

P53: Our Saviour and Our Destroyer

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ABSTRACT:

The p53 genome is a gene that has truly taken the scientific world by storm. Once referred to as an **oncogene**, p53 has proved itself to be more complex and dubious time and time again. This paper will talk about the beginning doubts and queries revolving around p53 and the discoveries that led to it being referred to as a **tumour suppressor gene**, rather than being mislabeled as an **oncogene**. It will touch upon the importance that p53 brings to our daily lives such as repairing the DNA, maintaining the cell cycle and starting **apoptosis**, as well as highlight the differences between the gene's two types: **wild-type p53** and **mutant p53**. Furthermore, it will talk about how a slight mutation in p53, can cause it to form many protein **isoforms**, a leading contributing factor in cancers. It will also mention the effects **post-translational modification** will have on the two types of p53. In addition to this, it will delve into the negative and potentially harmful effects that p53 could have on our bodies. Finally, it will also talk about p53-mediated therapy and targeting **mutant p53** with **autophagy**, which are 2 processes that have shown successful outcomes in reducing the complications caused by **mutant p53**.

INTRODUCTION:

Referred to as “The Emperor of all Maladies” by Dr Siddhartha Mukherjee in his book, cancer truly takes first place in being the king of all diseases. Cancer is one the oldest diseases known to man, having been around since 3000 BC. Yet it still doesn't have a cure. The reason why it doesn't have a cure is that no cancer is the same. This unpredictable uniqueness has stumped researchers and doctors for the past 5 millennia, making it difficult for those researching cancer and even more difficult for those battling the fatal disease. Nevertheless, many are determined in their pursuit to find a cure for this incurable disease. And there is no better way to begin this quest than to start at the beginning of all cancers.

Discovered in 1979, the **p53 gene** is becoming increasingly relevant in today's scientific world as well as giving scientists and researchers a better glimpse into the daily life of cancer. From helping maintain the cell's genomic character, to causing the uprise of malignant and cancerous cells, p53 has done it all. Due to its vast variety of abilities, there have been several advancements in understanding the biology and signalling in the p53 pathway, the p53 transcriptional readouts and the effects of an array of mutants. Yet the pathway remains a challenging realm of clinical transaction. Additionally, the role of **mutant p53** as a prognostic factor has been recognised, but the therapeutic modulation of its wild-type or mutant activities remains a work in progress. All in all, the ongoing research about the **p53 gene** will surely shed light on both the positive and negative effects of the gene. Therefore, it is only once enough light is shed, that can we decide whether the **p53 gene** is our saviour or rather, our destroyer.

THE BEGINNING OF P53:

TP53 is the gene that encodes for the p53 **tumour suppressor gene** and is commonly referred to as the guardian of the genome. The p53 protein is a transcription factor that controls the output of many biological processes. These are namely **oncogene** activation, DNA damage and replication stress. The output of such biological processes is determined by the type of cellular stress signal input. The protein is also known to play a role in **cell cycle arrest**, **apoptosis** and **senescence**. **Apoptosis** is a highly conserved mechanism by which eukaryotic cells willingly commit suicide to eliminate unwanted and defective cells. This results in **cell cycle arrest**, which is the stopping point in a cell cycle. **Senescence** is the biological ageing of a cell and can only occur once a permanent cell cycle is achieved. The TP53 gene is also one of the most commonly mutated genes in human cancer, with over 100,000 literature citations in PubMed. The p53 protein is also referred to as the **tumour suppressant gene**, meaning it can stop the formation of a **tumour**. A **tumour** is an abnormal mass of tissue that forms when cells grow and divide more than they should or do not die when they should. The gene itself has been mapped to chromosome 17, meaning its location has been found on the 17th chromosome. When bonded to DNA, p53 stimulates another gene to produce a protein known as p21. When p21 is complexed with cdk2 (a cell division stimulating protein), the cell cannot pass through the next stage of cell division. This causes p53 to not bind to the DNA effectively. As a consequence, the 21 protein is not made available to act as a stop signal for cell division. Therefore initiating cells divide uncontrollably and thus **tumours** start to form.

MORE ABOUT P53:

(A) DOUBTS ABOUT P53:

The **p53 gene** was originally thought to be an **oncogene**, by many scientists in the past. An **oncogene** is a photoncogene that has too many copies or is more active than normal. Since this is a mutated form of a gene, it may cause normal cells to become cancerous and grow inside the human body. Due to the time at which the research about p53 began as well as the evidence regarding the protein, much could not be deciphered about the gene. It was believed that **oncogenes** were the key to understanding cancer and had been identified in both RNA and DNA **tumour** viruses, while the existence and presence of **tumour suppressor genes** were barely on the radars of many cancer researchers. In addition, the **p53 gene** was bound to the SV40, a major oncogenic protein strongly suggesting it was the downstream effector of the larger t-antigen pathway. Furthermore, this was consistent with the high levels of p53 found in many cancers. One of the most remarkable discoveries was that the introduction of a “normal” **p53 gene** into a normal cell could transform it into a **tumour** cell. Nevertheless, several experimental observations did not fit the idea of p53 being an **oncogene** and by the mid to late 1980 there was little to no reason to believe otherwise.

(B) CONCLUSIONS ABOUT P53:

The year 1989 proved to be the major turning point in the search for a **tumour suppressor gene** on chromosome 17p. To formally exclude p53 as an **oncogene**, researchers decided to apply the two-hit test.

• **TWO HIT TEST:**

Tumour suppressor genes act like the brakes in a car(they stop **tumour** growth). But because each cell has 2 copies of each brake (one from the mother and one from the father), it is necessary to alter both copies, to get rid of the brake. In contrast, the mutant **oncogene** acts like an accelerator stuck to the car floor. As a result, only one accelerator needs to be stuck. Thus, this distinction allows a “two hit” test to distinguish whether a mutant gene is an **oncogene** or a **tumour suppressor gene**.

To put it simply, the **tumour suppressor gene** is one where both copies of the gene are altered while the **oncogene** has only one gene altered. Surprisingly, when this test was applied, the results were not as anticipated.

1. Firstly majority of the colorectal **tumours** were found to have only subtle mutations, for instance having a single base substitution, allowing for the formation of a new amino acid. Mutations, such as these had never been observed before.
2. Secondly, in virtually of cases, both **p53 genes** were altered. In these cases, one was generally altered while the other was completely deleted. This was the result expected of a **tumour suppressor gene** and not that of an **oncogene**.

By applying this test to many other **tumour** types, the results provide compelling evidence that p53 is a **tumour suppressor gene**.

(C) THE TYPES OF P53 GENES:

The **p53 gene** is a nuclear transcription factor with a **pro-apoptotic function**, meaning it promotes the cell to undergo **apoptosis**. Since 50% of human cancers carry loss of functioning mutations of p53, it is considered one of the classical **tumour suppressor genes**. The **p53 gene** is also known to have 2 different types: **mutant p53** and **wild-type p53**.

1. Mutant p53:

This is caused by a mutation in TP53 at an early stage of cancer progression and can occur as a result of being exposed to a **carcinogen**, which is a substance that may increase the likelihood of developing cancer. Most TP53 mutations change single amino acids in the p53 protein, which leads to an altered version of the protein. This altered version cannot control **cell proliferation** (an increase in the no of cells as a result of cell growth and cell division) and thus is unable to trigger **apoptosis** in cells with mutated or damaged DNA. As a result, DNA damage starts to accumulate within the cells, making it more difficult for the remaining normal **p53 genes** to repair the damage and maintain genomic integrity.

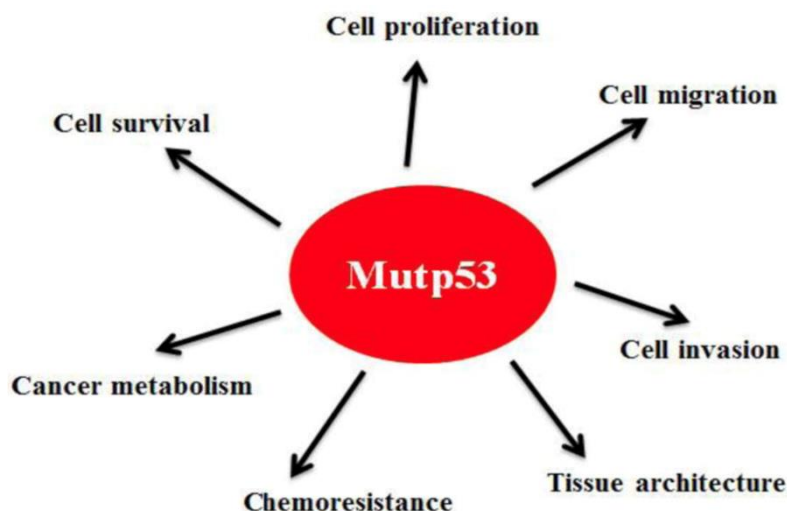


Figure 1: Mutant p53 (mutp53) regulates many cellular processes, including cell proliferation, cell migration, cell invasion, cell survival, cell metabolism, chemoresistance and tissue architecture, to promote tumour progression independently of wild-type p53.

2. Wild -Type p53:

This type of **p53 gene** is a sequence-specific transcription factor, that when activated by various stresses, promotes cellular outcomes, depending on the extent and context of stress.

The most important difference between the two is that **wild-type p53** is restricted to specific genomic sites, while **mutant p53** is not restricted to specific genomic sites.

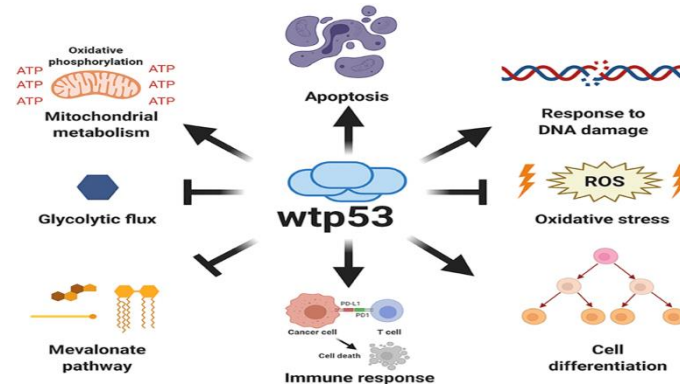


Figure 2: The canonical functions of wild type p53.

https://www.researchgate.net/figure/Canonical-functions-of-wild-type-p53-Wild-type-p53-is-a-major-tumor-suppressor-whose_fig2_349282391

P53's IMPORTANCE IN OUR DAILY LIVES:

The cells in our body are constantly being exposed to a variety of cellular stresses such as DNA damage, **oncogene** activation, etc. These cellular stresses finally start to introduce genomic aberrations like mutations, deletion, and/or translocation into the cellular genome, thereby inducing genomic instability. Accumulation of such aberrations can often result in the development of cancer. At times like these, it is important to have a proper stress response to maintain genomic coherence as well as protect cells from malignant transformations. Thus this is where p53 comes into play. Under normal conditions, the gene is expressed at an extremely low level. But upon DNA damage, p53 is induced to accumulate in the cell nucleus through post-translational modifications such as **phosphorylation** (the chemical process of adding the phosphate group to an organic compound) and **acetylation** (the organic esterification reaction, with acetic acid). After undergoing these chemical modifications, p53 is converted from a latent form to an active form. This functionally active p53 transactivates an appropriate set of its target genes to induce **cell cycle arrest** and/or **apoptosis**, depending on the extent and type of cell damage. Thus the p53 mediated **cell cycle arrest** allows cells to repair DNA damage.

When the DNA repair is complete, cells re-enter their normal cell cycle. On the other hand, when the cells are faced with severe DNA damage, p53 exerts its pro-apoptotic function to eliminate cells with severe DNA damage, therefore preventing the transfer of damaged DNA to the daughter cells. Thus allowing p53 to maintain the cell's genomic integrity. In addition to this, its DNA binding activity is tightly linked to its **tumour** suppressive function.

P53's ROLE IN CANCER:

(A) THE ISOFORMS OF THE P53 PROTEIN:

Like all other proteins present in the human body, the p53 protein also exists in multiple **isoforms** as a consequence of 2 promoters at the TP53 gene, post-transcriptional events such as alternative mRNA splicing and internal ribosome entry site. These protein **isoforms** are all generated from the same gene but with distinct amino acid sequences and biological roles. These isoforms also tend to arise when different

axons are combined through RNA splicing and are translated into proteins with distinct properties. To date, only 12 isoforms have been discovered - p53, p53 (beta and gamma), delta 40p53 (alpha, beta and gamma), delta 133p53 (alpha, beta and gamma) and 160p53 (alpha, beta and gamma). These isoforms denote the extent of their n-terminal deletions and beta and gamma have additional deletions at the c-terminal, yet they carry unique sequences due to the alternative splicing of intron 9.

The gene isoforms can be expressed in normal and tumour tissues and can simultaneously be expressed with a full-length p53. The delta isoforms have a dominant negative effect towards the full-length p53 and consequently prevent p53 mediated apoptosis. Beta isoforms on the other hand can enhance and improve, p53 target gene expression. Although specific roles in cancer of each p53 isoforms become less characterised. Nevertheless, some of these have been shown to correlate with decreased overall patient survival [delta 133p53(alpha, beta and gamma)] and progression-free survival [p53 gamma].

(B) POST-TRANSLATIONAL MODIFICATIONS OF THE P53 TYPES:

Both wild-type p53 and mutant p53 can be post-translationally modified. However, the biological response outcome of mutant p53 is different from that of wild-type p53. In sites commonly post-translationally modified in wild-type p53, many are rarely mutated across a variety of cancers. This is due to the common hotspot mutations in p53 that are not sites that are post-translationally modified. Irrespective of this occurrence, there are still sites including those across different p53 domains that are post-translationally modified and are found to be mutated in human tumours. Post-translational modification in wild-type p53 allows protein activation, specificity for cofactor interactions, binding to DNA and transactivation, and selectively of p53 target genes towards tumour suppression.

In the case of mutant p53, these modifications do not affect the ability of mutant p53 to bind to a specific DNA sequence for tumorigenesis functions. In fact, to date, there is not a defined DNA sequence that mutant p53 binds to sell as wild-type p53 binds to its corresponding p53RE. Instead, p53 interacts with other proteins like transcription factors and interferes with their transcriptional program.

In addition, cell stress signalling such as a glucose restriction can impact mutant p53 differently than wild-type p53. In this stress condition, mutant p53 is acetylated, resulting in cell metabolic requirements that provide a cell survival advantage. By contrast to wild-type p53, which were ubiquitylation targets for degradation to control its protein levels upon stress response resolution, mutant p53 is not frequently found to be ubiquitylated. This is mainly due to the lack of the MDM2 gene, transactivation and negative feedback loop. As a result, mutant p53 tends to become highly stable in a variety of tumour types. Nevertheless, there are certain types of mutant p53, that can interact with MDM2 and be targeted for degradation through ubiquitin ligase also found to regulate wild-type p53 stabilisation.

(C) THE NEGATIVE EFFECTS OF MUTANT P53:

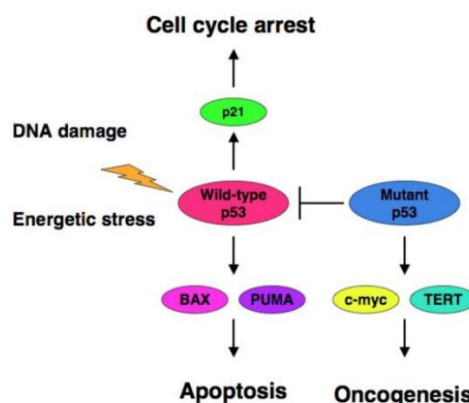


Figure 1. Dominant-negative effect of mutant p53 on wild-type p53. The pro-apoptotic function of p53 is significantly inhibited by certain p53 mutants which induce malignant transformation through up-regulation of c-myc and TERT.

Among the mutations, 95% of them are detectable within the genomic region, encoding the binding domain of p53. As a result, **mutant p53** lacks the pro-apoptotic function. **Mutant p53** also acts as the dominant negative inhibitor towards **wild-type p53**. This may prove to be difficult, as p53 plays a crucial role in the DNA damage response. In some cases, cancer cells bearing p53 mutations display a chemo-resistant phenotype. Furthermore, **mutant p53** does show some oncogenic potential. Therefore, it is important to develop a strategy that aims to eliminate the negative effect of **mutant p53**, on **wild-type p53** for efficient chemotherapy.

HOPE FOR A CURE:

(A) P53 MEDIATED THERAPY:

With all the good that p53 can do for us, there is certainly a lot of harm it can do as well. To combat this harm, more and more time has gone into researching mediated therapy. The therapeutic efficiency of anti-cancer agents depends strongly on their ability to trigger **apoptosis** in target cancer cells since the gene plays a pivotal role in the regulation of cell fate, in response to DNA damage, the therapeutic strategies that activate p-53-mediated pro-apoptotic pathways and/or eliminate the dominant negative effect of **mutant p53** on wild type p53, should be required. According to recent findings, MDM2 binds to the NH2 terminal region of p53 and inhibits its transcriptional as well as its pro-apoptotic function. The MDM2 also facilitates the **proteasomal degradation**(the degradation of proteins done by a specialised organelle referred to as the proteasome) of p53. Thus the MDM2 antagonist could activate p53, offering a newer therapeutic approach to cancer.

Vitali *et al.* described that a short peptide derived from the p53 coot-terminal region contains a Parc-binding domain that disrupts the integration between Parc and p53.treatment of this peptide causing the nuclear relocation of p53 and increases in sensitivity, to anti-ageing drugs in cancer, such as neuroblastoma with wild type cytoplasmic p53. Alternatively, the reactivation of **mutant p53** contributes too much more efficient treatments of cancers bearing the **mutant p53 gene**.

(B) THE CHALLENGES OF P53 TARGETED THERAPY:

It is recognised that directly targeting the **mutant p53 gene**, is difficult due to the structural diversity of **mutant p53**. It is also becoming exceedingly challenging to discover a compound that can target all **mutant p53**. Since p53's pathway is quite complex, restoring the p53 function in normal tissue can result in unpredictable adverse events, such as the development of **tumour** cells within the body. For instance, RG7112 is associated with at least one adverse event that is frequently linked with haematological toxicity in patients. In general, strategies that directly target **mutant p53**, require that their agents have a higher affinity for **mutant p53** to a greater and more successful anti-**tumour** effect and fewer events.

(C) TARGETING MUTANT P53 WITH AUTOPHAGY:

Autophagy is a housekeeping process that controls protein and organelle quality. It also recycles intracellular components such as misfolded proteins and dysfunctional mitochondria, as alternative resources to maintain normal biological activities, more often during nutritional deprivation. In tumours, **autophagy** has a dual effect: it inhibits tumour formation while also promoting tumour growth once it has begun. Although **autophagy** is thought to be suppressed by **mutant p53**, the pharmacological introduction of **autophagy** by mTOR inhibition or AMPK activation has been shown to have potential therapeutic value.

In addition to this, the activation of **autophagy** induced by mutp53 makes cancer cells more sensitive to the mTOR inhibitor everolimus, especially in cancers with p53 mutations, diminishing the growth of cancer cells caused by **mutant p53**. There are strategies to deplete mutp53 that also rely on **autophagy**. For example, Spautin-1 promotes chaperone-mediated **autophagy** to degrade mutp53. Though **autophagy** initiation is quite complicated, it is hypothesised that drugs that affect the **autophagy** process rather than their constituents are synthetically lethal for cancers with p53 mutations. But, it remains only a hypothesis.

CONCLUSION:

As time goes on, the no of mutations of p53 will certainly increase. With the potential harm portrayed by such mutations, there is an increased urgency for scientists and researchers alike to find a solution soon, if not a definitive cure. With more ideas such as p53 mediated therapy, p53 targeted therapy, chemotherapy and radiotherapy, the likelihood of cancer thriving within a body is slowly decreasing. But even with such successful outcomes, there are still more questions than answers. For instance, how do we successfully target the **mutant p53**, if its pathway is extremely complex? How do we go about finding a compound that can target virtually all **mutant p53**? How do we manoeuvre our way around the difficult structure of the **mutant p53**? How do we ensure that autophagy is effective against depleting **mutant p53** if it can easily be suppressed by the mutant gene? These questions and so many more, remain unanswered in the realm of p53 and its unpredictable side effects. But not all hope is lost. There are newer developments such as targeting **mutant p53** with cell cycle arrest, targeting it with energy metabolism, etc. These developments have shown successful results, pushing researchers a step closer to finding a definite cure. Furthermore, there is an increased desire to find a cure for cancer, and tackling the issue of **mutant p53**, might just be the answer we need.

REFERENCES:

(A) Key Definitions:

1. Acetylation- <https://www.news-medical.net/health/What-is-Acetylation.aspx>
2. Apoptosis- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1550296/#R1>.and <https://www.genome.gov/genetics-glossary/apoptosis>
3. Autophagy- <https://jhoonline.biomedcentral.com/articles/10.1186/s13045-021-01169-0#Sec2>
4. Carcinogen- <https://www.genome.gov/genetics-glossary/Carcinogen>
5. Cell cycle arrest and senescence- <https://www.sciencedirect.com/science/article/abs/pii/S1876162322000967>
6. Cell proliferation- <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/cell-proliferation>
7. Chemotherapy- <https://www.cancer.gov/about-cancer/treatment/types/chemotherapy>
8. Isoforms- <https://www.merriam-webster.com/dictionary/isoform>
9. Mutant p53- <https://medlineplus.gov/genetics/gene/tp53/>
10. Oncogene- <https://www.genome.gov/genetics-glossary/Oncogene>
11. P53- <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/p53-gene>
12. Post-translational modification- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8040245/>
13. Phosphorylation- <https://www.sigmaaldrich.com/IN/en/technical-documents/technical-article/protein-biology/protein-labeling-and-modification/phosphorylation>

14. Pro apoptotic function-
<https://www.merriam-webster.com/medical/proapoptotic>
15. Proteasomal degradation-
<https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2018.02738/full>
16. Radiotherapy- <https://www.cancer.gov/about-cancer/treatment/types/radiation-therapy>
17. Transactivation-<https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/transactivationand>
<https://www.oxfordreference.com/display/10.1093/oi/authority.20110803105309483>
18. Tumour- <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/tumor>
19. Tumour suppressor gene- <https://www.genome.gov/genetics-glossary/Tumor-Suppressor-Gene>
20. Ubiquitylation- <https://www.ncbi.nlm.nih.gov/books/NBK556052/>
21. Wild-type p53- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5287061/>

(B) INFORMATION AND STATISTICS:

1. <https://www.ncbi.nlm.nih.gov/books/NBK22268/>
2. <https://medlineplus.gov/genetics/gene/tp53/>
3. <https://www.nature.com/scitable/topicpage/p53-the-most-frequently-altered-gene-in-14192717>
4. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5287061>
5. <https://cancerbiomedcentral.com/articles/10.1186/s12935-021-02396-8>
6. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3756401/>
7. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8730328/>
8. <https://cancerbiomedcentral.com/articles/10.1186/s12935-021-02396-8#Sec1>
9. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8730328/#S10title>
10. <https://jhoonline.biomedcentral.com/articles/10.1186/s13045-021-01169-0#Sec17>
11. <https://www.sciencedirect.com/science/article/abs/pii/S0022283617301638?via%3Dihub>
12. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3135636/>
13. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3970583/>
14. <https://www.nature.com/articles/s41419-022-05408-1>
15. <https://www.nature.com/articles/s41418-018-0246-9>
16. <https://europepmc.org/article/med/28390900>
17. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1550296/#R1>
18. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4772082/#ack-a.e.atitle>
19. https://link.springer.com/chapter/10.1007/978-1-4020-2922-6_6
20. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3750306/>
21. <https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/transactivation>
22. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1831837/>
23. <https://www.ncbi.nlm.nih.gov/books/NBK9957/>
24. <https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2018.02738/full>
25. <https://jhoonline.biomedcentral.com/articles/10.1186/s13045-021-01169-0#Sec17>

(C) PHOTOS:

1. Figure 1- <https://europepmc.org/articles/PMC5663274/figure/F1/>
2. Figure 2- https://www.researchgate.net/figure/Canonical-functions-of-wild-type-p53-Wild-type-p53-is-a-major-tumor-suppressor-whose_fig2_349282391
3. Figure 3- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3756401/figure/f1-cancers-03-00994/>