In Silico Protein- Protein Interaction of Pterois Volitans Venom With Cancer Inducers of Helicobacter Pylori

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Research Article

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Abstract

Gastric cancer is a pathological condition induced by bacteria *Helicobacter pylori*. Targeting the key virulence factors of *H.pylori* causing gastric cancer is one such promising method for treating gastric cancer. Recently research has been focussed on analysing the adrenergic, cholinergic and anticancer properties of their venom proteins. Testing the anticancer activity of the lethal proteins in the venom of *P.volitans* not only provides a bioactive compound for cancer treatment but is also helpful to eliminate the ecological imbalance caused by this fish in marine environment. This study is focused on an in silico approach using Z-dock for analysing the bioactive prospective of the venom proteins of *P.volitans* against the key virulence proteins of *H.pylori* responsible for inducing cancer. Our in silico docking study using computational model of the venom proteins and *H.pylori* proteins has displayed the possible interactions between these proteins. The results revealed that the venom proteins of *P.volitans* hyaluronidase and PV toxin effectively interacts with *H.pylori* proteins Cag A, Cag L, GGT, Cag D and Urease and may be promising proteins in cancer therapy.

1. Introduction

The human body possesses around ten times more the microbial cells than human cells with a majority of them located in the human gut (1). The human gut serves as a natural habitat for several bacteria with vast quantities of nutrients available for their sustenance. An approximated estimate of 300 to 500 bacterial species inhabits the human gut and they may pose either beneficial or harmful effects on the host physiology and pathology (2). Apart from their beneficial role, certain bacteria are also liable for causing pathological conditions such as organ failure, cancer and bowel disease. The global cancer observatory reported that next to lung cancer, gastric cancer has the highest mortality rate (3, 4). With an average survival rate over a 5-year period of less than 20%, the prognosis is lamentable (5). Although there are various contributing factors to gastric cancer, infection via the *Helicobacter pylori* is said to be one of the major causes of the malignancy. In fact, *H.pylori* has been classified as a class 1 human carcinogen causing gastric cancer (6). This infectious agent generates reactive oxygenic species (ROS) capable of causing DNA mutations that may have carcinogenic effects. Although several treatment options like chemotherapy, immunotherapy, radiation therapy and surgery have been employed for treating gastric cancer, the problem of relapsed cancer remains irresolvable. Recent research data has provided more insight into the biological therapy for the treatment of gastric cancer. Targeting the key virulence factors of *H.pylori* that cause of cancer is one promising method in treating gastric cancer.

The enormous chemical diversity in the millions of plants, animals, marine species and micro-organisms has enabled natural products to be recognised as one of the most effective therapeutic candidates in treating malignancies (7). Marine organisms are one of the richest sources of natural products. Studies on marine organisms have revealed that fish venom is a source of various potent compounds possessing pharmacological significance in targeting vital processes of the body. However, there is less research focuses on this resource due to the difficulty in collecting the species and maintaining the stability of the proteins. The structural elucidation of the compounds and its synthesis are some of the challenging

factors. In fact, many compounds derived from marine organisms are well known for their cytotoxic properties against several types of malignancies.

Pterois volitans is a red lion fish native to the Indian and Western Pacific oceans. Their impressive growth rate combined with the absence of natural predators have resulted in their rapid breeding. There is a possibility of this lionfish invading the oceans and seas which can result in the disrupt the aquatic life, thus rising an ecological concern (8). Studies have reported the adrenergic, cholinergic and anticancer properties in their venom proteins (9, 10). Hence, analysing the pharmacological significance of this lionfish will not only result in a therapeutic agent but also reduce its invasion of the aquatic environment.

2. Materials And Methods

2.1. Retrieval of Protein Structures

Several studies have reported the role of the *H. pylori* proteins Cag A, GGT, Cag L, Cag D and urease in inducing gastric cancer (11, 12, 13, 14, 15, 16) and hence these proteins were particularly targeted during this study. The 3D structure of the proteins Cag A, GGT, Cag L, Cag D and urease were retrieved from RCSB PDB archive with the PDB ID: 3IEC, 3FNM, 4YVM, 3CWX and 1E9Y respectively (Fig:1i). The 3D structures of large biological molecules, including proteins and nucleic acids are available in the Protein Data Bank (PDB) archive. The lead compounds taken in this study, hyaluronidase and PV toxin-a are the venom proteins of *P. volitans*. The 3D structure of the lead compounds in this study is a predicted model obtained from the Swiss model repository (SMR). The Swiss model repository is a database that predicts the 3D structure of proteins based on homology modeling (17) Stefan et al 2016). The 3D structure of Hyaluronidase and PV toxin-A with the Uniprot ID K7ZMF5, F2ZAF0 respectively, were built based on the target-template alignment using ProMod3 (Fig. 1ii).

2.2. Preparation of Proteins:

The 3D structures of all proteins taken in the study are processed in the clean protein tool. The clean protein tool in Discovery Studio is used for correcting the 3D crystal structure of the proteins. It is used for inserting missing atoms in incomplete residues, modelling missing loop regions, deleting alternate conformations (disorder), optimizing side-chain conformations, removal of water, bond orders and missing side chains. It is also used to protonate the structure at the specified pH. Further energy minimization of the 3D structures was performed with charm M force field for 200 steps using smart minimizer algorithm (Table 1). Energy minimization is performed on structures prior to dynamics to relax the conformation and remove steric overlap that produces bad contacts.

Table 1
Energy minimization of proteins.

Name	Force field	Initial RMS Gradient (kcal/(mol x A)	Final RMS Gradient (kcal/(mol x A)
GGT	CharmM	148.9206	0.38648
Cag A	CharmM	115.73561	0.48607
Cag L	CharmM	58.16117	0.41586
Cag D	CharmM	541.33526	0.33297
Urease	CharmM	5886.11856	0.41106
Hyaluronidase	CharmM	36.1155	1.25262
PVtoxin-a	CharmM	74.6023	1.11856

2.3. Docking studies

Docking studies were performed by Z-dock through Discovery Studio version 2.5. All obtained docked protein complexes were analysed for their binding efficacy with the same software. On docking the energy minimized *H. pylori* proteins against hyaluronidase and PV toxin-a, 3600 docked poses were generated for each complex. Z-dock scored each pose based on shape complementarity, electrostatics and statistical potential to increase docking accuracy for better analysis (18). The top-docked complex was taken for analysing the binding efficacy of the proteins.

3. Results And Discussion

Z-docking reported 3600 docked poses for all of the inputted protein pairs. The interface cut-off distance was set at 6 with a surface area probe radius of 0.6. The maximum hydrogen bond distance, weak hydrogen bond distance and salt bridge distance parameters were set at 3.4, 3.8 and 4.0 respectively.

The docked proteins were analysed for their interactions and the resulting interactions are reported in Tables 2i and 2ii. The docked poses of the various protein complexes are given in Fig. 2i.

Table 2
i: Interaction of Hyaluronidase with the *H.pylori* proteins.

	Cag A	Cag L	GGT	Cag D	Urease
H- Bond interactions	36	7	7	17	12
Pi interactions	10	3	3	3	2
Salt bridge interactions	5				2

Table 2
ii: Interaction of PV toxin-a with the *H.pylori* proteins.

	Cag A	Cag L	GGT	Cag D	Urease
H- Bond interactions	16	29	7	8	57
Pi interactions	4		1	5	8
Salt bridge interactions		1			6

Protein-protein interaction depends upon the formation of a bond between the proteins. The binding of proteins involves the formation of weak non-covalent bonds, hydrogen bonds, ionic bonds, Vander Waal's and hydrophobic interactions. One protein can bind with another protein by the specific affinity of one rigid surface with that of another (19). There is a change in the structure of proteins when they bind with each other, and these changes play an important role in altering the cell regulatory process. The results of this study show that the interaction of the *H. pylori* proteins is more with the venom protein Hyaluronidase than that of the PV Toxin-a of *P. volitans*.

When few bonds form between proteins, they dissociate rapidly but when there is an increase in the number of bonds the interactions are stronger (20). It has been observed in this study that Cag A protein has a stronger interaction with the venom protein Hyaluronidase with 51 bonds (Fig. 2i). The 266th Lysine in the B chain of the Cag A protein forms an H-bond interaction at a distance of 1.6 Å with the 273rd Methionine in the A chain of the venom protein Hyaluronidase. The 953rd Histamine in the E chain of the Cag A protein forms a Pi interaction at a distance of 3.6 Å with the 214th Proline in the A chain of the venom protein Hyaluronidase. The 955th Lysine in the B chain of the Cag A protein forms a salt bridge interaction at a distance of 1.78 Å with the 193rd Aspartic acid in the A chain of the venom protein Hyaluronidase. Similarly, 35 other H-bond interactions, 9 other Pi interactions and 4 other salt bridge interactions have been observed within a maximum distance of 2.9 Å, 5.4 Å and 2.6 Å respectively.

Likewise, the urease protein has a stronger interaction with the venom protein PV toxin-a with 71 bonds (Fig. 2ii). It has been observed that the 22nd Arginine in the B chain of the Urease protein forms an H-bond at a distance of 1.7 Å with the 110th glutamic acid in the A chain of venom protein PV-Toxin a. The 60th asparagine in the B chain of the urease protein forms a Pi interaction at a distance of 2.3 Å with the 196th phenylalanine in the B chain of the venom protein PV toxin-a. The 1st methionine in B chain of the Urease protein forms a salt bridge interaction at a distance of 1.8 Å with the 167th aspartic acid in A chain of the venom protein PV-toxin a. Similarly, 56 other H-bond interactions, 7 other Pi interactions and 5 more salt bridge interactions are observed within a maximum distance of 2.8 Å, 5.2 Å and 2.7 Å respectively.

4. Conclusion

Since ancient times, various therapeutic agents produced by nature are recognised for their pharmacological properties. With better knowledge of the natural producers derived from marine

resources, studies have continued to produce novel drugs from the sea. In fact, the marine world has become a significant source of compounds possessing anticancer properties. Several reports have published the promising potential of the anticancer activity in marine products. The probability of the next decade witnessing an increase in the number of marine drugs for cancer treatment is extremely high. Both hyaluronidase and PV toxin-a have exhibited standard interactions with all the *H.pylori* proteins that were used in this study. It has been observed that the *H.pylori* protein Cag A and Urease had the most interaction with both the venom proteins. A deeper analysis on the *invivo* and *invitro* validation is needed to authenticate the in-silico results obtained via this study. This study was conducted with the hope that the computer-based structural interaction analysis of proteins could play a vital role in cancer treatment. The results of this study have led to the intriguing possibility that the interaction of venom proteins with key enzymes of *H. pylori* may possibly inhibit the cascade involved in gastric carcinogenesis. It may be concluded that the possibility of utilising venom proteins for gastric cancer treatment appears promising and will potentially open more opportunities for various cancer therapies.

Declarations

Ethical Approval- Not applicable

Consent to Participate- Not applicable

Consent to Publish- Not applicable

Authors Contributions- Guru Nivetha Ravi: Data curation, formal analysis, investigation, methodology, writing- original draft. Irfan Navabshan: Conceptualization, formal analysis, investigation, methodology. Sneha Unnikrishnan: Data curation, formal analysis, writing- original draft. Karthikeyan Ramalingam: Conceptualization, Supervision; Validation; Writing - review & editing.

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Availability of data and materials- Not applicable

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Figures

A

B

Figure 1

i: Cartoon representation of *H. pylori* proteins (A) cag A, (B) GGT, (C) cag L, (D) cag D and (E) urease ii: Cartoon representation of venom proteins of *P. volitans* (A) hyaluronidase and (B) PV toxin-a

Figure 2

i.: The docked poses of protein complexes (A) cag A and hyaluronidase (B) cag A and PV toxin-a (C) GGT and hyaluronidase (D) GGT and PV toxin-a (E) cag L and Hyaluronidase (F) cag L and PV toxin-a (G) cag D and hyaluronidase (H) cag D and PV toxin-a (I) urease and hyaluronidase (J) urease and PV toxin-a ii. Graphical representation for comparing the bonds formed by hyaluronidase and PV toxin-1 with *H. pylori* proteins