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Original Article

A Flippase-Like Domain-Containing Protein From Deltaproteobacteria Bacterium (Marine Sediment Metagenome) Has Anti-prostate Cancer Effects

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Abstract

Background: Prostate cancer is the second prevalent cancer and the fifth cause of deaths related to the men malignancies. The cancerous cell can travel from prostate to other parts of the body and cause metastasis in other organs such as bones and lymph nodes. For the treatment of the prostate cancer, chemotherapy and hormone therapy are being used. Hormone therapy in this disease is primarily based on the androgen suppression to prevent the growth and division of the cancerous cells. Different classes of drugs are used for hormone therapy. One group of these drugs is reducers of androgen production in adrenal glands. These drugs prevent the androgen production in adrenal glands and cancerous cells. Leuprorelin which is also known as leuprolide is a synthetic peptide that is used for treatment of prostate cancer.

Objectives: The aim of this study is to explore novel anti-prostate cancer compounds using proteomics of microorganisms.

Methods: Leuprorelin synthetic peptide was chosen as the template for amino acid sequence homology search using blast, and the resulting protein of the best candidate sequences were employed as a query to perform docking test against the gonadotropin-releasing hormone receptor (GRHR) using HDOCK web server.

Results: By employing an *in silico* approach, natural products with structure and function similar to leuprorelin were explored, and their features were characterized. A flippase-like domain-containing protein from Deltaproteobacteria bacterium showed strong binding to gonadotropin-releasing hormone receptor GRHR with docking score of -425. The absorption, distribution, metabolism, and excretion (ADME) tests showed no potential toxicity of this natural product to the body.

Conclusion: The present study demonstrated that the proteomics of living organisms contains natural compounds that can be considered a valuable source of medically important resources.

Keywords: Deltaproteobacteria, Flippase, Leuprorelin, Prostate cancer

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Background

Prostate cancer is a disease related to the malignancy of prostate gland cells. This disease is the second prevalent cancer and the fifth cause of deaths related to the men malignancies (1). This type of cancer is the most prevalent tumor in men in 84 countries, especially in developed states (2). The prostate tumor grows slowly, but in some cases, the rapid expansion of the cell was also reported. The cancerous cell can travel from the prostate to other parts of the body and cause metastasis in other organs such as bones and lymph nodes (3). In the early stages of the disease, there are no evident symptoms, but in the advanced phases, the patient may encounter difficulties related to urinating, hematuria, or pain in the pelvic and lumbar region during the urination (4). Some factors are related to high prevalence of the prostate cancer such as age, family history, and race. Almost 50% of all prostate cancers occur at ages above 50 years (5). If a first-degree family member gets prostate cancer, the incident of the cancer will be duplicated or triplicated for other members (6). Based on the statistics, the incident of this disease is higher in African-American people compared to white Americans (7). Other factors that affect the incidence of the prostate cancer are high meat diet and high consumption of dairy products (8). Cancer prostate is diagnosed by biopsy, and chemotherapy and hormone therapy are used for the treatment of the prostate cancer. Hormone therapy for this disease is primarily based on the androgen suppression to prevent the growth and division of cancerous cells (9). The most important

androgen hormones in the body are testosterone and dihydrotestosterone. In addition to testicles, adrenaline glands are also responsible for the production and secretion of androgens (10). Different classes of drugs are used for hormone therapy. The selection of the drug class and the time of their prescription are solely decided by patient's physician. One group of these drugs is reducers of androgen production in adrenal glands, which prevent the androgen production in adrenal glands and cancerous cells (11). Drugs in this category such as abiraterone inhibit the production of androgen by blocking the enzyme CYP17. This medication is used in the patients with advanced prostate cancer in which testosterone production is not inhibited by testicles, or in the cases in which the risk of metastasis exists. This drug is orally consumable and has complications, including high blood pressure and muscle pain (12). Luteinizing hormone-releasing hormone (LHRH) antagonists are other groups of drugs used for treatment of prostate cancer. These drugs have side effects such as decreasing the size of testicles, fatigue, and osteoporosis (13). Another group of these drugs is anti-androgens. These drugs are indeed antagonists of androgen receptors that prevent binding of androgens to the surface of cancerous cells. Some examples of these drugs are flutamide, bicalutamide, and nilutamide (14). The most important groups of these drugs are agonists of LHRH. These drugs reduce the production of testosterones by testicles. These medications decrease the size of testicles and simulate the tentacle resection surgery (15). Goserelin, lupride, and triptorelin are some members of these drugs (16). Leuprorelin, also known as leuprolide, is a gonadotropin-releasing hormone receptor (GRHR) agonist that is used for treatment of prostate cancer. The GRHR is a receptor protein that stimulates the secretion of gonadotropic hormones and is associated with G-protein, which activates a phosphatidylinositol-calcium second messenger system (Figure 1). This peptide is injected intramuscularly or subcutaneously (17). Due to its synthetic origin, leuprorelin has some side effects such as unstable mood, hot flashes, headache, trouble sleeping, and pain at the injection site. The other side effects include allergic reactions, high blood sugar, and problems with the pituitary glands (18). In this study, we used an *in silico* approach to find natural proteins and peptides which can simulate the leuprorelin action in the treatment of prostate cancer.

Materials and Methods

Sequence Homology Search

Leuprorelin synthetic peptide was chosen as the template for amino acid sequence homology search. The sequence and structure of the leuprorelin peptide were retrieved from the PubChem database (PubChem ID: 657181). In order to find proteins and peptides with sequences similar to leuprorelin, its sequence was multiple aligned with all amino acid sequences in the NCBI database using protein Blast. The similarity search was set against the whole

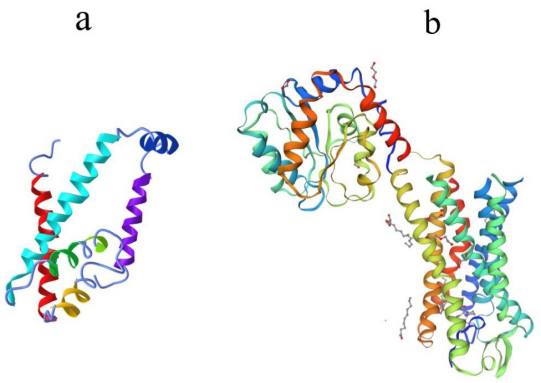

Figure 1. Structures of Ligand and Receptor. *Note.* GRHR: Gonadotropin-releasing hormone receptor. a) Tertiary structure of flippase-like domain-containing protein and b) tertiary structure of GRHR. The simulation is carried on solvated form of the protein

sequence; further, using the BLOSUM62 matrix, the expected threshold was 0.05, and gap costs of existence and extension were set to 11 and 1, respectively.

The Absorption, Distribution, Metabolism, and Excretion (ADME) Tests

Acute toxicity was calculated using toxicity profiler web-based tool (19). To predict toxicity of the proteins on different body organs and tissues, eMolTox online web-server was used (20). In addition, the metabolism and membrane transport of flippase-like domain-containing protein was predicted and evaluated using vNN-ADMET online web server (21). ADME@NCATS web server was also used to predict rat liver microsomal stability, parallel artificial membrane permeability assay solubility, human liver cytosolic stability, and *cytochrome* P450 toxicity tests of leginsulin (22).

Protein-Protein Docking

Drug leuprorelin was evaluated for its function and receptors in GoDrugBank, and the drug receptor sequence was retrieved from UniProt databank. Molecular docking was performed on the flippase-like domain-containing protein and the target receptor to study their interaction and the binding strength using the HDOCK web server (23).

Molecular Dynamic

Classical molecular dynamics simulations with flippase-like domain-containing protein bound to the GRHR were performed using GROMACS 2018 software. The initial native structure of flippase-like domain-containing protein was predicted using Phyre2 web server. The native structure of the growth hormone-releasing hormone receptor was also obtained from UniProt database (P30968). The water solvated forms of both proteins were obtained using TIP4P as the water model within a rhombic dodecahedron box, and the minimum distance was set at 3.0 nm between protein atoms and the box. In order to monitor the stability of the flippase-like domain-containing protein in their native motion, the root

mean square deviation (RMSD) was estimated. Contact analysis and the root mean square fluctuation were also determined for each complex. Moreover, constants for the pressure and temperature were set at 1.01325 and 300 k, respectively.

Natural Product Likeness Test

To examine the potential of flippase-like domain-containing protein to be considered as a natural product drug, the PDB file format of this peptide was converted to Mol file type, and the resulting data were uploaded in Natural Product Likeness Score calculator.

Results

Structural Similarity Search

The leuprorelin amino acid sequence aligned with all protein sequences in NCBI database and the most similar chemical structure with the shortest length were selected, which belonged to flippase-like domain-containing protein from a marine metagenomic study (NCBI accession ID: MBW2277380) (24). Figure 1 illustrates the general structure of the flippase-like domain-containing protein that is composed of 145 amino acids.

Protein-Protein Docking

Protein-protein docking was performed using HDOCK web server. This server uses hybrid algorithm for the prediction and affinity of the binding according to both template-based and template-free docking that leads to the accuracy of the prediction (23). The best molecular docking energy score for these two proteins was 425.31, and the ligand RMSD (Å) was calculated to be 339.61 and was named Model 1. The binding site for the flippase-like domain-containing protein was located at a region between amino acid 86-125 and for the GRHR was between amino acids 421-460. The most prominent amino acids in the binding region of flippase-like domain-containing protein were arginine and leucine. Figure 2 presents the binding schematic of Model 1 and other models of ligand-receptor interaction, and Table 1 depicts their affinity.

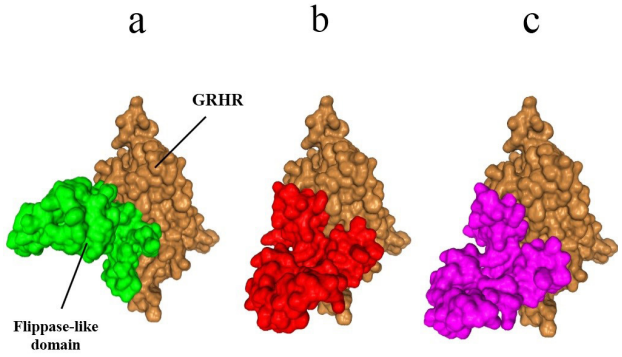

Figure 2. Three Models of Flippase-Like Domain-containing Protein and GRHR Interactions. *Note.* GRHR: Gonadotropin-releasing hormone receptor. a) Model 1 of interaction that had the best docking score, b, and c) Models 2 and 3 of ligand receptor interactions, respectively

Table 1. Docking Score and RMSD Quantities of Various Flippase-like Domain-Containing Protein and GRHR Interactions

Model	1	2	3
Docking score	-425.31	-423.85	-410.81
Ligand RMSD (A)	339.61	328.51	333.88

Note. RMSD: Root mean square deviation; GRHR: Gonadotropin-releasing hormone receptor.

Stability of the Flippase-Like Domain-containing Protein and GRHR Binding

The molecular dynamics technique was used to assess the stability of the flippase-like domain-containing protein and GRHR binding in triplicate for 150 ns. The results revealed that the flippase-like domain-containing protein and GRHR remained bound for more than 83 ns and the mean RMSD was ~ 1.9 Å (Figure 3). Analyzing the RMSD of the protein backbone, it was observed that an equilibrium is reached after 66 ns. Further, root mean square fluctuation analysis was performed to study the protein backbone flexibility.

Functional Simulations

The blood-brain barrier test showed that flippase-like domain-containing protein cannot be transmitted across the blood-brain barrier and is, therefore, safe for brain cells. The flippase-like domain-containing protein was found not to be a P-gp (glycoprotein) inhibitor or P-gp substrate. Rat liver microsomal stability module showed that flippase-like domain-containing protein is stable with the predicted class (probability) of 0.97. Acute toxicity for oral use was predicted based on the most similar compounds in toxicity database, and it was 0.45 (mmol/kg). Human liver cytosolic stability test indicated that this protein is very stable in the cytosol with a predicted class of 0.58, and CYP2C9-inhibitor and substrate tests were 0.54 and 0.56, respectively. For CYP2D6 inhibitor and substrate, the predicted class was calculated to be 0.48 and 0.53, respectively, and for CYP3A4 inhibitor and substrate,

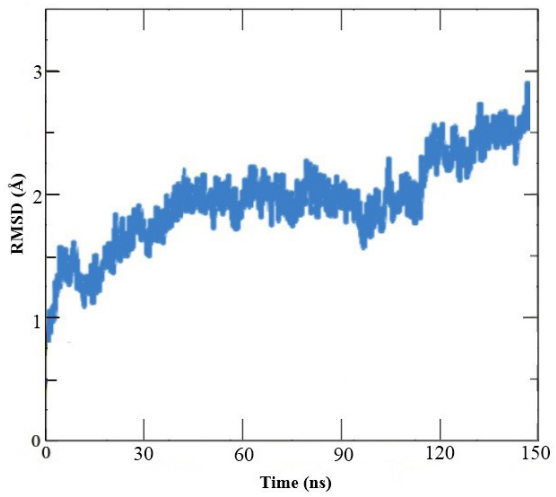

Figure 3. RMSD Results for the Docking of Flippase-like Domain-containing Protein and GRHR Based on Data From 150-ns MD Simulations. *Note.* RMSD: Root mean square deviation; GRHR: Gonadotropin-releasing hormone receptor; MD: *Molecular dynamics*

it was 0.52 and 0.53, respectively. The parallel artificial membrane permeability assay test measure was predicted to be high at pH 5, but it was low at pH 7.4 in the predicted class of 0.94. The results also demonstrated that it is highly soluble in the body fluids (predicted class of 0.96). It was shown the flippase-like domain-containing protein is not a matrix metalloproteinase inhibitor or a mutagenic chemical as its Ames Salmonella/microsome bacterial mutagenicity test was negative. It was also revealed that this natural peptide is not a hERG blocker. QSAR estimation of the maximum recommended therapeutic dose was calculated to be 1841. Table 2 presents the probable toxicity of the flippase-like domain-containing protein for different organs of the body.

The natural product-likeness scorer is developed based on the sum of frequency of compound fragments among natural products and synthetic molecules. This score is calculated for each atom in the molecular structure. The natural product score is used to predict the applicability of a compound or a putative structure from a library as a natural product. The score for flippase-like domain-containing protein was calculated to be 0.4.

Discussion

Similar to skin cancer, prostate cancer is the most common cancer among men in the United States. Based on the World Health Organization (WHO), more than 1.4 million new patients were diagnosed with prostate cancer in 2020 (25). Many new agents and medications have been developed and prescribed for the treatment of prostate cancer. Recently, natural compounds have attracted more attention in treating cancers due to their low side effects and low expenses they offer. In 2003, Holle et al studied the effectiveness of prostate cancer treatment using biotinylated melittin peptide coupled with avidin from bee venom. The prostate cancer cell line lysis occurred secondary to exposure of the cells to melittin-avidin-conjugate in an in vitro experiment (26). In another study, a conjugate of hecate and a 15-amino acid segment of beta-chain of LH was used for treatment of prostate cancer. The in vitro results indicated concentration dependent toxicity for different prostate cancer cell lines, while in vivo results showed the reduction of tumor size in mouse model (27). Curcumin is another natural product that is used for the treatment of prostate cancer. Curcumin is frequently used as a medicinal plant compound in south-east Asia and India (28). In 2000, Dorai et al conducted a study, examining the effects of curcumin on prostate cancerous androgen-sensitive cell line LNCaP and androgen-independent cell line PC-3. It was found that exposing cell lines to curcumin diminished the proliferative potential of human prostate cancer cells, down regulated the levels of apoptosis suppressor proteins, modulated the bax/bcl-2 ratio, and induced apoptosis in both androgen-dependent and androgen-independent manners (29). In another study on the cell line DU-145, it was observed that exposure to curcumin decreased the cell viability, proliferation, NF-kB protein activation, Bcl-2 protein, AP-1 protein, and bcl- xL protein, while increasing the apoptosis, **p**oly-**A**DP **r**ibose **p**olymerase cleavage, and procaspase -3 and -8 activity (30). In the present study, a flippase-like domain-containing protein from microorganism Deltaproteobacteria bacterium showed strong affinity and binding to GRHR which can act as a targeted hormone therapy in prostate cancer.

Table 2. The Possible Toxicity of the Flippase-Like Domain-containing Protein for Various Tissues of the Body

Target	Action	Organ	Confidence
Hepatotoxicity	Activators of the heat shock response signaling pathway	Liver	0.991
Nephrotoxicity	Cytotoxicity in HEK293 cells - 8 hour	Kidney	0.975
Cardiotoxicity	Cytotoxicity in HEK293 cells - 16 hour	Heart	0.965
Neurotoxicity	Agonist of the AR signaling pathway	Central nervous system	0.97
Reproduction toxicity	Agonist of the AR signaling pathway	Endocrine	0.96
Cell toxicity	Agonist of H2AX	DNA damage	0.983

Note. AR: Androgen receptor.

Conclusion

Flippases are transmembrane lipid transporter proteins that help the phospholipid molecules move between the two leaflets of the cell membrane. Using an in silico evaluation, the current study found that these groups of catalytic proteins from Deltaproteobacteria have the potential to be used as natural compounds for the management of prostate cancer. Further experimental studies are suggested to examine the therapeutic effects of these proteins.

Conflict of Interests

The authors reported no potential conflict of interests.

Ethical Issues

This study did not include any studies with human and animal subjects performed by any of the authors.

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References

1. Mehtälä J, Zong J, Vassilev Z, Brobert G, Gabarró MS, Stattin P, et al. Overall survival and second primary malignancies in men with metastatic prostate cancer. PLoS One. 2020;15(2):e0227552. doi: 10.1371/journal.pone.0227552.
2. Rawla P. Epidemiology of prostate cancer. World J Oncol. 2019;10(2):63-89. doi: 10.14740/wjon1191.
3. Jackson TL. A mathematical model of prostate tumor growth and androgen-independent relapse. Discrete & Continuous Dynamical Systems-B. 2004;4(1):187-201. doi: 10.3934/dcdsb.2004.4.187.
4. Smith JA Jr, Soloway MS, Young MJ. Complications of advanced prostate cancer. Urology. 1999;54(6A Suppl):8-14.

doi: 10.1016/s0090-4295(99)00448-3.
5. Delongchamps NB, Singh A, Haas GP. The role of prevalence in the diagnosis of prostate cancer. Cancer Control. 2006;13(3):158-68. doi: 10.1177/107327480601300302.
6. Steinberg GD, Carter BS, Beaty TH, Childs B, Walsh PC. Family history and the risk of prostate cancer. Prostate. 1990;17(4):337-47. doi: 10.1002/pros.2990170409.
7. Dess RT, Hartman HE, Mahal BA, Soni PD, Jackson WC, Cooperberg MR, et al. Association of black race with prostate cancer-specific and other-cause mortality. JAMA Oncol. 2019;5(7):975-83. doi: 10.1001/jamaoncol.2019.0826.
8. Patel AR, Klein EA. Risk factors for prostate cancer. Nat Clin Pract Urol. 2009;6(2):87-95. doi: 10.1038/ncpuro1290.
9. Litwin MS, Tan HJ. The diagnosis and treatment of prostate cancer: a review. JAMA. 2017;317(24):2532-42. doi: 10.1001/jama.2017.7248.
10. Heinlein CA, Chang C. Androgen receptor in prostate cancer. Endocr Rev. 2004;25(2):276-308. doi: 10.1210/er.2002-0032.
11. Crawford ED. Hormonal therapy in prostate cancer: historical approaches. Rev Urol. 2004;6(Suppl 7):S3-S11.
12. Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med. 2013;368(2):138-48. doi: 10.1056/NEJMoa1209096.
13. Kirby RS, Fitzpatrick JM, Clarke N. Abarelix and other gonadotrophin-releasing hormone antagonists in prostate cancer. BJU Int. 2009;104(11):1580-4. doi: 10.1111/j.1464-410X.2009.08924.x.
14. Ricci F, Buzzatti G, Rubagotti A, Boccardo F. Safety of antiandrogen therapy for treating prostate cancer. Expert Opin Drug Saf. 2014;13(11):1483-99. doi: 10.1517/14740338.2014.966686.
15. Engel JB, Schally AV. Drug Insight: clinical use of agonists and antagonists of luteinizing-hormone-releasing hormone. Nat Clin Pract Endocrinol Metab. 2007;3(2):157-67. doi: 10.1038/ncpendmet0399.
16. Vogelzang NJ, Chodak GW, Soloway MS, Block NL, Schellhammer PF, Smith JA Jr, et al. Goserelin versus orchiectomy in the treatment of advanced prostate cancer: final results of a randomized trial. Zoladex Prostate Study Group. Urology. 1995;46(2):220-6. doi: 10.1016/s0090-4295(99)80197-6.
17. Evans HC, Wagstaff AJ. Leuprorelin. Am J Cancer. 2004;3(3):197-201. doi: 10.2165/00024669-200403030-00008.
18. Chang JI, Bucci J. Unusual side effect from a luteinizing hormone-releasing hormone agonist, leuprorelin, in the treatment of prostate cancer: a case report. J Med Case Rep. 2016;10(1):323. doi: 10.1186/s13256-016-1110-5.
19. AbdulHameed MDM, Liu R, Schyman P, Sachs D, Xu Z, Desai V, et al. ToxProfiler: toxicity-target profiler based on chemical similarity. Comput Toxicol. 2021;18:100162. doi: 10.1016/j.comtox.2021.100162.
20. Ji C, Svensson F, Zoufir A, Bender A. eMolTox: prediction of molecular toxicity with confidence. Bioinformatics. 2018;34(14):2508-9. doi: 10.1093/bioinformatics/bty135.
21. Schyman P, Liu R, Desai V, Wallqvist A. vNN web server for ADMET predictions. Front Pharmacol. 2017;8:889. doi: 10.3389/fphar.2017.00889.
22. Gonzalez E, Jain S, Shah P, Torimoto-Katori N, Zakharov A, Nguyễn Đ T, et al. Development of robust quantitative structure-activity relationship models for CYP2C9, CYP2D6, and CYP3A4 catalysis and inhibition. Drug Metab Dispos. 2021;49(9):822-32. doi: 10.1124/dmd.120.000320.
23. Yan Y, Tao H, He J, Huang SY. The HDOCK server for integrated protein-protein docking. Nat Protoc. 2020;15(5):1829-52. doi: 10.1038/s41596-020-0312-x.
24. Flippase-like domain-containing protein, partial [Deltaproteobacteria bacterium]. 2021. Available from: https://www.ncbi.nlm.nih.gov/protein/MBW2277380.1?report=genbank&log$=prottop&blast_rank=3&RID=7XASK5JY013.
25. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, et al. Cancer statistics for the year 2020: An overview. Int J Cancer. 2021;149(4):778-89. doi: 10.1002/ijc.33588.
26. Holle L, Song W, Holle E, Wei Y, Wagner T, Yu X. A matrix metalloproteinase 2 cleavable melittin/avidin conjugate specifically targets tumor cells in vitro and in vivo. Int J Oncol. 2003;22(1):93-8.
27. Hansel W, Leuschner C, Enright F. Conjugates of lytic peptides and LHRH or betaCG target and cause necrosis of prostate cancers and metastases. Mol Cell Endocrinol. 2007;269(1-2):26-33. doi: 10.1016/j.mce.2006.06.017.
28. Hsu CH, Cheng AL. Clinical studies with curcumin. In: Aggarwal BB, Surh YJ, Shishodia S, eds. The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease. Boston, MA: Springer; 2007. p. 471-80. doi: 10.1007/978-0-387-46401-5_21.
29. Dorai T, Gehani N, Katz A. Therapeutic potential of curcumin in human prostate cancer-I. curcumin induces apoptosis in both androgen-dependent and androgen-independent prostate cancer cells. Prostate Cancer Prostatic Dis. 2000;3(2):84-93. doi: 10.1038/sj.pcan.4500399.
30. Nakamura K, Yasunaga Y, Segawa T, Ko D, Moul JW, Srivastava S, et al. Curcumin down-regulates AR gene expression and activation in prostate cancer cell lines. Int J Oncol. 2002;21(4):825-30.