The Detection of Inactivated p53 Protein by a Biosensor

Yuyang Cao, Yinan Wang

Abstract

P53 is a gene that closely correlates with human tumors. In this paper, we explore how various biology techniques can be applied to detect the malfunction of p53. Firstly, we insert the GFP gene, as correctly translated p53 protein will undergo fluorescence. Secondly, we apply artificial enzymes such as an antibody, and the structure of functional p53 protein differs from those that are incorrectly translated. The enzyme can detect this as its active site matches the structure of the functional p53 protein. Lastly, we aim to detect not the p53 protein but its side product. Caspase 3 is the one we will target to find. In this paper, we carefully compare and contrast these three methods, and the choice should always be considere with different experimental conditions. The detection using GFP should be the best method among the rest, as it gives a visually accessible result, is also easy, and can produce the result very quickly.

Keywords: molecular cloning, green fluorescent protein, P53, artificial enzyme.

1. Introduction

P53 gene is one of the genes with the highest correlation with human tumors [1]. It has always been a hotspot since Lane et al had found the p53 gene in 1979. In recent years, people's understanding of this gene has changed from tumor antigen to oncogene and then to tumor suppressor gene [2]. The more depth studies of this gene show the vital role of p53 in suppressing tumors because more than 50% of human tumors occur when p53 has been mutated, and the mutation of its sequence would cause inactive p53 protein. Therefore, its checkpoint function in the cell cycle fails, and the downstream p21/WAF1/CIP1 genes cannot be expressed to suppress cdks to go into S phase for DNA repair in some cases and directly go to apoptosis when the damage is unrepairable [3,4]. Thus, the checkpoint is malfunctioned and the cells with damaged DNA will keep on replicating which leads to benign or malignant tumors. The current way of detecting dysfunctional p53 protein is through hospital or third party p53 gene test. Under this circumstance, we propose some methods that can detect p53 in a biosensor.

In our work, we have proposed three methods which are possible to make biosensors to measure p53 functionality: insertion of GFP gene, shape determination using (artificial) enzyme, and the detection of side products to detect abnormal p53 protein in one's cell [5].

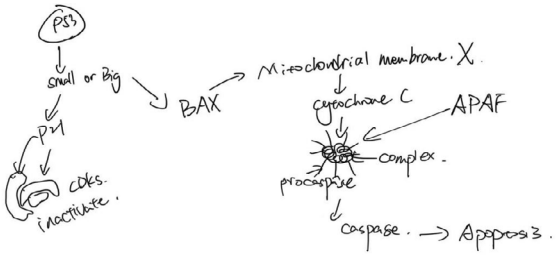


Figure 1. P53 pathways in regulating cell activities.

The figure 1 shows the possible pathways about how the p53 proteins play their role in cell regulation. The p53 proteins can sense how badly the DNA is been damaged and activate two downstream proteins for either small or big damage. When the damage is small, p53 is going to activate p21 protein, which is able to bind to CDKs(cyclin-dependent kinase) and inactivate them, making the cell stunning in the G0 phase(no DNA replication is been prevented). Besides, when the damage is big, BAX is been activated by p53 proteins. BAX is capable of dissolving mitochondrial membrane and releasing cytochrome C. Then cytochrome C binds with APAF and forms a complex that is able to cut procaspases to caspases, thus leading to apoptosis.

A. The basic introduction to the structure of the p53 protein.

The primary structure of p53 protein contains mainly 5

domains: 1~44 amino acids are the domain of transaction domain, 58~101 amino acids are the proline-rich domain, 102~292 is the DNA-binding domain, 324~355 is the tetramerization domain, and the last 363~393 is the regulatory domain[fig]. The transaction domains are attached to the RNA polymerase, regulatory domain and the proline-rich domain are pivotal in apoptotic activities whereas the DNA binding domain affects directly when this part of the sequence undergoes mutations. To illustrate, p53 serves the function of a transcription factor in other protein transcription. As a transcription factor, it needs to bind to the DNA sequence so that the RNA polymerase is able to transcript mRNAs. In this sense, the ability to bind to the DNA is important and it is even more crucial that p53 needs to form a homotetramer to become a transcription factor. Therefore, even one p53 is unable to bind to the DNA, the whole tetramer is dysfunctional.

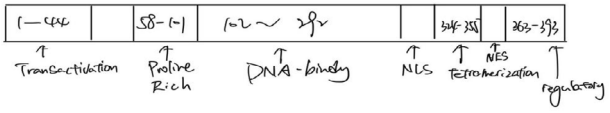


Figure 2. Domains in p53 genes.

The primary structure of the p53 protein is shown briefly in the above figure 2. P53 proteins contain 5 domains: a transaction domain, a proline-rich domain, a DNA-binding domain, a tetramerization domain, and a regulatory domain.

B. Common mutations on the DNA binding domain:

Over 95% of the mutations of the p53 protein in human cancer occur in the DNA-binding domain, with 175, 248, 249, 273, 282 having the highest possibility of being mutated [4]. And all of the mutation sites listed above are missense mutations (still code for an amino acid, but not the correct one) also called point mutations. [fig3] Although only one amino acid is being changed, it will have a profound effect on the structure of the protein, and it will not effectively bind to the target DNA sequence. To illustrate, it is doubtless that once two alleles of the p53 genes are all mutated, the p53 protein being produced is dysfunctional. However, it is the same even when one allele is well preserved and can produce fully functional p53 protein. Since p53 protein needs to form a tetramer transcription factor, one mutated p53 protein will make the whole tetramer dysfunctional. There is only 1/16 percent that four intact p53 protein forms a functional transcription factor. [fig4]

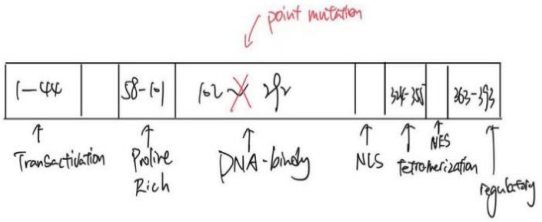


Figure 3. Detrimental point mutation mostly occurs on the DNA-binding domain.

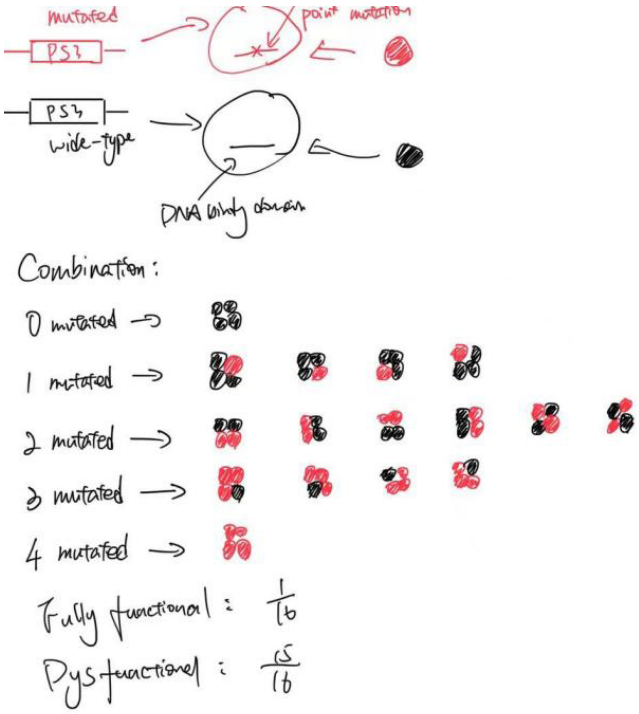
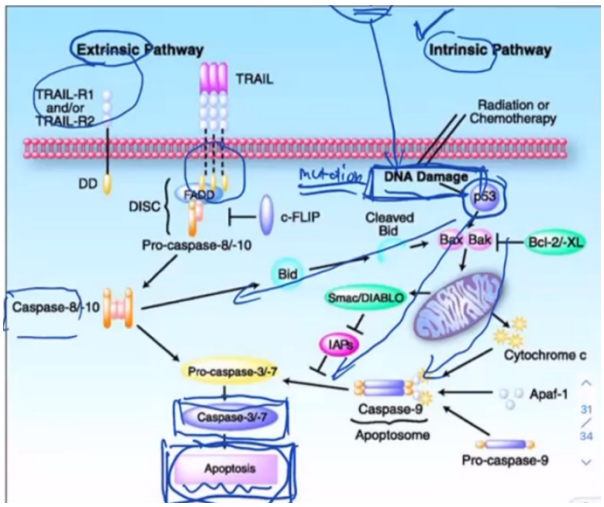


Figure 4. Possible outcomes involving one or more mutated p53 proteins in forming the homotetramer structure.

This above figure 4 shows what happens when one allele of the gene is mutated and another is wild-type. The mutated allele is presented in red lines and the protein it produces is in the red-filled circle and the white line represents the wild-type allele and the white-filled circle is the wild-type p53 protein. All the combinations of the tetramer under this circumstance is been represented and whatever a mutated protein combines with the other three proteins, the tetramer is inactive [2]. Therefore, only 1 out of 16 combinations of the tetramer is wild-type.

2. Methodology



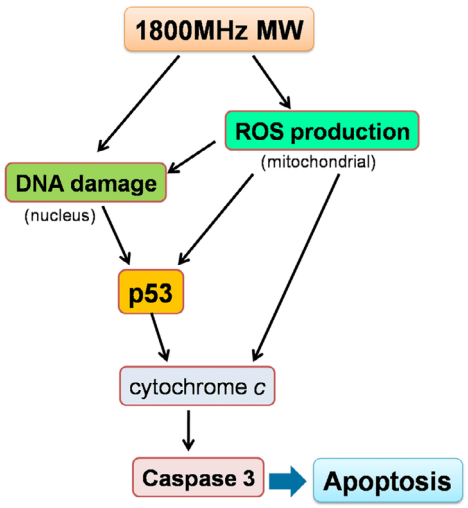


Figure 5. The overall mechanism of the p53 pathway [3],

Figure 5 shows the overall mechanism of the p53 pathway, from how it detects the mutation of the cell to its action to terminate cell growth [3]. The figure on the right shows the specific apoptosis pathway that is carried out by p53, this also includes our target protein, caspase 3.

A. Insertion of GFP gene

In the p53 gene sequence, we can insert a GFP gene, this allows us to visually see the presence of functional p53 being translated, this can help us detect the functional p53 [6]. If the malfunction p53 is translated, there will be no flourescence. GFP may affect the performance of the p53 protein for some extend, and this may require more in depth research to achieve the GFP insertion while maintaining normal p53 functions.

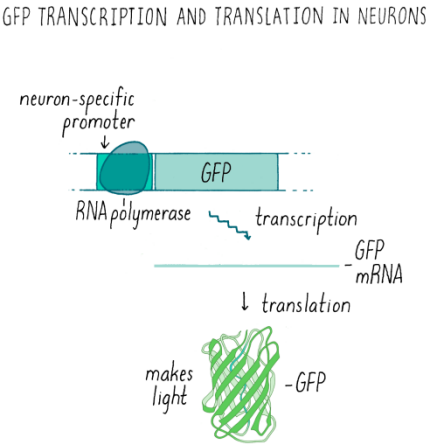


Figure 6. The mechanism of the GFP insertion

Figure 6 shows the mechanism of the GFP insertion, by inserting the GFP gene sequence into the desire gene, this allow the desired gene to be expressed along with the GFP gene, so that if the correct protein is being translated, we can see the fluorescence visually.

B. Shape determination using (artificial) enzyme

By using the recombinant DNA to make the enzyme that has the same shape of the active site to the p53 [7]. Place the enzyme on a biosensor chip, when p53 is successfully bind to the enzyme on the chip, signals can be detected. If the there is any mutation occured to the p53, the shape of the protein will be different which leads to the failure of binding to the recombinant enzyme and no signal will be detected. Artificial enzyme has problems with affinity, producing the protein with high affinity can be extremely challenging.

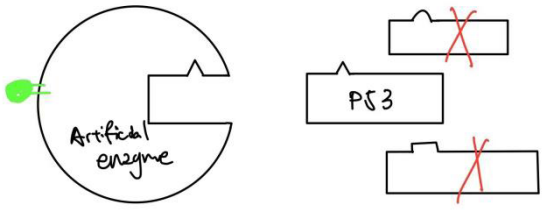


Figure 7. The artificial enzyme

Figure 7 shows that the artificial enzyme is based with fluorescent dye, once the p53 with the correct structure is bound with the enzyme, we can detect the fluorescence on our biosensor.

C. Detection of side products (Caspase 3)

As shown in the diagram, there is a large network of molecules and proteins associated with the p53, to determine whether the p53 is well-functioning or not, we will focus on several targets. The first protein of interest is Caspase 3 produced bu cytochrome c, this protein leads to direct apoptosis of the cell when normal functioning p53 had detected the abnormal of the gene. The problem with this technique is that sometimes side products can be hard to detect as they might be quickly degraded or transformed into another form of molecules, this can lead to false reading.

D. NMR and Mass spec analysis:

This is more like a proof of fidelity of the experiment, it can see the result much more clearly, but it takes longer time, therefore it is hard to be used to make the bio sensor.

3. conclusion

The detection of malfunction p53 is largely based on the ability of spotting the differences of side products from the ones produced by normal p53. This can be done by the recombinant enzyme, GFP and NMR and Mass spectrometry. The insertion of GFP genes allows easier detection when functional p53 is presented. And it can be done in a cheap way by using E.coli that had been genetically modified with the GFP gene inserted. Artificial enzyme can be hard to achieve, as it requires many trails to eventually making an enzyme that as the exact same shape of p53 or its side products with the active site of the enzyme. NMR and Mass spectrometry are the best way to assess the fidelity of the p53 protein, it allows accurate determination of the shape and atomic environment of protein, but it takes a long time to produce the result so it is not ideal to making a biosensor. We believe by combining these methods we can eventually generate a precise, low time and price cost biosensor to p53, and the outcome will be a gamechanger in the oncology and medicine field.

References

[1] Barton, M. C., and Lozano, G. (2022) P53 Activation Paradoxically Causes Liver Cancer. Cancer Res 82, 2824-2825

[2] Liu Yanmu. “Property and structure analysis of p53 protein.” Contemporary Chemical Research. 07 (2017): 112-114

[3] Li Wenjuan, Pan Qingjie, and Li Meiyu. “Research progress on p53 gene and its function.” Biotechnology Bulletin 25.02 (2014): 282-285

[4] Raj, N. , and L. D. Attardi . “The Transactivation Domains of the p53 Protein.” Cold Spring Harbor Persectives in Medicine (2017) 7(1):a026047.

[5] Fisher, D., and Krasinska, L. (2022) Explaining Redundancy in CDK-Mediated Control of the Cell Cycle Unifying the Continuum and Quantitative Models. Cells 11(13):2019.

[6] Zhang WW, Labrecque S, et al. “Development of a P53 responsive GFP reporter; identification of living cells with P53 activity.” Journal of Biotechnology. 84 (2000): 79-86

[7] Burnouf, T. “Recombinant plasma proteins.” Vox Sanguinis. 100(2011): 68-83