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An *in silico* study on plant-derived inhibitors against a prognostic Biomarker, Jab1

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ABSTRACT

Cancer kills millions of people worldwide every year. The main form of treatment at this point is chemotherapy, which comprises of systemic drug delivery so that they can kill the cancerous cells more effectively. But most of these drugs cause severe side effects in patients and, therefore there is a strong need to focus on identifying natural compounds as a potent phytoinhibitors using various *in silico* and *in vitro* approaches. Natural compounds pose low toxicity, hence render them to be an excellent alternative to the basis for the development of new anti-cancerous drugs. Our study considers an effective therapeutic target, Jab1 (c-Jun activation domain-binding protein-1) or a c-Jun coactivator, which has been implicated in multiple protein interactions that play a significant role in various stages of carcinogenesis. Hence we have performed screening of 1500 natural compounds having anticancerous activity by applying various *in silico* approaches including Lipinski rule of five, ADME, and various Molecular Docking tools. In this study, we have identified two potent phytoinhibitors against Jab1 which needs to be further validated through *in vitro* approaches.

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INTRODUCTION

Elevated level of Jab1 expression has been associated with several carcinomas including breast, thyroid, skin, ovarian, lung, and liver cancers (Li *et al.,* 2018; Pan *et al.,* 2017; Pan *et al.,* 2014; Kim *et al.,* 2016; Guo *et al.,* 2016; Liu *et al.,* 2018). However, several reports suggested that Jab1 down-regulation is directly linked within an increase in the p27 (tumor suppressor protein). NES domain of Jab1 is found to be responsible for p27 shuttling between the cytoplasm and the nucleus in a CRM1-dependent manner via NES (nuclear export signal-like sequence) sequences lying between amino acids 233 and 242 at its C-terminal end (Pan *et al.,* 2014). Inhibition of the NES sequence through inhibition of Jab1/CSN5 could thus represent a novel therapeutic approach for the treatment of Jab1-dependent cancers.

Recent advances in the developments of *in silico* (computational) approaches provide influential methods for identifying the pharmacological kinetics of plant-derived drugs (Fang *et al.,* 2017). *In silico* modelling is an alternative to the traditional methods of testing compounds for drug development. It is a very cost-effective and less time-consuming process. It is the novel approach towards the optimized screening of natural compounds as potential leads against target proteins by the use of computers to predict their binding to known target structures in the drug discovery process. A list of a wide range of software packages is available for molecular docking simulations like Auto Dock, PATCH DOCK, GOLD, FIRE DOCK and FlexX (Hanahan and Weinberg, 2000). Auto Dock 4.2 is the most commonly used version which has been extensively used for virtual screening. Molecular docking helps in determining the 3D conformation and binding interactions between protein and ligands.

MATERIALS AND METHODS

Requirements for in silico analysis

Windows XP or Windows 7, MGL tools, Cygwin, Discovery Studio Visualizer and Binary files

Target identification used in docking

The selected target for docking studies was Jab1, and its 3D structures were retrieved from the PDB (Protein Data Bank) (PDB ID: 4FNO, Res: 2.40 A°) (Figure 2). During molecular modelling, the water molecules were removed, and the energy of the protein structure was also minimized prior to docking.

Binding site: The binding site in Jab1 was determined using literature reviews. Jab1 comprises 334 amino acids and contains an NES domain that is responsible for the cytoplasmic degradation of p27 (Wang *et al.,* 2016; Oono *et al*., 2004). NES is located at the end of the C-terminal and contains leucine-rich nuclear export signal sequences which are highly conserved among different species. CRM1 exports p27 from nucleus to cytoplasm by interacting with the NES domain of Jab1 in LMB-sensitive manner. Sequences of the NES domain were selected for site-specific docking.

Ligand preparation: 1500 plant-derived compounds (phytoligands) were selected from NPACT Database for molecular docking analysis. Simplified Molecular Input Line Entry Specification notations (SMILES) of phytoligands were obtained from PubChem database which was then used to produce the 3D structures with the help of CORINA server. The structures of phytocompounds used in this study were downloaded from the PubChem compound database. These phytochemicals satisfied Lipinski's rule of five and ADME properties. These structures were then selected for performing docking program using Autodock 4.2.

Molecular docking: Receptor molecule (Jab1) was prepared in the AutoDock 4.2 program (Norgan *et al*., 2012), and protein-ligand docking was done as described by Rizvi *et al*, 2013. AutoDock Tools 4.0 was employed to find the most favourable binding conformations of the ligand in the protein (receptor) (Morris *et al.,* 1998). Analysis of the binding conformation of the ligand-protein complex was done by using a scoring function on the basis of free energy of binding (Huey *et al.,* 2007). Lamarckian Genetic Algorithm (LGA) was selected amongst the several search algorithms found in a suite of AutoDock for docking calculations (Solis and Wets, 1981). AutoDock 4.0 was used to develop the grid parameter file of Jab1 and various grid points in x, y, and z-axes were 60×60×60 Å. The distance between the two connecting grid points was 0.375 Å. LGA run terminated after 2500000 number of energy evaluations with about 27000 generations. Various parameters were selected according to the default values of the software.

RESULTS

Target Structure

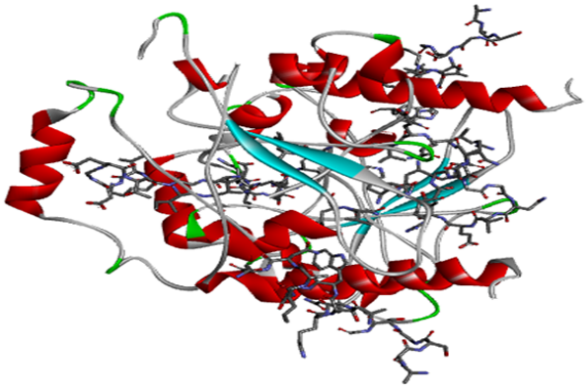

Figure 1: Jab1 Protein structure

The Crystal structure of human Jab1 protein was obtained from the RCSB protein data bank (PDB ID: 457O). Human Jab1 comprises of 334 amino acids having a molecular mass of 38KDa (Cirigliano *et al.,* 2016).

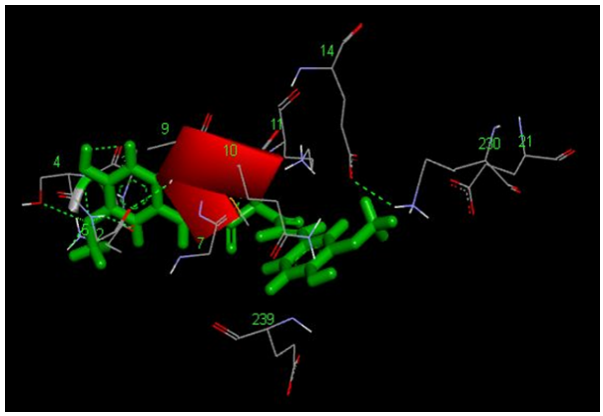

Figure 2: *In Silico* binding of Jab1 with Curcumin with binding Energy –5.69 Kcal/Mol

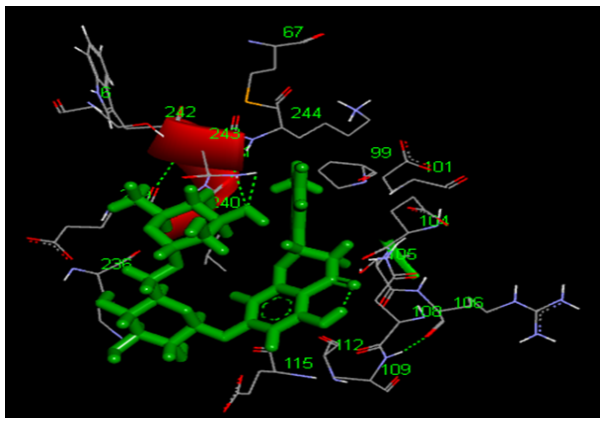

Figure 3: *In silico* binding of Jab1 with Hesperidin with binding Energy –7.73 Kcal /Mol

Table 1: Docking results of phytocompounds with Jab1 protein

PubChem ID	Name	Patch Dock (Score)	Firedock (Global energy)	Autodock			
				Binding Energy (kcal/mol)	No of H-bonds	Total Internal Energy	Estimated inhibition constant, Ki
969516	Curcumin	4496	-30.79	-5.69	1	-1.09	67.12
5280794	Stigmasterol	4848	-44.57	-7.06	1	-0.56	6.73
10621	Hesperidin	5460	-25.45	-7.73	2	-0.19	2.17

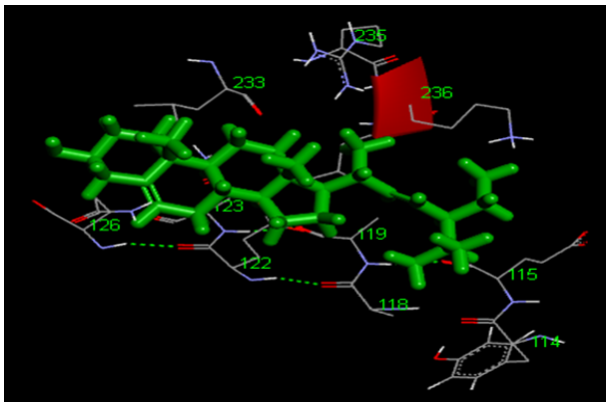

Figure 4: *In silico* binding of Jab1 with stigmasterol. Binding Energy –7.06 Kcal/Mol

Screening of Phytoligands for Jab1 by Molecular Docking Studies

Selected lists of 1500 compounds were used for this study using NPACT database (Mangal *et al*., 2012). After screening of 1500 compounds by using various *in silico* approaches including Lipinski rule of five, ADME, Molecular Docking and Patchdock, two potent phytoinhibitors were identified that have shown maximum inhibitory effects against Jab1 in comparison with curcumin (Liu *et al.,* 2013; Pan *et al.,* 2013). The molecular docking studies revealed that two significant phytoligands hesperidin and stigmasterol for Jab1 protein shown strong inhibition constant and better binding energy comparison with curcumin (Table 1). The binding energy of these screened phytoligands hesperidin, curcumin and stigmasterol with Jab1 were 7.73, 7.06 and 5.69 kcal/mol, respectively, and inhibition constant of 2.17, 6.73 and 67.12, respectively. The molecular docking of the two compounds showed good binding mode and interaction energy.

DISCUSSION

In our study, we selected the crystal structure of Jab1 protein and Phytocompounds to elucidate *in silico* small molecule inhibitors of Jab1 (Echalier *et al.,* 2013; Lingaraju *et al.,* 2014). A list of 1500 phytocompounds from NPACT database (Xie *et al.,* 2015) possessing anticancerous activity present in PubChem database was selected and docked into Jab1 structures using AutoDock Tools 4.0 to find potent phytoinhibitors for Jab1. The molecular docking analysis revealed that three potent phytocompounds curcumin, hesperidin and stigmasterol for Jab1 protein had shown better binding energy and promising inhibition constant amongst other compounds selected for this study (Table 1). All the screened natural compounds also satisfy the Lipinski's rule of five criteria without any violation depicted by molinspiration server. Results of molecular docking analysis of the target and screened molecules showed the evidence of efficient binding of the phytocompounds and interacting residues. Docking results were analyzed on the basis of binding energy and hydrogen bond formed, in order to find potent phytocompounds as the best ligands. The binding energy of these phytoligands hesperidin, curcumin and stigmasterol with Jab1 was 7.73, 7.06 and 5.69 kcal/mol, respectively which had minimum binding energy to potentiate the target protein amongst all ligands. All three screened compounds have been appeared to be a potential chemotherapeutic agent showing strong inhibition against the selected target. Thus, present study revealed a method which reduces the cost and time in the screening and development of a chemotherapeutic drug (Śledź *et al.,* 2018).

CONCLUSION

Jab1 is a multifunctional protein complex which has been involved in the modulation of gene transcription, signal transduction pathways and protein stability in cells and involved in control of cellular proliferation by inactivating various important negative regulatory proteins and tumor suppressors via their subcellular localization and degradation (such as p53, Smad 4/7, and the cyclin-dependent kinase inhibitor p27Kip1 (Tomoda *et al*., 2002). In the present scenario, there is an urgent need to elucidate effective anticancerous agents which can decimate the limitations of the presently existing chemotherapeutics. Our study also extends to the knowledge of phytoligands as a potential inhibitor against Jab1. Furthermore, in our study two potent phytocompounds, hesperidin and stigmasterol for Jab1 target (protein) produced strong inhibition constant and better binding energy amongst all other compounds considered for this study. All the three screened compounds have emerged as potential chemothera-

peutic agents showing strong inhibition against selected target, Jab1. The in silico results presented in the current study demonstrate that these phytocompounds exhibited much higher binding energy to inhibit Jab1, which provides a basis for the further investigation of these phytoligands in drug designing against Jab1.

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Conflict of Interest

I validate that this research paper authored by me/us is an original and genuine research work. It has neither been submitted for publication nor published elsewhere in any print/ electronic form.

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