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Research Article

IN SILICO DOCKING STUDIES ON THE ANTI-CANCER EFFECT OF THYMOQUINONE ON INTERACTION WITH PHOSPHATASE AND TENSIN HOMOLOG LOCATED ON CHROMOSOME 10q23: A REGULATOR OF PI3K/AKT PATHWAY

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ABSTRACT

Objective: The statistics on cancer imposes the urge to extend new methods to control this deadly form of the disease. Phosphatase and tensin homolog located on chromosome 10q23 (PTEN) is inactivated in a subset and AKT is frequently activated in cancer. The PTEN is the central negative regulator of the phosphatidylinositol 3-kinase (PI3K)/AKT signaling cascade that influences multiple cellular functions including cell growth, survival, proliferation and migration in a context-dependent manner. Dysregulation of this signaling pathway contributes to different types of cancers. The objective of the study is to explore the anti-cancer potential of thymoquinone (TQ) by analyzing the interaction between TQ with the target protein PTEN.

Methods: The three dimensional structure of TQ is designed using *in-silico* methods, and the structure of PTEN is obtained from National Center for Biotechnology Information against protein data bank. The query sequence from 8 to 353 amino acids was found to be 85% homologous to ID5R. For the target protein PTEN with 403 residues, protein families analysis covered the important domains in PTEN.

Result: TQ showed the binding energy of −7.37 Kcal/mol against PTEN with three hydrogen bonds.

Conclusion: Present study suggests that TQ might inhibit abnormal cell proliferation occurring in cancer by modulating the activity of PTEN, a negative regulator of PI3K/AKT pathway.

Keywords: Cancer, Thymoquinone, Phosphatase and tensin homolog located on chromosome 10q23, Docking and hydrogen bonds.

INTRODUCTION

Cancer is the leading cause of death in the world. It is progressively a deadly disease which is characterized by a persistent, abnormal and relatively autonomous proliferation of cells, as a result of permanent cellular defect that is passed on to the progeny [1]. The statistics on cancer imposes the urge to extend new methods to control this deadly form of cancer. Cancer chemoprevention involves the use of either natural or synthetic components to delay inhibit or reverse the development of cancer in normal or pre-neoplastic conditions. There have been considerable efforts to explore for naturally occurring substances that can curtail the multistage carcinogenesis. Wide arrays of substances present in the medicinal herbs or dietary plants have been screened for their ability to avert carcinogenicity.

Natural products have been established to exert anti-cancer activities partially based on their ability to quench reactive oxygen species and protect critical cellular components like DNA, proteins and lipids from oxidative damage [2]. They may also hamper with intracellular signaling pathways, such as those, which regulate proliferation, induction of apoptosis, and response to oxidative stress [3].

Recent studies have strongly indicated that certain daily-consumed dietary phytochemicals could have cancer protective effects against mice cancer models and cancers mediated by carcinogens, irradiations and carcinogenic metabolites derived from exogenous or endogenous sources. The cancer-protective effects elicited by these dietary compounds are believed to be due at least in part to the induction of cellular defense systems including the detoxifying and antioxidant enzymes system, as well as the inhibition of anti-inflammatory and anti-cell growth signaling pathways culminating in cell cycle arrest and/or cell death [4].

The phosphatase and tensin homolog located on chromosome 10q23 (PTEN)/phosphatidylinositol 3-kinase (PI3K)/AKT pathway is highly involved in tumorigenesis. PTEN is one of the most frequently inactivated tumor suppressors in different tumor types. Several mechanisms such as genetic mutation, promoter methylation, and post-transcriptional modification may contribute to PTEN inactivation.

The PTEN tumor suppressor gene is frequently deleted or mutated in a wide variety of human cancers, including glioblastoma, prostate cancer, breast cancer, lung cancer and endometrial cancer [5-8]. In addition, PTEN germline mutations are responsible for the development of Cowden disease, Bannayan–Zonana syndrome and Lhermitte–Duclos disease, in which disorganized hamartomas appear in various organs [9].

The PTEN/PI3K/AKT pathway is highly involved in tumorigenesis. PTEN is one of the most frequently inactivated tumor suppressor growths in different tumor types [10]. Several mechanisms such as genetic mutation, promoter methylation, and post-transcriptional modification may contribute to PTEN inactivation. In normal lung tissues, high levels of PTEN expression were detected in 100% [11]. While, in lung cancer cell lines, PTEN expression was lost in 44% and reduced in 29% and studies indicates that PTEN expression is lost in 50% of non-small cell lung cancer cells [12].

It has been reported that PTEN can induce apoptosis [13] and control cell invasion, migration [14] and angiogenesis [15] through interference with several signaling pathways. PTEN functions as a dual specificity phosphatase. Its key target is phosphatidylinositol 3,4,5-trisphosphate, the direct product of the PI3K.

The protein and ligand interaction takes an important part in protein function. Both ligand and its binding site are essential components for

understanding how the protein - ligand complex functions. Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the predominant binding model(s) of a ligand with a protein of known three-dimensional structure. Nowadays, molecular docking approaches are routinely used in modern drug design to help understand drug–receptor interaction. It has been shown in the literature that these computational techniques can strongly support and help the design of novel, more potent inhibitors by revealing the mechanism of drug-receptor interaction [16].

In docking, various algorithms are used to position a chemical from a virtual library into a specified target site or sites on the protein of interest. The objective of molecular docking is to determine the binding interactions between two molecules - either protein to protein or protein to ligand. Once a compound is docked, it is then scored using mathematical models. Scoring estimates the chemical interactions such as binding strength and energy state, between the ligand and protein to assist in ranking the efficacy of the compound being scored [17]. From these approaches, candidate phytochemicals that directly interact with target proteins can be identified.

The cancer preventive effects and interactions of selected phytochemicals must then be confirmed in validation steps using laboratory experiments, such as cell transformation assays, pull-down assays, reporter gene assays and animal studies. To further verify the specific interaction and binding site of a selected phytochemical and its target protein, X-ray crystallography, nuclear magnetic resonance and protein point mutation methods are used. Finally, modulation of candidate pathway and target proteins needs to be validated in patients, with tissue analysis before and after exposure to the agent, ideally in preneoplastic lesions. Promising candidate chemopreventive phytochemicals can then be fully evaluated in clinical trials to determine their suitability for use as cancer preventive agents. Hence, a study was planned to evaluate the interaction of the thymoquinone (TQ) (ligand) with PTEN (target protein), a negative regulator of AKT pathway.

TQ is a bioactive compound obtained from volatile oil of *Nigella sativa*. The volatile oil of *N. sativa* was shown to contain about 24% TQ. TQ is a pharmacologically active quinone, Studies were documented that TQ possess several medicinal properties including the analgesic, anti-inflammatory, protective effect on lipid peroxidation and oxidative damage, anticonvulsant and also antioxidant effect [18].

TQ may prove to be effective in treating prostate cancer, particularly in hormone refractory cases. TQ inhibits progression of prostate cancer cells from G1 to S phase [19]. TQ has been shown to induce apoptosis by p53-independent and p53 dependent pathways [20]. Growth inhibition of TQ is associated with inhibition of DNA synthesis and induction of cell cycle arrest. Overall, the promising effects of TQ against cancer metastasis and angiogenesis have been noted, and in-depth studies are needed to explain the molecular mechanism(s) involved in these effects.

However, no scientific reports were available on the literature for its interaction with PTEN, a negative regulator of PI3K/AKT signaling pathway. Hence, a study was planned to evaluate the interaction of TQ (ligand) with PTEN (target protein), a negative regulator of AKT pathway to explore its anti-cancer potential.

METHODS

Software and hardware

The bioinformatics tools used in this study were: Basic Local Alignment Search Tool (BLAST), FAST alignment (FASTA), protein families (P-fam), Q-site finder, Modeller9v8, PyMOL, 1.3 Molecular Graphics System, Auto DOCK software version 4, Weblab Viewer, ACD/ChemSketch (freeware version 10.00) installed on a desktop equipped with Pentium(R) Dual-E6600 at 3.05 GHz 3.06 GHz processor (2 GB RAM Core CPU) running the Ubuntu 10.04 (LINUX) and Windows XP SP3 operating system.

Ligand preparation

Chemical structure of ligand (TQ) was taken from Pubchem compound database, (http://www.ncbi.nlm.nih.gov/search). The three dimensional (3D) structure for TQ was generated using ACD/ChemSketch.

Target selection

Initially, it was ascertained that the 3D structure of mouse PTEN was not available in protein data bank (PDB) database [21]. An attempt had been made in the present study to determine the 3D structure. Mouse PTEN with accession number 008586 was chosen as the target protein from National Center for Biotechnology Information NCBI protein database after studying its metabolic pathway, significance in phosphorylating AKT and its relation to cancer [22].

Homology modeling

The FASTA format of the query sequence was used for homology modeling against PDB using NCBI BLAST [23,24]. Mouse 1D5R chain was taken as a template.

Domain analysis

Domain analysis was done for both the target and template proteins using P-fam database [25].

Modeling the protein

The 3D structure of the target protein was predicted using m align and model-default operations of the modeling tool Modeller9v1 [26].

Validation of the protein

The modeled protein was validated using combinatorial extension [27]. The alignment was calculated, downloaded as a PDB file, saved and then opened in Weblab viewer to obtain ball and stick model of the protein [28].

Energy minimization

Energy minimization was done to the protein using Swiss PDB viewer with operations “compute energy” initially and “energy minimization” finally [29].

Ramachandran plot

Ramachandran plots of the modeled protein before and after energy minimization were obtained using structural analysis and verification server of NIH MBI Laboratory [30]. Root mean square deviation and energy score values were observed.

Active site prediction

The active site of a model protein was found out using Q-Site finder [31].

Docking analysis

The ligand (TQ) was docked to the active site of the target (PTEN) using Auto Dock program, version 4.0. Polar hydrogen bonds (H-bonds) were added to the receptor, Kollaman charges were assigned and salvation parameters were added with the Addsol option in Autodock. The internal degree of freedom and torsions were defined using the “Ligand torsions” menu option of autodock. The grid maps representing the protein were calculated using “autogrid” option. The best ligand-receptor structure from the docked structures was chosen based on the lowest energy and number of H-bonds formed between the target and ligand. The results were visualized using visualization tool Weblab viewer.

RESULT

The query mouse PTEN protein was found to contain 403 amino acids. Upon homology modeling using NCBI BLAST against PDB, the query sequence from 8 to 353 amino acids was found to be 85% homologous to ID5R. For the target protein PTEN with 403 residues, P-fam - analysis covered the important domains in PTEN.

Among the 10 binding sites obtained from Q-Site Finder, site one was highly conserved and the most favorable site for docking. The residues in the active site were found to be GLU91, ASP92, HIS96, ASN94,

CYS124, LYS125, ALA126, GLY127, LYS128, GLY129, ARG130, GLY165, THR167, ILE168 and GLN171. Fig. 1 shows the structure of TQ.

Table 1 represents the physiochemical and pharmacophore properties of TQ it is very much clear that TQ obeys perfectly with the Lipinski's rule.

H-bonds are formed between the atoms in the active site residues of the target protein (PTEN) and the atoms in the ligand (TQ). Protein residues mentioned are the amino acids in its active site with their positions. Number of interactions between the protein and ligand denotes the number of H-bonds. More the number of H-bonds between the ligand-target better is the interaction. Table 2 depicts the interaction between PTEN and TQ.

The conformation with a minimum docking value was found to be stable. H-bonds and the residues involved in bonds between the target protein and the ligand, in the docked models, were illustrated by Weblab viewer. The final docked conformation obtained for the ligand was evaluated based on the docking score, and the number of H-bonds formed as given in Fig. 2.

DISCUSSION

In-silico molecular docking is one of the most powerful techniques to discover novel ligands for receptors of known structure and thus play a key role in structure-based drug design [32]. Molecular docking continues to holds great promise in the field of computer-based drug design which screens small molecules by orienting and scoring them in the binding site of protein. As a result, novel ligands for receptors of known structure are designed, and their interaction energies are calculated using the scoring functions [33].

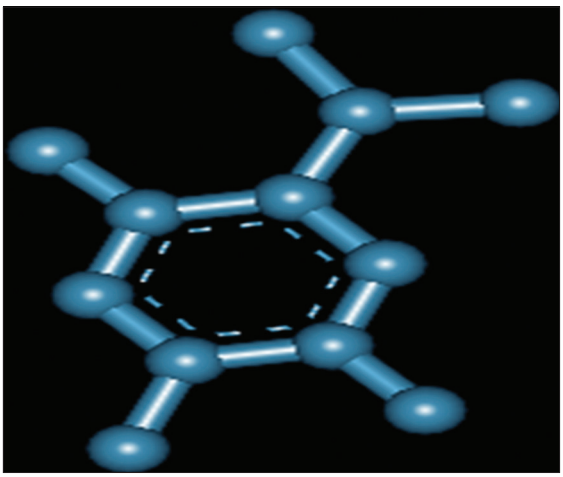

Fig. 1: Structure of thymoquinone

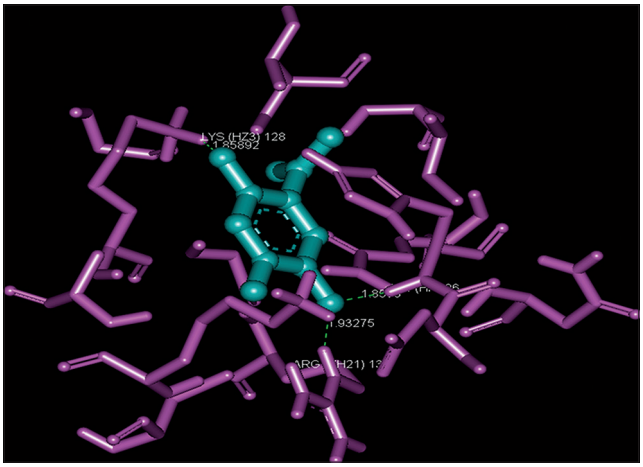

Fig. 2: Docked orientation of thymoquinone (ligand) with phosphatase and tensin homolog located on chromosome 10q23 (PTEN), ligand is denoted by ball and stick model bound to the active site of PTEN. H-bond formed are denoted by green dotted lines

Studies have shown that compounds should possess certain properties to be accepted as a drug [34]. Those properties were formulated by Lipinski *et al*. in 1997. It is a rule of thumb to evaluate drug likeness, or to determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely active drug [35]. It suggested that any pair wise combination of the following conditions: Molecular weight >500, LogP >5, H-bonds donors >5, and H-bonds acceptors >10, may result in compounds with poor permeability. As shown in Table 1, it is very much clear that TQ obeys perfectly with the Lipinski's rule of five and is considered to have a good absorption and permeability.

Predicting a drug candidate's pharmacokinetic and dynamic profile early in the drug development process is the key aspect of absorption, distribution, metabolism and excretion (ADME) testing. Profiling of ADME characteristics earlier in the drug discovery process especially in parallel with screening for activity has become a central focus for many drug discovery groups. ADME properties are recognized as key determinants of whether a molecule can be successfully developed as a drug or not. In this context, it is more obvious that TQ shows a good absorption, HIA, aqueous solubility level blood-brain barrier penetration without the hepato toxicity. These results confirm that TQ does not require further modifications to develop as drug for cancer.

Docking score is a measure of interaction of the ligand to the active site of the target [36]. More negative values indicate more effective stable conformation of the bound ligand-target. PTEN is a tumor suppressor gene. PTEN protein plays an important role in cell cycle progression, cell migration, DNA damage response, chromosome stability, protein-protein interaction, phosphatase activity both protein phosphatase and lipid phosphatase activity. The lipid phosphatase activity is essential for tumor suppressor activity. In the present study, the interaction of TQ with PTEN explores the anti-cancer potential of TQ by controlling various pathways that promotes cancer promotion and progression.

The energy value and the H-bonds obtained in the present docking study confirm that there is a stronger binding affinity between TQ and PTEN. This in-turn proves that the PTEN must have been promoted by TQ that further down regulates AKT and bring concurrent increase in

Table 1: Physiochemical and pharmacophore properties of TQ

Compound ID	10281
MW	164.20108 (g/mol)
Molecular formula	$C_{10}H_{12}O_2$
XLogP3	2
HDO	0
HAC	2

MW: Molecular weight, HDO: Hydrogen bond donor, HAC: Hydrogen bond acceptors, TQ: Thymoquinone

Table 2: Interaction between PTEN and TQ

PTEN		TQ	Distance Å
Residue	**Atom**	**Atom**	
HIS 96	HN	O	1.856
LYS 128	HZ3	O	I.858
ARG 130	HE	O	1.932

Docking score of the ligand and the target: −7.37 Kcal/mol, Number of hydrogen bonds formed: 3. PTEN: Phosphatase and tensin homolog located on chromosome 10q23, TQ: Thymoquinone

apoptosis by coinciding with the *in vivo* results. It is, therefore, essential to perform docking experiments, which can help in validating a target and adds support to the *in vivo* studies.

CONCLUSION

From this study, TQ could be considered as an efficient phytochemical in regulating the protein responsible for cancer and thereby preventing the cancer initiation and development, as a possible option for cancer control agenda. This study will help to understand how the target protein is regulated by the ligands and inhibiting the carcinogenic pathway. Further research is needed for refinement to enrich the activity of the ligands and destroying mechanism of cancer proteins, especially in the animal model system, and also to determine the dosage of safety levels, in order to explore this promising avenue for cancer control and to ensure the healthy state of humans.

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