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COMPARATIVE DOCKING ANALYSIS OF MARINE RED ALGAE AGAINST HEPATOCELLULAR CARCINOMA (HCC) 1IJX PROTEIN

Senthilraja P. *[1], Manikandaprabhu S.[1], Manju J.[1], Loganathan K.[2], Arulprakash A.[2], Prakash M.[2]

[1]Department of Zoology, Division of Bioinformatics, Annamalai University, Chidambaram, India.

[2] Department of Zoology, Annamalai University, Chidambaram, India.

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***Correspondence for Author:**
Dr. P. Senthilraja,
Assistant professor
Department of Zoology,
Division of Bioinformatics,
Annamalai University,
Chidambaram, Tamilnadu
lionbioinfo@gmail.com

ABSTRACT

Liver cancer is one of the most deadly deaths causing cancer leading third in the world. Hepatocellular carcinoma (HCC) starts from the main cell in the liver which is called hepatocytes. It is the most common types of primary liver cancer. Aberrant activation of several pathways such as EGFR, Ras/ERK, PI3K/MTOR, HGF/MET, Wnt, Hedgehog and apoptotic signaling induce the HCC. Wnt/β-catenin pathway is implicated in colon cancer and HCC. Unfortunately it constitutes the most undruggable pathway. Activation of Wnt pathway induces translocation of β-catenin into the nucleus, where it regulates specific oncogenes, including c-myc, cyclin D, and surviving. Mutations in β-catenin occur in and around 17% of cases. Natural compounds usually having biological activities are used for drug discovery and drug design. Many natural products have been clinically available as potent hepato-protective agents against commonly occurring liver diseases. With this reference we perform a docking analysis against HCC with marine red algae and we get the good docking energy value as -13.4575 for Pinnasterol compound.

Key Words: Hepatocellular carcinoma (HCC), Hepatocytes, Wnt/ β-catenin, Frizzled receptors, Marine red algae.

INTRODUCTION

Liver is made up of different cell, bile ducts, blood vessels and fat storing cells. Liver cancer originates from liver. Liver is responsible for the synthesis of serum proteins, intermediary

metabolism of amino acids, lipids, carbohydrates and detoxification of xenobiotic compounds. These functions are performed primarily by hepatocytes. Cancer starts in the liver called as Hepatocellular carcinoma or Hepatoma. Hepatocellular carcinoma (HCC) is the third leading cancer related deaths worldwide. It's the fifth most common cancer in world wide. It is often diagnosed at an advanced stage and hence typically has a poor prognosis [1, 2, 3].

Recent reports show that HCC is becoming more wide-spread and has dramatically increased in North America Western Europe and Japan [4, 5].Early liver cancer often doesn't showany symptoms. When the cancer grows larger, people may notice one or more of these common symptoms pain in the upper abdomen on the right side, a lump or a feeling of heaviness in the upper abdomen, swollen abdomen (bloating), Loss of appetite and feelings of fullness, weight loss, weakness or feeling very tired, nausea, vomiting, yellow skin, yellow eyes, pale stools, dark urine from jaundice and Fever [6].

HCC is generally a fatal disease; few patients are amenable to surgery because of late HCC diagnosis, and alternative treatments do not substantially improve the patients' prognosis when HCC is unresectable [7].The overall survival of patients with HCC has not significantly improved in the past two decades. Current treatments are only applicable at early stages of tumor development and include tumor resection, liver transplantation, chemoembolization and sorafenib administration [8]. Sorafenib was approved for HCC in 2007 [9].

The most important mechanism of liver cancer progression is cell Proliferation. Wnt/β-catenin activation observed in normal liver development, regeneration, and liver cancer [10].The Wnt/β-catenin pathway is implicated in colon cancer and HCC. Unfortunately it constitutes the most undruggable pathway. The signaling cascade is initiated extracellular, when Wnt ligands stimulate the Frizzled receptors. It is a family of G protein coupled receptor which signals β-catenin to uncouple from E-cadherin. The activation of Wnt pathway induces translocation of β-catenin into the nucleus, where it regulates specific oncogenes, including c-myc, cyclin D, and survivin. Mutations in β-catenin occur in and around 17% of cases [11, 12].Signaling by the Wnt family of secreted glycolipoproteins via the transcriptional co-activator β-catenin controls embryonic development and adult homeostasis. Wnt/β-catenin signaling mutation induces in human diseases including congenital malformations, cancer, and osteoporosis [13].

Wnt family of secreted ligands acts through many receptors to stimulate distinct intracellular signaling pathways in embryonic development in adults and in disease processes. Binding of Wnt to the Frizzled family of receptors and to low density lipoprotein receptor-related protein 5 (LRP5) or lipoprotein receptor-related protein 6 (LRP6) co-receptors stimulates the intracellular Wnt/β-catenin signaling pathway, regulates β-cateninstability and context-dependent transcription. This signaling pathway controls many processes, such as cell fate determination, cell proliferation and self-renewal of stem and progenitor cells [14]. Wnt/β-catenin and Hedgehog signaling pathways, and theVascular Endothelial Growth Factor Receptor (VEGFR) and Platelet-Derived Growth Factor Receptor (PDGFR) signaling cascades show altered activity in HCC, and agents targeting these pathways are under development [15].

Members of the Frizzled family of seven-pass transmembrane proteins serve as receptors for Wnt signaling proteins. Wnt proteins have important roles in the differentiation and patterning of diverse tissues during animal development, and inappropriate activation of Wnt signaling pathways is a key feature of many cancers. An extracellular cysteine-rich domain (CRD) at the amino terminus of Frizzled proteins binds Wnt proteins, as do homologous domains in soluble proteins-termed secreted Frizzled-related proteins-that function as antagonists of Wnt signaling. Recently, an LDL-receptor-related protein has been shown to function as a co-receptor for Wnt proteins and to bind to a Frizzled CRD in a Wnt dependent manner [16].

MATERIALS AND METHODS

Protein Structure

The targeted protein (ID: 1IJX), having the resolution of 1.80 Å was retrieved from theprotein data bank (PDB) (www.rcsb.org/pdb).

Chemicals screened

The Phytochemicals are identified from marine red algae, and the ligand molecules namely Pinnasterol, Costatol, Ecdysone, Gigartinine and Ornithine structures retrieved from pubchem database (http://pubchem.ncbi.nlm.nih.gov), were screened against the targeted protein of hepatocellular carcinoma.Structural and active site studies of the protein were done by using CASTP (Computed Atlas of Surface Topography of Proteins) and Pymol molecular visualization software. The selected chemical structureswere generated from SMILES

notation (Simplified Molecular Input Line Entry Specification) by using the Chemsketch Software (**www.acdlabs.com**).

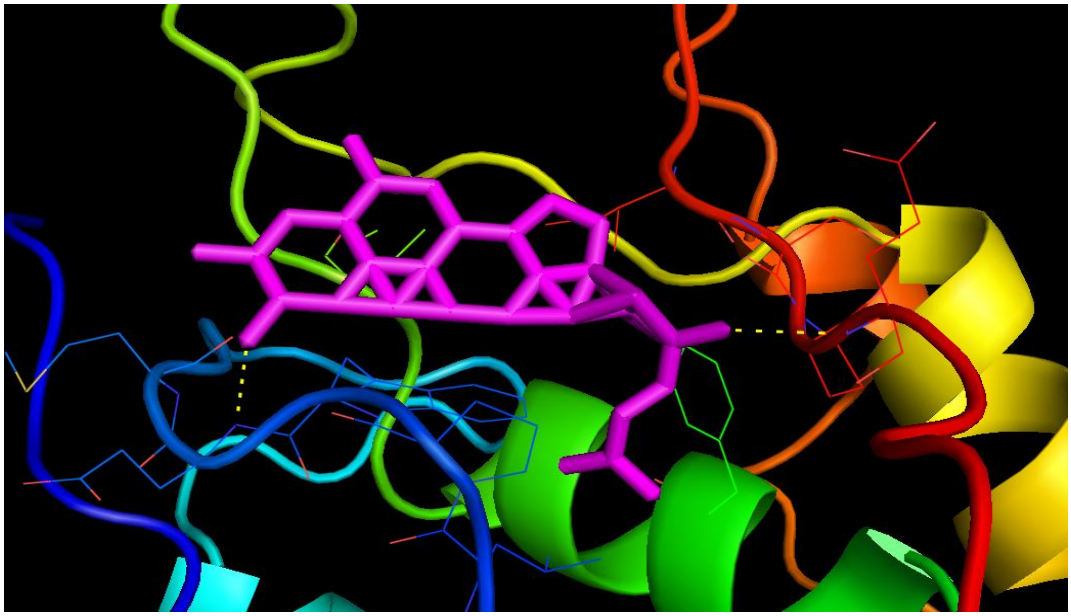
Table: 1 Amino acid binding site

Ligand molecule	Amino acid present in binding pocket	Chain
Pinnasterol	Leu14,Pro15,Trp16,Phe54,Ile63,Ile116,Ser117,Pro118	A
Ecdysone	Leu14,Pro15,Trp16, Leu53,Phe54, Ile63,Ile116, Ser117,Pro118	A
Costatol	Phe40,Leu43,Leu52,Phe55,Leu56,Leu91,Trp98,Pro99, Leu102	A
Gigartinine	Lys74,leu107,Pro108,Val109,Gly113,Val114,Cys115,	A
Ornithine	Lys74, Cys76, Pro75, Leu107, Pro08, Val109.	A

Docking methods

The molecular docking was performed using Argus Lab, widely distributed public domain molecular docking software.The inhibitor and target protein were geometrically optimizedand docked using docking engine Argus dock.

The binding interaction of pinnasterol ligand molecule with the target substrate 1IJX

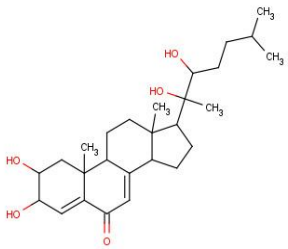
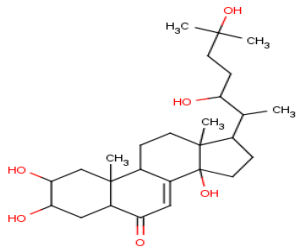
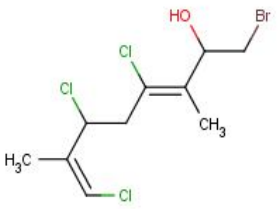


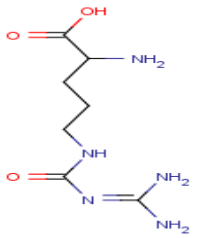
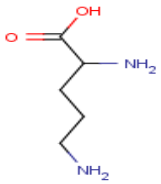
Magentas color indicate to Pinnasterol ligand molecule
Yellow dots line indicates hydrogen bond interaction between the ligand and target protein.

RESULTS

Drug discovery from natural resource has played an important role in the treatment of cancer and indeed most new clinical applications and their derivatives over the last half century have been appliedtowards combating cancer. Docking is a computation technique that samples confirmatory of small molecules in protein binding sites. The chemicals derived from marine red algae weredocked with protein responsible for hepatocellular carcinoma (HCC).The docked ligand molecules were selected based on dockingenergy and good interaction with the active site residues and the results are shown in Table 2. Three ligand molecules showed the activation energy of greater than 10 kcal/mol andthe remaining two molecules exhibited the values less than 10kcal/mol. The highest activation energy-13.4575Kcal/mol was observed in pinnasterol and the lowest activation energy -6.12065Kcal/mol was found with ornithine. From the *in-silico* docking results, it is quite evident that marine red algae compounds have the great potential against hepatocellular carcinoma.

Table 2: Docking results of marine red algae compounds against target protein

Compound Name	Pubchem ID	Compound structure	Molecular Weight (g/mol)	Hydrogen donor/ acceptor	Docking Energy Level (Kcal/mol)
Pinnasterol	CID: 52931315		446.6193	4,5	-13.4575
Ecdysone	CID: 19212		464.6346	5,6	-13.3253
Costatol	CID: 21630814		336.4805	1,1	-13.2725

Gigartinine	CID: 192847		217.2257	5,4	-7.01703
Ornithine	CID: 6262		132.1609	3,4	-6.12065

DISCUSSION

Marine compounds are rich in medicinal value. These compounds are eco-friendly and safer application. Natural products and their derivatives have been invaluable as a source of therapeutic agents it possess high chemical diversity, biochemical specificity and molecular diversity within the boundaries of reasonable drug-like properties, from these we make the marine derived compounds as attractive targets as lead structures for drug discovery.Our previous studies proved the efficacy of mangrove derived compounds against oncoprotein of cervical cancer, NS5 methyltransferase protein which is responsible for flavivirus, Myo-Inositol Oxygenase (MIOX) responsible for diabetes mellitus, breast cancer protein BRCA1 [17,18,19,20]. The results show the bestdocking score value and also the compounds weresatisfied Lipinski's rule of five [21]. The chemicals interaction between selected ligands and the target protein has been found to be good and has the best binding energy and interaction scores. We suggest that thePinnasterol, Costatol, Ecdysone have been potential to bedeveloped into a new class of liver cancer. Here we concluded the compounds derived from mangrove ecosystem Pinnasterol, Costatol, Ecdysone, Gigartinine, Ornithine could be a novel for inhibitor for hepatocellular carcinoma.

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REFERENCE

1. Dilek C, Chishti MA, Al-Bandary, Al-Bakheet, Al-Qahtani A, ShoukriMM, Goyns MH, Ozand PT, Quackenbush J, Park BH, Kaya N. Integrative and comparative genomics

analysis of early hepatocellular carcinoma differentiated from liver regeneration in young and old. Molecular Cancer, 2010; 9(146): 1-19.

2. Josep M, Llovet MD, JordiBruix MD. MolecularTargeted Therapies in Hepatocellular Carcinoma. Hepatology, 2008; 48(4): 1312–1327.
3. BoschXF, Ribes J, Diaz M, Cleries R. Primary liver cancer: Worldwide incidence and trends. J. Gastroenterology, 2004; 127(5) Supplements 1: S5-S16.
4. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality and survival trends in the United States from 1975 to 2005. J Clin Oncol, 2009; 27:1485-1491.
5. Nguyen MH, Whittemore AS, Garcia RT, Tawfeek SA, NingJ, Lam S, Wright TL, Keeffe EB. Role of ethnicity in risk for hepatocellular carcinoma in patients with chronic hepatitis C and cirrhosis.Clin Gastroenterol Hepatol, 2004; 2:820-824.
6. Duncan AW, Dorrell C, Grompe M. Stem Cells and Liver Regeneration. J.Gastroenterology, 2009; 137:466–481.
7. Calvisi DF, Wang C, Ho C, Ladu S, Lee SA, Mattu S, Destefanis G, Delogu S, Zimmermann A, Ericsson J, Brozzetti S, Staniscia T, Chen X, Dombrowski F, Evert M.Increased Lipogenesis, Induced by AKT-mTORC1-RPS6 Signaling, Promotes Development of Human Hepatocellular Carcinoma. J. Gastroenterology, 2011; 140:1071–1083.
8. Vara D, Salazar M, Olea-Herrero N, Guzman M, Velasco , Diaz-Laviada G. Anti-tumoral action of cannabinoids on hepatocellular carcinoma: role of AMPK-dependent activation of autophagy. Cell Death and Differentiation, 2001; 18:1099–1111.
9. Wilhelm SM, Adnane L, Newell P. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol Cancer Ther, 2008; 7: 3129–31140.
10. Tan X, Apte U, Micsenyi A, Kotsagrelos E, Luo J,Ranganathan S, Monga DK, Bell A, Michalopoulos GK, Satdarshan Monga PS, Epidermal Growth Factor Receptor: A Novel Target of the Wnt/β-Catenin Pathway in Liver. Journal Gastroenterology, 2005; 129: 285–302.
11. Moon RT, Kohn AD, Ferrari GV, Moon KA. WNT and betacateninsignalling: diseases and therapies. Nat Rev Genet, 2005; 5(9): 691–701.
12. Laurent-Puig P, Legoix P, Bluteau O. Genetic alterations associated with hepatocellular carcinomas defines distinct pathways of hepato carcinogenesis. J.Gastroenterology, 2001; 120(7): 1763–73.

13. MacDonald BT, Tamai K, He X.Wnt/beta-catenin signaling: components, mechanisms, and diseases.J. Dev Cell, 2009; 17(1): 9-26.
14. Angers S, Moon RT. Proximal events in Wnt signal transduction. Nat Rev Mol Cell Biol, 2009; 10(7):468-77.
15. Masatoshi Kudo. Signaling pathway/molecular targets and new targeted agents under development in hepatocellular carcinoma. World J Gastroenterology, 2012; 18(42): 6005-6017.
16. Dann CE, Hsieh JC, Rattner A, Sharma D, Nathans J, Leahy D.Crystal Structure of the Cysteine-Rich Domain of Secreted Frizzled-Related Protein 3 (SFRP-3; FZB). Journal Nature, 2001; 412: 86-90.
17. Senthilraja P, Kathiresan K, Sunil KS. *In-silico* docking analysis of mangrove-derived compounds against breastcancer protein (BRCA1). IRMJ-Health Sci, 2011; 1(1): 09-12.
18. Senthilraja P, Prakash M, Manikandaprabhu S. Potential of marine derived compounds against NS5Methyltransferase protein an *In-Silico* docking study. International Journal of Current Tropical Medicine and Health Research, 2013; 1(1): 006-008.
19. Senthiraja P, Kathiresan K. Computational selection of compounds derived from mangrove ecosystem for anticervical cancer activity. J. Recent Sci. Res, 2011; 2(4): 93-98.
20. Senthilraja P, Nyabuganda jean paul Aime, Manikandaprabhu S, Prakash M. Computational Screening and Docking Analysis of Natural Compounds Derived From Mangrove Plant against Type-2 Diabetes, Myo-Inositol Oxygenase Enzyme (Miox).Int. J. Pharm. Sci. Rev. Res, 2013; 20(2): 158-161.
21. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Del. Rev, 2001; 46: 3–26.