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Walking Through Elephant Cancer Resistance: What it can teach us about elephants, genetics and disease defenses

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ABSTRACT

Cancer is a disease that affects the whole animal kingdom, but it does not behave equally in all the species. Elephants are one of the animals that even though have a greater number of cells in their body they present a low cancer rate. This phenomenon is also known as the Peto's Paradox. Scientists have concluded that elephants have greater immunity to cancer because their genome has extra copies of tumor suppressor genes: TP53 and LIF. Even though elephants have a big immunity to cancer, they still get the disease, for example Asian elephants are more susceptible of getting reproductive tract neoplasia. Exploring this information is essential to understand how cancer behaves and how our own genes could help us fight the disease. This paper is a review of the actual knowledge the scientific community has regarding how cancer works in elephants, with the final goal of exploring the meaning this has into our understanding of genomics and how it could help us to develop a cure for cancer.

Introduction

Elephants are the biggest animal alive that walks on land. There are three elephant species: Elephas Maximus (Asian Elephant), Loxodonta Africana (African Bush Elephant) and Loxodonta Cyclotis (African Forest Elephant). Its body is full of cells that are constantly dividing and reproducing. As there is a bigger chance for mutations to develop logic will guide us to assume that they could be more prone to getting cancer: a disease that is present in all multicellular organisms on earth (Nery, Rennó, Picorelli and Ramos, 2022), and that occurs when there is a genetic mutation that causes cell reproduction to lose control. Surprisingly, scientific research findings have proved the contrary and scientists have shown that elephants only have a 3% chance of getting cancer (Callaway, 2015). This controversy is not new knowledge but it dates all the way back to epidemiologist Richard Peto of the University of Oxford, that in 1970 noted the little relationship there is between body size and cancer rate in certain species, creating what we call today the Peto's paradox. Even though there has been evidence of the little amount of cancer that elephants get, we start questioning ourselves: why? Why does such a big animal have such lower cancer rates? What makes it special?

Cancer is a wide disease which we are still trying to understand, and rates of cancer seem to not be related to the number of cells in a living body, but to how efficient cancer related mutations are in our body and what factors activate them. It has been proven that most if not all adults have cancer related mutations (Arney, 2020), but the true question is what and why they develop into tumors. Animals such as elephants possess a type of immunity to the disease that humans don't. Scientists have come to the conclusion that this is related to their efficient tumor suppressor genes. By continuing to develop research regarding the genome of this species we have gotten closer to understanding how evolution and tumor suppressors behave, allowing scientists to explore the possibility of developing new anti-cancer therapies and drugs.

Understanding Cancer

The scientific community already knows how to cure some cancers and how to treat others, making them stop their uncontrollable reproduction in the living organism body (Arney, 2020). Cancer kills thousands of humans every year, but this fact does not mean that it kills thousands of other species every year. Some animals are more prompt to get cancerogenous mutations and develop cancer than others. An example of this is that animals such as the caw, the human and the dog have more suitability for cancer than elephants, the naked mole rat or the whale (Arney, 2020). There are many theories as for why some animals get more cancer than others, but they vary within the species. Questions like: Does size matter? Does longevity matter? Does contamination influence? Are all questions that come to mind when discussing cancer rates on organisms, and the most accurate response to them would be yes, but we haven't found to what extent.

Following the definition of cancer, we are able to say that as an organism grows the probability of it getting cancer should increase, because cells of larger and older bodies have divided more times so there should be a higher risk of cancerous mutations (Callaway, 2015). According to scientific findings and Peto's Paradox this is not true between bigger and smaller animals of different species, however it does apply when talking about age. Between the same species, older individuals have a higher risk of presenting cancer than younger ones (Nery, Rennó, Picorelli and Ramos, 2022) (Preston et al, 2023).

Natural selection, the process that assures an animal evolves so its species survives, is a very important factor when solving Peto's paradox. Animals like elephants and whales live longer and are bigger but reproduce less often at an older age. Allowing their bodies to preserve as they do not wear out as much and enhancing protection against cancer as the species evolves (Nery, Rennó, Picorelli and Ramos, 2022). Natural selection would fail if animals got cancer and died before they got a chance to reproduce (Arney, 2020), meaning that bodies must have evolved or tried to evolve a kind of mechanism that combats cancer to an extent that ensures reproduction. Furthermore, genetics is also a very important factor when it comes to understanding cancer and solving Peto's paradox. Not only cancer is a disease that evolves because of damage in cells' genes but genetic material also is the place where the mechanism to combat cancer is. Elephants are a great example of this. In the elephant genome there are more copies of what is known as cancer suppressor genes. Elephants have 20 copies of the gene TP53 (Callaway, 2015), which is the main gene whose job involves preventing malign tumor development.

The Role of Tumor Suppressor Genes

Tumor suppressor genes are specific genes whose purpose is to protect the body against cancer. They are able to detect cell damage and then activate a series of responses such as: cell growth arrest, DNA damage repair, activation of oncogenes, telomere shortening, cell-cycle control and apoptosis (Voskarides and Giannopoulou, 2023). Some common tumor suppressor genes are BRD7, CNOT11, LIF, SOD1, TP53 and COX20, but there are more, and some may vary among species. Most mammals have just one copy of each gene, but a series of duplications have been found around organisms. The major occurrence of a tumor suppressor gene duplication has been of the T53 gene in elephants.

Gene TP53

The main tumor suppressor gene is TP53 which encodes for the protein p53. Since the start of evolution, the ancestral p53 protein's primary function has been protecting the DNA against all possible damage. The responses given by p53 to protect genetic material may be permanent or temporary, but its role is biologically essential for survival (Haupt and Haupt, 2017).

Humans, like most mammals, just have one gene of TP53 to protect us. Which is quite relevant as in 50% of cancer cases, a mutation in the TP53 gene is presented (Voskarides and Giannopoulou, 2023). We believe that elephants get so little cancer because they have developed extra TP53 copies. The African bush elephant contains 19 TP53 copies, the Asian elephant contains 20 copies, and the African forest elephant contains 21-24 copies, being the species with the highest quantity of TP53 copies currently alive. Some descendants of the actual elephants also had more than one TP53 copies such as: The extinct wooly mammoths who had 18-19 TP53 copies and the straight-tusked elephant who had 22-25 TP53 copies (Preston et al, 2023), as shown in (Figure 1). Scientists are also aware that elephant cells are more sensitive to DNA damage than other human cells but are twice as likely to commit apoptosis (cell death) than a healthy human cell (Gaughran et al., 2016).

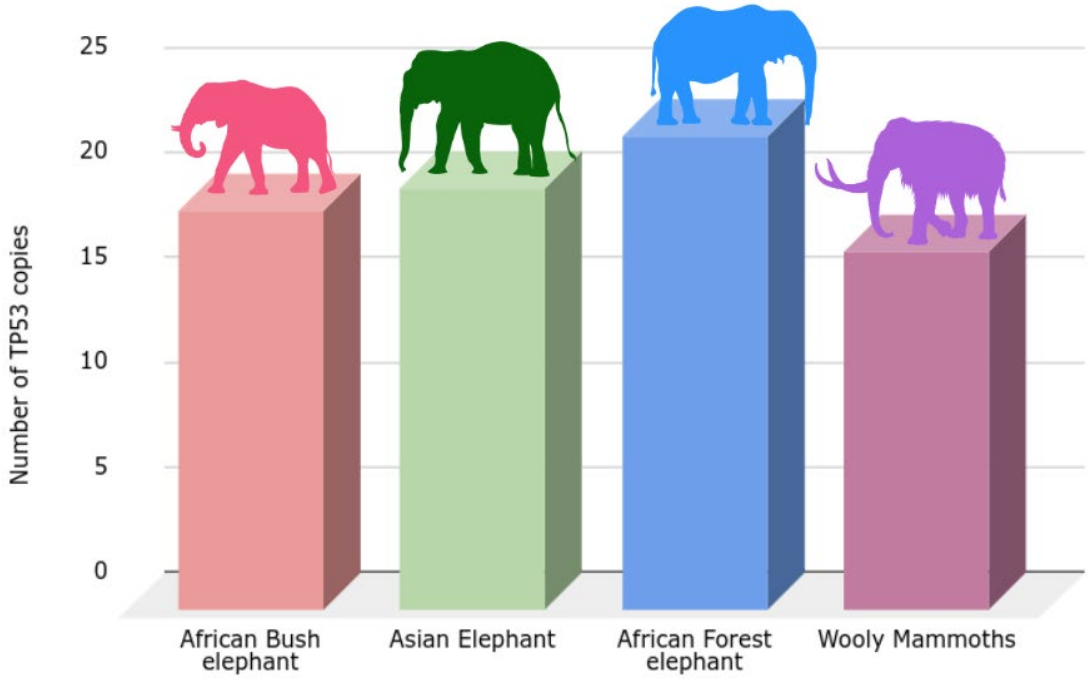

Figure 1. TP53 copies inside the genome of the respective elephant species or ancestor.

It is important to notice that these extra genes originated in the common ancestor of the elephants, manatees and the hyrax. But this feature was only passed to the elephant. In the same genetic line the number of TP53 genes increases as body size increases (Gaughran et al., 2016). This is related to natural selection and evolution. As the elephants were evolving into longer living creatures their body's way of finding defense became increasing TP53 genes. It is also known that TP53 is associated with fertility, aging and longevity (Voskarides and Giannopoulou, 2023). In addition, it is important to take into account that inside the elephant linage, the Afrotheria linage, the TP53 tumor suppressor duplication is not the only case that has been seen around evolution, as scientist have observed the duplication of other tumor suppressors in smaller animals related to the elephants (Vazquez and Lynch, 2021).

The extra copies of TP53 are called retrogenes (RTGs), because the extra copies originated after retrotransposition. This means that they were reverse transcribed, the RNA molecule had its introns removed and converted into DNA. Afterwards it reinserted into the genome over the course of millions of years, becoming retrocopies (Gaughran et al., 2016)(Callier, 2019). Retrocopies that have transcriptional activity are called retrogenes (Staszak and Makalowska, 2021). These retrogenes are not the same as the original TP53 gene. TP53 RGTs, have deletions and premature stop codons. These retrogens only code for a protein that is approximately 40% similar to the original one, having 157 of the 390 amino acids (Nunney, 2022). As the TP53 RGTs are not exact to the original and none

of them are complete, all presenting a premature stop codon, the accuracy of their ability to prevent cancer is unknown. Scientists question if the extra copies of tumor suppressor alone is what makes elephants have such a small chance of cancer (Nunney, 2022).

TP53 Retrogene-9

Out of all retrogenes, it is known that retrogene number 9 encodes for a protein called p53-R9. This protein has 180 amino acids, and its identity is 54.1% alike to the original p53 protein (Preston et al, 2023). This protein made by a retrogene has some deficiencies as the retrogene has a stop codon, that in this case, is located in the middle part of the code and prevents the resulting protein from having a tetramerization domain (a protein domain that allows/facilitates protein-DNA binding), and nuclear localization sequences. Even though this protein is not exactly like its original counterpart it was recently shown that it has the capacity to induce cell apoptosis.

In a recent study the p53-R9 gene was induced into human cancerogenous cells, with the purpose of studying if that retrogene 9 could induce apoptosis through caspase activation (Preston et al, 2023). The retrogene 9 interacts with the Tid1 protein (protein activated by cellular stress that is responsible for mitochondrial translocation of p53), releases cytochrome c and goes to the mitochondria. Once in the mitochondria it induces cell death by interacting with the apoptotic protein: Bax. These findings made scientists reach into the theory that in absence of p53, p53-R9 still has the ability to be part of a mitochondria-induced apoptosis.

LIF Gene

The multifunctional interleukin-6 class cytokine leukemia inhibitory factor, abbreviated as LIF is a tumor suppressor gene associated with elephant cancer. Elephants have 7-11 extra copies of LIF in their genomes (Nery, Rennó, Picorelli and Ramos, 2022). Most mammals only have one. Unlike with p53, these copies are not retrogenes and are also present in the elephant's closest living relatives, the manatee (Trichechus) and the hyrax (Hyracoidea).

LIF transcription is regulated by P53 through a binding site that is located in the intron 1 of the LIF gene (Vazquez et al., 2018). Talking about transcription, scientists have identified transcripts of LIF-D, LIF-M, and LIF-T. Also, they found a transcript of a duplicated LIF gene, named LIF6 in Asian Elephants (Vazquez et al., 2018). LIF6 encodes for a transcribed gene in elephants in its own way, the rest of the duplicated LIF genes encode transcription through distinct signals or they are pseudogenes.

LIF6 is a copied gene that is found in most elephant tissues and its transcription is upregulated by p53 in response to DNA damage and then moved to the mitochondria. LIF6 replicated gene in elephants has a putative TP53 binding site. LIF6 transcription outcome is to induce apoptosis. Similar to the TP53-retrogene 9, LIF6 induces cell death through a mitochondrial mechanism. What is currently known is that LIF6 induces apoptosis through a Bak/Bax mechanism. Both Bax and Bak are apoptosis related proteins that form large pores in the membrane of the mitochondria, from where cytochrome-c is released, leading to caspase mitochondrial dysfunction and finally cell death (Figure 2) (Vazquez et al., 2018).

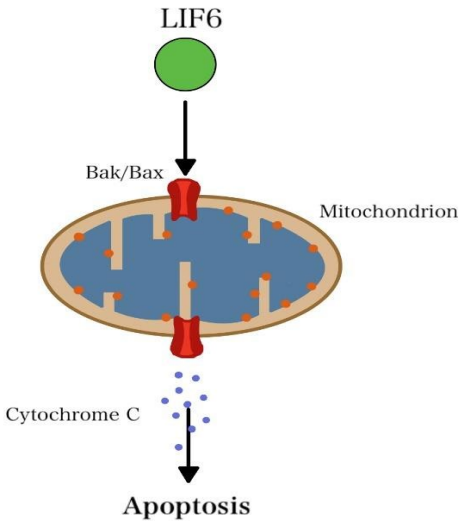

Figure 2. LIF inducing apoptosis through a bak/bax mechanism. Adapted from (Vazquez et al., 2018).

Elephants do get cancer

Any kind of cancer or tumors, both benign and malign aren't very common in elephants. Benign tumors are those that reproduce at a slower rate and are only dangerous in large quantities. Malign tumors are those that spread fastly around the body and are deadly. Even though elephants do present an immunity to this disease, in Asian elephants (Elephas Maximus) it is common to find reproductive tract neoplasia, a series of both benign and malignant tumors that commonly affect elephant reproduction. Using the cases of reproductive tract neoplasia, we are able to see how even though the cancer is only developed at low rates, it does exist in the species, and it may even be harmful for them.

There have been two recent studies that analyze reproductive neoplasia in elephants. The first one, shown in Tollis et al. (2021), compares any type of tumor between African (Loxodonta) and Asian (Elephas maximus) elephants. This study was done by analyzing data from 26 accredited zoos from the Association of Zoos and Aquariums (AZA), in the USA. The data was from 35 African elephants and 41 Asian elephants, 76 as a total. They found that 41.46% of Asian elephants and 5.71% of African elephants had neoplasia. Out of the Asian elephants with neoplasia, 35.3% were malign tumors and 64.7% were benign tumors. On the other hand, all of the neoplasias in African elephants were benign (shown in figure 3). Out of these tumors it is important to point out that more than half were in reproductive organs.

The second study, made by Landolfi, Gaffney et al. (2021), only talks about reproductive neoplasia in elephants. They looked at 80 female Asian elephants from USA zoos in the years of 1988-2019 and found reproductive neoplasia in 64 out of the 80 elephants. 63 of the cases were presented in the uterus, which were classified as: 57 being myometrial leiomyomas, 8 uterine adenocarcinoma and 15 uterine neoplasia (shown in figure 3). Out of all the cases only 19% were malignant (mostly adenocarcinomas), and they had metastasized in 67% of the cases.

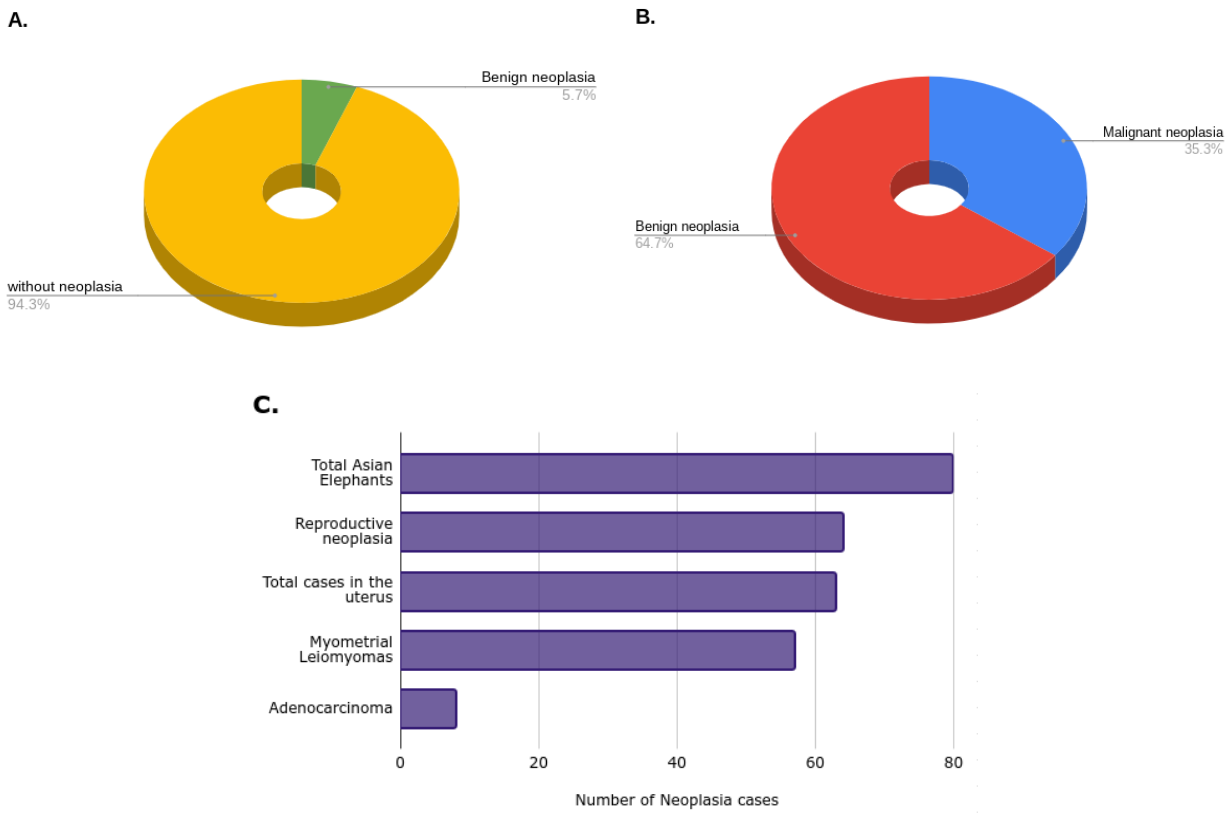

Figure 3. Graph A represents the percentages of benign neoplasia and no neoplasia in African elephants and Graph B represents the percentages of benign and malignant tumors in Asian elephants, the information is based on finding in (Tollis et al., 2021). Graph C represents the kinds of neoplasias found in elephants, as found in (Landolfi, Gaffney et al., 2021).

Analyzing the previously presented research we can point out that tumors in reproductive tissues are the most common in elephants. We can say that they form more often in Asian elephants than African elephants, and the most common form of reproductive tumors was leiomyomas. Furthermore, even though leiomyomas are benign, a significant percentage of malignant cancerous neoplasms were present in both studies, showing that elephants do get cancer that could potentially grow and impact their populations. Reproductive tumors can cause a big impact on elephant populations since they affect reproductive success. Neoplasias have a negative effect on conception success, pregnancy development and the nature of birth (Abegglen et al., 2022). This is quite an important factor when thinking about evolution, and preserving the species, which becomes important as elephants are an endangered species.

It is not clear at what point in their lives elephants get reproductive neoplasia, nor why it is more common than other types of tumors. There are records on elephants under human care getting leiomyomas when reaching older ages, giving insight that this tumor is probably more prevalent in large and older females. On the other hand, there is evidence of an Asian 15-year-old elephant dying from 5-10 cm uterine leiomyomas (Abegglen et al., 2022).

Something that should be looked further in, is the relationship between the tumor suppressor gene TP53 and reproductive tract neoplasia. As mentioned before, what scientists believe that gives elephants protection against cancer is extra copies of tumor suppressors, like p53. Taking this into account, a recent study claims that in humans the equivalent type of cancer to one of the elephant neoplasias (adenocarcinomas), is type 1 endometrioid carcinomas, which in humans are associated with mutations in some tumor suppressor genes, but usually not with mutations in the TP53 gene (Landolfi, Gaffney et al., 2021). If studied further this could give us more information on how TP53 protects elephants from cancer.

Drug Development

Considering that cancer is not all about genes and mutations, but also habits, health, hormones, and evolution, elephants showing cancer resistance is a promising model to study for the development of a cure. After all, p53 is a powerful tumor suppressor that works as a sequence-specific transcription factor with the ability to bind itself to certain DNA sequences activating the transcription of genes (Hassin and Oren, 2023). TP53 is currently crucial in protecting most mammals against cancer.

Since decades, scientists have been trying to develop drugs based on the p53 protein (Hassin and Oren, 2023). Unfortunately, only a few of the drug programs have reached clinical trials. Because of these failures, some consider p53 undruggable (Hassin and Oren, 2023). On a positive side, the process of exploring what can be developed with p53 has already started. Vaccines, antibiotics, a genetic focused drug, using p53 regulators, exploring immunotherapy and focusing on specific tumors are a range of the ways we are currently trying to develop a successful p53 oriented anti-cancer drug (Hassin and Oren, 2023) (Padariya et al., 2022).

Immunotherapy is one of the methods that seems promising, as they are trying to find a way to recruit the immune system to recognize and attack cancer cells that harbor TP53 mutations. This is good taking into account that loss of p53 function is related to cancer cells that evade more with efficiency, immune attacks (Hassin and Oren, 2023). Pursuing small molecules is also an interesting approach because small molecules protect p53 from negative regulators, can activate p53 signaling and can restore a mutated p53 protein (Hassin and Oren, 2023). Another important factor of the process of drug development that some scientists currently are and should continue to focus on is the MDM2-p53 interaction. MDM2 E3 ubiquitin liaise is a protein that regulates p53. In elephants, the TP53 retrogenes control the MDM2-p53 interaction so it is regulated by specific isoforms. They contribute to solving DNA damage and other types of cell stress (Padariya et al., 2022).

Other drug development techniques that are a focus of recent research are developing an mRNA vaccine and gene therapy (Hassin and Oren, 2023). The list of different drug techniques can continue, as there is other ways we can use the p53 protein to develop a cure, and hopefully more are going to come. Showing that as technology advances, we gain more knowledge into both how to develop drugs and how TP53 functions. Using p53 to find a cancer cure becomes promising.

Conclusion

Cancer is a disease that develops in most living organisms, but it is more abundant in some organisms than in others. Even though it would make sense for bigger animals to get more cancer, some of the biggest animals on earth such as whales and elephants get very little cancer, this is known as the Peto's paradox (Callaway, 2015). Elephants have shown to get very little cancer percentages through their lifetimes, scientists predict it is thanks to how natural selection and evolution affected their genomes causing elephants to have more copies of tumor suppressor genes such as TP53 and LIF. TP53 has a very important role in DNA damage repairing and acting against cellular stresses, but the TP53 elephant extra copies are all retrogenes making us doubt their complete functionality and capability to protect the body against tumor development (Nunney, 2022). We need further studies to analyze the exact role of every single one of the copies inside the elephant genome. Also, to further know the complete effect of TP53 RGTs in elephants, we shall consider how tumors behave in elephants. In this article we saw that elephants commonly develop reproductive tract neoplasia. Finally, we are able to realize the opportunity in developing a p53 focused drug to be able to fight cancer. Even though this seems like a difficult task, as technology is developed and new knowledge reached, the goal seems more possible.

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