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RES ARTICLE

Molecular interaction of *Survivin* and *Piperine* by computational docking analyses for neuroblastoma targeting

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KEY WORDS

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ABSTRACT

Background: Neuroblastoma (NB) is a childhood cancer causing significant mortality in at least 1% children worldwide. NB is an embryonically derived tumor. The causative agents include genetic predisposition and dysregulated signaling cascades. *Survivin* is an important anti-apoptotic protein that is significantly up-regulated in NB. In this study, a naturally occurring ligand - *Piperine* was assessed for its interaction with *Survivin* protein. **Purpose:** The study was undertaken in order to identify the experimental feasibility of *Survivin* inhibitor ligand *Piperine* as targeting treatment of NB. **Methods:** Protein sequences were retrieved and saved in PDB format. Similarly, the ligand data was processed using MGL (Molecular Graphics Laboratory) and chimera tools and saved in PDB format. Both protein and the ligand data were then uploaded to the docking server and docking parameters were set. **Results:** *In-silico* docking study of a protein ligand interaction resulted in -3.36 Kcal/mol free energy value for the ligand, with an involvement of 1 hydrogen bond, 7 hydrophobic interactions and 13 ionic interactions. The results were correlated with the existing free energy value of > -3 Kcal/mol which is established for a good inhibitor. **Conclusion:** The molecular docking study for mice *Survivin* and *Piperine* shows good inhibitory interaction effect and can, therefore, be considered as a molecule against *Survivin* enhanced tumor condition including NB.

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Introduction

Computational docking minimizes the time consuming process of molecular analyses for selecting a suitable ligand which could be then applied for wet lab investigations.[1] Wickbery and Coworkers used Bioinformatics to narrow down suitable ligands for biomedical research and drug design as structure based design shows precisely the location and orientation of bound inhibitors and their physico-chemical properties.[2] *Survivin* is an apoptosis pathway inhibitor protein. It has important roles in cell cycle and cell proliferation. In normal embryonic development, expression of *Survivin* was found to be high and it was also expressed in some adult's colonic epithelium, uterine, vascular endothelium and subventricular region of brain. In cancer cells, *Survivin* expression was found to be very high.[3-6] Previous works reported that *Survivin* mainly works as an inhibitor of apoptosis, blocking mitochondrial dependent apoptosis[7,8] (Figure 1). It was also reported later that it has other role as a mitotic checkpoint.[9] *Survivin* family of proteins are involved in control of mitosis and makes perfect cell division in normal cells.[10] It prevents the aneuploidy which normally occurs in malignant tissues.[11]

Neuroblastoma (NB) is a childhood cancer causing significant mortality of at least 1% of children worldwide. *Survivin* is known to be expressed at high levels in NB.[12] *Piperine* is a heterocyclic alkaloid that belongs to a family of nitrogenous compounds with marked physiological properties. It is non-genotoxic, but found to have anti-mutagenic and anti-tumor activity.[13]

Methods

The current study was focused towards developing understanding of *Survivin*, which has been reported to be up regulated in NB and in certain other tumors.[14] The PDB file of the protein was downloaded from RCSB (www.rcsb.org) which was then purified in a docking server. In the same server docking calculation were carried out. Also the ligand piperine, an alkaloid was drawn using ChemDraw tool v.4.0 and converted to PDB file format using MGL tool. The target protein and the ligand were subjected to docking.

Essential hydrogen atoms, Kollman united atom type charges, and salvation parameters were added with the aid of auto dock tools. The affinity (grid) map of XXÅ mid points 0.375Å spacing was generated using the autogrid program.[15] Auto dock parameters were set on distance dependent dielectric Van-der-waals and the electro static terms respectively.

Docking simulations were performed using the Lamarckian Genetic Algorithm (LGA) and the Solis and Wets local search method.[16] Initial position, orientation and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived

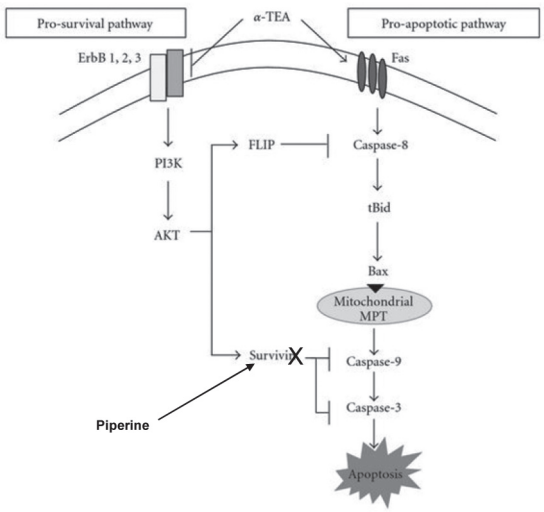

Fig. 1: Survivin Pathway.

from two different runs that were set to terminate after maximum of 25000 energy evaluations. The population size was set at 150. During the search, a translational step of 0.2Å and quaternion step of 5 were applied.[17]

Results

Molecule docking of *Survivin* with ligand *Piperine* has an outcome of good energy level calculations that suit drug modeling of the ligand (Figure 2). Free energy (ΔG) of −3.36 Kcal/mol, inhibition constant (K_i) of 3.42 mM, and electrostatic energy of −0.04 Kcal/mol (Table 1) was noted.

The protein–ligand interaction study showed 6 amino acid residues interaction with the ligand (12:Leu, 20:Ala, 21:Thr, 44:Ile, 46:Cys, 56:Gln) (Table 2). The interaction of ligand and protein was generated and is depicted in HB plot (Figure 3).

Discussion

NB is a hidden health risk for both the public and the researchers. Therefore, a drug that can inhibit the disorder will be helpful in better health management.

The signaling cascade molecules in NB need to be analyzed computationally for better ligand. For this purpose molecular docking is an ideal tool.[18] Faster and cheaper methods for drug designing at initial stages include molecular docking. In this study, the simulation of protein–ligand chemistry, binding and dissociation energy were focused upon. The energy and interaction details have been developed using Auto Dock. The free energy (ΔG) of interaction is −3.36 Kcal/mol, which is in good agreement with physiological protein-ligand (hormones, enzymes) interaction range of −2.00 Kcal/mol to −6.00 Kcal/mol[19] therefore; our result suggests a good candidate for protein–ligand interaction.

Inhibition constant (K_i) is an important force in molecular interaction. Obtained K_i[20] is favorable towards developing a novel drug

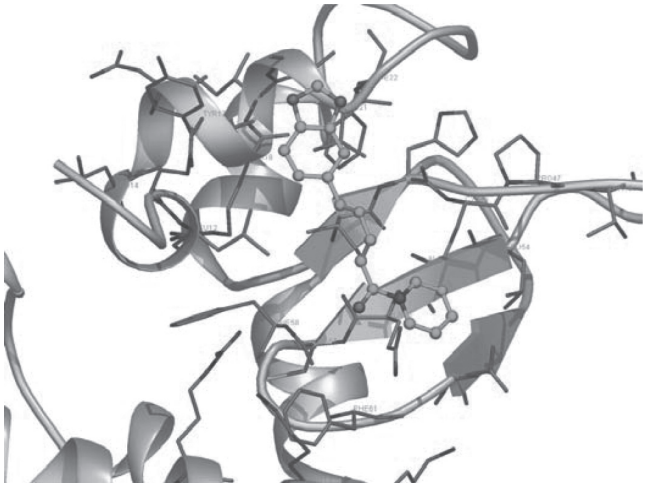

Fig. 2: Binding conformation of survivin and piperine.

Table 2: Protein and ligand interaction table of residues and atoms

Interaction Table		
Hydrogen bonds	**Hydrophobic**	**Other**
N1 0 – GLN56 (OE1) [3.02]	C12 0 – LEU12 (CD2) [3.20]	O1 0 – ALA20 (CB) [3.43]
	C13 0 – LEU12 (CD2) [3.56]	C15 – THR21 (CB, CG,OG1) [3.44]
	C17 0 – ALA20 (CB) [3.70]	C11 0 – THR21 (CG2,OG1) [3.70]
	C7 0 – ILE44 (CD1) [3.18]	C12 0 – THR21 (OG1) [3.57]
	C6 0 – ILE44 (CD1) [3.71]	C13 0 – THR21 (OG1) [3.42]
	C5 0 – ILE44 (CD1) [3.59]	C14 – THR21 (OG1) [3.33]
	C1 0 – CYS46 (CB,SG) [3.43]	C16 0 – THR21 (CG2 OG1) [3.38]
		C3 0 – GLN56 (CD,OE1) [3.15]
		C6 0 – GLN56 (OE1) [3.87]
		C4 0 – GLN56 (OE1) [3.08]
		C5 0 – GLN56 (OE1) [2.38]
		C1 0 – GLN56 (OE1) [3.04]

Table 1: Molecular docking energy level table

Result Table								
Rank	**Est. Free Energy of Binding**	**Est. Inhibition Constant, Ki**	**vdW + Hbond + desolv Energy**	**Electrostatic Energy**	**Total Intermolec. Engergy**	**Frequency**	**Interact. Surface**	**Download**
1	−3.36 kcal/mol	3.42 mM	−4.15 kcal/mol	−0.04 kcal/mol	−4.19 kcal/mol	50%	512.417	download
2	−3.09 kcal/mol	5.39 mM	−4.09 kcal/mol	+0.11 kcal/mol	−3.97 kcal/mol	50%	512.053	download

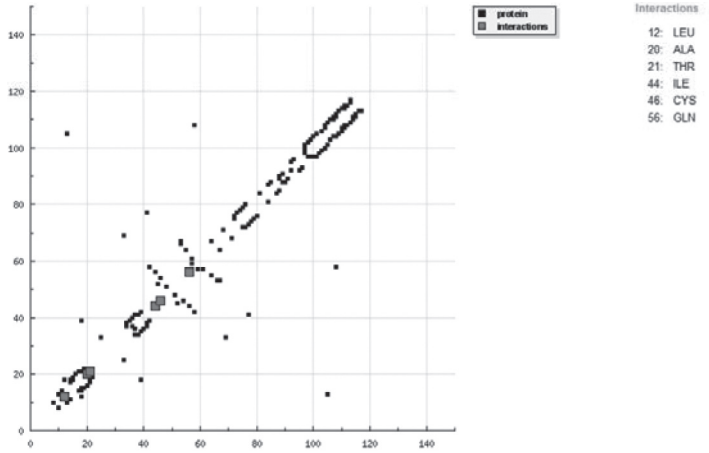

Fig. 3: Interaction of ligand and protein in HB plot.

molecule. Vander Waal's force, hydrogen bonds are the other factors which stabilize ligand-protein interaction in our docking study, in which the results for electrostatic force of molecules were significantly less, and it is a sign of a good protein-drug interaction. Docking results give binding site analysis for 6 amino acids, with the ligand which shows precise conformity. Three polar residues and 3 non-polar residues reflect a stable electrostatic interaction. Even though there is a single H-bond, the electrostatic force obtained in the result is significant enough for a strong bonding in case of a protein-drug interaction.[21] Also, the existence of rich number of ionic bond in the docking study suffices for a further more stable association. The ligand *Piperine* interacted well with the protein *Survivin* in the docking grid.

Conclusions

Molecular docking of surviving (mice) with ligand *Piperine* when subjected to docking analysis using AutoDock and docking server, predicted *in-silico* result with a free energy of −3.36 Kcal/mol which was agreed well with physiological range for protein-ligand interaction, making *Piperine* probable potent anti-survivin molecule. Therefore, it is expected that *Piperine* might participate by down regulating the levels of *Survivin* upon administration, making the NB cells pro-apoptotic, eventually leading to death of tumor cells.

The article complies with International Committee of Medical Journal Editor's uniform requirements for the manuscripts.

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