



Isolation and Amplification of P53 Gene from Cancer Blood sample and Designing a Potential Drug

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Abstract

Cancer being one of the dreadful diseases with no perfect treatment till date is laying a platform for the continuous research. Molecular methodologies for the diagnosis and analysis are gaining rapid importance and accuracy. The current work aimed to Collect the cancer blood samples followed by the extraction and purification of the DNA. The DNA thus obtained was used for the amplification of the P53 gene, one of the very important gene involved in the cancer cell metabolism. The amplification was performed using universal primers and the sequencing was performed. The sequence thus obtained was further used for insilico analysis. In the Insilico method the protein sequence corresponding to the obtained gene sequence was retrieved from the available Database Gene cards. The protein sequence was later used to develop the 3D structure of the protein as can be obtained from RCSB Protein Data Bank. This structure was targeted to dock with 10 of the 50 chemicals used for screening. The screening of the chemicals included the Drug likeliness, Ambiguity and Toxicity analysis. The docking was performed in HEX Software and the results were analyzed. Based on the results obtained it can be concluded that the chemical **p53 modulator 10d** with the docking energy of -275.77 is the best potent ligand used to bind with the receptor P53. Thus it can be used as one of the good drugs in treating the symptoms of cancer.

Keywords:

Insilico Drug Designing, P53 Modulator 10d, Docking

Introduction

Cancer also known as a malignant tumor or malignant neoplasm, is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Not all tumors are cancerous; benign tumors do not spread to other parts of the body. Possible signs and symptoms include: a new lump, abnormal bleeding, a prolonged cough, unexplained weight loss, and a change in bowel movements among others. While these symptoms may indicate cancer they may also occur due to other

issues. There are over 100 different known cancers that affect humans. The “war against cancer” is, in actuality, a battle against a large group of sometimes very different conditions caused by differing agents. Generally, cancer results from an altered balance between cell proliferation – growth and division – and cell death. A number of factors, including certain genes, viruses, chemicals or exposure to radiation, have been blamed for these normal activities gone awry. Mutations in identifiable genes are, as a group, the latest of these factors to be discovered. They have also served as fuel for excitement over potential clues to ways that drugs may be designed to battle the varied disease. Cancer is a genetic disease; it results from mutations in somatic cells. To understand it at a molecular level, we need to identify the relevant mutations and to discover how they give rise to cancerous cell behavior. Finding the mutations is easy in one respect: the mutant cells are favored by natural selection and call attention to them selves by giving rise to tumors. The hard task then begins: how are the genes with the carcinogenic mutations to be identified among all the other genes in the cancerous cells? A similar needle-in-haystack problem arises in any search for a gene underlying a given mutant phenotype, but for cancer the task is particularly complex. To make matters worse, most cancer cells will contain mutations that are accidental by-products of genetic instability, and it can be difficult to distinguish these from the mutations that have a causative role in the disease.

In the relatively short history of cancer genetics as is apparent from this, the accrual of p53 mutations equips emerging tumor cells with much more than just the loss of wtp53 tumor suppressor function. This poses mutp53 as a valid target for inactivation by prospective anticancer therapies. A number of such molecules have already been identified and described, and shown to exert antitumor effects in experimental mouse models. Yet, approaches that are more directly aimed toward elimination of mutp53, such as therapeutic administration of p53 siRNA to cancer patients whose tumors exhibit high mutp53 expression, are also worthy of consideration. Moreover, one may attempt to target proteins whose expression is markedly up-regulated by mutp53,

Particularly in cases in which such proteins are “druggable” targets, such as transmembrane or secreted proteins or enzymes whose catalytic activity is susceptible to inhibition by small molecular weight compounds.

Materials and Methods

1. Sample collection

The samples were collected from a nearest diagnostic Centre for the research purpose. 3 samples were collected from the cancer patients so as to analyze their P53 sequence. These samples were further used for the DNA extraction, Purification and sequencing.

2. Extraction of DNA from blood sample

DNA Extraction from the collected samples was done following Bunce method. Precipitation of the isolated DNA was performed using 70% chilled ethyl alcohol followed by storage in ice cold conditions for 24hrs. After the incubation time the DNA sample was centrifuged and the pellet was re washed with ethyl alcohol followed by centrifugation again. The final pellet obtained is stored in TE buffer to preserve the DNA and can be used for further analysis. Agarose Gel Electrophoresis was later performed on 0.7% agarose to confirm the presence of the DNA in the pellet based on the DNA bands obtained in the gel.

3. Purification of DNA

An equal volume of phenol: chloroform: isoamyl alcohol in the ratio of 13:12:5 was added to the samples. Gently vortexed and centrifused at 10000rpm for 10 minutes. The aqueous layer was taken and added equal volume of chloroform: iso amyl alcohol in the ratio of 12:5. Later vortexed and centrifuged at 10000 rpm for 10 minutes and the aqueous layer was taken. Added 100µl of 3M Sodium Acetate and equal volume of iso propanol. Then Incubated in ice cold condition for half an hour and centrifuged at 12000rpm for 12 minutes. Finally, the pellet was taken following the addition of 50 µl of TE buffer.

4. Amplification of DNA through PCR

PCR was employed to amplify the DNA obtained and can be used for further analysis. Universal primers were used for amplification. The reaction mixture was added to the PCR tube as follows:-3µl of forward primer, 3 µl of Reverse primer, 2 µl of dNTPs, was added and mixed. Then 2 µl of Taq DNA polymerase was added following the addition of 5 µl of Buffer and MgCl₂. 5 µl of template DNA was added that is blood DNA sample to the above solution. Before this the instrument was programmed for repeated heating and cooling cycles to the total of

35 times to produce a greater than million fold amplification of target DNA. Before cycle 1 can be initiated, the double stranded DNA target needs to be heat denatured to provide single strand region for primer annealing. This is called as pre PCR cycle.

The thermal cycle was adjusted as follows: Initial denaturation at 94°C for 5min, followed by 35 cycles of denaturation at 94°C for 1min, annealing at 70°C and extension at 72°C for 1min. Final extension was carried out at 72°C for 10min and the PCR products were stored in the thermal cycler at 4°C until they were collected.

5. Primer designing

For amplification of p53 mRNA specific for Homo sapiens, universal primers were used. The primers can be synthesized using oligonucleotide synthesizer. This step of the research was out sourced from the nearby laboratory.

6. Agarose Gel Electrophoresis

The PCR products were tested for positive amplification by Agarose Gel Electrophoresis using 1.5kb molecular weight markers. The DNA extracts were loaded 2% W/V gel for electrophoresis and checked the bands using ladder under UV Transilluminator (Fig1).

7. Sample sequencing

All the 10 DNA samples which were isolated and purified were subjected to PCR Amplification and post PCR Electrophoresis. Later the samples were sent for sequencing using universal primers. Sanger’s method was employed for the sequencing of the samples.

8. Structural analysis and Drug designing against cancer causing P53 protein through Bioinformatic tools

Based on the sequences obtained after sequencing the comparative study was performed to identify any SNP Present in the sequences. CLUSTAL W multiple sequence alignment was performed to identify the SNP and the conserved patterns.

3D Structure selection from RCSB PDB:

Based on the sequence obtained after sequencing one among the several protein structures available at RCSB PDB was selected for further analysis. The PDB ID selected as the structure representative was **3Q05 corresponding to human P53**. This structure was later used for analysis.

Chemical compound selection:

Pubchem database was extensively screened to identify the chemicals that have the capability to

control cell division or act against cell cancer. A total of 50 chemicals were selected based on their functionality. All these were screened for their Drug likeliness based on Lipinski rule of five. The selected chemicals were further screened for toxicity using Mol inspiration and Ambiguity based on Dundee Pro Drg server.

Docking using Hex Software:

Hex is automated user friendly software that performs non targeted docking. All the selected chemicals were further used for docking with the 3D structure of protein in the HEX Software. After the dockings the energies were screened and the best chemical based on the lowest energy was finalized to be the best drug for targeting P53 in cancer samples.

Results and Discussion

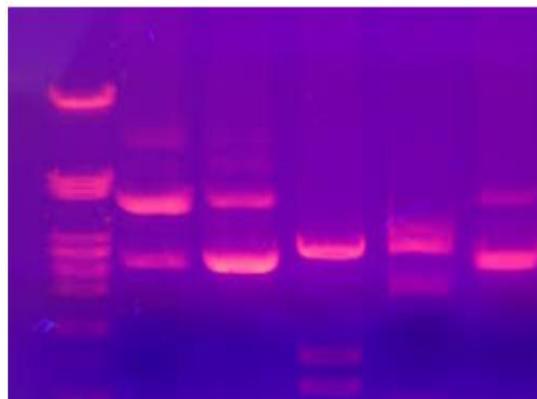
In the above research work the major aim in collecting the patient blood samples and sequencing is to identify any natural SNP present among the individuals which may be one of the causes if cancer. The results of the work can be summarized as below.

Post PCR Gel Electrophoresis:

To confirm the amplification of the DNA samples extracted AGE was performed along with the ladder to compare the molecular weights. The results indicate that the amplification was perfect. For those

samples which were not showing the amplification the experiment was repeated.

Fig 1: The GEL picture showing 5 of the samples Post Gel Electrophoresis results.

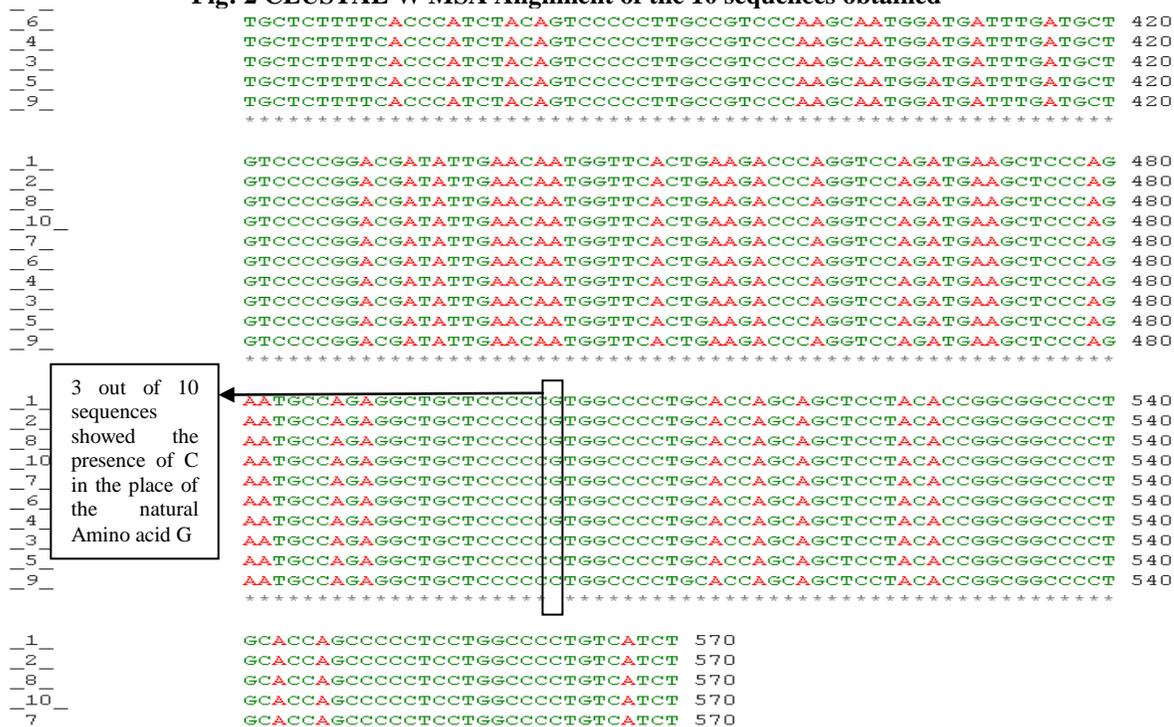


From the above picture it can be concluded that the 5 samples were amplified and can be further sent for sequencing.

Sequencing and Analysis of the Sequences:

All the 10 samples obtained after sequencing were further subjected for MSA using Clustal W program available at SDSC Biology work bench to identify the conservation and SNP sites. The results of the alignment are depicted in the Fig 2.

Fig: 2 CLUSTAL W MSA Alignment of the 10 sequences obtained



The Above figure shows that out of a total number of 10 sequences obtained 3 of the samples showed the presence of Camino acid instead of the wild type G. This is an SNP at this position

Based on the above results it cannot be concluded that the disease is due to the SNP as only 3 out of the 10 patient samples showed the mutation inspite of the fact that all of them are the patient samples. Thus to confirm the same the work can be extended with increased sample size.

Structure Analysis of the P53 and visualization in Rasmol:

Based upon the sequence obtained the best structure for the P53 has been collected from the RCSB PDB whose PDB ID is **3Q05**. The structure of it is visualized in RASMOL Software as shown below.

Fig: 3 3D structure of protein as seen in RASMOL

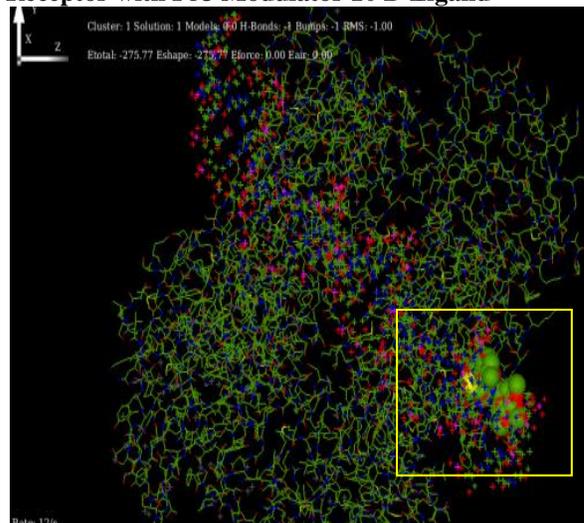


The above figure shows the 3D structure of P53 bound to DNA as seen in Rasmol.

Ligand Screening and Docking of the selected chemical with the Receptor P53:

PubChem data base has been used to screen and select the chemicals that can act against P53 receptor and inhibit the abnormal activity. A total number of 50 chemicals were selected which were subjected for several screening steps as mentioned above, Only these chemicals that have passed the screening of Drug likeliness, Toxicity, Ambiguity etc. were selected for the final Docking Step. Out of the total.....chemicals the ligand p53 modulator 10d has been selected as the best ligand due to its lowest docking energy of -275.77. The docking has been shown in the Fig 4.

Fig 4: The Figure showing the Docking of P53 Receptor with P53 Modulator 10 D Ligand



From the above figure 4 it can be observed that the docking of this selected pair showed the docking energy of -275.77 which the lowest energy among the screened chemicals. Thus it can be selected as the best ligand to act against the P53 protein.

Conclusions:

The current work was aimed to identify the SNP if any present in the P53 gene of the Homo sapiens that has a major role in cell division and onset of cancer. The blood samples were collected from 10 patients and it was found that at some point in the gene there was a change in the amino acid G to C in 3 of the 10 samples. These results cannot be taken as conclusive evidence for the role of SNP in diseased condition as only 3 out of the 10 samples were showing the mutant allele in spite of the fact that all the fact that all the samples were from the effected patients. Some patients were having the mutate allele where as the others possessed normal allele in spite of their diseased condition. Thus a complete conclusion cannot be drawn from the data; the work can be extended with an increased sample size to obtain conclusive evidence. Further the work was processed for *Insilico* Drug development against the P53 protein involved in Cancer. The structure of the protein was obtained from the Public Database **RCSB PDB**. The structure was further used for docking with the ligand selected from the Pubchem Database. The ligand was obtained from the Pubchem after screening for several parameters. The ligand that was selected after docking was P53 Modulator 10d due to its lowest docking energy of -257.77. Thus its can be concluded that this ligand can be used in the treatment of Cancer for targeting the P53 protein.

References

1. Strachan, T.. *Human molecular genetics 2*. New York: Wiley; 1999. ISBN 0-471-33061-2. Chapter 18: Cancer Genetics.
2. Cho Y, Gorina S, Jeffrey PD, Pavletich NP (1994). "Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations". *Science* **265** (5170): 346–55. doi:10.1126/science.8023157. PMID 8023157.
3. Bykov et al. 2002; Bykov et al. 2005; Foster et al. 1999; Lambert et al. 2009
4. Emanuel BS, Givol D, Oren M, Croce CM. Localization of gene for human p53 tumour antigen to band 17p13. *Nature*. 1986;320(6057):84–5. doi:10.1038/320084a0. PMID 3456488.
5. Matlashewski G, Lamb P, Pim D, Peacock J, Crawford L, Benchimol S. Isolation and characterization of a human p53 cDNA clone: expression of the human p53 gene. *EMBO J.* 1984;3(13):3257–62. PMID 6396087.