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Docking studies between Natural unsaturated Oleic-acidand a *de novo* lung cancer peptide of CHRNA3 (Cholinergic Receptor Nicotinic Alpha 3) using Insilico protocols

LN. Aadya[1]

[1]B.Tech 1st year
Department of Biotechnology, Alagappa College of Technology, Anna University, Chennai – 600025

Abstract

Lung cancer, according to current clinical pharmacological studies, is among the most prevalent types of cancer. Polymorphisms in CHRNA3 gene have been associated with an increased risk of smoking initiation and an increased susceptibility to lung cancer. In the current Insilico study, there were two major findings. First, the novel 3D peptide was designed from the genomic sequence of CHRNA3. Next, we observe how Oleic acid interacts with the derived cancer peptide using Insilico tools. The exonic sequence of CHRNA3 was converted into a protein sequence which subject to peptide designing using immuno-medicine protocols. The peptide was modelled using an automated homology modelling server. The binding affinities between the control drug, 5-Fluorouracil and the test compound, Oleic-acidwith CHRNA3 can be found using CB Dock, an automated drug docking server. The docking results clearly elucidated that when compared to the existing anti-cancer drug, 5-flourouracil, Oleic acid showed a higher binding affinity with CHRNA3. Hence, Oleic acid can be added as a supplementary drug to existing drugs to enhance their efficiency. Oleic acid, being a natural compound, one can expect lesser side-effects. Our 3D novel peptide structure will be available in PDB-Dev (https://www.modelarchive.org/doi/10.5452/ma-m1plw). Our study clearly revealed that Oleic acid is a potential therapeutic agent for Lung Carcinoma.

Keywords: CHRNA3 (Cholinergic Receptor Nicotinic Alpha 3), Oleic-acid, Drug Docking

INTRODUCTION

(Siegel, R.L et al., 2020) Lung cancer continues to be the second most prevalent diagnosis. Lung cancer has a 59% 5-year survival probability if it is discovered while the disease is still localized in the lungs, however only 16% of cases are identified early (Siegel, R.L et al., 2021). There is a 6% 5-year survival rate for distant metastases. Metastatic illness is the primary cause of cancer-related fatalities, accounting for 57% of all patients with a diagnosis. The high risk of metastasis and delayed diagnosis are the main causes of the high death rate. Although lung cancer has historically been thought to be a disease that is resistant to chemotherapy and radiation, individuals with locally progressed and advanced lung cancer are now more likely to survive thanks to chemoradiotherapy and novel therapeutic approaches including

immunotherapy and targeted medicines. Patients with advanced lung cancer typically get palliative treatment in the absence of a known curative therapy. In order to lessen the burden of lung cancer by comprehending the elements influencing its early development, it is imperative to look into and comprehend the underlying biology and molecular mechanisms of lung cancer risk (Yang, I.A et al., 2013).There is growing evidence that lung carcinogenesis may be linked to gene alterations that alter pulmonary surfactant balance, even though tobacco smoke exposure accounts for the majority of lung cancer risk. (Honda, T et al., 2018, Takamiya, R et al., 2017)

The FinnGen study, the ILCCO study, and the TRICL study provided data on lung cancer and its subtypes. According to McKay JD et al. (2017), all participants in the TRICL and ILCCO studies were of European ancestry. There were 29,266 patients with European ancestry and 56,450 controls. Of these, 11,273 cases and 55,483 controls had lung adenocarcinoma, 7,426 cases and 55,627 controls had lung squamous cell carcinoma, and 2,664 cases and 21,444 controls had small cell lung cancer. The publicly available R10 data of the FinnGen study, which comprised 1,590 cases and 314,193 controls for lung adenocarcinoma, 1,510 cases and 314,193 controls for lung squamous cell carcinoma, 717 cases and 314,193 controls for small cell lung cancer, and 5,315 cases and 314,193 controls for non-small cell lung cancer, were used to generate summary data on the genetic associations of lung cancer and its subtypes [Kurki MI et al., 2023].Lung cancer patients, today, are subject to the severe side-effects of chemotherapic drugs. Our current study aims at identifying natural compounds which are capable of controlling Lung Carcinoma. The molecular drug docking of oleic acid, the most abundant unsaturated fatty acid, with CHRNA3 (Cholinergic Receptor Nicotinic Alpha 3) is the primary focus of current lung cancer research. As a result, the full docking study demonstrated how oleic acid affected the CHRNA3 protein.

MATERIALSANDMETHODS

Protein Sequence Selection: The proteomics database was used to locate the Ensemble genome browser of [Source:HGNC Symbol;Acc:HGNC:1957]. Immune medicine tool (Kolaskar AS, Tongaonkar PC (1990) (http://imed.med.ucm.es/Tools/antigenic.pl) was used to construct the peptide, and Swissmodel server was used to model the peptide sequence [Waterhouse A et al., 2018] (https://swissmodel.expasy.org/).Oleic-acid(CID:445639) (https://pubchem.ncbi.nlm.nih.gov/compound/Oleic-Acid) and the control drug 5-Fluorouracil (CID: 3385) (https://pubchem.ncbi.nlm.nih.gov/compound/3385) was obtained from PubChem Compound Database to do a molecular drug docking study. Three-dimensional structures were predicted using Discovery Studio Software, a potent molecular visualization tool. Docking of Molecular Drugs and 3D Interactions: The CB-Dock server's curvature-based cavity identification algorithm and AutoDock Vina-based molecular docking mechanism are carried over into CB-Dock2, an enhanced protein-ligand blind docking tool. Molecular drug docking research have made use of the automated molecular drug docking service CB dock (https://cadd.labshare.cn/cb-dock2/index.php) [Yang Liu et al., 2022, Xiaocong, Yang et al., 2022]. The molecular affinities of oleic acid and CHRNA3 (Cholinergic Receptor Nicotinic Alpha 3) were determined using a 3D Ligand-Protein docking technique. The post-docking research was carried out using the Discovery Studio program. The 3D image (3D H-bond/Electrostatic interactions) was thoroughly analysed using the molecular dynamics concept based on the docking score.

Results

Gene name	Protein name	Chromosome position	Protein length	Identified peptide
CHRNA3-*Cholinergic Receptor Nicotinic Alpha 3*	(CHRNA3 [Source:HGNC Symbol;Acc:HGNC:1957)	7	325 aa	AEAPLRCRALKSELLQPRRVQRLQGGLPLPGRDVWLLPP

Table :1

CCWGFPGGRQDQSLTQVHWGGDLDTSGHLELLNRRDLLPVLPKLYHEVRFLVLRGENRSGPD
RLFHEPQGLLGERRVGHHQSPRLQTRHQVQLLRGDLPRHHILAVHPAPALVLHHQPHHPLPAH
LLPHCARLLPALRLREGDPVHFCPPLPDGVSPGDHDHPFHLAGHPPDWRVPPVHHDFCNLVHR
HHRLRAQRALQNPDDTHNALMGEDCILEPAPQGHVHDQANKQRGQRSEAEAPLRCRALKSEL
LQPRRVQRLQGGLPLPGRDVWLLPPPQDKNLQFQCPHEKLFICCCAVPLCFVTRNQRSHPKCQV
YCKYESTKSQR

Fig.1: Protein sequence derived from the genomic sequence of CHRNA3 using Ensembl genome browser

There are 13 antigenic determinants in your sequence:

n	Start Position	Sequence	End Position
1	12	QSLTQVHW	19
2	36	DLLPVLPKLYHEVRFLVL	53
3	61	PDRLFHEPQG	70
4	76	RVGHHQSP	83
5	85	LQTRHQVQLLRG	96
6	98	LPRHHILAVHPAPALVLHHQPHHPLPAHLLPHCARLLPALR	138
7	141	EGDPVHFCPPLPDG	154
8	161	DHPFHLAG	168
9	171	PDWRVPPVHHDFCNLVHRHHRLRAQRA	197
10	209	MGEDCILEPAPQGHVHD	225
11	237	AEAPLRCRALKSELLQPRRVQRLQGGLPLPGRDVWLLPP	275
12	281	LQFQCPHEKLFICCCAVPLCFV	302
13	308	SHPKCQVYCKY	318

Fig.2: Various antigenic peptides present in CHRNA3 protein sequence. We select the potential peptide sequence ranging from 237 to 275 amino acid position.

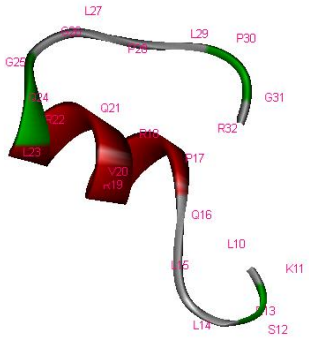

Fig.3: 3D peptide modelling from Swiss-model server and viewed in Discovery Studio software with coloured secondary structure and amino acid labels

DRUG NAME	IUPAC NAME	COMPOUND ID	MOLECULAR FORMULA	MOLECULAR WEIGHT	SUMMARY
Oleic acid	(Z)-octadec-9-enoic acid	445639	$C_{18}H_{34}O_2$	282.5g/mol	An unsaturated fatty acid that is the most widely distributed and abundant fatty acid in nature.

Table.2: Molecular properties of the chemical test compound, Oleic acid, retrieved from, PubChem compound database

DRUG NAME	IUPAC NAME	COMPOUND ID	MOLECULAR FORMULA	MOLECULAR WEIGHT	SUMMARY
5-Fluorouracil	5-fluoro-1H-pyrimidine-2,4-dione	3385	$C_4H_3FN_2O_2$	130.08g/mol	Anti Neoplastic Drug

Table.3:Molecular properties of the chemical test compound, 5-Fluorouracil, retrieved from, PubChem compound database

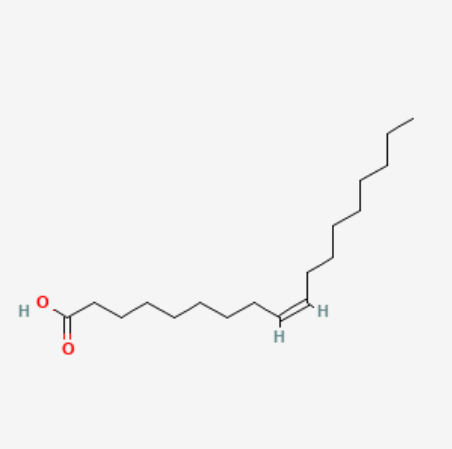

Fig:4 Oleic acid's 2D structure, displaying the corresponding atoms, obtained from PubChem compound database.

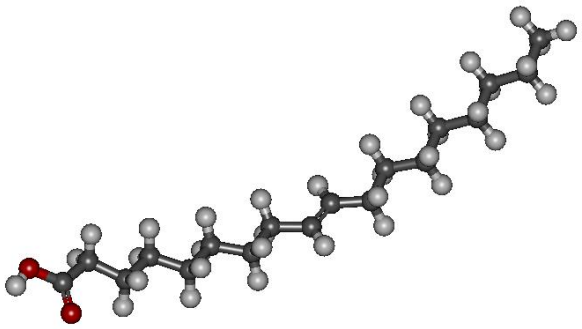

Fig.5: Oleic acid's 3D structure, displaying the corresponding atoms,viewed in Discovery Studio software.

Fig.6:5-fluorouracil's 2D structure, displaying the corresponding atoms, obtained from PubChem compound database.

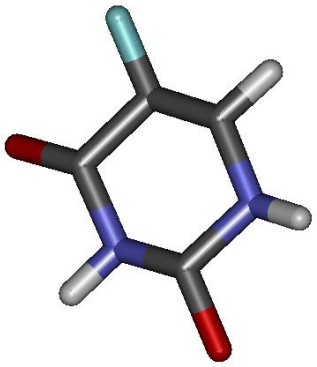

Fig.7: 5-fluorouracil's 3D structure, displaying the corresponding atoms,viewed in Discovery Studio software

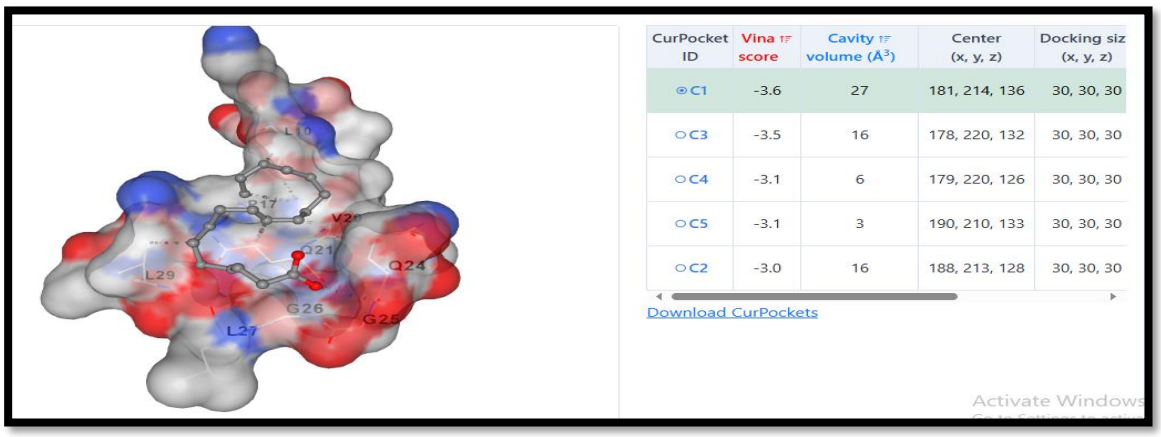

Fig.8: Molecular docking studies done using CB Dock server with respective binding score ofCHRNA3 peptide against Oleic-acid

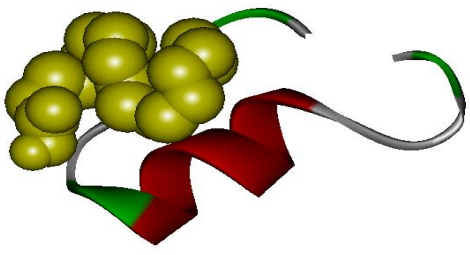

Fig:9. 3D complex form ofCHRNA3 peptide – Oleic-acid viewed using Discovery Studio software. Yellow color indicates Oleic-acid molecule bound to the respectiveCHRNA3 binding cavities

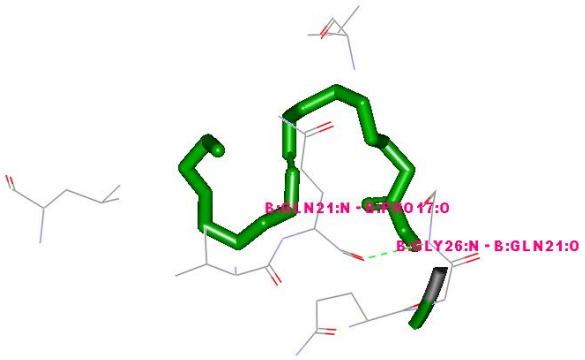

Fig:10. H-bond interaction betweenCHRNA3 peptide and Oleic-acid viewed using Discovery Studio software. Green colored structure indicates Oleic-acid molecule bound to the respective binding cavities with respective amino acid position labels

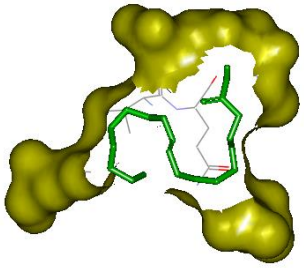

Fig: 11. Vander Waals' interaction betweenCHRNA3 peptide and Oleic-acid viewed using Discovery Studio software. Green colored structure indicates Oleic-acid molecule bound to the respectiveCHRNA3 binding cavities

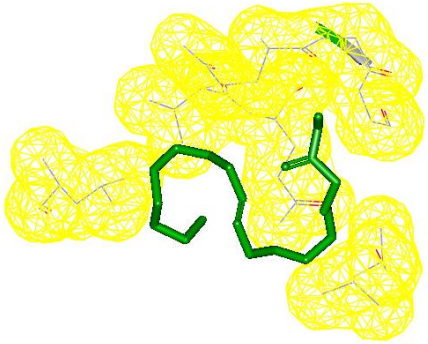

Fig: 12. Electrostatic interaction force betweenCHRNA3 peptide and Oleic-acid viewed using Discovery Studio software. Green colored structure indicates Oleic-acid molecule bound to the respectiveCHRNA3 binding cavities with electrostatic force

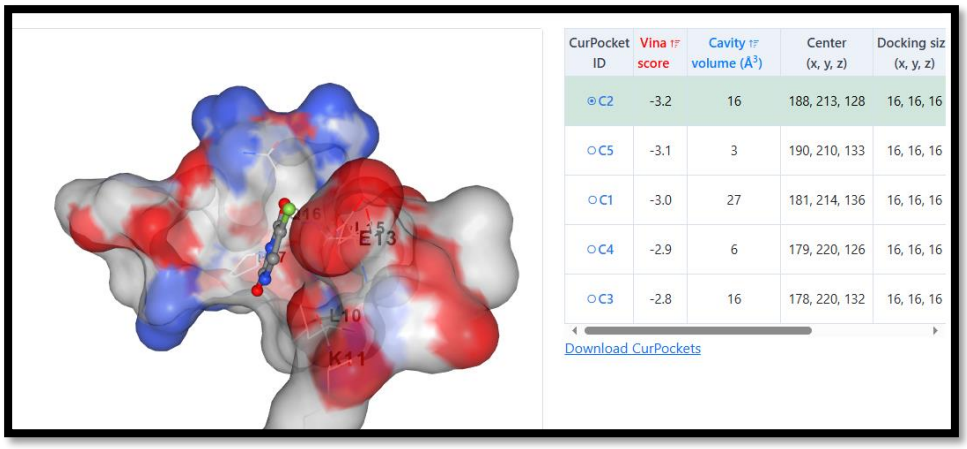

Fig.13: Molecular docking studies done using CB Dock server with respective binding score ofCHRNA3 peptide against 5-fluorouracil

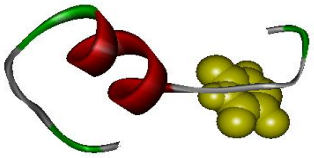

Fig: 14. 3D complex form ofCHRNA3 peptide – 5-fluorouracil viewed using Discovery Studio software. Yellow color indicates 5-fluorouracil molecule bound to the respectiveCHRNA3 binding cavities

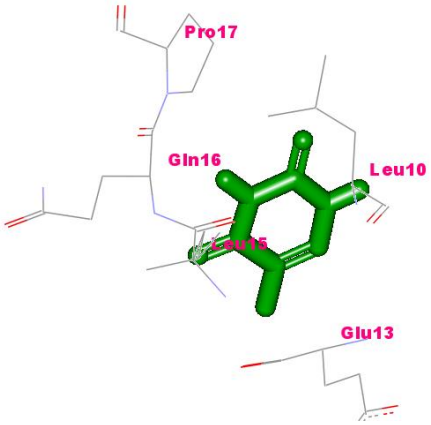

Fig:15. H-bond interaction betweenCHRNA3 peptide and 5-fluorouracil viewed using Discovery Studio software. Green colored structure indicates 5-fluorouracil molecule bound to the respective binding cavities with respective amino acid position labels

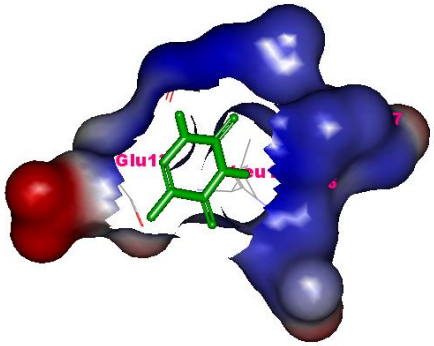

Fig: 16. Vander Waals' interaction betweenCHRNA3 peptide and 5-fluorouracil viewed using Discovery Studio software. Green colored structure indicates 5-fluorouracil molecule bound to the respectiveCHRNA3 binding cavities

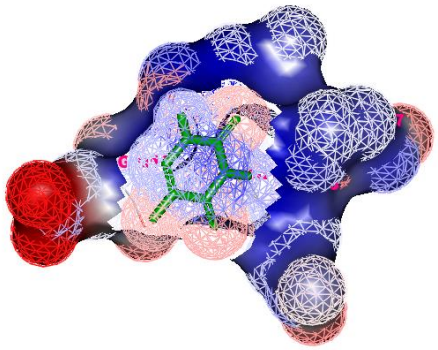

Fig: 17. Electrostatic interaction force between CHRNA3 peptide and 5-fluorouracil viewed using Discovery Studio software. Green colored structure indicates 5-fluorouracil molecule bound to the respective CHRNA3 binding cavities with electrostatic force

	Test Compound	**Control Drug**
Protein Target	Oleic acid (Compound CID: 445639)	5-Fluorouracil (Compound CID: 3385)
CHRNA3 (*Cholinergic Receptor Nicotinic Alpha 3*) (CHRNA3 [Source:HGNC Symbol;Acc:HGNC:1957) designed peptide	-3.6 *kcal/mol*	-3.2 *kcal/mol*

Table 4: Drug Docking Scores between the test compound, Oleic-acid and the control drug, 5-flurouracil. Higher negative value indicates higher binding affinity between the lung cancer peptide of CHRNA3 (CB dock server) and the selected compounds. In the above table, we find that Oleic acid has greater affinity with CHRNA3 peptide.

DISCUSSION

The Ensembl genomic sequence of the CHRNA3 lung cancer susceptibility gene (Yang, L et al., 2024)which is present in the chromosome was chosen (Table:1). The product of the exon sequence product and the translated corresponding amino acids were analysed. The potential antigenic peptide was designed using Immuno medicine tool, modelled using Swiss modelled server and visualized using Discovery studio software. In this docking study, the CB Dock server was used to dock theCHRNA3 peptide sequence with Oleic-acidand 5-Fluorouracil. Figure 4 shows the 3D peptide structure of CHRNA3, which may be viewed in secondary structure color using the Discovery Studio software (Fig :1-3).

The chromosome 15q25.1 locus has been found to be a susceptibility region for smoking habit, nicotine dependency (ND), and LC in some populations by a number of GWAS and case-control studies [Ji X et al., 2018]. Three nicotinic acetylcholine receptors (nAChRs), including CHRNA3, CHRNA5, and CHRNB4, are clustered together at this locus [Kita-Milczarska K et al., 2016]. It has been demonstrated that the synonymous nucleotide change caused by the SNP rs1051730C > T, which is found in exon5 of CHRNA3 (Cholinergic Receptor Nicotinic Alpha 3 Subunit), is substantially linked to an elevated risk of ND and LC. [H. Kupiainen and others, 2016].Additionally, our analysis is based on prior work that explores the state-of-the-art for Insilico docking exams.

In this study, the test compound, Oleic acid is chosen as it has anti-oxidant properties according to the research works of Menendez JA et al., 2005 (Fig .4-5) (Table:2). The double bond at C-9 in oleic acid, an octadec-9-enoic acid, possesses Z (cis) stereochemistry

The control drug molecule in this instance is 5-fluorouracil, a medication frequently used to treat a variety of malignancies. [S. Ghafouri-Fard and colleagues, 2021](Fig .6-7) (Table: 3).5-fluorouracil is a nucleobase analogue of uracil in which fluorine is substituted for the hydrogen atom at position 5. It is an antineoplastic drug that functions as an antimetabolite; after being converted to the active deoxynucleotide, it inhibits DNA synthesis by preventing the cellular enzyme thymidylate synthetase from converting deoxyuridylic acid to thymidylic acid, which reduces the growth of tumors. It functions as an antimetabolite, xenobiotic, radio sensitizing agent, immunosuppressive agent, antineoplastic agent, and environmental pollutant. It is an organofluorine chemical and an analogue of a nucleobase. It shares functional similarities with uracil.

Cavity detection-guided blind docking, which looks for potential binding pockets using cavity detection algorithms such as Fpocket and P2Rank [Röhrig UF et al., 2023, Goullieux M et al., 2023]. Furthermore, COACH-D and GalaxySite use blind docking to find binding sites instead of cavity discovery. After identifying the binding site, cavity detection-guided blind docking methods such as CB-Dock and EDock are used to perform local docking at the anticipated binding sites. [Zhang W *et al*., 2020],[Maithreyee S, and Prabha V, (2023), Nijanthi, P., S and Munivelan, B. (2023), Grace, H *et al*., 2022, Zashumo, K. J *et al*., 2023]

Figures 8–17 illustrate the interactions between oleic acid and the CHRNA3 protein at various binding amino acid locations. The drug-receptor complex view and associated drug binding scores for CHRNA3 and oleic acid are displayed in the figures. The binding affinities of the human CHRNA3 peptide to oleic acid are (-3.6 kcal/mol). Conversely, the binding affinities of the CHRNA3 peptide to

oleic acid are (-3.2 kcal/mol). H-bond interactions demonstrate that the lung cancer gene, CHRNA3 peptidecontains the regions where it interacts with Oleic acid (LEU10 LEU15 PRO17 VAL20 GLN21 LEU23 GLN24 GLY25 GLY26 LEU27 PRO28 LEU29 ARG32).H-bond interactions demonstrate that CHRNA3 peptide contains the regions where it interacts with the control drug, 5-fluorouracil(LEU10 LYS11 GLU13 LEU14 LEU15 GLN16 PRO17 GLY31 ARG32).These interactions suggest that the oleic acid molecule interacts with the functional motif region of the CHRNA3 peptide.

CONCLUSION

Oleic acid, a chemical substance, suppresses the expression of the protein that causes lung carcinoma by directly binding to the identified antigenic peptide ofCHRNA3. Based on docking scores, the 3D molecular interaction between the CHRNA3 peptide and the test compound, oleic acid shows a higher score when compared to that between CHRNA3 peptide and the control drug, 5-Fluorouracil. Thus, we conclude that the oleic acid molecule can be used as an extra medication to manage lung carcinoma in Homo sapiens. In this research work, we clearly show how the derived lung cancer peptide inhibits the natural compound, Oleic acid.Thus, it can be demonstrated that oleic acid potentially down regulates CHRNA3 and that it has pharmacological actions against the human susceptible lung-cancer gene, CHRNA3. The docking data and modelled structures are available inPDB-Devat the following link: https://www.modelarchive.org/doi/10.5452/ma-m1plw). To sum up, our research unequivocally demonstrated that oleic acid is a promising treatment for lung cancer.

Conflict of interest:

The author declares that there are no conflicts of interest with regard to this article.

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REFERENCE

1. Siegel, Rebecca L et al. “Cancer statistics, 2020.” *CA: a cancer journal for clinicians* vol. 70,1 (2020): 7-30. doi:10.3322/caac.21590
2. Siegel, Rebecca L et al. “Cancer Statistics, 2021.” *CA: a cancer journal for clinicians* vol. 71,1 (2021): 7-33. doi:10.3322/caac.21654
3. Yang, Ian A et al. “Genetic susceptibility to lung cancer and co-morbidities.” *Journal of thoracic disease* vol. 5 Suppl 5,Suppl 5 (2013): S454-62. doi:10.3978/j.issn.2072-1439.2013.08.06
4. Honda, Takayuki et al. “Deleterious Pulmonary Surfactant System Gene Mutations in Lung Adenocarcinomas Associated With Usual Interstitial Pneumonia.” *JCO precision oncology* vol. 2 (2018): 1-24. doi:10.1200/PO.17.00301
5. Takamiya, Rina et al. “Disruption of the structural and functional features of surfactant protein A by acrolein in cigarette smoke.” *Scientific reports* vol. 7,1 8304. 16 Aug. 2017, doi:10.1038/s41598-017-08588-5

6. McKay, James D et al. "Large-scale association analysis identifies new lung cancer susceptibility loci and heterogeneity in genetic susceptibility across histological subtypes." *Nature genetics* vol. 49,7 (2017): 1126-1132. doi:10.1038/ng.3892
7. Kurki, Mitja I et al. "FinnGen provides genetic insights from a well-phenotyped isolated population." *Nature* vol. 613,7944 (2023): 508-518. doi:10.1038/s41586-022-05473-8
8. McKay JD, Hung RJ, Han Y, et al. Large-scale association analysis identifies new lung cancer susceptibility loci and heterogeneity in genetic susceptibility across histological subtypes. Nat Genet. 2017;49(7):1126–1132. doi: 10.1038/ng.3892. *[DOI] [PMC free article] [PubMed]* [Google Scholar]
9. Kurki MI, Karjalainen J, Palta P, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. Nature. 2023;613:508–518. doi: 10.1038/s41586-022-05473-8. [DOI] [PMC free article] [PubMed] [Google Scholar]
10. Kolaskar AS, Tongaonkar PC. A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS Lett*. 1990;276(1-2):172-174. doi:10.1016/0014-5793(90)80535-q
11. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L, Lepore R, Schwede T.SWISS-MODEL: homology modelling of protein structures and complexes.Nucleic Acids Res 46, W296-W303. (2018) PubMed logo29788355DOI logo10.1093/nar/gky427
12. Liu Y, Yang X, Gan J, Chen S, Xiao ZX, Cao Y. CB-Dock2: improved protein-ligand blind docking by integrating cavity detection, docking and homologous template fitting. *Nucleic Acids Res*. 2022;50(W1):W159-W164. doi:10.1093/nar/gkac394.
13. Liu Y, Grimm M, Dai WT, Hou MC, Xiao ZX, Cao Y. CB-Dock: a web server for cavity detection-guided protein-ligand blind docking. *Acta Pharmacol Sin*. 2020;41(1):138-144. doi:10.1038/s41401-019-0228-6.
14. Yang, Lei et al. "Epidemiological evidence for associations between variants in CHRNA genes and risk of lung cancer and chronic obstructive pulmonary disease." *Frontiers in oncology* vol. 12 1001864. 6 Oct. 2022, doi:10.3389/fonc.2022.1001864
15. Ji X, Bossé Y, Landi MT, et al. Identification of susceptibility pathways for the role of chromosome 15q25.1 in modifying lung cancer risk. Nature Communications. 2018 Aug;9(1):3221. DOI: 10.1038/s41467-018-05074-y. PMID: 30104567; PMCID: PMC6089967
16. Kita-Milczarska K., Sieminska A., Jassem E. Association between CHRNA3 and CHRNA5 nicotine receptor subunit gene variants and nicotine dependence in an isolated populationof Kashubians in Poland. Med. Sci. Monit. 2016;22:1442–1450. [Europe PMC free article] [Abstract] [Google Scholar]
17. Kupiainen H., Kuokkanen M., Kontto J. CHRNA5/CHRNA3 locus associates with increased mortality among smokers. J. Chronic Obstr. Pulm. Dis. 2016;13(4):464–470. [Abstract] [Google Scholar]
18. Goullieux M, Zoete V, Röhrig UF. Two-Step Covalent Docking with Attracting Cavities. *J Chem Inf Model*. 2023;63(24):7847-7859. doi:10.1021/acs.jcim.3c01055.
19. Zhang W, Bell EW, Yin M, Zhang Y. EDock: blind protein-ligand docking by replica-exchange monte carlo simulation. *J Cheminform*. 2020;12(1):37. Published 2020 May 27. doi:10.1186/s13321-020-00440-9

20. Maithreyee S, Prabha V. Molecular interactions between anti-inflammatory drug with colorectal cancer (MSH2) protein using in-silico studies. Solovyov Studies ISPU. 2023;71(10):171-86.
21. P Nijanthi, Santhi S, Balaji Munivelan. Molecular dynamics studies on the arginine kinase protein of Aedes sollicitans: Against the natural chemical compound, Gedunin. Int J Mosq Res 2023;10(2):10-14. DOI: https://doi.org/10.22271/23487941.2023.v10.i2a.665.
22. Grace H, VM ZK, Leelavathi D. Plant Derived Compound-Luteolin Promising Role against SARS-CoV-2 Protein. Research Journal of Agricultural Sciences [an international journal]. 2022;13(05):1449-53.
23. Zashumo KJ, Leelavathi D, Grace H. Antiobesity property of Indian Tulsi plant (Ocimum sanctum) using In silico docking techniques. InBiol Forum–An Int J 2023 (Vol. 15, No. 2, pp. 9-14).
24. Menendez JA, Vellon L, Colomer R, Lupu R. Oleic acid, the main monounsaturated fatty acid of olive oil, suppresses Her-2/neu (erbB-2) expression and synergistically enhances the growth inhibitory effects of trastuzumab (Herceptin) in breast cancer cells with Her-2/neu oncogene amplification. *Ann Oncol*. 2005;16(3):359-371. doi:10.1093/annonc/mdi090.
25. Ghafouri-Fard, S., Abak, A., Tondro Anamag, F., Shoorei, H., Fattahi, F., Javadinia, S. A., Basiri, A., & Taheri, M. (2021). 5-Fluorouracil: A Narrative Review on the Role of Regulatory Mechanisms in Driving Resistance to This Chemotherapeutic Agent. *Frontiers in oncology*, *11*, 658636. https://doi.org/10.3389/fonc.2021.658636