy in elephants, gathered and assessed pathology information from 26 AZA-accredited zoos across the United States. This dataset encompassed diagnoses from a total of 76 elephants, with 35 being African elephants and 41 being Asian elephants. The results revealed that neoplasia was identified in 5.71% of African elephants and a significantly higher percentage of 41.46% in Asian elephants *(Tollis M., et al., 2020).* Out of all the tumors found in elephants, 69% were non-cancerous, while 14.63% of Asian elephants were identified as having malignant tumors, a contrast to the absence of such tumors in African elephants (see **table 1**) *(Tollis M., et al., 2020)*. In contrast, humans are confronted with a substantial lifetime risk of 39.5% for developing malignant cancer *(Howlander N., et al., 2020).* Furthermore, the risk of benign tumor formation is even more prevalent, with 70%-80% of women experiencing the development of uterine fibroids *(leiomyomas)* during their lifetime *(Zimmermann A., et al., 2012).*

The findings from their study validate two key points. Firstly, elephants exhibit lower rates of malignant cancer compared to humans, and secondly, Asian elephants in zoo settings are more frequently diagnosed with both neoplasia and malignancies when contrasted with their African counterparts *(Tollis M., et al., 2020).*

Neoplasia and Malignant Prevalence					
Species	Total Individuals	Neoplasia	Neoplasia	Malignant	Malignant
		Cases	%	Cases	%
Asian elephant	41	17	41.46	6	14.63
African elephant	35	2	5.71	0	0
Total elephants	76	19	25	6	7.89

Table 1. Incidence and frequency of cancer in African and Asian elephants (Tollis M., et al., 2020)

3.4 Discussion

The study examined revealed that elephant tumors generally display a benign nature, fortified by robust genetic mechanisms that ward off malignant changes. The research highlighted a higher incidence of both benign tumors and malignant cancers in Asian elephants compared to their African counterparts hence, it might be advantageous to enhance tumor surveillance in such cases.

Although earlier research implies that TP53 copy numbers increase with body mass throughout proboscidean evolution in response to heightened cancer susceptibility, the current study revealed that some of the highest TP53 copy numbers were actually found in the smallest elephants *(Sulak M. et al., 2016).* Utilizing the existing sequence information, their analysis projected around 19 to 21 instances of TP53 gene copy variations within the ancient woolly mammoth genome, which dates back approximately 44,800 years and hails from Oimyakon, Russia. Intriguingly, in a more recent context, the genome of the mammoth from Wrangel Island, aged about 4,300 years, exhibited a noticeably higher count of TP53 copies, approximately 1.3 times greater than the previous.

The rise in TP53 gene copies observed in the Wrangel Island mammoth is possibly linked to the stochastic integration of retrogenes within the population, rather than being a result of selection influenced by body size *(Rogers R. L., et al., 2017).*

Despite the smaller physical stature of forest elephants, their genomes were found to harbor a substantial number of TP53 copy number variations, ranging approximately from 21 to 24. This count surpasses the estimates for bush elephants. New genomic findings indicate a stronger genetic affinity between forest elephants and straight-tusked elephants, as opposed to bush elephants *(Meyer M., et al., 2017; Palkopoulou E., et al., 2018).*

The higher estimated TP53 copy number in forest elephants relative to bush elephants may not be related to modern differences in body mass between species (and possible protection from increased cancer risk), but instead may be due to complicated evolutionary and demographic histories which include migration that can dramatically affect the dynamics of repetitive genomic elements such as retrogenes *(Deceliere G., et al., 2005).*

4. CONCLUSION

Cancer, a complex disease characterized by uncontrolled cell growth and proliferation, has long been a challenge for both humans and various animal species. The phenomenon of cancer development defies a straightforward explanation, as demonstrated by Peto's paradox, which questions why larger and longer-lived animals do not proportionally exhibit higher cancer rates. This enigma has driven researchers to explore the development of cancer resistance mechanisms in mammals.

Among these remarkable adaptations, long-lived bats have evolved unique genetic mechanisms that confer resistance to tumorigenesis and promote cellular damage repair. These mechanisms, involving DNA repair pathways and specialized proteins, contribute significantly to their extended lifespan and cancer resistance. Similarly, the naked mole rat and blind mole rat have unveiled extraordinary strategies to combat cancer. The naked mole rat employs high-molecular-mass hyaluronan (HMM-HA) as a tumor-suppressing compound, early contact inhibition, unique INK4 locus and epigenome stability. While the blind mole rat showcases an interferon-based 'concerted cell death' (CCD) mechanism. Whales , instead, developed specific mutation in DNA repair genes.
These adaptations highlight nature's ingenuity in developing diverse approaches to block cancer.
Conversely, elephants exhibit their own intriguing mechanisms for cancer resistance. Their expanded TP53 gene family and enhanced p53-dependent DNA damage response contribute to the elimination of damaged cells before they progress into precancerous stages (see **table 2**).

	Species	Mechanisms	Lifestyle and ecological adaptation
Cancer	Naked mole rat	High-molecular-mass Hyaluronan (HMM-HA); Early contact inhibition; unique INK4 locus; epigenome stability	Subterranean burrows; hypoxia; protected environment
	Blind mole rat	Interferon mediated concerted cell death	Subterranean; burrows; hypoxia; protected environment
	Elephant	Multiple *p53* and *LIF* copies	Large size; lack of non-human predators
	Whale	Whale specific mutations in DNA repair genes	Large size; Lack of non-human predators; deep water diving; cold

Table 2. List of neoplastic mechanisms in mammals and their adaptive responses.

The distinction between Asian and African elephants in terms of cancer susceptibility further emphasizes the complexity of cancer resistance mechanisms among closely related species.
In light of these discoveries, understanding the intricacies of tumor susceptibility in Asian elephants presents a valuable avenue for future research.
Ultimately, the study of cancer resistance mechanisms in various mammals underscores the awe-inspiring diversity of nature's solutions to a common biological challenge.

In closing, the study of cancer resistance mechanisms in mammals transcends species boundaries to offer profound insights into the fundamental biology of cancer. These discoveries possess the potential to redefine the landscape of human cancer research and treatment, ushering in an era marked by more effective, personalized, and targeted approaches to combat one of the most challenging aspects of medical science.

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