Review of Literature on the p53 Protein and how it is Beneficial to Cancer Research and Funding

Malave Bettinger

And

Jami Sanchez

EH301-05

Dr. Joseph W. Robertshaw

University of Alabama in Huntsville

25 November 2019

Table of Contents

Table of Contents 2

Abstract 3

Introduction. 4

Body 6

Cancer Statistics 6

Polymerase Chain Reaction 6

p53 Protein 8

Motif for p53 Cancer Mutants 8

Conclusion 11

Resources 12

Abstract

Cancer affects millions of people yearly, and there are few successful treatments for many different types of cancers. The consequences of too many treatments result in adverse or harsh side effects. The p53 protein is responsible for many cancers because when it is deactivated or mutated, it cannot regulate cell reproduction. Research conducted by Washington State University School of Medicine shows hope for treatments for cancer using the p53 protein by helping them regain function with specific amino acids. The article is found on Proceedings of the National Academy of Sciences of the United States of America. A review of literature on the p53 protein could be beneficial to others not familiar with science terminology. Many people who fund research are not familiar with the language of science; therefore, this may help reach more people to aid in cancer research and funding so that the findings on the p53 protein are not overlooked.

Introduction

Cancer is an epidemic around the world. Millions of people will get cancer next year in the United States, and thousands diagnosed with cancer are expected to die. Cancer treatments are available, and the success rate varies greatly depending on the type of cancer. Some treatments such as chemotherapy have harsh side effects. Killing cancer cells with potent chemicals or radiation can be detrimental to one's own healthy cells. New research and treatment should be funded in order to alleviate this epidemic.

One thing that is common amongst most people diagnosed with cancer is a mutation in the p53 protein. The p53 protein is responsible for regulating cell production. When the p53 protein is mutated or deactivated, cells overproduce, leading to cancer. The p53 protein has been successfully activated using specific amino acids, which was discovered by using polymerase chain reaction (PCR).

The literature reviewed was written between the years 2004 and 2018. "Cancer statistics, 2019", is a scholarly article on research conducted by the American Cancer Society regarding the statistics about cancer in the United States. This includes estimations on the rates of cancer and mortality rate based on previous data collected by the National Cancer Institute since the year 1930 up to the year 2016. These statistics are broken down based on socioeconomics, gender, type of cancer, and state. "The power of real time PCR", is a scholarly article by the American Physiological Society regarding what PCR is, its background, and what and how it can be used. An overview of the p53 protein, based on the previous 25 years of research, was published as a scholarly article. This article, "p53: 25 years after its discovery" describes what functions the p53 protein has and why it should be a target for cancer therapy. Lastly, "A global suppressor motif for p53 cancer mutants" is a research article by Washington School of Medicine reporting amino

acids that rescued over half of the common p53 cancer mutants tested. The p53 protein and the eight most common p53 mutations were extracted by PCR. After review of this literature, it is ascertained that the p53 protein research can be a breakthrough for the cancer epidemic.

Body

Cancer Statistics[3]

Cancer statistics and projections were compiled by the American Cancer Society, and have appalling results. Cancer is the second leading cause of death in the United States, with heart disease being the first. It was projected that 1,262,450 new cancer cases and 606,880 cancer deaths would occur in the year 2019. Over eleven thousand of those cases are expected to be children, with 1,190 children dying from cancer in the year 2019. The statistics were broken down for gender, type of cancer, ethnicity, and socioeconomic. Prostate is the most common cancer for men (20% of cancer cases for men). Breast cancer is the most common cancer for women (30% of cancer cases for women). Survival rates are lowest for cancers of the pancreas, liver, esophagus, and lung, all being less than 20%. African Americans are 33% more likely to die than Caucasians after being diagnosed with cancer. Native Americans are 51% more likely to die from cancer than Caucasians. People in poor areas are 20% more likely to die from cancer than wealthy areas. The racial gap for cancer is slowly decreasing; however, the socioeconomic gap for cancer is widening. Cancer is common in the United States as well as other areas of the world and we are in desperate need of a cure.

PCR[4]

In 2005, the American Physiological Society published “The power of real-time PCR”. Kary Mullis discovered PCR in the 1980s. It is used to amplify DNA more than a billion fold. PCR is common in the biology and medical community. PCR amplifies targeted DNA sequences by exploiting DNA polymerases. Sequence specific oligonucleotides act as primers. Polymerase is heat resistant and produces new strands of DNA using a DNA template and primers. The

double stranded DNA is denatured at high temperatures, at about 95 degrees Celsius depending on the device used. The reaction is then cooled to elongate the DNA by the polymerase enzyme and adding their complementary nucleotides to form a new strand of DNA. Then new double stranded DNA is created. The cycle of denaturation by heating and elongation by cooling is done repeatedly. The DNA is amplified by 2^n with n being the number of cycles. In perfect conditions, the amount of DNA is doubled each cycle but eventually reaches a plateau. RNA can also be used in PCR if it is paired with reverse transcriptase so that it can generate complementary DNA from the RNA. PCR is ideal because it can detect less than five copies of a target sequence, it is quick, and does not allow much room for error because DNA is unique and specific. The drawbacks of PCR are that compounds such as urea can inhibit it, and human error can occur during assays or data analyzation. PCR can be used for things such as cancer detection, measure bacterial loads, cloning, and etcetera.

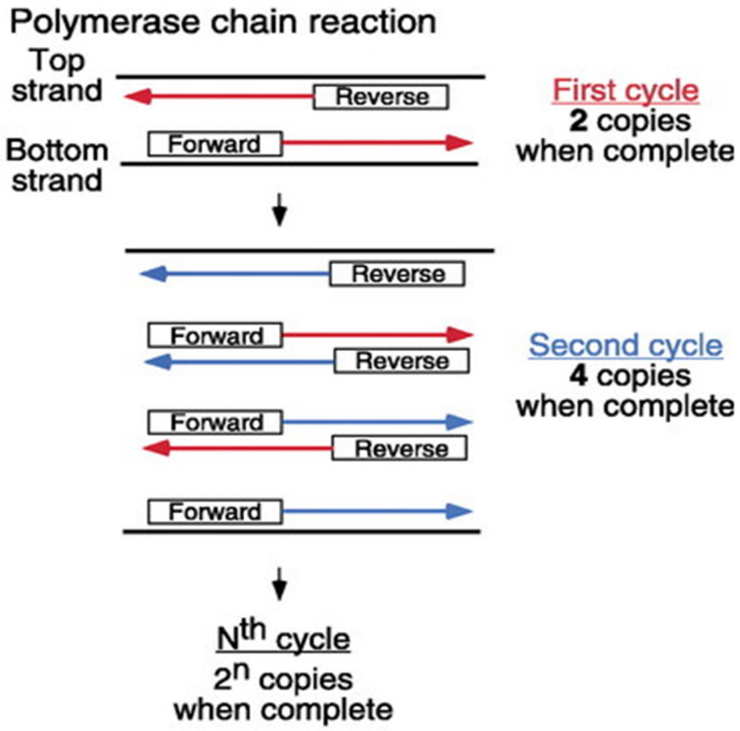

Fig 1: PCR Cycle

The p53 Protein[2]

In the scholarly article, "p53: 25 years after it discovery", the p53 protein is discussed as a tumor suppressor for stress response pathways. The p53 protein is essential for the repair and survival of cells and gets rid of severely damaged cells. The p53 protein is responsible for regulating many functions involved with cells such as apoptosis, development, differentiation, gene amplification, DNA recombination, chromosomal segregation, cellular senescence, cell-cycle regulation, and DNA repair by nucleotide excision repair and base excision repair. P53 is at on the crossroads of multiple cellular stress pathways. These stressors include DNA damage, hypoxia, and oncogenes. The biochemical mechanisms and pathways for p53 are not completely characterized and require further research. There is a high frequency of p53 mutants, 75%, in human cancers. p53 can be used as an intermediate biomarker for carcinogenesis because the p53 has a specific molecular signature based on the type of cancer. The p53 protein alterations are amino acid substitutions, and could be detected in early cancer stages for target tissues or surrogate fluids. The p53 molecule can also be used as cancer therapy instead of radiation and chemical therapies that are traditional treatments by restoring the protein's function. Cancer treatments would include restoring the wild type p53 protein, increasing its capacity, reactivation of the protein, or using molecules that mimic p53.

Motif for p53 Cancer Mutants[1]

The p53 protein is a transcription factor and tumor suppressor that is inactive in many cancer cases. Over 13,000 documentations of p53 mutations exist; with over 71% of them include one amino acid substitution within the p53 core domain. Tumors in the lungs, neck, colon, and pancreas, are resistant to common cancer treatments and p53 mutations are commonly

found in these cancers. PCR was used to obtain a cancer mutation. The p53 protein is responsible for apoptosis, also known as programmed cell death. Apoptosis prevents cancer because when there is cancer cell growth; apoptosis can kill off these cells. A benefit to reactivating the p53 protein would be fixing an essential apoptotic pathway. The p53 protein also signals target genes with stress signals when DNA needs to be repaired. The Washington University School of Medicine's "A global suppressor motif for p53 cancer mutants", identifies a global suppressor motif. These include the codons 235, 239, and 240. Out of the 30 most common p53 mutants, 16 were restored in function by these codons. In this experiment, the Washington University School of Medicine tested cancer mutations in mutagenic conditions in the RBy377 strain of yeast-by-yeast colony PCR. PCR was also used to determine the specific restriction site provided by the cancer mutation codon. The yeast colonies expressed plasmids of 30 p53 mutants. Some of the plasmids reverted back to the wild type p53 by homologous recombination. Yeast that repaired the p53 protein was separated by using a selective plate with histidine and uracil so that these strains did not cause error in the experiment. Yeast cells lacking histidine indicates that the gapped plasmid was repaired and cells lacking uracil suggest that the p53 was repaired. PCR was used to confirm the retention of cancer mutations on p53 in the remaining yeast. It was hypothesized that the 239/240 are of the core domain represented a suppressor motif to successfully restore the function of the p53 protein. There could be 19 amino acid changes in codons 239 and 240. The suppressor amino acids in their experiment were found to restore function to p53 cancer mutants. This was done by improving the stability of p53 and/or restoring its conformation needed to bind to DNA. These findings were tested in mammalian cells, and in the p53 mutants R158L, V173M, Y205C, and Y220C, there was only 20% wild type p53 activity. In twelve other p53 cancer mutants in mammalian cells, the wild type p53 activity was

restored to 40% up to 130%. This experiment confirms that codons 239 and 240 are an important global suppressor motif. Their effect was enhanced by the codon 235. This was discovered through reporter gene and apoptosis assays. This rescue mechanism is not completely understood. Understanding this mechanism could potentially aid in other rescue mechanisms on the p53 protein.

Conclusion

Cancer is rampant, but there are hopeful findings. When mutated or deactivated, the p53 protein is responsible for many cancers. Using PCR, it was discovered that it could regain function with the codons 235, 239, and 240 in over half of the most common p53 mutations. This opens many doors for cancer research, where harsh radiation and chemical treatments can be replaced with targeted p53 therapy. The p53 protein is still in need of research. This complex protein needs to be analyzed by people of different fields such as chemists, medical doctors, biochemists, molecular biologists, computer scientists, etcetera, in order to get an in depth understanding of the protein. Research could start in identifying the suppressor regions in the core domain of the p53 protein and discovering what codon or combination of codons rescues the other most common p53 mutations since a majority of p53 mutations occur in the core domain.

References

(1) Baroni, T. E.; Wang, T.; Qian, H.; Dearth, L. R.; Truong , L. N.; Zeng , J.; Denes , A. E.; Chen, S. W.; Brachmann , R. K. A Global Suppressor Motif for p53 Cancer Mutants . *Proceedings of the National Academy of Sciences of the United States of America* **2004**, *101* (14), 4930–4935 .

(2) Hofseth, L. J.; Hussain, S. P.; Harris , C. C. p53: 25 Years after Its Discovery. *Trends in Pharmacological Sciences* **2004**, *25* (4), 177–181.

(3) Siegel , R. L.; Miller, K. D.; Jemal, A. Cancer Statistics, 2019. *CA: A Cancer Journal for Clinicians* **2019**, *69* (1).

(4) Valasek, M. A.; Repa, J. J. The Power of Real-Time PCR . *Advances in Physiology Education* **2005**, *29* (3), 151–159.