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Inhibition of breast cancer progression by targeting β-catenin protein using a novel anticancer peptide

S Kanitha[1], T Malarvili[2]
[1] Department of Biochemistry, Government Arts and Science College for Women (Affiliated to Bharathidasan University, Tiruchirappalli), Orathanadu, Tamil Nadu, India
[2] Bharathidasan University Constituent College, Srirangam, Tiruchirappalli, Tamil Nadu, India

Abstract
Breast cancer stands as one of the major causes for global elevated death rate. The deregulated Wnt signaling pathway is accountable in innumerable types of cancers, especially in breast cancer. Though enumerable strategies have emerged in the recent past to target the Wnt signaling pathway, regrettably none have succeeded in clearing the clinical trials till now. Antimicrobial Peptides have attracted much of attention as anticancer peptides. Most of these peptides are cationic in nature and hence are attracted towards the cancerous cells which possess negative charge due to the presence of phosphatidylserine on the cell membrane. Hence in this present study we tried to arrest the Wnt signaling pathway by inhibiting the activity of β-catenin protein, the major component which accelerates the rate of Wnt signaling pathway using a novel antimicrobial peptide possessing anticancer property. The anticancer property of the peptide was studied and also by adopting insilico studies we have proved that our peptide is able to target the β-catenin protein specifically and could prove as a potential therapeutic agent against breast cancer after further invitro and *in vivo* studies.

Keywords: breast cancer, antimicrobial peptides, anticancer peptides, β-catenin, Wnt signaling pathway

Introduction

Very recently cancer has emerged as a consequential threat for the human life [1]. The commonly recognized treatment for cancer includes radiation therapy, surgery, and chemotherapy. The combination therapy has manifested to prolong the survival rate of cancer patients to an appreciable extent. Despite various advances in the treatment adopted for cancer, still scientists are facing challenges which includes metastasis and recurrence [2, 3]. In the present scenario targeted therapy has been appreciated which affords greater survival rate promisingly.

Taking account of this a detailed knowledge at the signaling pathways that act at the molecular level in the development and prognosis of breast cancer would pave way for structured targeted therapy. Amidst various signaling pathways in breast cancer, Wnt signaling pathway has come to the fore as a propitious one. The Wnt signaling pathway is an essentially unchanged pathway through evolution that modulates the expression of diverse group of genes that are involved in cell proliferation and differentiation [4].

It is evident from previous studies that the genetic modifications connected with Wnt signaling pathway leads to the development and progression of breast cancer over a period of 10 years [5].

The Wnt signaling pathway could be classified into 2 types namely canonical and non-canonical pathways. The canonical Wnt pathway is also known as the Wnt/β-catenin signaling pathway. This pathway is primarily dependent on β-catenin which regulates the expression of various genes involved in the cell cycle, cell division, apoptosis etc. [6]. In the absence of Wnt ligands, β-catenin undergoes degradation by a multimeric protein complex consisting of axis inhibitor (Axin), adenomatous polyposis coli (APC), glycogen synthase kinase-3 β (GSK3β), protein phosphatase 2 A, and casein kinase 1 (CK1) thereby turning "off" the Wnt signaling pathway. Alternatively in the presence of Wnt ligands, the Wnt signaling pathway gets turned "on" and subsequently inhibits the activity of the destruction complex which leads to the stabilization of cytosolic β-catenin. The accumulated β-catenin thereafter enters the nucleus where along with T-cell factor (TCF)/lymphoid enhancer binding factors (LEF) family regulates the expression of the genes that encompass the stemness, proliferation and differentiation of a cell [7–10]. Wnt ligands are a large family of 19 secreted glycoproteins produced in the endoplasmic reticulum that relay signals from the extracellular environment to the cell via cell surface receptors [11]. Therefore targeting β-catenin could be a best choice to hamper the Wnt signaling pathway thereby inhibiting the growth and development of cancer.

Though enormous efforts have been taken to develop anticancer drugs that could prove potential enough, the drugs that are currently in use are not free from numerous drawbacks, the resistance developed against the drugs being the primmest one. Consequently there is an urgent necessity to develop a drug that surpasses the inadequacy of the current drugs.

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 [12] 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 [13]. 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 [14]. Few authors 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. 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.

Materials and methods

Collection of antimicrobial peptides

The sequences of antimicrobial peptides were retrived from Data base of Anuran Defense peptides (DADp) (link split4. pmfst.hr/dadp/) [15], Collection of Antimicrobial Peptides (CAMP) [16] and Antimicrobial Peptide Database (AMP) (http://aps.unmc.edu/ AP/main.php) [17].

Selection of antimicrobial peptides

One of the prime requisite of possessing anticancer property of the antimicrobial peptide is to have amino acid composition from 5-30 [18]. Accordingly 40 antimicrobial peptides which had 5-30 amino acids were selected for our study.

Screening for anticancer peptides

It has been proven in earlier studies that certain amino acid residues dominated in anticancer peptides when compared to non-anticancer peptides and antimicrobial peptides [18]. Therefore the selected peptides were then deeply studied for the composition of amino acids that they are made of. Four best peptides which showed maximum number of preferred amino acids were finally selected for further studies. Subsequently the anticancer properties of the selected antimicrobial peptides were further confirmed using the online Anti CP [https://webs.iiitd.edu.in/raghava/ anticp/ submission. php] web portal.

Molecular Docking

Studying the molecular interaction through molecular docking is an advantage to decipher molecular recognition like signaling, metabolism and disorder. Identified anticancer peptide and β-catenin receptor were docked using HPEPDOCK program. HPEPDOCK program is developed to facilitate the peptide-protein docking as user friendly [19]. Algorithm for peptide-protein docking is based on blind and user defined method. HPEPDOCK has been robustly describing the binding site in a protein by clustering and ranking approach. The algorithm utilizes a ranking system and request based clustering and cycles to predict binding affinity.

Results

Collection of antimicrobial peptides

Around 4000 antimicrobial peptides were collected from various repositories like Collection of Antimicrobial Peptides [CAMP], Antimicrobial Peptide Database [AMP] [http://aps.unmc.edu/AP/main.php], Database of Anuran Defense peptides [DADp] [link_split4.pmfst.hr/dadp/]. Subsequently from the collected peptides, we then selected 25 antimicrobial peptides in such a way that they possessed 5-30 amino acids. The antimicrobial peptides chosen for our study are listed out in Table-1.

Table 1: List of antimicrobial peptides and their sequences

S. No.	*Source*	Sequence
1.	*Ranaareolata*	GFISTVKNLA
2.	*Oreumenes decorates*	SLLSLIRKLIT
3.	*Myxinidin(P11)*	GIHDILKYGKAS
4.	*Myxinidin (S12)*	GIHDILKYGKPA
5.	*Litoria Rubella*	GLGDILGLDLGL
6.	*Litoria dahlia*	GLFDIIKNIVSTL
7.	*Crabrolin*	FLPLILRKIVTAL
8.	*Ranacatesbeiana*	FISAIASMLGKFL
9.	*Ranaridibunda*	FLKPLFNAALKLLP
10.	*Hypsiboas punctatus*	GILDAIKAIAKAAG
11.	*Polybiapaulista*	IDWLKLGKMVMDVL
12.	*Urodacusyaschenkoi*	GFWGKLWEGVKNAI
13.	*Odorranagraham*	GFSPNLPGKGLRIS
14.	*Litoriaaurea*	FDIVKKVVGALGSL
15.	*Grammistessex lineatus*	IGGIISFFKRLF
16.	*Harmoniaaxyridis*	IGGYCSWLRL
17.	*Anopliussamariensis*	GLLKRIKTLL
18.	*Orancistrocerusdrewseni*	ILGIITSLLKSL
19.	*Litoria infrafrenata*	GLLDALSGILGL
20.	*Mesobuthusmartensii*	FIGAVAGLLSKIF
21.	*Vespa bicolor*	INMKASAAVAKKLL
22.	*Polybiapaulista*	IDWLKLGKMVIDAL
23.	*Ranachensinensis*	ILPILSLIGGLLGK
24.	*Eumenesrubronotatus*	LNLKGIFKKVASLLT
25.	*MD4R*	GIHRILKYGKPS

Screening for anticancer peptides

Certain amino acid residues like Gly, Cys, Lys, Phe, Trp and Ile are observed to dominate others in anticancer peptides when compared to non-anticancer and anti-microbial peptides. Also it is observed that for an antimicrobial peptide to be an anticancer peptide, it should posses glycine or isoleucine in first position of N-terminal which is not specific and should posses leucine, lysine, alanine and phenyl alanine in C-terminal. Therefore all the collected antimicrobial peptides were then thoroughly studied for the composition of those amino acid residues. Details are shown in Table-2

Table 2: Percentage of amino acids with anticancer properties in each peptide

S. No.	*Source*	Sequence	% of preferred amino acids
1.	*Ranaareolata*	GFISTVKNLA	60
2.	*Oreumenes decorates*	SLLSLIRKLIT	64
3.	*Myxinidin(P11)*	GIHDILKYGKAS	58
4.	*Myxinidin (S12)*	GIHDILKYGKPA	58
5.	*Litoria Rubella*	GLGDILGLDLGL	92
6.	*Litoria dahlia*	GLFDIIKNIVSTL	62
7.	*Crabrolin*	FLPLILRKIVTAL	54
8.	*Ranacatesbeiana*	FISAIASMLGKFL	69
9.	*Ranaridibunda*	FLKPLFNAALKLLP	79
10.	*Hypsiboas punctatus*	GILDAIKAIAKAAG	86
11.	*Polybiapaulista*	IDWLKLGKMVMDVL	50
12.	*Urodacusyaschenkoi*	GFWGKLWEGVKNAI	50
13.	*Odorranagraham*	GFSPNLPGKGLRIS	36
14.	*Litoriaaurea*	FDIVKKVVGALGSL	57
15.	*Grammistessex lineatus*	IGGIISFFKRLF	83
16.	*Harmoniaaxyridis*	IGGYCSWLRL	50
17.	*Anopliussamariensis*	GLLKRIKTLL	80
18.	*Orancistrocerusdrewseni*	ILGIITSLLKSL	75
19.	*Litoria infrafrenata*	GLLDALSGILGL	83
20.	*Mesobuthusmartensii*	FIGAVAGLLSKIF	69
21.	*Vespa bicolor*	INMKASAAVAKKLL	71
22.	*Polybiapaulista*	IDWLKLGKMVIDAL	64
23.	*Ranachensinensis*	ILPILSLIGGLLGK	79
24.	*Eumenesrubronotatus*	LNLKGIFKKVASLLT	73
25.	*MD4R*	GIHRILKYGKPS	42

From the above results it is clearly observed that the peptides GLGDILGLDLGL (92%), GILDAIKAIAKAAG (86%), IGGIISFFKRLF (83%) and GLLDALSGILGL (83%), showed maximum percentage of the preferred amino acid residues. Therefore the above 4 peptides were then analysed for their anticancer property were confirmed.

Estimation of anticancer property and toxicity of the selected peptide

From the previous results we could infer that the 4 peptides GLGDILGLDLGL GILDAIKAIAKAAG, IGGIISFFKRLF and GLLDALSGILGL possessed maximum number of desired amino acids. These peptides GLGDILGLDLGL, GILDAIKAIAKAAG, IGGIISFFKRLF and GLLDALSGILGL were named as AAACP001, AAACP002, AAACP003 and AAACP004 respectively for convinience. Table-3 shows the details regarding the anticancer activity of the 4 peptides. The anticancer property of the selected 4 peptides were further evaluated using AntiCP[https://webs.iiitd.edu.in/raghava/anticp/submission.php] server. It was confirmed that out of the 4 peptides only AAACP003 possessed anticancer activity. Therefore AAACP003 was chosen for our further studies. So it could be inferred from these results that the selected peptide could be further evaluated for its mechanism of action to be recognized as a potential anticancer drug.

Table 3: Results obtained from the web server Anti CP

S. No	Name of the peptide	Peptide Sequence	Anti CP	
			Prediction	SVM score
1.	AAACP001	GLGDILGLDLGL	Non-Anticp	-0.01
2.	AAACP002	GILDAIKAIAKAAG	Non-Anticp	-0.43
3.	AAACP003	IGGIISFFKRLF	Anticp	1.11
4.	AAACP004	GLLDALSGILGL	Non-Anticp	-0.45

Protein-peptide interactions

The selected peptide was docked against the target protein β-catenin using HPEPDOCK method. HPEPDOCK is supporting blind docking method and hierarchical algorithm. Generally, it considers 100 cycle per docking for local conformation and dictates ranking system. The Table-4 shows the docking scores with RMSD value for interaction. Negative scores have been considered for best pose and interaction in most methods. Docking score of AAACP003 was found to be promising as it showed good affinity score with β-catenin protein. Consequently, interaction of peptide with protein is shown Figure 1.

Table 4: The peptide was docked with the target β-catenin receptor using HPEPDOCK web server.

S. No	Protein	Peptides	Peptide Sequences	Docking Score	RMSD
1	β-catenin	AAACP003	IGGIISFFKRLF	-196.582	62.103

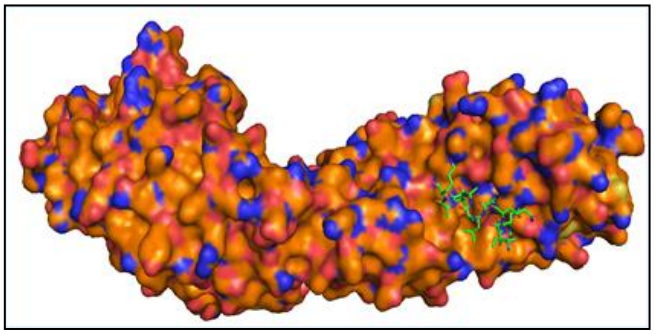

Fig 1: Docking of AAACP003 with β-catenin

Discussion

Miscellaneous therapeutic approaches for various types of cancers are under study presently. The Antimicrobial peptides have been recognized recently as one of the most efficient tool to be used against cancer [20]. The AMPs with anticancer activity known as anticancer peptides (ACP) are good enough in targeting the cancerous cells alone without affecting the normal cells [21]. These ACPs are able to recognize the dissimilarities prevailing between the malignant cells and the normal cells, hence attacks the cancer cells easily [21]. Consequently the ACPs can emerge as a potential anticancer agent for various types of cancers. In this context, we collected 5000 antimicrobial peptides from various web portals like Collection of Antimicrobial Peptides (CAMP) [16], Data base of Anuran Defense peptides (DADp) (link split4. pmfst.hr/dadp/) [15] and Antimicrobial Peptide Database (AMP) (http://aps.unmc.edu/ AP/main.php) [17]. The cytotoxic activity of a peptide depends not only on its structure but also on the length of the peptide [22]. Previous studies have proved that the ACPs are short peptides, which are made up of 5-30 amino acids only. Hence we chose 25 such peptides which were made up of 5-30 amino acid residues. Another crucial factor that decides the anticancer property of an AMP is its amino acid composition. Few amino acids like Ile, Gly, Cys, Trp, Lys and Phe dominate other amino acids in ACPs. In addition to that the N-terminal and the C-terminal amino acid composition of a peptide also plays a very important role in determining the cytotoxic activity of a peptide [18]. For that reason we comprehensively investigated the amino acid composition of all the 25 AMPs which we had collected for our study. We observed that 4 of the selected AMPs were made up of the preferred amino acid residues. Those peptides were named as AAACP001, AAACP002, AAACP003 and AAACP004. Consequently many bioinformatic tools have emerged latterly which are able to carry out the whole process accurately within a short span of time. Among the various bioinformatic tools available we chose Anti CP web portal for exploring the anticancer activity of the finally selected 4 peptides. Our results confirmed that out of the 4 peptides only AAACP003 was observed to possess anticancer activity. Finally we took the help of insilico approach for evaluating the targeting of the selected peptide against the target protein β-catenin. It was clearly evident from the docking results that the peptide AAACP005 had good binding score with β-catenin. The noncovalent interactions between the β-catenin and peptide-AAACP003 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.

Altogether it can be clearly inferred from the present study that the peptide AAACP005 may be considered as a prospective candidate for the treatment of breast cancer after careful evaluation of its effect through invitro and *in vivo* approaches.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A, *et al*. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018; 68(6):394-424.
2. Mantyh PW, Clohisy DR, Koltzenburg M, Hunt SP. Molecular mechanisms of cancer pain. Nature Reviews Cancer. 2002; 2(3):201-9.
3. Ghafoor A, Jemal A, Ward E, Cokkinides V, Smith R, Thun M, *et al*. Trends in breast cancer by race and ethnicity. CA: a cancer journal for clinicians. 2003; 53(6):342-55.
4. Clevers H, Nusse R. Wnt/β-catenin signaling and disease. Cell. 2012; 149(6):1192-205.
5. Khramtsov AI, Khramtsova GF, Tretiakova M, Huo D, Olopade OI, Goss KH, *et al*. Wnt/β-catenin pathway activation is enriched in basal-like breast cancers and predicts poor outcome. The American journal of pathology. 2010; 176(6):2911-20.
6. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases. Developmental cell. 2009; 17(1):9-26.
7. Rubinfeld B, Souza B, Albert I, Muller O, Chamberlain SH, Masiarz FR, *et al*. Association of the APC gene product with beta-catenin. Science. 1993; 262(5140):1731-4.
8. Su LK, Vogelstein B, Kinzler KW. Association of the APC tumor suppressor protein with catenins. Science. 1993; 262(5140):1734-7.
9. Behrens J, von Kries JP, Kühl M, Bruhn L, Wedlich D, Grosschedl R, *et al*. Functional interaction of β-catenin with the transcription factor LEF-1. Nature. 1996; 382(6592):638-42.
10. Bhanot P, Brink M, Samos CH, Hsieh JC, Wang Y, Macke JP, *et al*. A new member of the frizzled family from Drosophila functions as a Wingless receptor. Nature. 1996; 382(6588):225-30.
11. Komiya Y, Habas R. Wnt signal transduction pathways. Organogenesis. 2008; 4(2):68-75.
12. Harris F. On the selectivity and efficacy of defense peptides with respect to cancer cells. Medicinal Research Reviews. 2011; 29(6):1292–1327.
13. Schweizer F. Cationic amphiphilic peptides with cancer-selective toxicity. European journal of pharmacology. 2009; 625(1-3):190-4.
14. Deslouches B, Di YP. Antimicrobial peptides with selective antitumor mechanisms: prospect for anticancer applications. Oncotarget. 2017; 8:46635.
15. Novković M, Simunić J, Bojović V, Tossi A, Juretić D. DADP: the database of anuran defense peptides. Bioinformatics. 2012; 28:1406-7.
16. Waghu FH, Barai RS, Gurung P, Idicula-Thomas S. CAMPR3: a database on sequences, structures and signatures of antimicrobial peptides. Nucleic acids research. 2016; 44:D1094-7.

17. Wang G, Li X, Wang Z. APD3: the antimicrobial peptide database as a tool for research and education. Nucleic acids research. 2016; 44:D1087-93.
18. Tyagi A. In silico models for designing and discovering novel anticancer peptides. Scientific reports. 2013; 3:2984.
19. Zhou P, Jin B, Li H, Huang SY. HPEPDOCK: a web server for blind peptide–protein docking based on a hierarchical algorithm. Nucleic acids research. 2018; 46(W1):W443-50.
20. Mader JS, Hoskin DW. Cationic antimicrobial peptides as novel cytotoxic agents for cancer treatment. Expert opinion on investigational drugs. 2006; 15(8):933.
21. Hoskin DW, Ramamoorthy A. Studies on anticancer activities of antimicrobial peptides. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2008; 1778(2):357-75.
22. Koszałka P, Kamysz E, Wejda M, Kamysz W, Bigda J. Antitumor activity of antimicrobial peptides against U937 histiocytic cell line. Acta Biochimica Polonica. 2011, 58(1).