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Drug Design for Cancer-Causing PI3K (P110 α) subunit Mutant Protein

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Abstract

The phosphoinositide 3- kinase (PI3K) pathway is consider to have a vital role in wide range of cancer such as breast, ovarian, myeloid leukemia, lung cancer. Therefor PI3K is the mostly used protein as target for the cancer. PI3K have three subunit classes such as p110 α, p110 β, p110γ. The vast majority of PI3K mutations in human carcinogenesis occur in subunit P110 α codon 545 that leads to an altered regulation of cell proliferation and malignant transformation. In this context, the use of cost-effective computational tools to predict potential anticancer drug/inhibitor molecules is gaining importance in the recent years. The Ramachandran map analysis indicated that the PI3K (P110α) subunit protein model constructed were in stable conformations. Active sites near to the mutational site of codon 545 were further detected in all PI3K (P110 α) subunit protein models. *In-Silico* drug designing approaches follow for molecular docking studies using PyRx (0.9-Linux-x86- a virtual screening) software used for computational drug discovery. Out of drug molecule inhibitors identified according to their lowest docking energies for blocking the mutated PI3K (P110 α) subunit protein conformations we got 10 best derivatives of pyrazolo pyrimidine, 5a: -21. 44 kcal/mol; pyrazolo pyrimidine, 10: -17.67kcal/mol; pyrimidine, 25: -16.44 kcal/mol; Sorafenib, : -13.4 kcal/mol; xl147:-11.8 kcal/mol; PLX4032: -10.7 kcal/mol; D_87503:-9.4 kcal/mol; NVP_PEZ235: -9.01 kcal/mol; ly294002: -8.05 kcal/mol;3-Aminopropanesulphonic acid :-3.01 kcal/mol Further the five best-docked found to obey the Lipinski's rule of five and can be considered as a good drug molecule to inhibit lysine specific mutations of PI3K (P110 α) subunit protein.

Key words: *PI3K (P110 α) subunit, Modeller, Molecular docking, PyRx*

Introduction

PI3K (Phosphoinositid-3-Kinase) is a proto oncogene which have an essential roles in controlling the activity of several crucial cell signaling pathways that enhance the stimulation of cellular replication and cell proliferation and to inhibit growth and apoptosis(Cantley 2002; Zunde *et al.*, 2008). The PI3K proteins family includes a group of eight member classified according to their sequence, domain structure and mode of regulation. to three groups *i.e.*, Class I PI3K, Class II PI3K and class III PI3K.(Maira et al., 2008) Class I PI3K has three subunit classes *viz;* p110 α, p110 β and p110γ which plays an important role in cancer. Mutations in PI3K family are very common in class I PI3K only.

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The vast majority of class I PI3K mutations found in human cancer occur in p110α among all three subunit classes of PI3K class I. it was reported, p110α subunit is mostly responsible to be frequently mutated protein in several type of cancers such as breast (27%), endometrial (23%), urinary tract (17%), colorectal (14%) and ovarian (8%) cancers(Engelman et al., 2006; Yuan and Cantley 2008; Zunder et al., 2008; Miller et al., 2011). A mutation in PI3K (P110α) subunit protein at codon 542, 545,and 1047 the most common mutational events in human carcinogenesis and has been found on a variety of human cancers, with the frequent replacement of the amino acids , Glu by Lys in codon 542 and Glu by Lys or Gln in codon 545 and His by Arg or Leu in codon 1047.(Samuels and Velculescu 2004; Vogt et al., 2011). Moreover, the mutations in PI3K (P110α) subunit leads to constitutive activation of downstream pathways resulting in the altered regulation of cellular proliferation and malignant transformation which makes it a very attractive drug target for cancer biology.

Drug based in target design and discovery involves early validation and the identifying of disease-associated target. Mutation occurring in the PIK3CA gene(s) which code PI3K (P110α) subunit lead to uncontrolled cell growth and proliferation and prevent cell apoptosis. When we think through treatment for cancer today, they depend on the type and stages of cancer development. Chemotherapy, surgery, radiation therapy, and hormonal therapy are the various treatments that currently take place (Vincent and Djulbegovic 2005). But these types of treatments except for the target based, cannot differentiate between normal and tumorous cell. Therefore, healthy cells are generally damaged in the process of treatments, which results in side effects. Many therapeutic agents currently being evaluated have multiple targets and their antitumor effects may not be due to specific mutated PI3K (P110α) subunit inhibition. Myriads of attempts have been carried out to utilize PI3K (P110α) subunit as anticancer target protein. In this context, target-based drug Design is considered to be highly potential (Sams-Dood 2005; Pearce et al., 2008). Therefore, the present investigation was carried out to utilize PI3K (P110α) subunit as an canonically relevant anticancer drug target in cancer therapy.

Material and Methods

The protein architecture of PI3K (P110 α) subunit was traced in *Homo sapiens* by generating Boolean query against UniProt database (*http://www.uniprot.org*) Furthermore, the Protein query sequence (*P42336*) in fasta format was downloaded from UniProt (*(http://www.uniprot.org/uniprot/)*) databases. The 3D structures of all wild type and mutational protein were predicted using Modeller 9.17 software (Sali et al., 1995)**.** All possible(hotspot) mutations reported for codons 542, 545 and 1047 of the PI3K (P110 α) subunit proto-oncogene were retrieved through literature survey (Engelman et al., 2006; Yuan and Cantley 2008; Miller et al., 2011; Janku et al., 2011). The structural models were further evaluated using Structural Analysis and Verification Server's PROCHECK tool which checks stereochemical quality of a protein structure utilizing Ramachandran plot (Laskowski et al., 1993) The best models from each individual protein (wild type as well as mutated) were taken under consideration for further analysis. A prediction of active site of all mutated PI3K (P110 α) subunit conformations was performed with putative active sites with spheres software, PASS (Brady et al., 2000). For further utilized for virtual screening. A library of anticancer drugs was prepared on the basis of literature retrieved from different sources *viz.*, PubChem (*http://www.pubchem.ncbi.nlm.nih.gov/*) and ChemSpider of the Royal Society of Chemistry (*http://www.chemspider.com/*). *In silico* virtual screening was carried out using PyRx (Jacob et al., 2012). software to rank all the library molecules under study according to their affinity towards the active site of PI3K (P110 α) subunit mutated protein conformations. All the library molecules (*i.e.*, top ten putative library molecules) were ranked according to their affinity towards the mutated PI3K (P110 α) subunit conformations. The molecules were further subjected to analyze their likeness as drug using SCFBIO's Lipinski filter software(*http://www.scfbio-iitd.res.in/utility/LipinskiFilters.jsp*). Lipinski rule of 5 predicts that poor absorption or permeation is more likely when there are more than 5 H bond donors, 10 H bond acceptors, the molecular weight is more than 500 Daltons and the calculated Log P is greater than 5.

Results and Discussion

The structure of PI3K (P110 α)subunit protein wild type and five mutated models have determined by using homology modeling protocol. By using Modeller 9.17 software. firstly BLASTP search was performed against PDB with default parameters to find suitable templates for homology modeling. Based on the maximum identity with high score and lower e-value (Template) were used as the template for homology modeling. The final stable structure of PI3K (P110 α) subunit protein is shown in Figure 1,2 and 3. The mutations were selected according to the earlier literature (Samuels et al., 2004; Lee et al., 2005; Ikenoue et al., 2005; Isakoff et al., 2005; Kang et al., 2005; Zhao et al.,2005; Bader et al., 2006; Samuels and Ericson, 2006; Zhao and Vogt 2008). For each protein three models were generated. The selected protein models for wild type and other five mutated codon 542, 545and 1047 PI3K (P110 α) subunit conformations are illustrated in Figure 1,2 and 3. The mutations occurring in codon 542, 545and 1047 due to the amino acid changes are clearly shown in Figure.(1,2A,B and 3A,B). The structure of PI3K (P110 α)subunit protein wild type and five mutated models have determined by using homology modeling protocol. By using Modeller 9.17 software. firstly BLASTP search was performed against PDB with default parameters to find suitable templates for homology modeling. Based on the maximum identity with high score and lower e-value (Template) were used as the template for homology modeling. The

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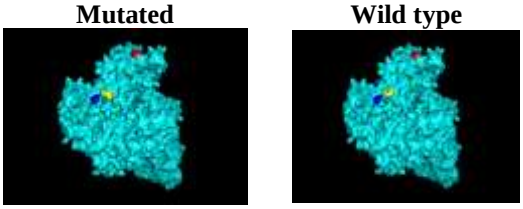

Fig.1: Protein models constructed for wild type (Glu) and mutated PI3K (P110 α) subunit of Codon 545 (A: mutated by Lys; B: mutated by Gln)in blue color

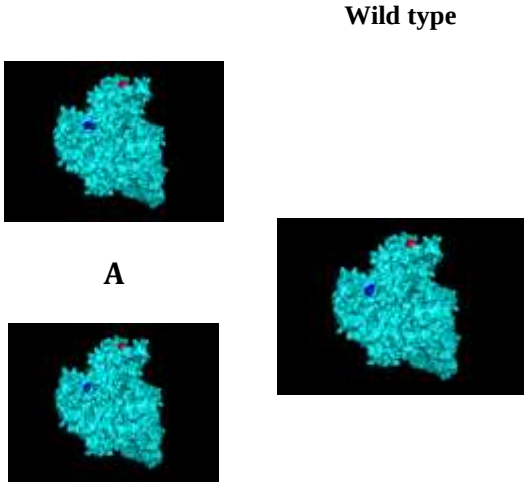

Fig 2: Protein models constructed for wild type (Glu) and mutated PI3K (P110 α) subunit of Codon 545 (A: mutated by Lys; B: mutated by Gln)in blue color

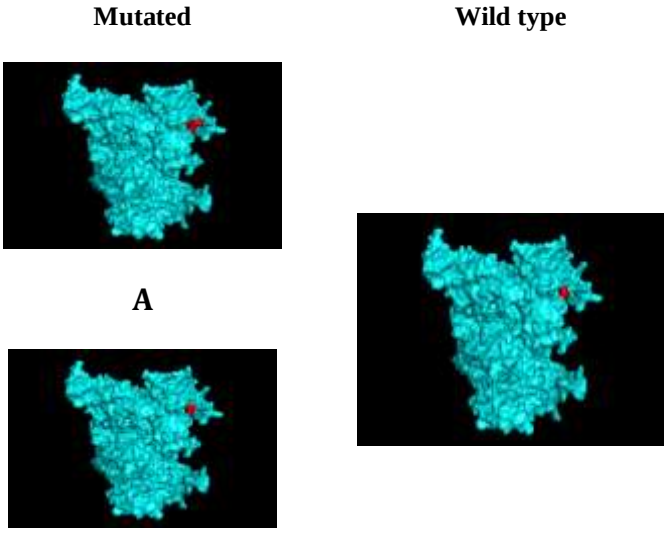

Fig 3: Protein models constructed for wild type (Glu) and mutated PI3K (P110 α) subunit of Codon 1047 (A: mutated by Leu; B: mutated by Arg)in red color

The models were analyzed online by submitting to NIH MBI Laboratory for Structural Genomics and Proteomics' SAVES server. Validity reports generated by PROCHECK and Verfiy_3D judged accuracy of the protein models. A comparison of the results obtained from the above mentioned validation tools, showed that one of the models generated by Modeller is more acceptable in comparison to the others. So, one of every three most valid model was selected (Table 1) for each protein model to use for further studies. It was found that the phi/psi angles of 85.1 to 87.3% of the residues fell in the most favored regions, 9.1 to 11.7% of the residues fell in the additional allowed regions, 2.0 to 2.4 % fell in the generously allowed regions, and 0.8 to 1.7 of the residues fell in the disallowed regions in all PI3K (P110α) subunit wild type and all other proteins (Table 1; Figure 4). A score of >50% in the most favored regions are acceptable for a reasonable protein model and the score obtained more than 80% indicate the quality of all selected PI3K (P110 α) subunit protein models. Once the stable protein models of wild type and mutated PI3K (P110 α) subunit conformations were constructed, three possible binding sites were detected in the protein models using PASS software. The probe (representing the cavities present in the protein

molecule) having maximum number of residues was chosen as the active site.

Table 1: Validation of PI3K (P110 α) subunit structure

Selected **PI3K (P110α)** protein models	Ramachandran map analysis phi/psi angles %	Additional allowed regions	Additional disallowed regions	Generously allowed regions
Model 1of **PI3K (P110α)**	87.3	9.1	1.7	2.0
Model 2of **PI3K (P110α)**	85.1	11.7	0.8	2.4
Model 3 of **PI3K (P110α)**	85.5	10.8	1.6	2.2

545 in PI3K (P110 α) subunit protein models. These possible binding sites obtained in the PI3K (P110 α) subunit protein models are illustrated in Figure 5. It is reported that ILE800, LEU807, LEU814, TYR836, GLY837, CYS838, ILE848 residues were identified as active site in the PI3K (P110 α) subunit (Shah et al., 2002; von Bubnoff et al., 2005; Zunder et al., 2008; Sujatha and Silja 2011; Chaudhary and Singh 2012). This confirms the binding affinity pocket of our study having the same residues. so the active site which we have observed is considered to be the region that interacts with the target ligand molecule of the PI3K (P110 α) subunit protein.

The identification of active site in the mutated protein models was followed by the screening and identification of potential inhibitor molecule targeting active site toward mutant codon 545, 542 of PI3K (P110 α) subunit protein. Towards finding suitable inhibitor(s), ten probable inhibitor molecules with lower docking energies were chosen individually for PI3K (P110 α) subunit mutations. Each inhibitor molecule was viewed and the moieties having lower docking energies were chosen as the possible PI3K (P110 α) subunit inhibitor molecules and their ranking according to the lowest docking energies are as shown in Table 2. The computational approach that dock small molecules into the structures of macromolecular targets and score their potential complementary to binding sites are now widely used in hit identification and lead optimization (Kitchen et al., 2004; Kroemer 2007).

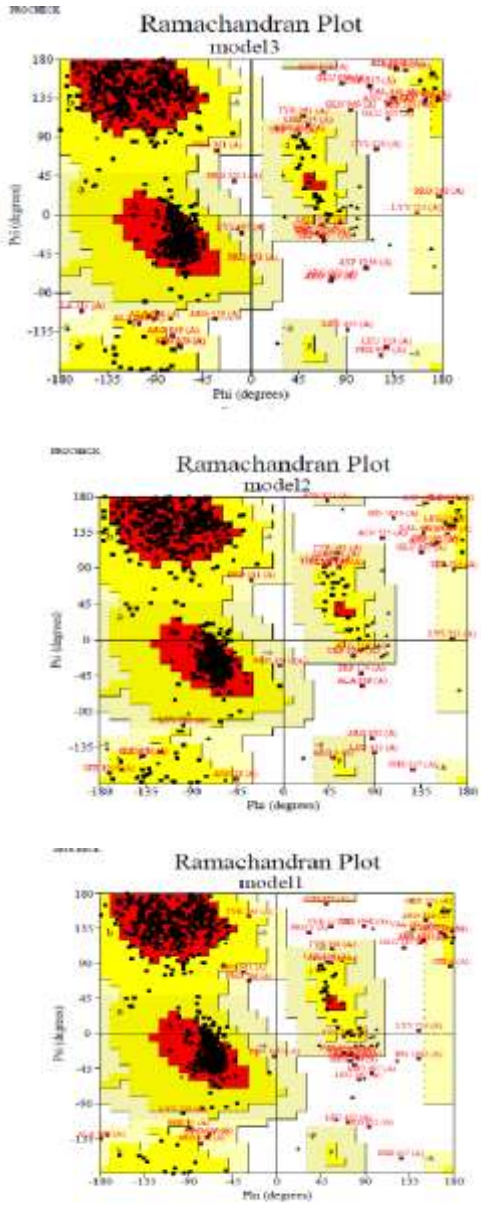

Fig 4: Shows Ramachandran plot of PI3K (P110 α) subunit wild type proteins models to validate the structure

Fig 5: The active site was identified by PASS yellow and green colors show Point mutations at codon 542,545 and 1047,and red and blue colors show active site point at codons 800,814,836,837and 838 of PI3K (P110 α) subunit

Table 2: Docked Energy and Lipinski's Values of Ligand Molecules

Ligand molecules	Molecular formula	Docking energy (kcal/mol)	Xlog P <5	H-Bond donor <5	H-Bond Acceptor <10	Molecular weight (g/mol) <500
pyrazolo pyrimidine, 5a	C20H24N6O2	-21.44	1.248	1	2	376.00
pyrazolo pyrimidine, 10	C28H33N9O2	-17.67	2.145	5	3	528.00
pyrazolo pyrimidine, 25	C28H31N9O4	-16.44	1.440	3	4	559.00
Sorafenib	C21H16ClF3N4O3	-13.40	4.200	3	7	464.50
xl147	C21H16N6O2S2	-11.80	0.4303	0	2	443.00
PLX4032	C23H18ClF2N3O3S	-10.70	2.6878	1	3	466.00
D_87503	C17H15N5OS	-9.4	0.2718	0	1	335.00
NVP_PEZ235	C30H23N5O	-9.01	-0.6025	1	1	463.00
ly294002	C19H17NO3	-8.05	0.8341	0	3	301.00
3-Aminopropanesulphonic acid	C3H9NO3S	-3.01	0.643	2	7	130.1

The present results on identification of potential inhibitor molecules based on the lowest docking energies are in agreement with Garcia-Echeverria and Sellers (2008); Sujatha, S. and Silja (2011) and Chaudhary and Singh (2012) in their Docking studies of PI3K (P110 α) subunit with various compounds revealed against selected active site which was the best to be targeted by all hits and showed good docking score just similar to our docking score.

The Lipinski's 'rule of five' is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely active drug in humans. As per the Absorption, distribution, metabolism and excretion (ADME) parameters of the Lipinski's 'rule of five', it was observed that the inhibitor molecules were found to obey Lipinski's rule of five as shown in t Table 2. These molecular properties that are important for a drugs pharmacokinetics in human body include ADME parameters (Lipinski et al., 1997). The molecule that obey Lipinski's 'rule of 5' which include solubility, partition coefficient, drug score, molecular weight etc. can be considered as a good drug molecule and may be selected for further research.

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Abbreviations

PDB = Protein data Bank
PI3K= Phosphatidylinositol 3-OH kinase
PDB= Protein data bank
Scfbio= Supercomputing facility for bioinformatics
SAVS= Structural analysis and verification server
Uniprot = Universal protein resources
PASS= Putative active sites with spheres
BLAST= Basic local alignment search tool

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