Interactions and stability of tryptophan, proline and arginine rich peptides with EGFR kinase and its anticancer activities – insilico approaches

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Research Article

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Abstract

Lung cancer is a major public health concern due to its high prevalence and mortality, and it is also the second leading cause of cancer death worldwide. Non-small cell lung cancer accounts for more than 80% of lung cancers. In recent years, the use of bioactive peptides in cancer therapy has widened the treatment regimen. They have been confirmed to selectively target and destroy cancer cells while leaving healthy cells unaffected. Furthermore, antimicrobial peptides can cause cytotoxic activity in human cancer cells by binding to negatively charged phosphatidylserine moieties on the outer surface of cancer cell plasma membranes. In the present study, we predicted the interactions of tryptophan, proline, and arginine-rich peptides with EGFR kinase and evaluated the protein-peptide complex stability through molecular dynamics simulations. From the computational study, we selected the potential peptides based on the docking score, the number of hydrogen bonds formed between the protein-peptide complexes and the stability. These results suggest that antimicrobial peptides show potential for application in lung cancer therapies.

1. Introduction

Due to their clinical potential, antimicrobial peptides (AMPs) have piqued the interest of researchers, health professionals, and pharmaceutical companies in recent years. These peptides have a low molecular weight and exhibit a broad spectrum of antimicrobial and immunomodulatory activity against gram-positive and negative bacteria, viruses, and fungi. Additionally, these peptides exhibit enhanced efficacy, increased specificity, decreased drug interaction, low-level toxicity, direct attacking capabilities, and biological diversity. As a result, pharmaceutical companies are conducting clinical trials to determine the potential therapeutic value of these peptides [1]. By interacting with negatively charged phospholipids, cationic AMPs can destabilize microbial cell membranes. Additionally, several peptides can cause cytotoxicity in human cancer cells by binding to negatively charged phosphatidylserine moieties on the outer surface of cancer cell plasma membranes [2]. AMPs are a new source of anticancer drugs that are low in toxicity and have the potential to overcome tumor resistance to conventional chemotherapy [3].

Although the mechanism of action of host defense peptides is unknown, electrostatic attraction between negatively charged cancer cell components and positively charged AMPs is required for efficient binding and degradation of cancer cell membranes [4]. Natural AMPs are found in a wide range of organisms, are unstable with a short half-life, and have the potential to cause hemolysis. It is vital and essential to produce synthetic and long-lasting AMP analogues that overcome the inherent limitations of their natural peptides and also address potential issues for therapeutic candidates [5]. Reduced cholesterol levels in cancer cells may result in increased membrane fluidity, which aids AMP-induced apoptosis [6]. AMPs have obtained a lot of interest as anticancer drugs as they can selectively target cancer cells instead of healthy cells [7, 8].

Antimicrobial peptides containing tryptophan have been shown to improve cytotoxicity against non-small cell lung adenocarcinoma A549 cells [9]. Proline-rich AMPs (p1922) have been investigated in a primary

gingival fibroblast cell line and a squamous cancer cell line [10]. PR-39 is an antimicrobial peptide with a high proline-arginine content derived from neutrophils. It is a curly antimicrobial peptide with an irregular shape. It has a moderate inhibitory effect on tumour cells but a strong inhibitory effect on normal human embryonic kidney 293T cells [11]. The tryptophan residue on cell-penetrating peptides is critical for their entry into cancer cells, which involves an endocytic pathway and binding to the nuclear DNA's primary groove [12]. The epidermal growth factor receptor (EGFR), a member of the ErbB receptor tyrosine kinase (TK) family, plays an important role in the development and progression of non-small-cell lung cancer (NSCLC). Tyrosine kinase inhibitors (TKIs) targeting the human EGFR has now have a standard treatment regimen in the clinic for patients with advanced EGFR mutant NSCLC. Since EGFR-mutated tumors rely on EGFR activity to stimulate downstream signalling pathways, there is a chance that such cancers can be treated with peptide-based TKIs that block EGFR signalling [13]. We addressed significant advancements in the importance of tryptophan, proline, and arginine-rich AMPs as anticancer agents, as well as their binding affinity for the EGFR kinase and estimation of stable kinetics using molecular dynamics simulations. In addition, we did bilayer dynamics between the screened peptide and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylserine (POPS) to figure out stable conformations.

2. Materials And Methods

2.1 Peptides and Protein preparation

Previously, antimicrobial peptides with high tryptophan, proline, and arginine residues were collected and their three-dimensional structures evaluated. For this study, we used those three-dimensional peptide structures [14]. The crystal structure of the EGFR kinase domain protein was used as the target protein (PDB ID: 6ZJ0). The structure of the target protein was obtained from the Protein Data Bank (<https://www.rcsb.org/>).

2.2 Peptide-protein docking and simulations

The native structure of the EGFR kinase, with a resolution of 2.86, was obtained from the PDB. Before docking, the protein and peptide structures were prepared by removing water molecules and adding hydrogen atoms. For protein-peptide docking, the CASTp server is used to determine the target protein's binding sites [15]. Docking was performed using the ClusPro server [16, 17, 18, 19]. The LIGPLOT was used to visualize the hydrogen bond interaction between the protein and peptide [20]. Additionally, molecular dynamics calculations were performed using GROMACS [21]. A molecular dynamics simulation of the docked complex was performed for a time period of 50 ns using the CHARMM force field. Energy minimization was performed for 50K steps. Subsequently, 100 ps NVT and 100 ps NPT MD simulations at a temperature of 300 K were performed. XMGRACE software was used to make the RMSD, RMS fluctuation, and radius of gyration plots [22].

2.3 Peptide-lipid bilayer simulations

A coarse-grained molecular simulation was performed to evaluate the peptide-lipid interaction. In this study, we used POPS as a lipid model. The modelled peptides in the lipid bilayer are constructed using the CHARMM-GUI input generator [23, 24]. For coarse-grained molecular simulations, the MARTINI force field was used. VMD was used to visualise the molecular dynamics production run [25].

3. Result And Discussion

3.1 Interactions between the docked complexes

To evaluate peptide-protein interactions, we docked 41 antimicrobial peptides with the EGFR kinase to determine which peptides have a high affinity for binding and interaction with the EGFR kinase. The EGFR kinase binding sites are found between the residue numbers (716–876). Figure 1. Represent the EGFR kinase's binding pocket (orange colour). The binding score of the EGFR kinase against 41 antimicrobial peptides was predicted through docking studies in order to find a potent peptide molecule. The peptides Tet009, Retrocyclin3, Tet033 showing abundant hydrogen bond interactions with the target protein and the binding residues of the target protein with the potent peptides (Tet009, Retrocyclin3 and Tet033) were tabulated in Table 1.

Table 1
Scoring of docked models based on rank of EGFR kinase with selected peptides

Antimicrobial peptides	Sequence	ClusPro Scoring System with EGFR kinase	
		Center	Lowest
Apidaecin IA	GNNRPVYIPQPRPPHPRI	-1253	-1276.7
Apidaecin IB	GNNRPVYIPQPRPPHPRL	-884.7	-1059.9
Apidaecin II	GNNRPIYIPQPRPPHPRL	-1058.4	-1227.2
Astacidin 2	RPRPNYRPRPIYRP	-846.3	-1045.1
Combi2	FRWWHR	-1018.7	-1227.3
Bradykinin	RPPGFSPFR	-757.2	-805.2
Bucath4lc	RIRFPWPWRWPWRRVRG	-1088.8	-1222.3
Combi1	RRWWRF	-1059.8	-1211
cyclic_dodecadopeptide	RICRIIFLRVCR	-908.3	-1018.1
Idr-1010	IRWRIRVWVRR	-1144.2	-1281.9
hlf	GRRRRSVQWCA	-786	-895.2
Formaecin1	GRPNPVNNKPTPHPR	-815	-898.5
Formaecin2	GRPNPVNTKPTPYPR	-891.1	-1160.4
inverso	CKRWWKWIRW	-944.7	-1084
metalkowinIIA	VDKPDYRPRPWPRPN	-684.5	-860.1
metalkowinIII	VDKPDYRPRPWPRPNM	-684.5	-860.1
Paf26	RKKWFW	-885.6	-1007.2
Retrocyclin3	RICRCICGRRICRCICGR	-1237.6	-1569.9
tet003	RLARIVPIRVAR	-917.2	-1107.1
Tet008	RRWRIVVIRVRR	-1053.5	-1355.6
Tet009	RRWKIVVIRWRR	-945.2	-1062.2
Rlid12.1	TPPQS	-484.2	-484.2
Rtd2	GVCRCCLCRRGVCRCCLCRR	-1051.7	-1321.8
Tet013	VRFRIRVAVIRA	-839.9	-963.1
Tet024	RRRWIVVIWRRR	-1292	-1524
Tet032	RRWRRWWRRWRR	-1064.4	-1160.2

Antimicrobial peptides	Sequence	ClusPro Scoring System with EGFR kinase	
		Center	Lowest
Tet033	RRRWWWRWWRR	-957	-1044.2
Tet037	IVRVAVALRRIR	-796.3	-999.9
Tet052	RRAAVVLIVIRR	-964.8	-1010.7
Tet091	ILKWKWPWWPWRR	-1136	-1309.5
Tet092	FIKWKFRWWKWRK	-1013.5	-1072.6
Tet099	FIRWRFRWWRWRR	-1212.3	-1377.6
Bactenin	RLCRIVVIRVCR	-1026.9	-1288.1
PP30	YVPPVQKPHPNGPKFPTFP	-881.5	-1054.2
Pac-525	KWRRWVRWI	-874.1	-921.8
Tet110	RWRWWRWRR	-992	-1149.1
Tet112	KWKWWKWKK	-784.7	-890.3
Tet124	KLWWMIRRW	-942.7	-998.6
Tet127	KRWWKWWRR	-901.5	-1010.8
Tet213	KRWWKWWRRC	-970.2	-1059.3
TetraF2K-RK	WWWLRLKIW	-809.4	-938.3

Computational protein-peptide docking studies identify the top scored binding of the given antimicrobial peptides with EGFR kinase. The protein-peptides (Tet009, Retrocyclin3 and Tet033) complex got the highest docking score, which shown in Table 1. Figure 2. Three potent antimicrobial peptides against EGFR kinase. After docking, the EGFR kinase protein shows 17 hydrogen bonds with Tet009, Retrocyclin3 and 16 hydrogen bonds with Tet033. Figure 3.A shows the hydrogen bond interactions with bond length between the EGFR kinase- Tet009, Fig. 3.B shows the hydrogen bond interactions with bond length between the EGFR kinase- Retrocyclin3, Fig. 3.C shows the hydrogen bond interactions with bond length between the EGFR kinase- Tet033. In summary, the docking results concluded that the three potent peptides (Tet009, Retrocyclin3 and Tet033) shows good binding with EGFR kinase protein. Based on the binding sites, we found Rectrocyclin3 and Tet033 is perfectly binds with the target protein.

3.2 Stable conformation of docked complexes

The stability of the docked peptide (Tet009, Retrocyclin3 and Tet033) - EGFR kinase complexes was further investigated by performing molecular dynamics (MD) simulations of the complexes in an explicit water box at 300 K for a period of 50 nanoseconds. Exploration on RMSD parameters produces complete structural information in understanding the conformational stability of protein-peptide complexes. The

root mean square deviation of the complex structures shows the range from 0.1 to 1.5 Å. To examine the stability of the docked complexes, Fig. 4.A shows the root mean square deviation of the docked complexes, black colour RMSD indicates the plain structure. Green colour denotes the EGFR kinase-tet009, red colour denotes the EGFR kinase- Retrocyclin3 and navy blue denotes the EGFR kinase- tet033. Based on the RMSD plot, EGFR kinase- Retrocyclin3 is more stable than EGFR kinase- tet009 and EGFR kinase- tet033. At the time of the simulation, from 15ns parallel deviations was observed between the EGFR kinase- Retrocyclin3 and EGFR kinase- tet033 complexes. Higher RMSD value of EGFR kinase-tet009 shows the unstable structure throughout the simulations. As the further step to conform the backbone stability, RMS fluctuation and compactness analysis was carried out.

Figure 4.B portrays the RMS fluctuation of atomic position. As observed, the peak values lies between 0.1 to 3.0 nm. Similarly, RMS fluctuation of atom positions shows that EGFR kinase- Retrocyclin3 and EGFR kinase- tet033 lesser fluctuations pattern than EGFR kinase- tet009. Both RMSD and RMSF results, indicate EGFR kinase- Retrocyclin3 and EGFR kinase- tet033 complexes were stable throughout the simulations. The radius of gyration (Rg) represents the compactness of the protein and protein-peptide complexes. Comparative analysis between the protein and protein-peptide complexes was performed to elucidate the compactness, for the period of 50ns. Figure 4.C shows the radius of gyration between the complexes. EGFR kinase- Retrocyclin3 and EGFR kinase were found similar deviation pattern in the radius of gyration plot. From the plot, we interpreted EGFR kinase- Retrocyclin3 shows the least Rg value from 2.2nm and decrease to the lesser value of 2.05 nm. From the results of RMSD, RMS fluctuations and Rg clearly interpret that EGFR kinase- Retrocyclin3, EGFR kinase- Tet033 is more stable over the simulation. Figure 4.D shows the hydrogen bonds plot formed during the 50ns simulations, in comparison of EGFR kinase-peptide complexes, EGFR kinase- Tet033 and EGFR kinase- Retrocyclin3 has the highest number of hydrogen bonds formed. This study shows retrocyclin3 and tet033 as a potent anticancer drug for further investigation.

3.3 Stable peptides on lipid bilayers

The coarse-grained dynamics simulations explained the peptide aggregation and stability in the lipid bilayer environment. The three peptides (Tet009, Retrocyclin3 and Tet033) was placed over the POPS lipid bilayer along Z axis 6 angstrom using CHARMM-GUI server. Both peptide-lipid bilayer system was ionized with a suitable amount of 0.15 M of NaCl buffer. Distance ions replacing method was used to faster the ions placement. The final coarse-grained models of POPS lipid bilayer containing different peptide-lipid were subjected to at least 500 ns of CG MD simulations at 310 K. The total simulation time is up to 500ns, the snapshot was saved every 100 ns for the follow-up analysis. In this section, we compared the results found for Tet009, Retrocyclin3 and Tet033 peptides in their interaction with POPC lipid structure. In each of simulations, the peptides started to interact with the POPS bilayers at 100s. After 100ns of martini coarse grained simulations, we have observed the peptides anchoring to the bilayers and internalization of peptide starts from 300ns.

In addition, the Arginine residues are shown in red colour, Proline residues shown in black colour and Tryptophan residues show in pink colour. Cysteine and isoleucine indicate the yellow and green colour

respectively in Fig. 5. In this study, we found that the peptides (Tet009, Retrocyclin3 and Tet033) were showing stable conformations with the bilayers till 500ns. In summary, peptides (Retrocyclin3 and Tet03300) show it preference to stable internalization with the POPS bilayer from 200ns onwards.

4. Conclusion

For this study, we chose 41 previously published antimicrobial peptide structures. The 41 antimicrobial peptides docked with EGFR kinase and the interaction, stability of the protein-peptide complex were determined using molecular docking and dynamics simulations. In addition, we used coarse-grained molecular dynamics simulation to predict the molecular interactions of three antimicrobial peptides with POPS lipid structures. Based on the docking score system, the number of hydrogen bonds formed between the protein-peptide complexes, and the protein-peptide stability, we chose three peptides (Tet033, Tet009, Retrocyclin 3) from the computational docking and molecular dynamics studies. It is worth noting that molecular dynamics simulation studies show that EGFR kinase-Retrocyclin 3 and EGFR kinase-Tet033 complexes have good stability. These findings suggest that Retrocyclin 3 and Tet033 have the potential to be used in lung cancer therapies.

Declarations

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Conflicts of interest / competing interests

Author declares no conflicts of interest / competing interests

Availability of data and material

All the data are available upon request

Code availability

Used open source software's

Authors' contributions

SKM SL devised the work, methodology, investigation, writing original draft, review, editing, and project administration. KN SP participated in the manuscript preparation.

References

1. Boparai JK, Sharma PK (2019) Mini Review on Antimicrobial Peptides, Sources, Mechanism and Recent Applications. *Protein Pept Lett* 27:4–16.
<https://doi.org/10.2174/0929866526666190822165812>.
2. Tornesello AL, Borrelli A, Buonaguro L, et al (2020) Antimicrobial Peptides as Anticancer Agents: Functional Properties and Biological Activities. *Molecules* 25.
<https://doi.org/10.3390/molecules25122850>.
3. Housman G, Byler S, Heerboth S, et al (2014) Drug resistance in cancer: An overview. *Cancers (Basel)*. 6:1769–1792. <https://doi.org/10.3390/cancers6031769>.
4. Scocchi M, Mardirossian M, Runti G, Benincasa M (2015) Non-Membrane Permeabilizing Modes of Action of Antimicrobial Peptides on Bacteria. *Curr Top Med Chem* 16:76–88.
<https://doi.org/10.2174/1568026615666150703121009>.
5. Lei J, Sun LC, Huang S, et al (2019) The antimicrobial peptides and their potential clinical applications. *Am. J. Transl. Res.* 11:3919–3931.
6. Beloribi-Djefafli S, Vasseur S, Guillaumond F (2016) Lipid metabolic reprogramming in cancer cells. *Oncogenesis* 5:e189–e189. <https://doi.org/10.1038/oncsis.2015.49>
7. Hoskin DW, Ramamoorthy A (2008) Studies on anticancer activities of antimicrobial peptides. *Biochim. Biophys. Acta - Biomembr.* 1778:357–375 <https://doi.org/10.1016/j.bbamem.2007.11.008>.
8. Kunda NK (2020) Antimicrobial peptides as novel therapeutics for non-small cell lung cancer. *Drug Discov. Today* 25:238–247. <https://doi.org/10.1016/j.drudis.2019.11.012>.
9. Conlon JM, Mechkarska M, Prajeep M, et al (2013) Transformation of the naturally occurring frog skin peptide, alyteserin-2a into a potent, non-toxic anti-cancer agent. *Amino Acids* 44:715–723.
<https://doi.org/10.1007/s00726-012-1395-7>
10. G. Radicioni, A. Stringaro, A. Molinari, G. Nocca, R. Longhi, D. Pirolli, E. Scarano, F. Iavarone, B. Manconi, T. Cabras, I. Messina, M. Castagnola, A. Vitali, Characterization of the cell penetrating properties of a human salivary proline-rich peptide, *Biochim. Biophys. Acta - Biomembr.* 1848 (2015) 2868–2877. <https://doi.org/10.1016/j.bbamem.2015.08.019>.
11. Veldhuizen EJA, Schneider VAF, Agustiandari H, et al (2014) Antimicrobial and Immunomodulatory Activities of PR-39 Derived Peptides. *PLoS One* 9:e95939.
<https://doi.org/10.1371/journal.pone.0095939>.
12. Jeon H, Le MT, Ahn B, et al (2019) Copy number variation of PR-39 cathelicidin, and identification of PR-35, a natural variant of PR-39 with reduced mammalian cytotoxicity. *Gene* 692:88–93.
<https://doi.org/10.1016/j.gene.2018.12.065>

13. Lin SY, Chang Hsu Y, Peng YH, et al (2019) Discovery of a Furanopyrimidine-Based Epidermal Growth Factor Receptor Inhibitor (DBPR112) as a Clinical Candidate for the Treatment of Non-Small Cell Lung Cancer. *J Med Chem* 62:10108–10123. <https://doi.org/10.1021/ACS.JMEDCHEM.9B00722>.
14. Marimuthu SK, Nagarajan K, Perumal SK, et al (2020) Structural stability of antimicrobial peptides rich in tryptophan, proline and arginine: a computational study. *J Biomol Struct Dyn*. <https://doi.org/10.1080/07391102.2020.1848631>
15. Tian W, Chen C, Lei X, et al (2018) CASTp 3.0: computed atlas of surface topography of proteins. *Nucleic Acids Res* 46:W363–W367. <https://doi.org/10.1093/NAR/GKY473>.
16. Desta IT, Porter KA, Xia B, et al (2020) Performance and Its Limits in Rigid Body Protein-Protein Docking. *Structure* 28:1071-1081.e3. <https://doi.org/10.1016/J.STR.2020.06.006>.
17. Vajda S, Yueh C, Beglov D, et al New Additions to the ClusPro Server Motivated by CAPRI. <https://doi.org/10.1002/prot.25219>
18. Kozakov D, Hall DR, Xia B, et al (2017) The ClusPro web server for protein–protein docking. *Nat Protoc* 12:255–278. <https://doi.org/10.1038/nprot.2016.169>.
19. Kozakov D, Beglov D, Bohnuud T, et al How Good is Automated Protein Docking? <https://doi.org/10.1002/prot.24403>
20. Wallace AC, Laskowski RA, Thornton JM (1995), LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions, *Protein Eng.* 8. 127–134. <https://doi.org/10.1093/PROTEIN/8.2.127>.
21. Spoel DVD, Lindahl E, Hess B, Groenhof G, et al (2005) GROMACS: fast, flexible, and free, *J. Comput. Chem.* 26 (2005) 1701–1718. <https://doi.org/10.1002/JCC.20291>.
22. Turner: XMGRACE, Version 5.1. 19
23. Qi Y, Ingolfsson HI, Cheng X, et al (2015) CHARMM-GUI Martini Maker for Coarse-Grained Simulations with the Martini Force Field. *J Chem Theory Comput* 11:4486–4494. <https://doi.org/10.1021/ACS.JCTC.5B00513>.
24. Hsu P-C, Bruininks BMH, Jefferies D, et al (2017) CHARMM-GUI Martini Maker for modeling and simulation of complex bacterial membranes with lipopolysaccharides. *J Comput Chem* 38:2354–2363. <https://doi.org/10.1002/JCC.24895>
25. Humphrey W, Dalke A, Schulten K (1996) VMD: visual molecular dynamics. *J Mol Graph* 14:33–38. [https://doi.org/10.1016/0263-7855\(96\)00018-5](https://doi.org/10.1016/0263-7855(96)00018-5)

Figures

Figure 1

Binding pocket of the EGFR kinase

Retrocyclin 3

Tet009

Tet033

Figure 2

Potent AMP structure against EGFR kinase

Figure 3

A Hydrogen bond interactions between the EGFR kinase- Tet009, B Hydrogen bond interactions between the EGFR kinase- Retrocyclin3, C Hydrogen bond interactions between the EGFR kinase- Tet033.

Figure 4

A. Root mean square deviation plot of EGFR kinase-peptide complexes, B. Root mean square fluctuation plot of EGFR kinase-peptide complexes, C. Radius of gyration of EGFR kinase-peptide complexes.

Figure 5

Snapshots of side views of peptide structure anchoring to the POPS bilayer during the simulations at 100 ns intervals.

Supplementary Files

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