Invitro And Insilico Analysis Of The Marine Seaweed-Derived Compound Fucoidan Against EMT Markers

Sivaranjani Ganapathy, Shafna Asmy VS, Jeyakumar Natarajan, Uthra Selvaraj, Somasundaram Thirugnanasambandam Somasundaram

Abstract: In the current study, fucoidan from marine seaweed Turbinaria conoides were investigated for its cytotoxicity by MTT assay. The IC50 value of fucoidan was found to be 288.58μg/ml. To study the mechanism of action, selected EMT proteins like PLEKHA7, Snail1, E-cadherin, β-catenin and PI3K responsible for metastasis were subjected for docking with fucoidan and its structure activity relationship were investigated. Insilico studies by Schrodinger software revealed that fucoidan possess better interactions with PLEKHA7 (hydrogen-bonded with 4 amino acid moieties) and PI3K (hydrogen-bonded with 3 amino acid moieties) based on hydrogen bond length, number of interactions. Thus, from the insilico studies, it’s been proved that fucoidan interacts with EMT markers that are responsible for cancer metastasis.

Index Terms: Fucoidan, Docking, PLEKHA7, Epithelial-mesenchymal markers

1. INTRODUCTION
Seaweeds are the rich source of polysaccharides and polyphenols that contains numerous biological potential in treating various diseases. Seaweed derived drugs possesses good therapeutic value and so few drugs were approved and currently used by the public for various ailments. One of the natural molecules called fucoidan, a sulphated polysaccharide found in brown algae have enormous potential as anti-inflammatory, anti-coagulant, anti-viral, antioxidant, anti-cancer [1], [2]. The fucoidan from Turbinaria conoides were extensively studied for anticancer properties in different cancer diseases but still anti-metastasis property is not been explored. More than 80% of human cancers are carcinomas, which originate in epithelial tissues. The majority of cancer patients succumb to carcinomas due to the development of metastases and therapy resistance or tumor relapse. Recent evidences demonstrate that cancer stem cells (CSCs) can be generated via the activation of epithelial-mesenchymal transition (EMT). E-cadherin/catenin system localized on the epithelial cell surface maintains the homeostasis of the cell. Deregulation in this system may result in phenotypic change, which may create an opportunity for tumor cells to differentiate, metastasize and invade neighbouring tissue. More recently, it was found that E-cadherin/catenin system interacts with PLEKHA7 (Plekstrin Homology Domain containing familyA7) and miRNA processing complex that suppresses proliferation and mesenchymal functions [3]. It was also noted that there is a loss of PLEKHA7 protein in cancerous cells compared to normal cell. It has been understood from the above facts that the homeostatic signaling of the cell at the epithelial junction is maintained indirectly by PLEKHA7. There are evidences from literature that fucoidan demonstrated two significant criteria similar to PLEKHA7 [4] viz., upregulation of epithelial markers (E-cadherin) and downregulation of mesenchymal markers (β-catenin, Snail1).

Based on these facts, insilico approaches are made to study anti metastatic potential of fucoidan by analysing their interactions with Epithelial–mesenchymal proteins namely PLEKHA7, E-Cadherin, β-catenin, Snail 1 and PI3K.

2. MATERIALS AND METHODS:

2.1 Cell line and Culture Conditions:
A human ductal epithelioid carcinoma PANC-1 cell line was obtained from National Centre for Cell Science, Pune, India and used for the present study. The cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) high glucose (#AL111, Himedia) with 10% Fetal Bovine Serum (#RM10432, Himedia) and 100 mg/ml streptomycin. Then, the cells were incubated in5% CO2 incubator at 37°C until 70-80% confluency was observed. Cancer stem cell population CD133 cells were isolated from PANC-1 cell culture by flow cytometry. The isolated cancerstem cells were cultured alpha minimal essential medium with 10% fetal bovine serum incubated in 5% CO2 incubator at 37°C.

2.2 Cytotoxicity Study:
Cells were seeded in 96-well plate at 20,000 cells per well in 200 μl medium and allowed to attach for 24 hours. Attached cells were treated with 31.5, 62.5, 125, 250 and 500μg/ml of fucoidan and curcumin separately and incubated for 24 hrs at 37°C in a 5% CO2 atmosphere. After the incubation, media was removed and MTT reagent to a final concentration of 0.5mg/ml of total volume was added and incubated in dark for 3 hrs. Subsequently, DMSO was added to each well after the removal of MTT from the wells. The absorbance was read on ELISA reader at 570nm and 630nm was used as reference wavelength [5]. The IC50 value was determined by using linear regression equation i.e. Y = Mx +C. Here, Y = 50, M and C values were derived from the viability graph.

2.3 Docking studies:
The insilico studies for the five proteins namely PLEKHA7, β-catenin, E-cadherin, PI3K and Snail1 from human protein database was studied. Among the 5 proteins, PLEKHA7 was devoid of a 3D structure and so further subjected to molecular modeling. The molecular modeling of PLEKHA7 was performed using ITASSER SERVER [6] from yhangs lab

Authors 1, 3, 5 - Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipetata - 608502, Tamil Nadu, India
• Authors 2, 4 - Data mining and text mining lab, Department of Bioinformatics, Bharathiar University, Coimbatore –641046, Tamil Nadu, India

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showing a 71.5% of the residues in the allowed region in structure validation using PROCHECK [7]. The molecular docking of the 5 proteins with fucoidan was done using Schrodinger software [8]. The conformers were generated using Ligprep. The docking score and interaction was comparatively best for PLEKHA7 (-6.766) and PI3K (-8.645). The interactions studies was also done in Schrodinger with PLEKHA7 (hydrogen bonded with 4 amino acid moieties) and PI3K (hydrogen bonded with 3 amino acid moieties) based on hydrogen bond length and other interaction.

3 Results and Discussion:

3.1 Cytotoxicity study:
The effects of fucoidan on the growth of cancer stem cells were determined by evaluating the viability of PANC-1 CSCs CD133+ cells by MTT assay. The growth inhibition was observed for 24h after treated with fucoidan and found to be dose dependent (31.25, 62.50, 125, 250 & 500 μg/ml). The viability percentage of cells after treatment with fucoidan showed 31.5% at 500 μg/ml (Figure 1a) compared to the standard Curcumin (27.4%) at 500μg (Figure 1b). The IC50 value of fucoidan and Curcumin was determined to be 288.58μg/ml and 189.44μg/ml (Figure 1c).

3.2 Molecular Modelling of PLEKHA7:
The molecular modeling of PLEKHA7 was performed due to non availability of the 3D structure. The 3D structure modelling was done using Iasser server (fig 2). The Ramachandran plot of the modeled structure was obtained with 71.5% of the residues in the most favored regions and 4.2% in the disallowed regions (fig 3).

3.3 Fucoidan:
Fucoidan, a sulphated polysaccharide from marine seaweed T. conoides satisfied the Lipinski rule of five, which is very essential for the ligand to pass the criteria such as Mass: 242.000000, Hydrogen bond donor: 3, Hydrogen bond acceptors: 7, LOGP: -0.215800, Molar Refractivity: 48.488388.

3.4 Conformer generation of fucoidan:
The compound fucoidan was run for creating 32 conformers shown in fig 3. All the 32 conformers were considered for the interaction studies with the 5 proteins. The best 5 conformers were considered after the docking analysis. After the analysis of the five best conformers one best was finalized based on the interaction and the docking score and glide score.
Fig 4: Conformers of fucoidan.

The best conformer after the study is the first conformer or the original structure of fucoidan (fig 4) because it showed the best interaction and the best docking score among the 32 conformers.

Fig 5: The best conformer of fucoidan

3.5 Molecular docking

The 5 proteins PLEKHA7, β-catenin, E-cadherin, PI3K and Snail1 were considered for further analysis. The molecular docking of fucoidan was done with all the 32 conformers generated with each of the protein respectively. From the scores and the interaction studies of all the 32 conformers, the best conformer (first conformer) showed the best score in all the 5 proteins. The docking and gliding scores of proteins were tabulated in table 1.

<table>
<thead>
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<th>S.NO</th>
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<tr>
<td>1</td>
<td>PLEKHA7</td>
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<tr>
<td>2</td>
<td>β-catenin</td>
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<tr>
<td>3</td>
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<td>5</td>
<td>Snail1</td>
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Table 1: Docking score for EMT markers

3.6 Interaction Studies:

The 2D and 3D interaction of fucoidan with PLEKHA7 is shown in fig 6A. The hydroxyl group of fucoidan interacts with PRO913 by hydrogen bond with a length of 1.58Å. The hydrogen bond length between fucoidan and MET632 is 2.08 Å. There are two more hydrogen bonding between hydroxyl groups of fucoidan and GLH927 with bond length of 1.86 Å and 2.13Å respectively. The 2D and 3D interaction studies of fucoidan with β-catenin showed the two strong hydrogen bonding between hydroxyl group of fucoidan and ASN426 with a bond length 1.64Å and GLU462 with 1.73Å respectively (fig 6B). One more hydrogen bonding was observed between oxygen atom of fucoidan and ARG469 with a bond length of 2.07Å. The 2D and 3D interaction studies of fucoidan with E-Cadherin showed strong hydrogen bonding between two hydroxyl groups of fucoidan with TRP109 with the bond length of 2.15Å and 1.68Å. Another hydrogen bonding was observed between hydroxyl group of fucoidan and ASP107 in E-cadherin with the bond length 1.73 Å (fig 6C). The interaction studies of fucoidan with SNAIL1 was depicted in fig 6D. It showed only two hydrogen bond interactions between hydroxyl groups of fucoidan with PRO207 and SER209 of snail1 with 1.88 and 2.18 Å bond lengths respectively. The interaction studies between fucoidan and PI3K were shown in fig 6E. Three strong hydrogen bonding was observed between hydroxyl groups of fucoidan and TYR813, VAL828 and GLU826 of PI3K protein. VAL828 and GLU826 interacted with same hydroxyl moiety of fucoidan with a bond length of 2.09 and 1.86Å respectively. The bond length between each with the following residues and the respective bond length hydroxyl moiety of fucoidan and TYR813 was measured to be 2.38Å.

4. CONCLUSION

In conclusion, the cytotoxicity study revealed that fucoidan can be used as a potent drug to treat cancer. Insilico analysis of fucoidan with the 5 EMT proteins PLEKHA7, β-catenin, E-cadherin, PI3K and Snail1 were studied. The result of docking and interaction studies demonstrated that the proteins PLEKHA7 and PI3K showed better interaction and docking score with fucoidan when compared to the other three proteins. The proteins PLEKHA7 and PI3K showed 4 and 3 hydrogen bonds respectively with better docking scores -6.766 and -8.645 respectively. The hydrogen bond length was also below 3 and showed best interaction along with other disulfide bonds. Furthermore, this study concludes that PLEKHA7 and PI3K can be considered as target proteins for in vitro gene expression analysis in cancer cells treated with fucoidan.
Fig 6: Interactions of fucoidan with A) PLEKHA7, B) β-catenin, C) E-cadherin, D)Snail1, E) PI3K

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