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Exploring The Connection Between the Spontaneous Regression Seen in Neuroblastomas, Hypertumors, and Reactive Oxygen Species

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A Review of the Literature

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Introduction

Peto's Paradox

Despite having 2000 times more cells than humans, blue whales have not been found to have tumors, or cancer in general (Caulin & Maley, 2019). This suggests that blue whales have a mechanism at suppressing cancer at least 2000 times more effective than humans. If an animal is larger or lives for a longer time, that animal would have an increase in the number of cells dividing. Under the assumption that all cells have equal risk of cancer, this would mean an animal like a human would have more cancer than an animal like a rat. However, as shown in figure 1 the observed cancer rate remains the same across animals with varying lifespans and body mass. This variation in expected and observed cancer rate is the basis of Peto's Paradox.

This is the phenomenon that larger animals like blue whales should have higher rates of cancer when compared to a human due to their increased lifespan and bodymass, but that is not what is observed. There are a multitude of possible explanations as to why this may occur, but this paper seeks to specifically explore hypertumors and metabolism.

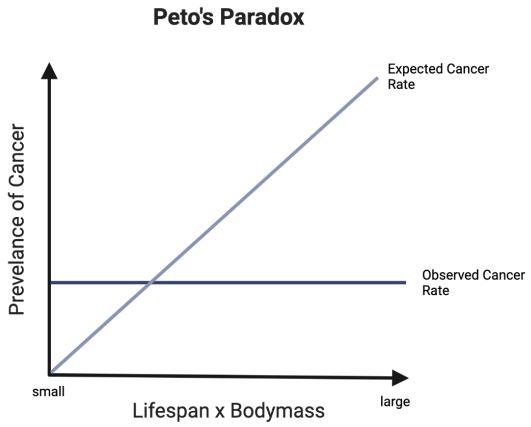

Figure 1*. This figure illustrates the expected rate of cancer vs. the observed rate of cancer.

Hypertumors

A possible explanation for the negative correlation between body size and rate of cancer in animals is the ability of tumors to form cheater cells known as hypertumors which have not yet been proven to exist. Hypertumors allow the size of the tumors to be maintained at sublethal sizes. Clinical disease in humans was defined as reaching 1.2 kg in weight. In a study done by Nagy (2004), the mathematical cell simulations showed hypertumors held a sublethal size for years, while only a small portion of the simulated tumors would have been deemed as clinical disease in humans. Hypertumors are theorized to be "cheater cells" because they develop and proliferate within a pre-existing tumor, depleting the original tumor's resources and blood supply. This trend can be seen in Figure 2 that depicts the decrease and eventual extinction of hypertumor 1's growth mass as hypertumor 2 begins to form. The formation of hypertumors would deplete the original tumor of its blood supply and nutrients, leading to spontaneous cell death. The hypertumor would no longer have a source of nutrients and die as well. It is postulated that the common phenomenon of spontaneous necrosis or apoptosis in malignant tumors, such as those seen in some neuroblastomas, could actually be the formation of hypertumors (Nagy, 2004).

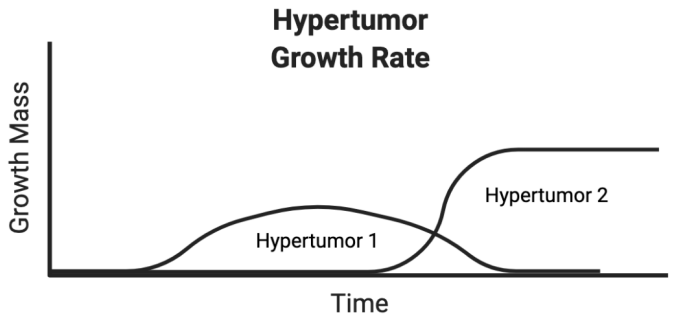

Figure 2*. This figure illustrates the growth rate of hypertumors.

Neuroblastomas

The progression of how neuroblastoma development can be compared to the hypothesized formation of hypertumors. Neuroblastomas are the second most common malignant tumor in childhood. Children with this disease have a poor prognosis with the 5-year survival rate being less than 60% (Garaventa et.al., 2003). Even with intense chemoradiotherapy, children have a high fatal relapse rate and long-term survival rate of merely 25%. Little improvement in prognosis has been made by additional treatments such as cytokines, monoclonal antibodies, and radioactive metabolic drugs (Garaventa et.al., 2003). In its natural course, neuroblastomas have been observed to go through spontaneous regression, a characteristic expected to be seen in hypertumors. For this reason, it is hypothesized that neuroblastomas are a form of hypertumors. It has also been noted that neuroblastomas that exhibit overexpression of the RAS g-protein, a well-known oncogene, show morphological changes indicative of cell death, another feature found in hypertumors (Nagy, 2004).

Metabolism

One of the pathways that has been studied in metabolism is the increase of reactive oxygen species (ROS). This includes hydrogen peroxide (H_2O_2) and a number of free radicals. ROS are highly reactive intracellular chemical species and are derivatives of O_2. ROS also plays an important role in metabolism, including regulation of apoptosis and vascular cell proliferation. ROS are byproducts of metabolism and can cause DNA damage which can lead to aging and cancer. Elevated ROS levels can lead to pathologic conditions, including tumor growth as well as spontaneous cell death, a characteristic of hypertumors (DeBerardinis and Chandel, 2016). Larger animals have lower basal metabolic rate (BMR), indicative of the amount of oxidative damage which is also low in large animals (Caulin and Maley, 2011). This leads to less

endogenous DNA damage and somatic mutation rates, decreasing the rates of cancer before it even forms (Caulin and Maley et.al., 2011). However, it is now accepted that moderate levels of ROS are needed for many cellular functions.

How Hypertumors Explain Peto's Paradox

Characteristics of Hypertumors

If hypertumors were witnessed in a clinical setting spontaneous cell death of portions of a tumor would be visible. This may present itself as necrosis or large regions of apoptosis, a cause of which may be disruption of the tumor's blood supply without further explanation. This spontaneous cell death is linked to the overexpression of the RAS g-protein which is associated with the activation of the JNK and p38 MAPK pathways (Wagner and Nebreda, 2009). These characteristics are present in neuroblastomas making them a target of research as possible hypertumors. An anticancer drug, Fenretinide, is used to treat neuroblastomas and also induces overexpression of RAS. Depending on the pathway and dosage taken, this can lead to cell death or cancer further providing evidence for the existence of hypertumors.

RAS and JNK/p38

The RAS g-protein activates the JNK and p38 MAPK pathways which are integral in the control of cell proliferation and differentiation by regulating cell cycle progression. In particular, the JNK and p38 MAPK pathways are associated with cancer growth in humans.

JNK's role in cell cycle progression and cell growth has been well defined, however, the pathway has yet to be fully realized. JNK can initiate cell growth and survival by being activated transiently, however, its prolonged activation leads to necrosis that will be seen in the mechanism

for fenretinide (Maurer et.al., 1999). One possible pathway for prolonged JNK activation involves the mediation of the tumor necrosis factor-α (TNFα)-dependent apoptosis in a caspase-dependent manner, more specifically the caspase 8 activation pathway. Further, the second possible pathway for JNK works in a caspase-independent manner by inhibiting the caspase 8 pathway. This pathway involves E3 ubiquitin ligase iTCH which leads to the inhibition of the caspase pathway and FADD-like apoptosis regulator through JNK1, a MAPK, phosphorylation of iTCH31. The JNK1 pathway involves the regulation of proteins p53, activating transcription factor 2 (ATF2), and ElK1. The third possible mechanism involves the tumor necrosis factor-α (TNFα)-dependent apoptosis and caspase 8- independent cleavage of BH3-interacting domain death agonist (BiD). The purpose of this is to relieve the inhibition on caspase 8 (Wagner and Nebreda, 2009).

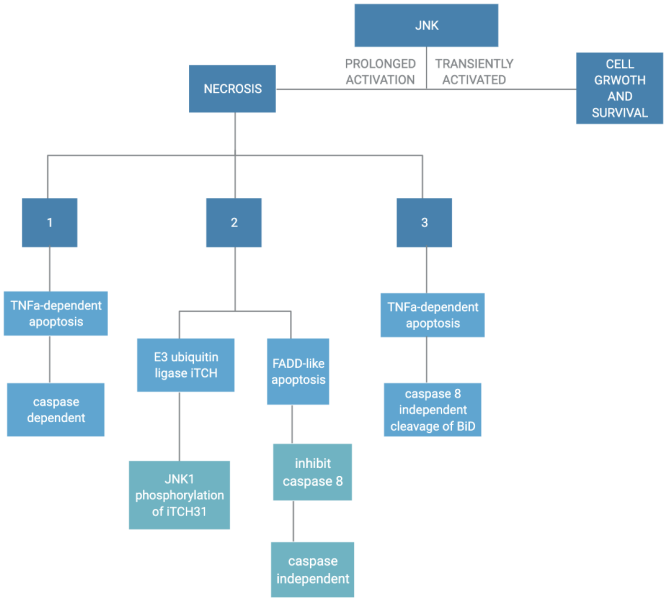

Figure 3*. The possible JNK pathways.

In contrast to the JNK pathway, the p38 MAPK pathway has been established with a special focus on p38α. Currently, it has been discovered that there are four genes that encode for

the p38 MAPK pathway, including MAPK14 encoding for p38a, which is the most researched and highly abundant (Wagner and Nebreda, 2009). This pathway is activated by upstream MKK3 and MKK6 kinases as shown in the Figure 4. The major proteins of interest that are regulated by this pathway include p53, ATF2, and ElK1. It should also be noted that these are the same proteins regulated by the JNK1 pathway as well. This pathway, like the JNK pathway, can increase tumor growth and promote angiogenesis of a tumor. However, under correct conditions such as activation due to MKK6, the p38 pathways have been shown to suppress tumor metastasis. In addition, it has been shown that apoptotic stimuli can activate p38α. This has been linked to the production of ROS and is likely important to p38a tumor suppression. In immortalized cells, p38α triggers apoptosis in response to increased expression of ROS-inducing oncogenes.

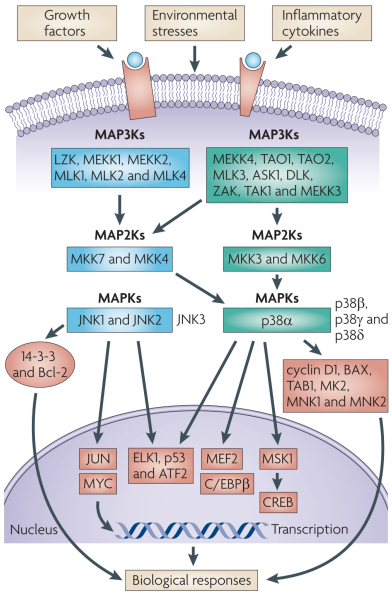

Figure 4. The p38 MAPK Pathway (Wagner et al., 2009)

Sustained activation of the JNK and p38 MAPK has been shown to induce cell death. These pathways can be activated by a number of stimuli including oxidative stress and chemical

agents. In KP-N-TK (neuroblastoma) cells, the RAS g-protein activates these pathways in an ROS-dependent manner.

Neuroblastomas

Neuroblastomas exhibit characteristics seen in mathematical models of hypertumors including programmed cell death and overexpression of the RAS g-protein (Nagy, 2004). Research has examined the role of RAS in tumor regression, showing high expression of RAS in favorable outcomes (Kitanaka et al., 2002). Degenerating tumor cells also showed nuclear condensation, no activation of caspase cascade, and no apoptotic DNA fragmentation. This means neuroblastoma cells with overexpression of RAS follow a caspase-independent, non-apoptotic cell death. It was also tested and confirmed that autophagic degeneration, a nonapoptotic type of programmed cell death, can be induced by RAS overexpression. Thus, increased levels of RAS have been correlated to aggressive cell proliferation which may be causing the spontaneous regression seen (Kitanaka et.al., 2002). For this reason, neuroblastomas and their mechanisms are being further studied to determine correlation to the possible existence of hypertumors.

Fenretinide

N-(4-Hydroxyphenyl)- retinamide (4HPR), commonly known as Fenretinide, is a retinoid anti-cancer drug that has been recently studied in correlation to the treatment of neuroblastomas. Fenretinide induces apoptotic cell death in neuroblastoma cells. Studies have shown that this occurs due to sustained activation of the p38 MAPK and JNK pathways of the RAS g-protein in an ROS-dependent manner. In resistant neuroblastoma cells, Fenretinide did not produce intracellular ROS and thus activated the kinase pathways. (Osone et.al., 2004).

Retinoids have been researched extensively in vitro regarding neuroblastomas and in recent years have been used in clinical investigation. In vitro studies demonstrated that Fenretinide can suppress malignant tumor growth associated with induced apoptosis (Lovat et.al., 2000). This includes neuroblastomas, and it is believed that administration of this drug over a long period may prevent relapse of the disease. This is essential in neuroblastoma patients, where the high rate of relapse can be fatal. In a phase I drug trial, disease progression was also measured and showed signs of disease stabilization. In addition, the majority of patients had manageable, reversible toxicities showing promise for the clinical use of the drug. This is indicative that Fenretinide has the potential to provide prolonged stabilization within neuroblastoma patients and should be further researched.

Metabolism is Another Pathway of Peto's Paradox

ROS

Fenretinide has multiple distinct pathways that allow it to induce apoptosis. One of the pathways that have been studied is the increase of ROS (Lovat et.al., 2000). In humans, moderate to low levels of ROS are needed for many cellular functions. Elevated ROS levels can be seen in tumor cells and can lead to increased tumor growth. Paradoxically, once a toxicity threshold of ROS levels is reached, this triggers apoptosis of cancer cells, as seen in Fenretinide, without affecting normal cells. The effect of ROS levels is shown in Figure 5. A decrease in ROS levels would lead to less formation of cancer, but if cancer were to form it would lead to an adapted cancer cell that can survive in lower ROS conditions. An increase in ROS levels above optimal range for cancer cells would lead to cancer cell death.Therefore, manipulating ROS levels can be used to treat cancer (DeBerardinis and Chandel, 2016).

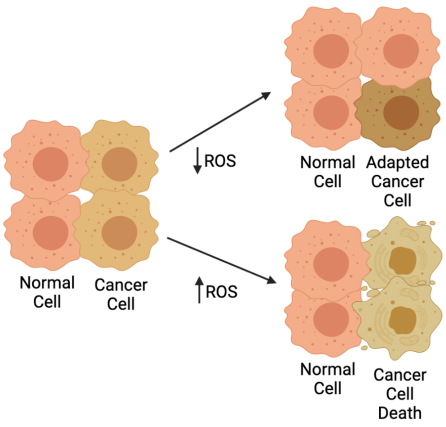

Figure 5*. This figure illustrates the effect of a decrease and increase in ROS levels.

Previous studies claimed that metabolism and hypertumors were two different pathways that serve to explain Peto's Paradox, however, given the literature review in this paper, it can be hypothesized that the effects of metabolism and the existence of hypertumors are linked via ROS. Despite low levels of ROS being optimal for both large animals and humans, higher levels of ROS above the normal level has been shown to be beneficial. The trials involving Fenretinide support this (Perillo et al., 2020).

Redox

Reactive Oxygen Species include hydrogen peroxide (H_2O_2) and a number of free radicals. One of the ways metabolism controls signaling is by manipulating ROS levels. Low levels of ROS are integral to basic cell function, cell proliferation and cellular adaptation due to metabolic stress. In cancer cells, H_2O_2 levels increase. This leads to activation of signaling pathways and conversion of H_2O_2 to OH radicals, which directly damage DNA, proteins, and lipids (DeBerardinis and Chandel, 2016). In addition, signaling pathways and transcription factors necessary for tumorigenesis are activated. This is done by activation of oncogenes, and

by losing tumor suppressors. Cancer cells need increased ROS levels, but substantially increased levels of ROS can lead to spontaneous cell death. Increased ROS levels can be induced by disabling the antioxidant capacity of cancer cells which allows ROS levels to rise and induce cancer cell death. To prevent this from happening, cancer cells induce the NRF2- dependent genes. In other words, this gene acts as a redox balance for cancer cells, making sure ROS levels stay within an optimal range for proliferation (DeBerardinis and Chandel, 2016).

Discussion/Conclusion

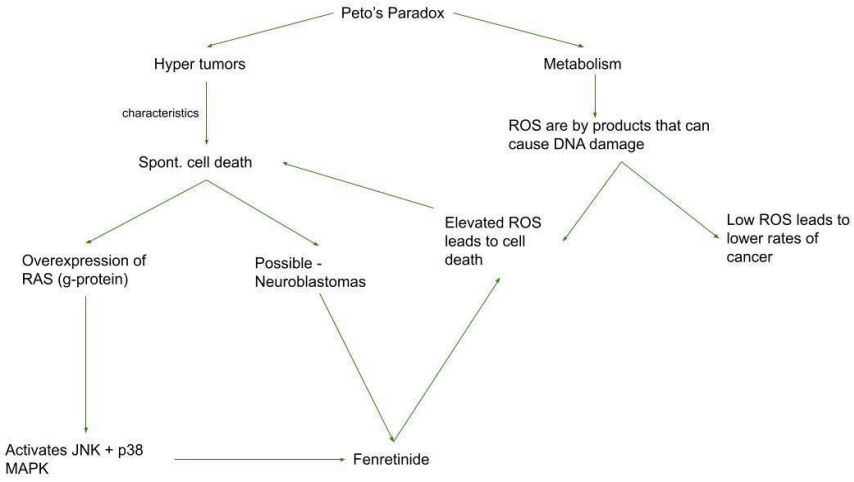

Figure 6. This flowchart illustrates the connection made throughout this paper in a consolidated manner.

In this paper, we postulate that the formation of hypertumors leads to overexpression of the RAS g-protein, ultimately causing spontaneous cell death. In the study done by Kitanaka (2002), it was stated that hypertumors would be expressed as regions of spontaneous cell death, something that is seen in neuroblastomas (Kitanaka et. al., 2002). Further research provides

evidence that the cause of this cell death is due to overexpression of the RAS g-protein leading to sustained activation of the JNK and p38 MAPK pathways in an ROS dependent manner (Osone et.al., 2004). If ROS levels increase beyond the optimal range, the cancer cells try to regulate it by using NRF2 - dependent genes, and the cells die as a result. In this case, ROS levels rose too high leading to cell death. However, lower ROS levels can prevent oxidative and DNA damage beforehand, leading to less cancer overall. The metabolism standpoint serves to explain that larger animals have lower BMRs and thus ROS, leading to less overall rates of cancer. Based on these connections, we believe hypertumors follow a similar pathway to that of Fenretinide in which it leads to over expression of the RAS g - protein in the original tumor. This results in cell death alongside depleting the original tumor of its blood supply.

Not only does the existence of hypertumors serve as a possible explanation for Peto's Paradox, but the similarities in mechanisms involved in hypertumors, fenretinide and metabolism serve as a basis for treatment of neuroblastomas and potentially other aggressive cancers. However, it is important to note that hypertumors and metabolism are not the only possible explanations for Peto's Paradox. It could also be an adaptation of highly advantageous characteristics, or evolution. The increased number of cells and mutations led whales to have an increased number of p53 genes also known as tumor suppressor genes (Tollis et al., 2017). The solution to Peto's Paradox could be hypertumors, evolution or both.

Hypertumors stall the progression of cancer growth, lengthening the original prognosis for many cancer patients. Since the stage of cancer at diagnosis is one of the most significant prognostic factors, the stabilization of cancer growth may result in better outcomes than the current methods used to approach cancer treatment. This paper strongly support further research into the existence of hypertumors and their potential use against cancer growth. This includes

drug trials of Fenretinide for treatment of neuroblastomas and further investigating into the sustained activation of the JNK and MAPK pathways in an ROS dependent manner.

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Legend

(*) - This figure was created using BioRender.