IN SILICO DOCKING ANALYSIS OF A NOVEL ANTIMICROBIAL PEPTIDE AGAINST HUMAN BREAST CANCER TARGETING β-CATENIN

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ABSTRACT

Breast cancer is the serious health concern in India causing the highest mortality rate in females, which occurs due to uncontrolled cell division and can metastasize to other parts of the human body. β-catenin can be involved in breast cancer formation and/or progression through Wnt signaling pathway and may serve as a target for breast cancer therapy. Most of the drugs currently used for the breast cancer treatment produce various side effects and hence we focused on antimicrobial peptides. Structure of β-catenin was retrieved from the Protein Data Bank and the structures of antimicrobial peptides have been collected from PubChem database. Molecular docking and drug likeness studies were performed for those antimicrobial peptides to evaluate and analyze the anti-breast cancer activity. The results of this study can be implemented in the drug designing pipeline. It is concluded that the antimicrobial peptide (SKACP003) was the ideal one for more effective targeting.

Keywords: Breast cancer, Antimicrobial peptides, β-catenin, Protein Data Bank, PubChem database

INTRODUCTION

Anticancer peptides (antimicrobial peptides with anticancer property or simply ACP) ought to be potential candidates which can promote the target-specific cancer therapy at a larger scale. As anticancer peptides, comprise of a group of small molecules (b50 amino acids), have the ability to destabilize only the cancer cells by either distorting the membrane or disrupting the mitochondria (Harris *et al.*, 2013) without harming the nearby normal cells, one can make them as target-specific weapons to eliminate the cancerous cells from the affected regions. Owing to the fact that the anticancer peptides possess the positive charge, it is quite natural to get attracted by cancerous cell membrane which has a net negative charge due to electrostatic attraction (Schweizer, 2009). On the other hand, normal cell membranes have Zwitterionic property and hence the anticancer peptides simply ignore them. Moreover these peptides can penetrate through the cancer cell membranes, because of their amphiphilicity levels in addition to the hydrophobic arc size, and thereby destabilize the membrane integrity (Dennison et al., 2006; Huang et al., 2011). The authors in ref. (Hilchie *et al.*, 2011) have shown that a peptide (pleurocidin-like) present in fishes destroyed breast cancer cells as well as human mammary epithelial cells by damaging the cancerous cell membrane with subtle harm to human fibroblasts.

Recently, SA12, an anticancer peptide, has been re- ported to induce apoptosis on SKBr-3 breast cancer cells (Yang *et al.*, 2015). In a nutshell, it is concluded that the anti- cancer peptides posses the ability to act as impeccable drugs in the field of target-specific cancer therapy and therefore have a vivid future.

For a more protective cancer therapy, the selection of particular target is of paramount importance. In this aspect, understanding of Wnt signaling helps in devising novel therapeutic approaches. The canonical Wnt signal transduction pathway (or simply CWSTP) is the cause of concern as it was found onset in most of the tumor types, including colorectal tumors and tumors in breast (Clevers and Nusse, 2012; Prosperi and Goss, 2010; Prosperi *et al.*, 2011; Rahmani *et al.*, 2020). Presumably, β-catenin, the major component of the CWTSP, is found to be dominant in tumors in the form of either Wnt ligand overexpression, or loss of the Adenomatous polyposis coli (APC) tumor suppressor or down-regulation of Wnt ligand antagonists (King *et al.*, 2012). Consequently, β-catenin shifts into the nucleus and starts controlling the gene expression by using its association with T cell factor (TCF) members. A few of the significant β-catenin/TCF transcriptional targets are cell cycle regulators cyclin D1 (Lin et al., 2000) and c-Myc (He *et al.*, 1998), stem cell gene Bmi-1 (Kirstetter et al., 2006), matrix metalloproteinase Mmp-7 (Hovanes *et al.*, 2001), and Wnt pathway component Axin2 (Yook *et al.*, 2006). Moreover, accumulation of β-catenin inside the cytoplasm and overflow of the excessive β-catenin into the nucleus of the cancer cells enhance the cancer cell growth. This, in fact, reduces the chance of survival of breast cancer patients (Khramtsov *et al.*, 2010). With this motivation, this study is intended to exactly target the β- catenin protein by a novel anticancer peptide in order to deactivate it.

MATERIALS AND METHODS

Collection of antimicrobial peptides

Around 5000 antimicrobial peptides were collected from various repositories which include Antimicrobial Peptide Database (AMP) (http: // aps.unmc.edu / AP/ main.php) (Wang and Wang, 2015), Collection of Antimicrobial Peptides (CAMP) (Waghu et al., 2015) and Data base of Anuran Defense peptides (DADp) (link split4. pmfst.hr/dadp/) (Novkovi´c et al., 2012).

Screening for anticancer peptides

From the collected peptides, those peptides which were made up of 5-30 amino acid residues were selected for our study. The selected peptides were then analyzed thoroughly for the possession of a specific number of anticancer property providing amino acids (Mader and Honskin, 2006). Thus 5 peptides that contained the maximum percentage of amino acids with anticancer properties were chosen for further studies. The selected antimicrobial peptides were then evaluated for anticancer properties using the online AntiCP (https: //webs.iiitd.edu.in/raghava/ anticp/ submission. php) web site.

***In silico* studies:**

Protein-Peptide Docking: All computational studies were carried out by using Maestro, Schr¨odinger, LLC, New York, 2015-4 (Release, 2017) and GROMACS version 4.6.3 (van der Spoel *et al.*, 2012) software packages on 3.40 GHz Intel CoreT M i7 processor loaded with CentOS 7.0.

Protein preparation: A three dimensional crystal structure of β-catenin protein [PDB ID: 1JDH] was determined by using X-ray diffractiometer with 1.9˚A resolution for our study (Graham *et al.*, 2001). The target protein structure with the help of protein preparation wizard, Schr¨odinger LLC, 2015-4 was prepared. Subsequently, the fine protein preparation processes which involve, assignment of bond orders, incorporation of hydrogen atoms in the protein molecule, and

optimization of hydrogen bonds and water molecules in each structure were accomplished (Loganathan, and Muthusamy, 2019). Nonreactive water molecules and cofactors that had no role in protein function were filtered out from the target protein. Finally the protein structure was minimized with the help of OPLS 2005 force field by fixing the backbone atoms.

Peptide preparation: All the 5 selected peptides were converted into the pdb structure format using the Pymol tool and the peptide structure files were taken for preparation using the same protein preparation wizard. The peptide was simulated by adopting the same protein preparation method.

Molecular docking: Protein-peptide molecular docking studies is a vital tool which provides a better understanding of the structural relationship and residue interactions between the molecules. Protein-peptide docking was carried out to find the best peptide which has greater binding affinity as well as more interaction energy with the receptor. The protein and peptide were docked using HADDOCK Protein-Peptide docking algorithm (HADDOCK version 2.2) (Van Zundert *et al.,* 2016). The overall docking process was done in several stages. Initially, a number of possible arrangements around 2000 were generated and then by using rigid-body docking some useful structures to the tune of 400 were identified upon optimization for further scrutiny. The identified structures were later subjected to a reasonably flexible docking approach and tested for water solubility. Among such 400 identified structures, only half of them were retained after the HADDOCK solvation technique. The protein and solvent metrics were assigned by using de- fault HADDOCK parameters during the entire docking process (Trellet *et al.,* 2013). A cluster analysis was carried out by computing Root Mean Square Deviation (RMSD) for docked structures as provided in HADDOCK.

Molecular dynamics simulation (MDS): The conformational features of protein-peptide were analyzed by performing molecular dynamics simulations using GROMACS version 4.6.4 with GROMOS9643a1 force field (Loganathan and Muthusamy, 2018). Then the complexes were allowed to relax for the purpose of eliminating unwanted atomic contacts and then they were placed in a dodecahedron water container with space point charge 216 water model. Neutralization of the system was done by the addition of apropriate charge carriers. Before the start of MDS the system was minimized by removing unwanted van der Waals contacts with the aid of steepest descent algorithms. Then Particle Mesh Ewald (PME) method was adopted to irradiate the system in a normal cut off wavelength of 0.9 nm. The temperature and pressure of the system were maintained at 300 K and 1bar, respectively along with the maintenance of Berendesen thermostat with coupling times of 0.1 and 1.0 ps. During the equilibrium period, free MDS of 100 ps were simulated with an application of force constant 1 MJ mol^{-1} nm^{-2}. By using the LINCS algorithm the covalent bond lengths containing hydrogen atoms were restricted. Ultimately, a free MDS of 30ns was run for the predominant protein-peptide composites identified earlier using docking. Origin (pro8 version) package was used for the statistical analysis of all the complexes (Seifert, 2014).

RESULTS

Peptide Collection and Screening for anticancer properties

From the collected peptides of around 5000, we short- listed such peptides which are made up of five to thirty amino acids for the preliminary studies. Further filtering process was done on the basis of maximum number of amino acids present in the peptide with anticancer property [ACP] for the next level of study (Tyagi *et al.,* 2013). The chosen peptides were tested for anticancer properties using the AntiCP web site. Consequently, a set of five peptides with ACP rich amino acids were selected and listed in Table 1.

Table 1 List of peptides selected for our study with their anti-cancer property prediction results

SI. No	Peptides	Sequence of peptide	Molecular weight (g/mol)	Anticancer property rich amino acids	Prediction
1.	SKACP001	FLPLLLAGL PKLLCLFFKKC	2278.3	Phe, Leu, Pro, Ala, Gly, Lys, Cys	ANTICANCER
2.	SKACP002	GGLGGLLGPLL GGGGGGGGGL L	1706.37	Gly, Leu, Pro	ANTICANCER
3.	SKACP003	FPLPCAYKGTY C	1362.78	Phe, Pro, Leu, Cys, Ala, Tyr, Lys, Gly, Thr	ANTICANCER
4.	SKACP004	LPPWIG	681.92	Leu, Pro, Trp, Isoleu, Gly	ANTICANCER
5.	SKACP005	NLCASLRARHTI PQCRKFGRR	2484.24	Asp, Leu, Cys, Ala, Ser, Arg, His, Thr, Isoleu, Pro, Glu, Lys, Phe, Gly	ANTICANCER

Protein-Peptide Docking

In protein-peptide docking analysis, the optimized and energy minimized structure of β-catenin protein (PDB ID: 1JDH) and 3-D structure of five peptides listed in Table 1 were docked using HADDOCK package V.2.2. The receptor site was defined as the crystal structure of β-catenin and ligands as peptides. All the five peptides were docked individually using the standard protocol. Since the active site of the β-catenin protein was found to be present in Armadillo (ARM) domain as re- ported in ref. (Valent *et al.,* 2012), the constraints were given based on the amino acids present in the ARM domain of the receptor (protein). The active sites were fixed by using the location of active residues and passive residues where the former located in the ARM domain and the latter resided around the former as amino acids.

Table 2 Results of the protein-peptide docking using Haddock

Receptor (β-catenin)	Peptide	Haddock Score	RMSD	Electrostatic energy	Z- score
1JDH	SKACP001	-91.2	7.4	-107.2	-2.0
1JDH	SKACP002	-82.7	10.1	-101.5	-1.8
1JDH	SKACP003	-95.9	5.4	-237.3	-1.8
1JDH	SKACP004	-59.7	1.6	-80.7	-1.4
1JDH	SKACP005	-95.5	10.8	-111.7	-1.6

The results were found to be extremely convincing based on their HADDOCK score, electrostatic energy, and Z-score (Table 2). The docked protein-peptide complex was taken for the structural and binding mode analysis to find the binding conformation of the peptides with the receptor. Among the five peptides, SKACP001, SKACP003 and SKACP005 showed prominenence and, in particular, the SKACP003 had the highest HADDOCK score as well as electrostatic energy (Please see Table 2 and Fig. 1). It was predicted from Fig. 2 and Fig. 3 that SKACP003 had docked at the ARM domain, which is a large cleft at the bottom of the receptor. Further, the docked protein- peptide was taken for hydrogen bond analysis and the additional number of hydrogen bonds were observed between the β-catenin and SKACP003. To be precise, Cys466, Arg565, Asn516, Arg474, Asn609 and Asn640 residues were found to form hydrogen bonds (Fig. 3).

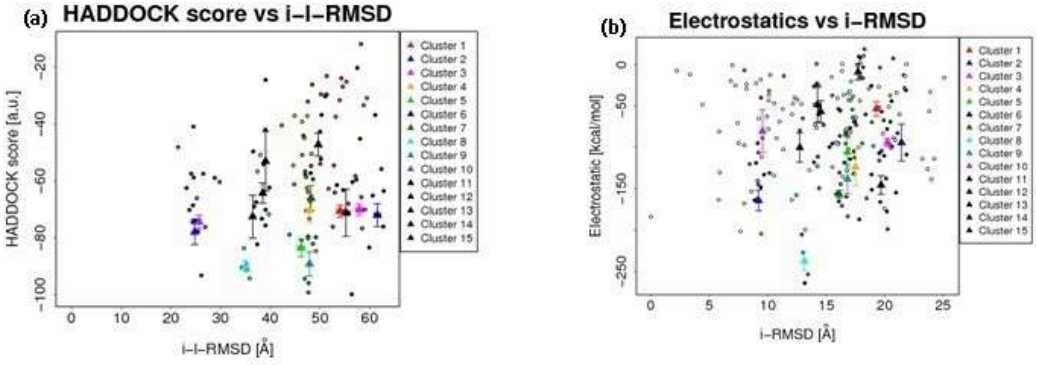

Fig. 1 The plots represent (a) HADDOCK scores vs i-l-RMSD, and (b) Electrostatic energy values vs i- RMSD comparatively in 15 clusters for the peptide SKACP003. The structure and interaction figures were plotted using the program PyMOL, 2017.

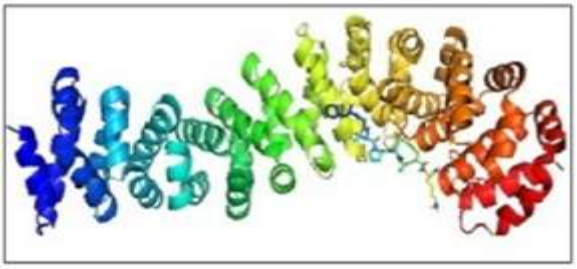

Fig. 2 3-dimensional representation of β-catenin in complex with peptide SKACP003

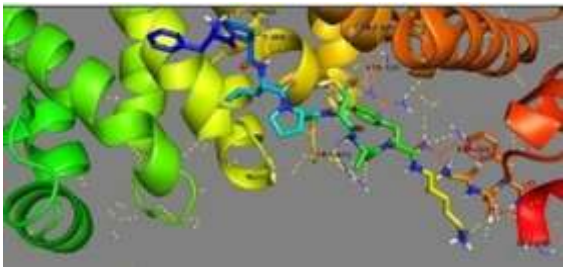

Fig. 3 Hydrogen interaction observed between peptide SKACP003 and β-catenin in the active site region

Molecular Dynamics Simulation [MDS] studies

The docked complex of protein with peptide molecules were taken for MDS to analyze and understand the structural changes and binding affinity between them. All the three complexes were highly unstable upto 15 ns because they needed some time to attain equilibrium state, but after 20 ns all the complexes remained stable with very feeble deviation (Fig 4). The 30 ns MDS run provided a better knowledge of the peptide- receptor protein binding. The RMSD values that correspond to backbone atom of protein and peptide were plotted to find the structural variation and conformational changes. The best complex 1, 3, and 5 were taken into consideration based on their high docking scores. All the three complex molecules were found to be stable and had good RMSD value in the range of 0.3 to 0.7nm. The complex 3 was found to have the greatest attraction and very stable as well with an RMSD value between 0.3 and 0.5 nm. The other two complexes were stabilized within 0.7 nm and the conformational changes were noted to get decreased at the end of the simulation. Therefore, it is concluded that the third complex (SKACP003) was the ideal one for more effective targeting.

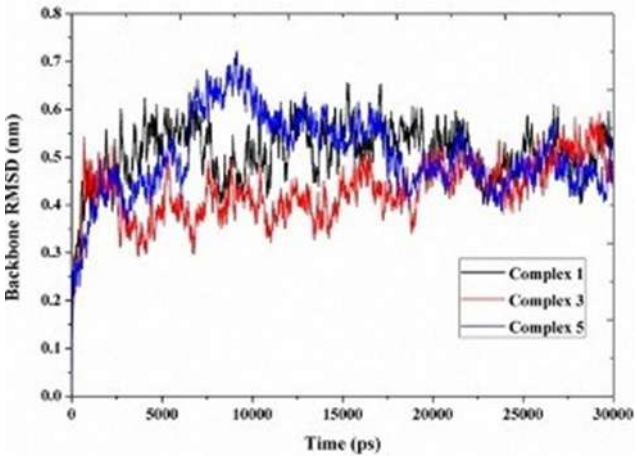

Fig. 4 Backbone RMSD of the protein-peptide complex for 30 ns molecular dynamic simulation

DISCUSSION

Certain antimicrobial peptides showed anticancer activity and so we opted for an antimicrobial peptide which could be used as a potential weapon to target the β- catenin protein as it is considered as a primary element in the development and prognosis of breast cancer. As we mentioned already, 5000 antimicrobial peptides were chosen as precursors from various available databases like AMP, CAMP, DADp, and so on. The smaller the peptide, the more it possesses anticancer activity (Lim *et al.*, 2013). Hence the peptides with 5 to 30 amino acids were shortlisted from the collected peptides. We chose 59 peptides which were predicted to possess anticancer activity depending upon their amino acid composition and number of amino acids present within. As the presence of certain amino acids like Cys, Gly, Ile, Lys, Phe, and Trp increases the anticancer property of the peptides, we screened out five peptides that contained the above mentioned amino acids within them. The anticancer activity of all the five peptides was ascertained with the help of the ANTICP web portal. Furthermore, in-order-to analyze the targeting and binding efficiencies of the peptides, the peptides were docked against the β-catenin protein and observed that the peptide SKACP003 had a strong binding efficacy among the 5 peptides. The complex structure consisting of protein β-catenin and peptide-SKACP003 was analyzed through molecular dynamics simulation studies. The results expressed the structural stability of the complex

during the 30 ns simulation run. The non-covalent interactions between the β-catenin and peptide- SKACP003 were considered to be crucial in the inhibition mechanism. Notably, selective residues forming H-bonds with residues of the peptide elevates the binding efficiency and stability of the complex.

CONCLUSION

In conclusion, based on the present study, the newly identified antimicrobial peptide SKACP003 successfully targeted and destroyed the β-catenin protein.

Acknowledgements:
Authors acknowledge the help received from Dr.V.Ravikumar Department of Biochemistry, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India.

Compliance with Ethical Standards

Conflict of interest

The authors SK, TM, LL, and MK declare that they have no conflict of interest.

Funding

The work of LL and MK was supported by Ministry of Human Resources and Development, Government of India in the form of a Major Project (MHRD-RUSA 2.0 - F.24/ 51/ 2014-U).

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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