MediPharm

International Journal of MediPharm Research

ISSN:2395-423X

www.medipharmsai.com

Vol.02, No.02, pp 91-97, 2016

Simulation studies on homology Modeling, Docking and Pharmacophore of Ras Protein in Cancer

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Abstract : The main objective of this study is to identify one of the essential Ras protein in cancer and predicted its 3d structure by homology modeling method with various softwares like discovery studio and Molsoft and model verification is carried out by using various methods like Ramachandran plot analysis Profiles 3d. Docking studies have been performed with various docking algorithams like CDOCKER, LIP DOCKetc by using anti-Ras agents or inhibitors and pharmacophore generation.
Key words: Cancer, Metastatis, Ras protein, Homology, Docking

Introduction

Cancer is a class of diseases in which a group of cells display uncontrolled growth, invasion, and sometimes metastasis. The Ras GTPase proteins and their down-stream effectors regulate specific intracellular signalling pathways involved in numerous biological processes. Their actions directly influence progression through the cell cycle and the delicate balance of pro- and anti-apoptotic factors[1-4]. The variety of functions controlled by Ras, and the emerging evidence indicating that aberrations in Ras as well as at multiple points in downstream signalling pathways contribute to tumourigenesis, suggest that Ras signal transduction mechanisms have significant potential as anti-cancer therapeutic targets. The RAS protein controls signaling pathway are major player in cell growth, its regulation and malignant transformation. Any activation in RAS brings alteration in upstream or downstream signaling component.Ras-specific guanine nucleotide-releasing factor 2 is a protein that in humans is encoded by the RASGRF2 gene. RAS (MIM 190020) GTPases cycle between an inactive GDP-bound state and an active GTP-bound state. Guanine-nucleotide exchange factors (GEFs), such as RASGRFs, stimulate the conversion of the GDP-bound form into the active form[5-8].

N.A.D.H. is one of the most important coenzyme catalyzing more than a thousand of metabolic reactions in the human body, the most important of which is the production of ATP (Adenosin-Tri-Phosphate). The more ATP energy a cell has available the better it can function and the longer itcan live. The question is can we increase the ATP production in the cells by exposing them tocoenzyme-1 (NADH). The answer is yes, we can, as has been shown on isolated heart cells. Whenhearts cells are incubated with NADH a 30% increase in ATP production is observed[9-14]. Due to this the vitality and lifespan of these heart cells are increased. As most cancer cells exhibit an ATP deficiency and NADH can increase ATP energy in cells and also repairs altered DNA and damaged cells this coenzyme was given to cancer patients in an form of an open label trial. 17 prostate cancer patients with pathologically proven carcinoma have been cured in 3 to 5 months with a daily dose of 40 mg of NADH. A number of patients with mammary carcinoma and small cell lung cancer have been cured in between 6 months with the same daily dose of NADH. More than 60 cancer patients have been treated so far with NADH most of them are disease free or show no tumour progression(2). The increase in physical and mental energy was reported from all the cancer patients after taking NADH. The beneficial effects of NADH may be based on various mechanism Most likely it is the elevation of the intracellular ATP level, which allows the cell

to produce sufficient amounts of components regulating the cell cycle. NADH may convert cancer cells to normal by repairing the damaged or altered DNA in the cancer cells. Furthermore NADH as one on the strongest biological antioxidants can scavenge free radicals and thus protect the cells from further damage or alteration. Taking all these facts into account NADH seems to be a new but reasonable and effective strategy for cancer therapy[15-17].

The main objective of our project work we have to identify one of the essential Ras protein in cancer and predicted its 3d structure by homology modeling method with various software like discovery studio and Molsoft and model verification is carried out by using various methods like ramachandran plot analysis Profiles 3d,And also perform docking studies with various docking algorithams like CDOCKER, LIP DOCKetc by using anti-Ras agents or inhibitors and pharmacophore generation.

Materials and Methods:

The following databases have been used for the study

1. NCBI (National Center for biotechnology information):

http://www.ncbi.nlm.nih.gov/

2. PDB:

The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. (See also crystallographic database). The data, typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, can be accessed at no charge on the internet. The PDB is overseen by an organization called the Worldwide Protein Data Bank (www.pdb.org).

Swiss-Prot: Swiss-Prot is a manually curated biological database of protein sequences. Swiss-Prot was created in 1986 by Amos Bairoch during his PhD and developed by the Swiss Institute of Bioinformatics and the European Bioinformatics Institute. Swiss-Prot strives to provide reliable protein sequences associated with a high level of annotation (such as the description of the function of a protein, its domains structure, post-translational modifications, variants, etc.), a minimal level of redundancy and high level of integration with other databases (**http://expasy.org/sprot/)**

Drug Bank:

The Drug Bank database is a unique bioinformatics and cheminformatics resource that combines detailed drug (i.e. chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e. sequence, structure, and pathway) information. The database contains nearly 4800 drug entries including >1,350 FDA-approved small molecule drugs, 123 FDA-approved biotech (protein/peptide) drugs, 71 nutraceuticals and >3,243 experimental drugs. Additionally, more than 2,500 non-redundant protein (i.e. drug target) sequences are linked to these FDA approved drug entries. Each Drug Card entry contains more than 100 data fields with half of the information being devoted to drug/chemical data and the other half devoted to drug target or protein data (**http://www.drugbank.ca/)**

Pubchem:

PubChem is a database of chemical molecules. The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health (NIH). PubChem can be accessed for free through a web user interface. Millions of compound structures and descriptive datasets can be freely downloaded via FTP. PubChem contains substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds. The American Chemical Society tried to get the U.S. Congress to restrict the operation of PubChem, because they claim it competes with their Chemical Abstracts Service[1]. More than 80 database vendors contribute to the growing PubChem database (**http://pubchem.ncbi.nlm.nih.gov/)**

Web Based Tools:

1.BLAST: Blast(Basic Local alignmen search tool)

BLAST searches for high scoring sequence alignments between the query sequence and sequences in the database using a heuristic approach that approximates the Smith-Waterman algorithm.

2.SOPMA

SOPMA is a secondary structure prediction method. SOPMA (Self-Optimized Prediction Method with Alignment) is an improvement of SOPM method. These methods are based on the homologue method of Levin et al. SOPMA correctly predicts 69.5% of amino acids for a three-state description of the secondary structure (alpha-helix, beta-sheet and coil) in a whole database containing 126 chains of non-homologous (less than 25% identity) proteins. Joint prediction with SOPMA and a neural networks method (PHD) correctly predicts 82.2% of residues for 74% of co-predicted amino acids

Web Based Servers:

1.RAPPER (Ramachandran plot analysis) RAPPER is an *ab initio* conformational search algorithm for restraint-based protein modelling. It has been used for all-atom loop modelling, whole protein modelling under limited restraints, comparative modelling, *ab initio* structure prediction, structure validation and experimental structure determination with X-ray and nuclear magnetic resonance spectroscopy.

2.**TMHMM (Prediction of Transmembrane Helices in Proteins)**

TMHMM is a program of prediction of transmembrane helices in proteins. It was rated best in an independent comparison of programs for prediction of TM helices in July 2001 (Bioinformatics, 17:646-653). TMHMM is a membrane protein topology prediction method based on a hidden Markov model.

Software:

1.BIOEDIT

BioEdit is a mouse-driven, easy-to-use sequence alignment editor and sequence analysis program designed and written by a graduate student who knows how frustrating and time consuming it can be to rely upon word-processors and command-line programs for sequence manipulation.

2.MOLSOFT

Molsoft is a La Jolla, California based company that is a primary source of new breakthrough technologies in: molecular graphics and visualization, molecular modeling, docking and virtual screening, computational biology and cheminformatics.

3.Catalyst

Catalyst is an open source web application framework written in Perl, which closely follows the model-view-controller (MVC) architecture, and supports a number of experimental web patterns. It is written using Moose, a modern object system for Perl. It's heavily inspired by such frameworks as Ruby on Rails, Maypole, and spring. Catalyst is primarily distributed through the CPAN, which is the official distribution channel for Perl libraries and applications.

Discovery Studio

Discovery Studio® provides the most advanced software solutions for life science researchers available today. From project conception to lead optimization, Discovery Studio includes a diverse collection of sophisticated software applications to take your research to the next level, all conveniently packaged into a single, easy-to-use Linux- or Windows-based environment. Because Discovery Studio is built upon SciTegic® Pipeline Pilot™, Accelrys' scientific operating platform, any software that you need can be integrated into the research environment, whether it's software from Accelrys, in-house developers, or other vendors.

Methodology:

Retrieval of Target Sequence:

The protein sequence of the protein HUMAN Ras-specific guanine nucleotide-releasing factor system protein of mycoplasma pneumoniae had been retrieved from UniProt, and saved in fasta format that gives the specific information regarding the number of amino acids in the sequence and other sequence related information

Performing Template Search:

The protein sequence of the protein HUMAN Ras-specific guanine nucleotide-releasing factor protein had been retrieved from UniProt and the search for the template had been done using *BlAST* algoritham Template selection by blast searching Algorithm

Homology Modeling Using Molsoft:

The query is loaded by going to windows and clicking on sequence editor. Single letter residues are then displayed. color residues are displayed next.

PDB search is done is done by going to homology and giving the query. First template in the result is loaded. Then the two sequences are and aligned by freeze in align of homology. The model is build by going to homology and selecting homology model.

Homology Modeling

Homology modeling, also known as comparative modeling of protein refers to constructing an atomic-resolution model of the "target" protein from its amino acid sequence and an experimental three-dimensional structure of a related homologous protein. Homology modeling relies on the identification of one or more known protein structures likely to resemble the structure of the query sequence, and on the production of an alignment that maps residues in the query sequence to residues in the template sequence. The sequence alignment and template structure are then used to produce a structural model of the target. Because protein structures are more conserved than DNA sequences, detectable levels of sequence similarity usually imply significant structural similarity.

The quality of the homology model is dependent on the quality of the sequence alignment and template structure. The approach can be complicated by the presence of alignment gaps (commonly called indels) that indicate a structural region present in the target but not in the template, and by structure gaps in the template that arise from poor resolution in the experimental procedure (usually X-ray crystallography) used to solve the structure.

Homology modeling can produce high-quality structural models when the target and template are closely related, which has inspired the formation of a structural genomics.

Results and discussion:

A typical Retrieval of Query sequence from Swissprot databank is given below:

>sp|O14827|RGRF2_HUMAN Ras-specific guanine nucleotide-releasing factor 2 OS=Homo sapiens GN=RASGRF2 PE=1 SV=2

SAMELAEQITLLDHVIFRSIPYEEFLGQGWMKLDKNERTPYIMKTSQHFNDMSNLVASQIMNYADVSS
RANAIEKWVAVADICRCLHNYNGVLEITSALNRSAIYRLKKTWAKVSKQTKALMDKLQKTVSSEGRF
KNLRETLKNCNPPAVPYLGMYLTDLAFIEEGTPNFTEEGLVNFSKMRMISHIIREIRQFQQTSYRIDHQP
KVAQYLLDKDLIIDEDTLYELSLKIEPR

TMHMM SERVER

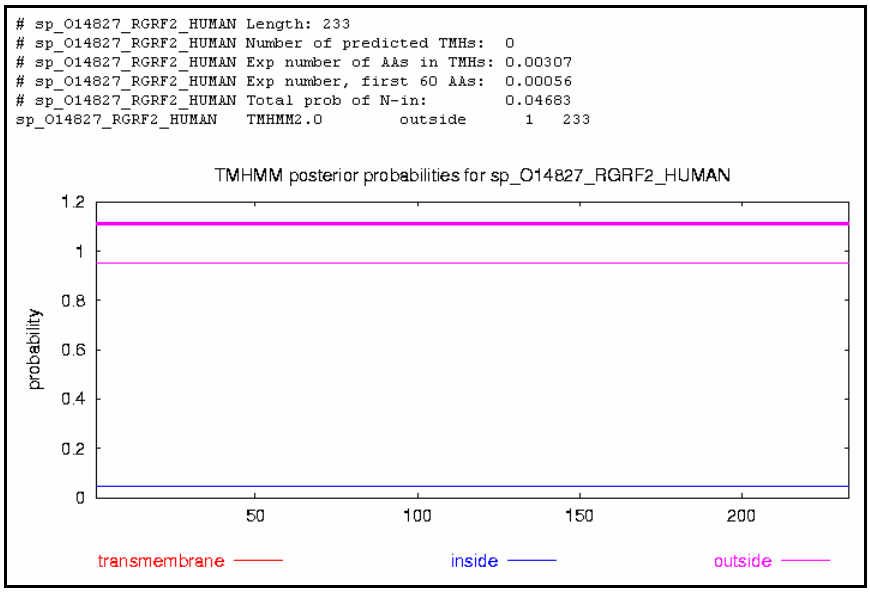
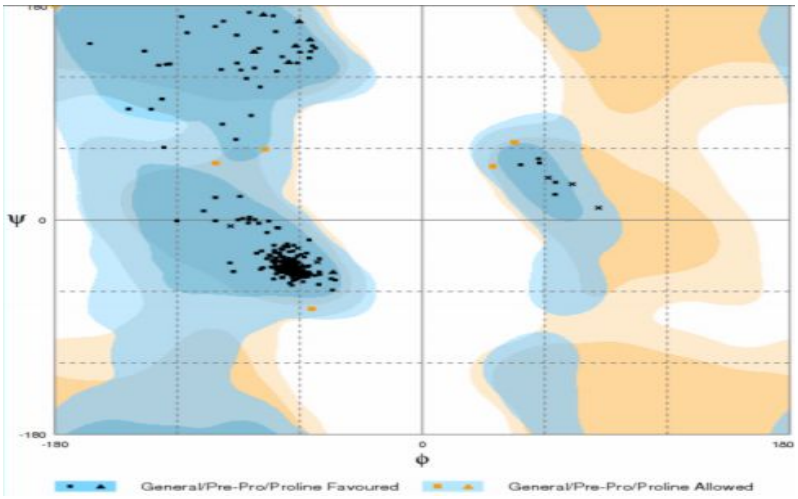
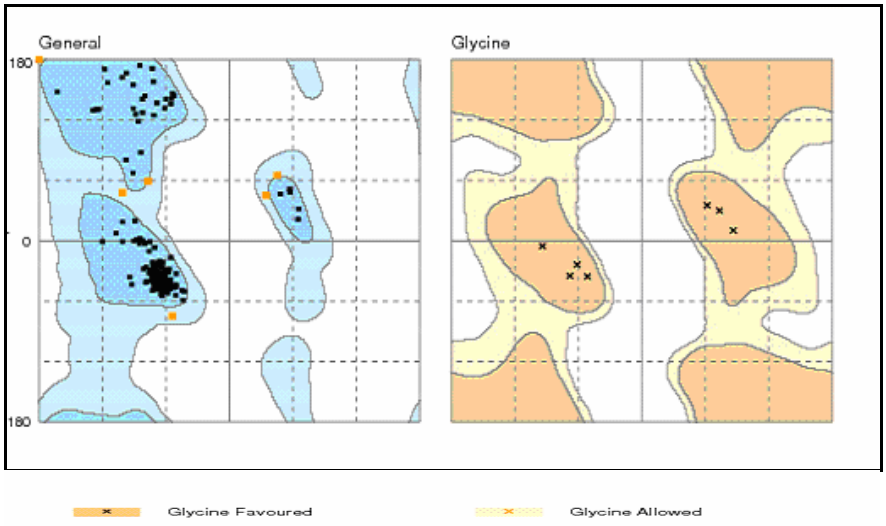


Figure: TMHMM posteriors probabilities of sp_0014827_RGRF2_HUMAN

Model verification was done by RAPPER (Ramchandran plot analysis server):





Typical LIBDOCK RESULT is shown below:

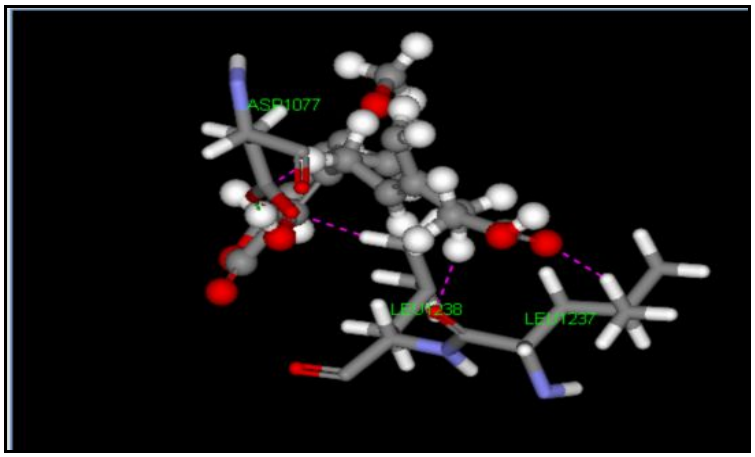


Figure: Libdock results which is a fastest docking method,In which ligand molecule was interacting with LEU 1238,ASP1077,LEU1237..

Summary and Conclusions

In the present study, Ras Guanine nucleotide releasing factor 2 proteins which is well known for its down-stream effectors regulate specific intracellular signaling pathways involved in numerous biological process has been under taken. Because of its unique function and lack of 3d structure it got significance attention by researchers to know the 3d structure. To reveal the 3D structure, we performed homology modeling by taking template as 2IJE and modeled it s structure using MOLSOFT software. Modeled structure was shown very less RMSD difference with its template suggests that modeled structure more similar to the template. Further We have performed docking studies in Accelrys discovery studio with algorithams like Cdocker and Libdock by taking anticancer drugs and Coenzymes such as Hydrocortisone, Mycophenolic acid, Thioguanine and Coenzymes NADH, biotin,. Among all of them Hydrocortisone shows least energy -65.5. Which is showing Hbond interactions with LYS1240,LEU 1291,HIS 1074 After that we have generated Interaction map and pharmacophore studies the drug molecule hydrocortisone was shows best fit score.

References:

1. Böhm, H.-J. 1992. The computer program LUDI: A new method for the de novo design of enzyme inhibitors. *J. Comp-Aided Molec. Design* 6:61- 78
2. Böhm, H. J. 1992. LUDI: rule based automatic design of new substituents for enzyme inhibitor leads. *J. Comp-Aided Molec. Design* 6:593-606
3. Böhm, H. J. 1994. The development of a simple empirical scoring function to estimate the binding constant for a protein-ligand complex of known three-dimensional structure. *J. Comp-Aided Molec. Design* 8:243-56
4. Böhm, H. J. 1994. On the use of LUDI to search the Fine Chemicals Directory for ligands of proteins of known three-dimensional structure. *J. Comp-Aided Molec. Design* 8:623-32
5. Böhm, H. J. 1998. Prediction of binding constants of protein ligands: a fast method for the prioritization of hits obtained from de novo design or 3D database search programs. *J. Comp-Aided Molec. Design* 12:1-15.
6. Nagar B, Bornmann WG, Pellicena P, Schindler T, Veach DR, et al. 2002. Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res* 62: 4236
7. Pendergast AM, Gishizky ML, Havlik MH, Witte ON. 1993. SH1 domain autophosphorylation of P210 BCR/ABL is required for transformation but not growth factor independence. *Mol Cell Biol* 13: 1728
8. Böhm HJ. 1992. LUDI: rule based automatic design of new substituents for enzyme inhibitor leads. *J Comp-Aided Molec Design* 6: 593
9. Böhm HJ. 1994. The development of a simple empirical scoring function to estimate binding constant for a protein-ligand complex of known three-dimensional structure. *JComp-Aided Molec Design* 8: 243

10. Böhm HJ. 1994. On the use of LUDI to search the Fine Chemicals Directory for ligands of proteins of known three-dimensional structure. *J Comp-Aided Molec Design* 8: 623
11. Böhm HJ. 1998. Prediction of binding constants of protein ligands: a fast method for the prioritization of hits obtained from de novo design or 3D database search programs. *J Comp-Aided Molec Design* 12: 309
12. Böhm H-J. 1992. The computer program LUDI: A new method for the de novo design of enzyme inhibitors. *J Comp-Aided Molec Design* 6: 61
13. Dorsey JF, Jove R, Kraker AJ, Wu J. 2000. The pyrido[2,3-d]pyrimidine derivative PD180970 inhibits p210Bcr-Abl tyrosine kinase and induces apoptosis of K562 leukemic cells. *Cancer Res* 60: 3127
14. Nagar B, Bornmann WG, Pellicena P, Schindler T, Veach DR, et al. 2002. Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res* 62: 4236
15. Pendergast AM, Gishizky ML, Havlik MH, Witte ON. 1993. SH1 domain autophosphorylation of P210 BCR/ABL is required for transformation but not growth factor independence. *Mol Cell Biol* 13: 1728
16. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, et al. 2000. The Protein Data Bank. *Nucleic Acids Res* 28: 235
17. Wright AJ. 1999. The penicillins. *Mayo Clin Proc* 74: 290
18. Connolly ML. 1983. Analytical molecular surface calculation. *J Appl Cryst* 16: 548Connolly ML. 1983. Solvent-accessible surfaces of proteins and nucleic acids. *Science*221: 709
19. Ringhofer S, Kallen J, Dutzler R, Billich A, Visser AJ, et al. 1999. X-ray structure and conformational dynamics of the HIV-1 protease in complex with the inhibitor SDZ283-910: agreement of time-resolved spectroscopy and molecular dynamics simulations. *Mol Biol* 286: 1147

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