The Evolution of Tumor Suppressing Genes in Multicellular Organisms: Nature's Prevention of Oncogenesis

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The p53 gene family, a well-known group of genes, is the primary propagator of tumor-suppressing mechanisms in multicellular organisms. Although they are currently critical drug targets in cancer, the p53 family also serves specific functions in the development of multicellular organisms. In this paper, the current function, origin, and evolutionary purpose of the p53 family are reviewed in the evolution of multicellular organisms. The TP53 gene induces cellular responses such as apoptosis as a way to combat detrimental environmental and cellular factors that can damage the integrity of a cell's DNA. The other two members of the p53 family are the TP63/TP73 genes. The TP63/TP73 genes are involved in the embryonic development of limbs and the neuronal system, respectively. It has been discovered that these three genes originated as an ancestral gene that later separated individually throughout the evolution of higher functioning vertebrates from invertebrates. The p53 family provides complex networks of tumor-suppressor genes that allow for the prevention of oncogenesis in multicellular organisms. The ultimate evolutionary goal of the p53 family is to preserve the integrity of their organisms from unwanted mutations, which in turn also assists their own fitness. However, the genomic evidence presented in the current literature contains gaps. Further research is needed to fill in these gaps in the genomes of multicellular organisms, in order to have a cohesive understanding of the evolutionary purpose of the p53 family, not only for the benefit of humans but for other species as well.

Introduction

Diseases, like cancer, are shown to be related to the evolutionary histories of humans. Further understanding of these evolutionary ties would improve our strategies for preventing, managing, and treating diseases (Casás- Selves & DeGregori, 2011). The majority of cancers stem from mutations in Tumor Suppressing Genes (TSGs) that help cells recover from stressors such as ultraviolet exposure, oxidative stress, and carcinogens (Grandér, 1998). This makes it critical for us to understand tumor suppression mechanisms from an evolutionary perspective. The most common TSGs are found within the p53 family, which consists of TP53, TP63, and TP73. Although these TSGs are highly known for their current role in tumor prevention, the p53 family appears recurrently alongside the evolution of multicellular organisms (Belyi et al., 2010). The evolution of long-lived and large organisms required tumor-suppressing mechanisms to conserve the fitness of these organisms. Since large-bodied and long-lived organisms have an increased number of cells compared to smaller organisms, they have evolved more specialized tumor-suppressing mechanisms to compensate for the additional cells (Seluanov et al., 2018).

Present day, we have a high number of estimated cases of cancer in the U.S., and at least half of these cases are associated with TP53 mutations, which is leading to the development of many genetic-based therapeutics to combat this disease (Sigal & Rotter, 2000). Yet, we should question the extent to which these implemented therapeutics will influence the future evolution of the p53 family. So far, we know the divergence of the p53 family due to accumulated genetic evidence, but how will our current "unnatural" selection impact the evolutionary timeline and how will this impact the future of therapeutic development? The aim of this paper is to address the current functions, origin, and evolutionary purpose of the p53 family in the evolution of multicellular organisms.

The Function and Mechanism of the TP53 Gene

The TP53 gene is famously known as the "guardian of the genome" for its ability to induce apoptosis, cell cycle arrest, and DNA repair when environmental or cellular stressors target a cell's genome. TP53 serves as the "general manager" of the cell, keeping a close eye on proteins to make sure everything is running smoothly within the cell. The TP53 gene produces p53 proteins, which act as transcription factors that regulate the expression of a vast number of target genes (Vogelstein et al., 2000). p53 proteins are mostly found in the nucleus of cells, but a portion of them are also harbored in the cytoplasm (**Figure 1**). These proteins can be considered the "executive housekeeper" for the other proteins within the nucleus and the "front office manager" for those roaming around the cytoplasm. Within the nucleus, p53's first target is p21, a cyclin-dependent kinase that regulates the progression of the cell cycle (Horn & Vousden, 2007). Meanwhile, p53 in the cytoplasm targets pro-apoptotic proteins and the anti-apoptotic protein BclxL (B-cell lymphoma-extra large) (Horn & Vousden, 2007). Some key proapoptotic proteins are Bax (BCL2-associated X), Bak (BCL2 antagonist/killer), and PUMA (p53-Upregulated Modulator of Apoptosis). Cytoplasmic p53 is regulated by MDM2 (Mouse Double Minute 2 homolog) through ubiquitination, where ubiquitin molecules are attached to protein substrates for protein degradation through a proteasome (Pitolli et al., 2019). Ubiquitination by

MDM2 is similar to getting flagged for not doing a certain task properly and eventually getting fired from getting flagged numerous times in a row. Much is known about the TP53 gene and its respective protein, but the same cannot be expected from the rest of the p53 family since TP63 and TP73 and their products have only recently been studied. Additionally, past studies prove that the p53 family came from the triplication of a common ancestral gene, which explains their homology to each other (Pflaum et al., 2014).

Figure 1

The Role of Wildtype P53 in the Nucleus and Cytoplasm
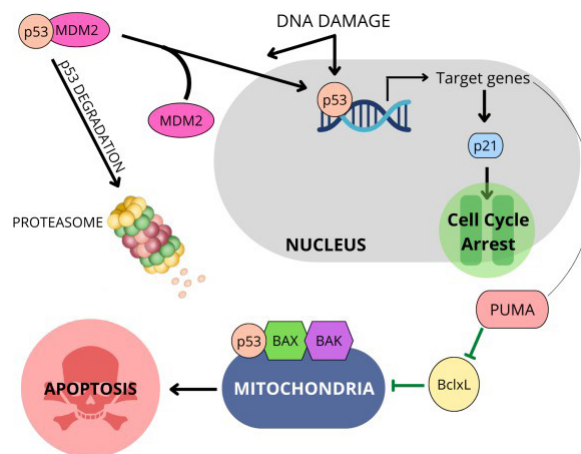
Note: Although not much is known about the normal function of TP63 and TP73's protein products (p63 and p73,respectively) in this pathway, it is proposed that they also participate in the activation of target genes, leading to cycle arrest and apoptosis.

Evolution of the p53 Family

The p53 family arose from a common ancestral gene and is found in most invertebrates (Pflaum et al., 2014). Eventually, a separation/duplication occurred within the gene, leading to two independent genes (TP53 and TP63/TP73), which can be found in early vertebrates (Belyi et al., 2010). An additional separation/duplication led to the separation of TP63 and TP73, which can be seen in bony fish and higher-order organisms (Belyi et al., 2010). It can be assumed that the expansion of the p53 family ancestral gene allowed for the differentiation of function between the three family members. It has previously been discussed that p53 regulates cellular responses to external stressors. However, TP63 is noted to be responsible for embryonic development of the ectoderm and, similarly, TP73 is involved in the neuronal development of embryos (Levrero et al., 2000). Diseases such as ectrodactyly- ectodermal dysplasia-clefting syndrome (EEC) are linked to mutations in the human TP63 gene. EEC is an inherited disease that causes the absence of some fingers and/or toes, as well as the appearance of a cleft lip, palate, and/or flat nasal tip. On the other hand, mutations in the TP73 gene have recently been associated with autoimmune disorders (inflammatory bowel, Celiac, etc.) and neurodegenerative diseases (Alzheimer's, Huntington's, etc.), affecting hundreds of thousands in the U.S alone (Gonzalez-Cano et al., 2010; Ren et al., 2020).

Evolution of Multicellular Organisms with Respect to the Function of the p53 Family

The starlet sea anemone (Nematostella vectensis), fruit flies (Drosophila), and roundworms (Caenorhabditis) are the earliest organisms that display the presence and functions of the p53 family (Belyi et al., 2010). A p53/p63/p73 gene found in sea anemones is responsible for the induction of apoptosis to protect their gametes and germ line cells from environmental stressors (Pankow and Bamberger 2007). Adult fruit flies similarly have an ancestral gene that induces apoptosis. However, this hybrid gene plays another significant role in preserving genome integrity by preventing birth defects in its offspring (Sogame et al., 2003). In roundworms, the ancestral gene is also involved in using apoptosis to regulate germline cells from external factors, as well as allowing for the homeostasis of egg production (Conradt and Horvitz, 1998; Schumacher et al., 2005). Ultimately, the advantage these simpler organisms have from the hybrid p53/p63/p73 gene is the preservation of the integrity of their genome for themselves and their future generations. This makes it seem as though there could be a possible positive selection at play for this gene in more developed organisms since the role of the gene is preserved in more complex organisms by genetic homologs.

When vertebrates emerged, the p53/p63/p73 ancestral gene duplicated, producing a separate TP53 gene and facilitating a new body plan. This new body plan consisted of somatic stem cells that allowed for the replenishing of tissues and organs throughout the organism's life span. TP53 became involved in preventing tumor growth in somatic cells that were rapidly replicating by preserving DNA from cellular and environmental damage. As bony fish, like lobe-finned fish, appeared, another duplication event occurred resulting in the separation of the hybrid p63/p73. The separation of this hybrid gene allowed for each additional gene to gain specialized functions, similar to the TP53 gene. TP63 had gained the responsibility for embryonic limb growth and craniofacial development while TP73 specialized in the neuronal development of embryos (Yang et al., 2000; Yang et al., 1999).

Today, we now see amphibians, reptiles, and mammals (including humans) contain all three genes (TP53, TP63, TP73). In general, as organisms within the vertebrate clade continued to evolve, the duplication of the p53/p63/p73 ancestry gene overtime gave these organisms the ability to develop limbs, craniofacial features, neurogenesis, and maintain genome integrity (**Figure 2**). However, the ability to maintain genome integrity does not completely protect organisms, including humans, from being subjected to oncogenesis. Factors such as carcinogens, age, and natural selection are contributors to cancer.

Figure 2

Evolution of Multicellular Organisms in Respect to the Function of the P53 Family
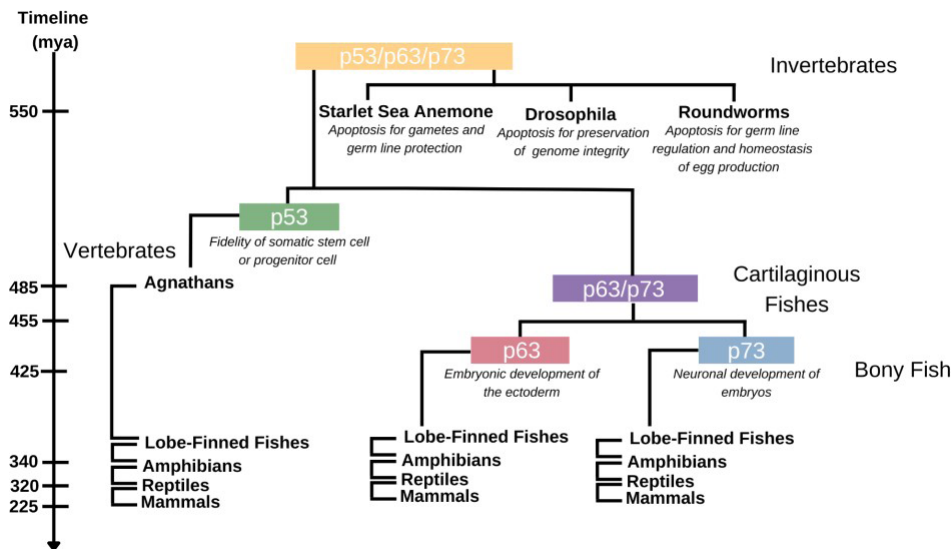
The Role of the p53 Family in Oncogenesis

Worldwide, cancer is a major public health issue. According to the American Cancer Society, the estimated new cases of cancer are close to two million, and the estimated death toll was over half a million individuals for 2023 (Siegel et al., 2023). Since cancer is such a prominent health issue, understanding the causes and influences of oncogenesis is vital for the development of better diagnostic, prognostic, and treatment techniques.

Oncogenesis occurs when there is an accumulation of mutations that change specific genes within a cell's DNA that leads to a cascade effect on the proteins produced by said genes. Targets for mutations can be either TSGs or oncogenes. As previously mentioned, TSGs assist the cell in recovering from external and internal stressors by restricting the cell cycle. On the other hand, oncogenes promote the cell cycle, signaling for more cells to divide and spread. TSG mutations typically result in a nonfunctional or gain-of-function gene, whereas oncogenes are always active.

In the p53 family, TP63 and TP73 genes are rarely mutated in human cancers (Levrero et al., 2000). The same cannot be said for TP53. Over 50% of all cancers involve a mutated TP53 gene, which influences their aggression (Sigal & Rotter, 2000). Mutations in the TP53 gene are majorly due to exposure to carcinogens over time. Additionally, cancer incidents are seen more often in older individuals than in younger ones.

It has been proposed that long-lived multicellular organisms have reductions in fitness due to age and exposure to carcinogens (DeGregori, 2011). Promoting selection for adaptive mutations that could be passed down through generations can increase fitness. Using natural selection as a mechanism of evolution, cells can select for adaptive mutations as a fitness strategy to prevent oncogenesis. However, the goal of natural selection is to allow organisms to have the maximum reproductive health even if it results in a decrease in health and longevity (Dolgava & Lao, 2019).

Tumor Surpressing Strategies for Mutant p53

Several studies have determined that there are three man-driven tumor suppressing strategies influenced by interactions between mutant p53 and p63/p73 proteins, respectively: 1) p53 mutant down-regulation, 2) p63/p73 over-expression, and 3) small-molecule inhibition of the protein-to-protein interaction between mutant p53 and p63/p73 (Li & Prives, 2007). Downregulation of mutant protein can be done by using small, non-coding RNAs that can target messenger RNA or DNA and inhibit further activity (Hu et al., 2020). Over-expression can be accomplished by using genetic material (DNA/RNA) that promotes more transcription/translation or by implementing CRISPR (clustered regularly interspaced short palindromic repeats)/Cas (CRISPR-associated protein) to directly edit genes (Li et al., 2023). If the ancestral gene never duplicated over time to produce the p53 family in multicellular organisms, these strategies would not be viable. Within the last decade, massive quantities of molecules have been produced to target mutant p53. However, p53 reactivation and induction of massive apoptosis (PRIMA-1) and its methylated counterpart, eprenetapopt (APR-246/PRIMA-1MET), have gained the most spotlight. These two molecules target mutant p53 proteins by restoring them back into their wild-type conformation, which facilitates normal function as well.

Although a multitude of therapeutics have been and are being developed constantly to overcome diseases linked to the mutant p53 family, it is important we understand the potential evolutionary implications of using these therapeutics on patients. A recent strong natural selection that occurred in humans is lactose tolerance after weaning, also known as lactose persistence (LP). Many believe that LP coevolved with the spread of domesticating animals throughout Europe and Africa. However, recent evidence supports the culture-historical hypothesis which argues that LP was selected for after the spread of dairy consumption (Gerbault et al., 2011). This example of natural selection shows that external pressures can still result in genetic drifts in humans. If this type of environmental event in human history affected a subpopulation of individuals, then it is not far fetch to propose that using gene based therapy over time in patient populations would not result in some sort of genetic shift as well?

Limitations in the Evolutionary Perspective of TSGs

Given that the focus of the paper is TSGs, there is a heavy reliance on existing genomes of multiple organisms. However, recovered genomes are not always complete; some are missing certain genes or have been poorly assembled. Additionally, even if there are complete or mostly complete genomes of certain organisms, it is difficult to compare them to other organisms that don't have a complete genome. It has recently been announced that the human genome is completed (Nurk et al., 2022). Yet, the genomes of most other vertebrates are not. Comparing the completed human genome to one that is incomplete would not allow for the proper understanding of the functions of the p53 family in other organisms. With this limitation present, we should assume that the previously mentioned appearances of the p53 family in multicellular evolution are estimates.

Conclusion

The p53 family plays a pivotal role in the evolution of multicellular organisms. The TP53 gene serves

as a massive regulator of DNA to preserve its genes from being mutated, which can allow for diseases such as cancer to occur. The p53 family was derived from an ancestral gene through the evolution of vertebrates from invertebrates. This family of genes have been conserved over generations for its ability to correct and maintain DNA from damages which could be harmful for organisms and their future offsprings.

Even though the p53 family evolved for specialized prevention of oncogenesis, there is still an alarming number of individuals in the U.S. with cancer initiated from a TP53 mutation. This has led to the production and usage of more gene-based therapies in the patient population that could potentially influence the further evolution of the p53 family, similar to lactose persistence. However, evolution will ultimately weigh the costs and benefits of tumor suppression, even if it compromises overall health (DeGregori, 2011).It is important to note that the evidence present within this review is limited due to incomplete genomes of certain species. Future studies should focus on finding the incomplete pieces of genomes from organisms in the evolutionary tree of multicellular organisms to further understand the history of the p53 family and gain new insights for better treatments, not only for the benefit of humans, but even for other multicellular species.

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