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SYSTEMATIC SCREENING FOR ANTICANCER ACTIVITY OF ANTIMICROBIAL PEPTIDES

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ABSTRACT

Cancer has become one of the predominant causes of elevated death rate globally. In spite of exhaustive research in devising new therapeutic approaches for treating cancer, regrettably the discomfort and the difficulties still persists. The unfocused action of anticancer drugs and the drug resistance developed by the cancer cells, prevail as the two most vital drawbacks of the current therapy. For this reason there is a frantic need for the optional targeted therapeutic approaches. Presently the Antimicrobial Peptides have come under the lime lights as anticancer agents. In our present study we collected antimicrobial peptides from various repositories. As the length of the peptide decides its anticancer property, we then selected 40 antimicrobial peptides with 5-30 amino acid residues. Those peptides were then thoroughly studied for their physico-chemical properties, following which 15 peptides with greater positive charge were selected. Further the 15 peptides were analysed for their amino acid composition and finally for our further studies we chose 6 best peptides namely NSAAACP001, NSAAACP002, NSAAACP003, NSAAACP004, NSAAACP005 and NSAAACP006. AntiCP and ToxinPred web portals were then made use of to confirm the anticancer property and toxic activity of the selected 6 peptides respectively and all the peptides were observed to be anti-cancerous and non-toxic in nature.

Key words: Cancer, Antimicrobial peptides, Anticancer peptides, Targeted therapy, AntiCP, ToxinPred

INTRODUCTION

Cancer devastates and shortens the life span of human beings world-wide. There is also an imbalance in the mortality to incidence ratio among urban and rural population. The most alarming fact is that there is an exponential increase in the number of affected individuals as per the forecast of this worrisome issue **[1]**.

As far as the treatment is concerned, varied approaches are followed for curing the aliment. Chemotherapy stands as a popular therapeutic approach. Nevertheless it faces a few hurdles like lacking target specific action against cancerous cells which eventually leads to the destruction of nearby healthy cells thereby causing deleterious side effects **[2, 3]**. On the other hand, immunotherapy, a forward looking approach for cancer therapy, which also induces a few post complications like toxicity and reverse autoimmunity fails to intrude into the target specific tissues on most of the occasions **[4]**. In addition to these demerits, modern day cancer therapy finds it difficult to deal with the development of multidrug resistance by the cancerous cells when they become metastatic **[5]**.

Consequently there is a desperate need for seeking a non-conventional therapy with an oncolytic agent as a prospective mode of targeted therapy against cancer cells. In this direction antimicrobial peptides (AMPs) can be considered as good candidates because of the fact that AMPs are proved to provide the required immunity against a wide range of pathogens. More importantly bacteria, fungi, plants and animals such as arthropods, fish, amphibians and mammals produce these antimicrobial peptides naturally. Few of such AMPs are found to be cationic and hydrophobic in nature **[6, 7]**. Furthermore, recently reported reviews on the anticancer activities, selectivity and efficacy of AMPs, particularly from animals, compliment in a great manner the notion of AMPs can be used as a novel oncolytic agent for the treatment of cancer **[8-11]**.

Anticancer peptides (ACPs), the antimicrobial peptides with anticancer properties, are small peptides containing 5 to 40 amino acids. Generally ACPs have low molecular weight and are cationic in nature at physiological pH. These ACPs are focused with great precision in their action and they can penetrate the walls of the tumor at ease. Moreover they can be easily synthesized in the lab also for bulk requirement. With the above mentioned features and also the ability to interact only with the cancerous cells leaving the healthy cells unaffected, the ACPs can be considered to be more vital

element in the realm of cancer treatment **[7]**. By invoking the abundance of antimicrobial peptides with anticancer property, designing of new drugs can be taken up for targeted therapy. Recently few ACPs have been clinically approved to be used as anticancer drugs, motivating the researchers to search for more such peptides with improved performance **[12]**.

The bioinformatic tools could be exploited for the prediction of anticancer property of the ACPs as they prove to be far more advantageous than the conventional methodologies presently under use. Few of the bioinformatic tools available are AntiCP **[7]**, Hajisharifi *et al*.'s method **[13]**, ACPP **[14]**, iACP **[15]**, Wang's method **[16]**, Li and iACP-GAEnsC **[17]**, MLACP **[18]**, SAP **[19]** and TargetACP **[20]**.

With this motivation we studied the anticancer property of the antimicrobial peptides by taking the aid of the bioinformatic tools which would prove as both time and cost effective along with more accurate prediction results.

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/) **[21]**, Collection of Antimicrobial Peptides (CAMP) **[22]** and Antimicrobial Peptide Database (AMP) (http://aps.unmc.edu/ AP/main.php) **[23]**.

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 **[24]**. Accordingly 40 antimicrobial peptides which had 5-30 amino acids were selected for our study.

Evaluating the physico-chemical properties of peptides

The physico-chemical properties of the antimicrobial peptides play a very important role in determining the anticancer activity of the respective peptide. Hence the physico-chemical properties of each antimicrobial peptide like their charge, hydrophobicity, hydrophilicity, amphipathicity, hydro-pathycity, net hydrogen and molecular weight were calculated using the AntiCP web portal. Thereafter depending upon their net positive charge 15 best peptides were chosen for further studies.

Screening for amino acid composition

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 **[7]**. Therefore the selected 15 peptides were then deeply studied for the composition of amino acids that they are made of.

Analysing the anticancer property and toxicity of the peptides

Six 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 AntiCP [https://webs.iiitd.edu.in/raghava/ anticp/ submission. php] web portal. Through the ToxinPred **[25]** web portal we checked their toxic effects also.

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 40 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**.

Evaluating the physico-chemical properties of peptides

A number of antimicrobial peptides possessing anticancer properties have been identified recently. The physico-chemical properties of these peptides ascertain its activity towards the cancerous cells. The values of the hydrophobicity, hydrophilicity, amphipathicity and the hydrophathycity of the peptides shows its penetrating capacity towards the cancerous cells. **Table 2** shows the details of the physico-chemical properties of the selected peptides.

The peptides with more of positive charge gets attracted towards the negatively charged cancerous cell membrane to a great extent specifically. Hence from the above results we collected 15 antimicrobial peptides with a greater net positive charge for our further studies. The peptides selected are listed in **Table 3**.

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 4**.

From the above results it is clearly observed that the peptides ALYKKFKKKLLKSLKRLG (77.7%), ALYKKFKKKLLKSLKRL (76.47%), KWKLFKKIPKFLHSAKKF (66.67), GLRKRLRKFRNKIKEKLKKI (65%), GRFKRFRKKFKKLFKKLS (61.11%) and KNLRRITRKIIHIIKKYG (61.11%) showed maximum percentage of the preferred amino acid residues. Therefore the above 6 peptides were then analysed for their toxic effects and their anticancer property were confirmed thereafter.

Estimation of anticancer property and toxicity of the selected peptide:

From the previous results, 6 peptides ALYKKFKKKLLKSLKRLG (77.7%), ALYKKFKKKLLKSLKRL (76.47%), KWKLFKKIPKFLHSAKKF (66.67), GLRKRLRKFRNKIKEKLKKI (65%), GRFKRFRKKFKKLFKKLS (61.11%) and KNLRRITRKIIHIIKKYG (61.11%) possessed maximum number of desired amino acids. These peptides ALYKKFKKKLLKSLKRLG, ALYKKFKKKLLKSLKRL, KWKLFKKIPKFLHSAKKF, GLRKRLRKFRNKIKEKLKKI, GRFKRFRKKFKKLFKKLS and KNLRRITRKIIHIIKKYG were named as NSAAACP001, NSAAACP002, NSAAACP003, NSAAACP004, NSAAACP005 and NSAAACP006 respectively. **Table 5** shows the details regarding the anticancer activity and the toxicity of the 6 peptides. The anticancer property of the selected 6 peptides were further evaluated and confirmed using AntiCP [https://webs.iiitd.edu.in/raghava/anticp/submission.php] server. It was confirmed that all the peptides possessed anticancer activity. Also with the help of ToxinPred web portal we analysed the toxicity of these selected peptides. None of the peptides showed to possess toxic effect. So it could be inferred from these results that all the selected peptides could be further evaluated for their mechanism of action to be recognized as a potential anticancer drug.

Table 1: List of antimicrobial peptides and their sequences

S. No.	PEPTIDE SEQUENCE	SOURCE
1:	MRTGNAN	*Escherichia coli*
2:	CTTCECCCSCS	*Streptomyces actuosus*
3:	KRIVQRIKDFLR	Synthetic construct
4:	HPLKQYWWRPSI	Synthetic construct
5:	FKRIVQRIKDFLR	Synthetic construct
6:	ILGKIWEGIKSLF	*Opisthacanthus madagascariensis*
7:	VRRFPWWWPFLRR	*Sus scrofa*
8:	ILAWKWAWWAWRX	Synthetic construct
9:	RLFDKIRQVIRKF	Synthetic construct
10:	GLFDIIKKIAESF	*Litoria raniformis*
11:	GLFDKLKSLVSDF	Synthetic construct
12:	GLFDIVKKLVSDF	Synthetic construct
13:	ILPWKWPWWPWRR	*Bos taurus*
14:	VAIALKAAHYHTHKE	*Homo sapiens*
15:	VGALAVVVWLWLWLW	*Bacillus brevis*
16:	KWKLFKKIGAVLKVL	Synthetic construct
17:	ALYKKFKKKLLKSLKRL	Synthetic construct
18:	KWCFRVCYRGICYRRCR	*Tachypleus tridentatus*
19:	KYYGNGVHCGKHSCTVDW	*Lactobacillus sakei*
20:	RGGRLCYCRRRFCVCVGR	*Sus scrofa*
21:	RGGGLCYCRRRFCVCVGR	*Sus scrofa*
22:	GFCRCLCRRGVCRCIC TR	*Macaca Mulatta*
23:	GRFKRFRKKFKKLFKKLS	*Bos taurus*
24:	RRWCFRVCYRGFCYR KCR	*Limulus polyphemus*
25:	ALYKKFKKKLLKSLKR LG	Synthetic construct
26:	KWKLFKKIPKFLHSAK KF	Synthetic construct
27:	RCVCRRGVCRCVCRR GVC	*Papin Anubis*
28:	ECRRLCYKQRCVTYCR GR	*Acanthoscurria gomesiana*
29:	KNLRRITRKIIHIIKKYG	Synthetic construct
30:	ZCRRLCYKQRCVTYCR GR	*Acanthoscurria gomesiana*
31:	AKKVFKRLEKLFSKIQ NDK	*Helicobacter pylore*
32:	FLSLIPHAINAVSAIAK HN	*Phyllomedusa hypochondriatis*
33:	ICIFCCGCCHRSKCGM CCKT	*Homosapiens*
34:	CTFTLPGGGGVCTLTS ECIC	*BacillusSp.strain H*
35:	VDKPPYLPRPPPPRRIY NNR	*Oncopeltus fasciatus*
36:	GLRKRLRKFRNKIKEK LKKI	*Oryctolagus cuniculus*
37:	ACAAHCLLRGNRGGY CNGKG	*Protophormia terraenovae*
38:	IIGPVLGLVGSALGGLL KKI	*Bombina Variegata*
39:	GSKKPVPIIYCNRRTGK CQRM	*Podisus maculiventis*
40:	GCRFCCNCCPNMSGCG VCCRF	*Moronechrysops×Morone Saxatitis*

Table 2: Physico-chemical properties of the selected peptides

Peptide Sequence	Hydrophobicity	Hydrophilicity	Amphipathicity	Hydropathycity	Net Hydrogen	Charge	Mol. Wt
MRTGNAN	0.32	-1.27	0.35	0.17	1.29	1	762.93
CTTCECCCSCS	-0.11	0.77	0.12	-0.29	0.45	-1	1142.4
KRIVQRIKDFLR	-0.48	-0.71	1.33	0.73	1.58	4	1572.11
HPLKQYWWRPSI	-0.19	-1.16	0.73	-0.56	1.08	2.5	1611.06
FKRIVQRIKDFLR	-0.4	-0.44	1.23	0.48	1.46	4	1719.3
ILGKIWEGIKSLF	0.11	0.78	0.66	-0.43	0.54	1	1504
VRRFPWWWPFLRR	-0.29	-0.79	0.75	-0.5	1.46	4	1902.46
ILAWKWAWWAWRX	0.08	0.06	0.47	-1.24	0.85	2	1829
RLFDKIRQVIRKF	-0.4	0.44	1.23	0.48	1.46	4	1719.3
GLFDIIKKIAESF	0.04	0.67	0.66	-0.03	0.54	0	1480.96
GLFDKLKSLVSDF	0.04	0.67	0.66	-0.03	0.54	0	1480.96
GLFDIVKKLVSDF	0.03	0.78	0.56	-0.08	0.54	0	1480
ILPWKWPWWPWRR	-0.13	-1.07	0.66	-0.89	1.15	3	1907.4
VAIALKAAHYHTHKE	-0.09	-0.21	0.86	-0.15	0.67	2.5	1689.1
VGALAVVVWLWLWLW	0.43	2.11	0	-1.55	0.27	0	1825.52
KWKLFKKIGAVLKVL	-0.09	0.28	1.31	-0.01	0.79	5	1672
ALYKKFKKKLLKSLKRL	-0.36	-0.61	1.66	0.59	1.18	8	2105
KWCFRVCYRGICYRRCR	-0.43	-0.52	0.94	0.01	1.47	6	2269
KYYGNGVHCGKHSCT VDW	-0.15	-0.78	0.57	-0.27	0.78	2	2054.55
RGGRLCYCRRRFCVCVGR	-0.43	-0.25	0.82	0.24	1.39	6	2161
RGGGLCYCRRRFCVCVTR	-0.32	-0.02	0.68	0.08	1.17	5	2105.79
GFCRCLCRRGVCRCICTR	-0.33	0.35	0.68	0.06	1.17	5	2106
GRFKRFRKKFKKLFKKLS	-0.53	-1.29	1.84	0.93	1.5	10	2343
RRWCFRVCYRGFCYRKCR	-0.51	0.83	1.02	0.14	1.61	7	2459
ALYKKFKKKLLKSLKRLG	0.33	-0.59	1.56	0.58	1.11	8	2163
KWKLFKKIPKFLHSAKKF	-0.23	-0.64	1.51	0.22	0.94	7.5	2275
RCVCRRGVCRCVCRRGVC	-0.44	0.22	0.82	0.33	1.33	6	2085
ECRRLCYKQRCVTYCRGR	-0.55	-1.06	1.02	0.59	1.56	5	2294
KNLRRITRKIIHIIKKYG	-0.36	-0.66	1.3	0.4	1.39	7.5	2251
ZCRRLCYKQRCVTYCRGR	-0.52	-0.87	0.95	0.33	1.5	6	2147
AKKVFKRLEKLFSKIQNDK	-0.39	-1	1.42	0.81	1.21	8	2321
FLSLIPHAINAVSAIAKHN	0.09	0.78	0.35	-0.63	0.53	2	2017
ICIFCCGCCHRSKCGMCCKT	-0.09	0.8	0.56	-0.35	0.55	3.5	2200
CTFTLPGGGGVCTLTS ECIC	0.1	0.94	0.06	-0.59	0.3	-1	1963
VDKPPYLPRPPPPRRIYNNR	-0.44	-1.69	0.67	0.44	1.25	4	2446
GLRKRLRKFRNKIKEKLKKI	-0.61	-1.47	1.84	1.23	1.65	10	2554
ACAAHCLLRGNRGGYCNGKG	-0.15	-0.31	1.36	0.11	0.85	7.5	2022
IIGPVLGLVGSALGGLLKKI	0.22	1.53	0.37	-0.58	0.25	2	1919
GSKKPVPIIYCNRRTGKCQRM	-0.37	-0.9	0.93	0.36	1.19	6	2436
GCRFCCNCCPNMSGCGVCCRF	-0.11	0.58	0.23	-0.43	0.62	2	2264

Table 3: List of peptides with more of positive charge

S. No	Peptide Sequence	Net Hydrogen	Charge
1.	FKRIVQRIKDFLR	1.46	4
2.	VRRFPWWWPFLRR	1.46	4
3.	ALYKKFKKKLLKSLKRL	1.18	8
4.	RGGRLCYCRRRFCVCVGR	1.39	6
5.	RGGGLCYCRRRFCVCVTR	1.17	5
6.	GFCRCLCRRGVCRCICTR	1.17	5
7.	GRFKRFRKKFKKLFKKLS	1.5	10
8.	RRWCFRVCYRGFCYRKCR	1.61	7
9.	ALYKKFKKKLLKSLKRLG	1.11	8
10.	KWKLFKKIPKFLHSAKKF	0.94	7.5
11.	RCVCRRGVCRCVCRRGVC	1.33	6
12.	KNLRRITRKIIHIIKKYG	1.39	7.5
13.	ZCRRLCYKQRCVTYCRGR	1.5	6
14.	AKKVFKRLEKLFSKIQNDK	1.21	8
15.	GLRKRLRKFRNKIKEKLKKI	1.65	10

Table 4: Percentage of amino acids with anticancer properties in each peptide

S. No.	Peptide Sequence	Percentage of amino acids with anticancer properties
1.	FKRIVQRIKDFLR	38.46
2.	VRRFPWWWPFLRR	15.38
3.	ALYKKFKKKLLKSLKRL	76.47
4.	RGGRLCYCRRRFCVCVGR	22.22
5.	RGGGLCYCRRRFCVCVTR	22.22
6.	GFCRCLCRRGVCRCICTR	22.22
7.	GRFKRFRKKFKKLFKKLS	61.11
8.	RRWCFRVCYRGFCYRKCR	11.11
9.	ALYKKFKKKLLKSLKRLG	77.78
10.	KWKLFKKIPKFLHSAKKF	66.67
11.	RCVCRRGVCRCVCRRGVC	11.11
12.	KNLRRITRKIIHIIKKYG	61.11
13.	ZCRRLCYKQRCVTYCRGR	16.67
14.	AKKVFKRLEKLFSKIQNDK	57.90
15.	GLRKRLRKFRNKIKEKLKKI	65.00

Table 5: Results obtained from the web server AntiCP and ToxinPred

S. No.	Name of the peptide	Peptide Sequence	ToxinPred		AntiCP	
			Prediction	SVM score	Prediction	SVM score
1.	NSAAACP001	ALYKKFKKKLLKSLKRLG	Non-toxin	-0.94	Anticp	0.64
2.	NSAAACP002	ALYKKFKKKLLKSLKRL	Non-toxin	-0.48	Anticp	0.63
3.	NSAAACP003	KWKLFKKIPKFLHSAKKF	Non-toxin	-0.09	Anticp	0.93
4.	NSAAACP004	GLRKRLRKFRNKIKEKLKKI	Non-toxin	-1.14	Anticp	0.39
5.	NSAAACP005	GRFKRFRKKFKKLFKKLS	Non-toxin	-1.11	Anticp	0.86
6.	NSAAACP006	KNLRRITRKIIHIIKKYG	Non-toxin	-1.04	Anticp	0.89

DISCUSSION

There is an urgent need to develop a novel therapeutic strategy which would overcome all the drawbacks present in the currently adopted approaches so that both the quality of the life of the patient as well as the survival rate of the patient improves dramatically. In the recent past the Antimicrobial peptides have been successful in attracting the researchers towards itself to be used as an anticancer agent. Most of the AMPs with anticancer property are found to be cationic in nature **[26]**. These anticancer peptides are greatly specific towards the malignant cells leaving the normal cells unhurt **[27]**. The cell membrane of the cancerous cell differs from the normal cell membrane in various aspects which drives the ACPs towards the cancerous cells easily **[27]**. Therefore the ACPs may be considered as an emerging anticancer agent for targeted therapy for various types of cancers. In view of this, we collected 4000 antimicrobial peptides from various web portals like Data base of Anuran Defense peptides (DADp) (link split4. pmfst.hr/dadp/) **[21]**, Collection of Antimicrobial Peptides (CAMP) **[22]** and Antimicrobial Peptide Database (AMP) (http://aps.unmc.edu/ AP/main.php) **[23]**. Only few of them which possess few properties specific to anticancer peptides show anticancer activity. It has been observed that all the AMPs do not show anticancer activity. Generally the shorter peptides with 5-30 amino acid residues are more anti-cancerous than the longer peptide **[28]**. Hence for the study 40 such peptides were selected with 5-30 amino acid residues . The physico-chemical properties of the AMPs like the hydrophobicity, hydrophilicity, amphipathicity and the hydropathycity decides the penetration capacity of the peptides. Therefore the physico-chemical properties of the peptides were studied using the Anti-CP web portal. All the selected peptides were found to

possess appreciable level of penetration property. Another most predominant factor that determines the anticancer activity of the AMPs is the net positive charge possessed by the peptide which directs the ACPs towards the malignant cells specifically which in turn are anionic in nature. Consequently 15 peptides with a good amount of net positive charge were selected for our further studies. Few of the amino acids dominate the others in the ACPs than in the AMPs and the Non-ACPs. In ACPs, the amino acids like Ile, Gly, Cys, Trp, Lys and Phe are observed frequently than the other amino acids **[7]**. Hence each peptide was then thoroughly analyzed for the presence of these unique amino acids and the percentage of these amino acids were calculated thereafter. We observed that out of the 15 peptides, 6 peptides showed appreciable quantum of preferred amino acids within them. Those peptides were named as NSAAACP001, NSAAACP002, NSAAACP003, NSAAACP004, NSAAACP005 and NSAAACP006. Conventional procedures through which the anticancer property of the peptide could be confirmed would be too laborious **[7]**. Subsequently many bioinformatic tools have now emerged which prove to be greatly advantageous than the traditional methodologies. Therefore the AntiCP web portal was made use of for confirming the anticancer properties of the selected peptides. The SVM score revealed the anticancer property of our peptides accurately. Further to confirm the non-toxic effect of the chosen peptides towards the normal cells, the ToxinPred web portal was made use of. All the 6 peptides were found to be non-toxic which was confirmed using their SVM score.

In a nutshell, NSAAACP001, NSAAACP002, NSAAACP003, NSAAACP004, NSAAACP005 and NSAAACP006 stands successful in every aspect of acting as a potential anticancer agent with additional efficiency and a side effect free drug which can be used courteously in cancer therapy. Along these lines further investigations like insilico, invitro and clinical trials may be continued in the near future.

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