Original Article

Molecular Docking Studies on Cystic Fibrosis Transmembrane Conductance Regulator Protein and Corrector Molecules

Sandhiya. E[a1], Vinitha. E[a1], Nishaandhini. K. S[a1], Harini. E[a1], Susmita Roy[a*]

A Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam. Chennai, Tamil Nadu.

Ms. Sandhiya E. is pursuing her final year of undergraduate in Medical Biotechnology program in the Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam. Chennai, Tamil Nadu.

Corresponding author - Susmita Roy – (sushmiroy96@gmail.com)

Chettinad Health City Medical Journal 2020; 9(4): 224 - 227

DOI: https://doi.org/10.36503/chcmj9(4)-05

Abstract

The cystic fibrosis transmembrane conductance regulator (CFTR) protein is an ATP binding cassette (ABC) transporter found in the cell membrane. CFTR functions as the regulator of chloride ion conductance. The ΔF508 mutation reduces chloride transportation leading to the accumulation of thick mucus outside the epithelial membrane in the lungs. The Lumacaftor is a commercially available pharmaceutical drug that functions as protein folding chaperone and helps CFTR trafficking to the surface of cell. In this study, an effort as taken to optimize the structure of Lumacaftor by attaching various functional groups (methyl –CH3, amine –NH2 and, hydroxyl -OH) based on the amino acids found at the active site. From the docking calculations it was found that among these functional groups, the OH group at the 7-position of the Lumacaftor showed better binding energy -10.0 kcal/mol compared to Lumacaftor with CFTR protein with binding energy of -8.2 kcal/mol. This modified derivative might reduce the drug dosage due to its better binding affinity and might work as a better choice of drug to treat cystic fibrosis (CF).

Introduction

Cystic fibrosis is an autosomal recessive genetic disorder affecting around 70,000 people worldwide but mostly the Caucasian due to various mutations.[1] The CFTR associated with the ABC transporter is embedded in the membrane. The function of CFTR protein is to transport the Cl- ions across the cell membrane.[2] The pathogenesis of cystic fibrosis affects multiple organ systems such as lungs, intestine and, pancreas. In cystic fibrosis, the chronic infection of the bronchioles is the most common symptom.[3]

The ABC transporter consists of two membrane spanning domains (MSDs) that function as a channel pore, two nucleotide binding domains (NBDs) that hydrolysis the ATP to control the channel gating and, the regulatory domain (R) regulates the channel to open. The CFTR channel opens when the R domain is phosphorylated with the hydrolysis of ATP.[4] Various mutations in CFTR gene are linked to cystic fibrosis that ensues because of functional derangements of CFTR protein at different levels.[5]

The most common mutation is F508del that affects about 90% of human beings. This mutation leads to the misfolding of CFTR protein and consequently the loss of channel gating. This mutation that occurs in the NBD1 which conducts the thermal instability and, kinetics. It also influences the functions of biosynthesis of channel and the trafficking of CFTR protein.[6]

Current treatment has focused on potentiator and corrector group of drugs, wherein Klydeco is targeting G551D and ORKAMBI targeting ΔF508 mutations.[7] The Lumacaftor or VX-809 is a corrector that reduces the misfolding of mutant CFTR. However, it is effective when taken in combination with the VX-770.[8] Lumacaftor treatment leads to chest discomfort and the respiratory abnormality often associated with hypersensitivity reaction, pulmonary exacerbation, pneumonia, dyspnea and hemoptysis (9). Therefore, an alternative drug with increased potency might be beneficial for the treatment of cystic fibrosis. In this report, we employ computer-aided drug design (CADD) approach (Figure 1) to optimize the CFTR corrector molecule.

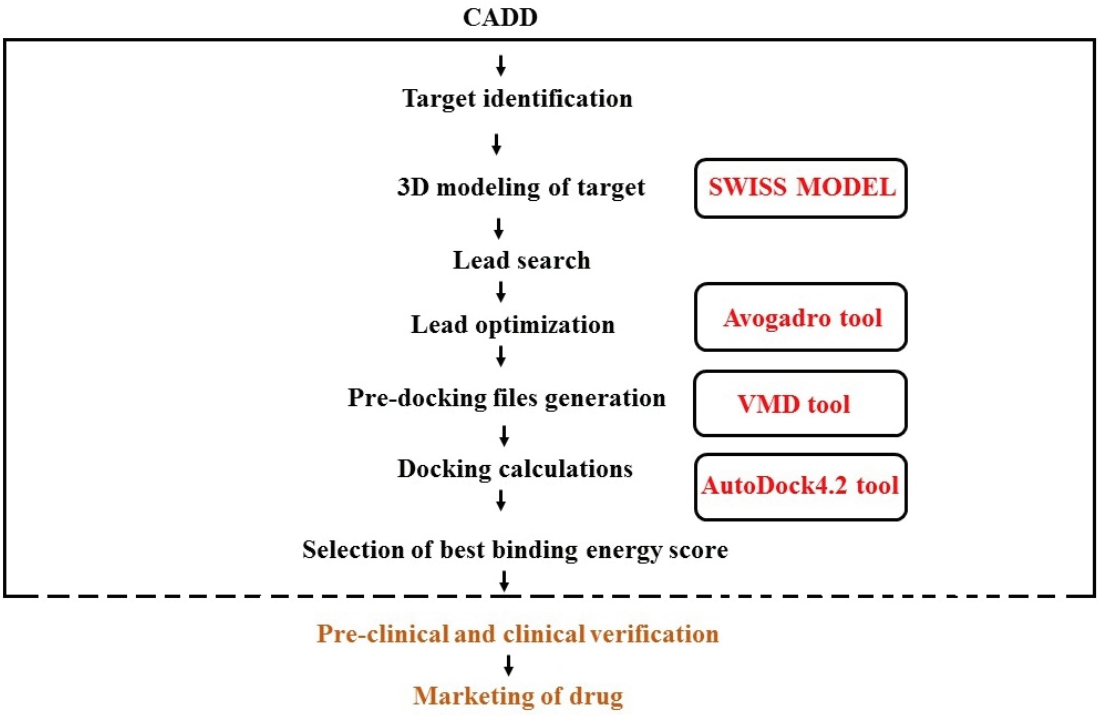

Figure 1: Computer aided drug designing (CADD) approach.

Methodology

Homology modeling of ΔF508 mutant

SWISS MODEL is an online tool widely used for building the 3D protein structures by homology modeling. The wildtype protein sequence of NBD1 was retrieved from PDB database with PDB ID (2PZE). The FASTA sequence was used for constructing the 3D model of F508 mutant through SWISS MODEL online server. This construct was later used for the interaction study with ligand Lumacaftor.[10]

Modeling of small molecules

Avogadro program was used to construct and optimize the structure of lumacaftor and its derivatives.

Molecular docking

The molecular docking is designed for the prediction of the interaction between NBD1 of CFTR and ligand (lumacaftor). The macromolecule and the ligand files were prepared using AutoDock Tools. The Grid box was used to specify the region for docking of the ligand. In this study, the Grid box covered the whole protein allowing the Ligand to freely move and locate the binding site with highest affinity. The Genetic algorithm was set with population size of 50 for the docking studies.[12]

Lead optimization

The lead optimization was done by adding different functional groups (methyl –CH3, amine –NH3 and hydroxyl -OH) to various sites[13] and the compounds were docked with NBD1 domain of ΔF508 mutant.

Result and discussion

First, we employed homology modeling to generate the structure of ΔF508 mutant using the wild type protein (2PZE). The structure of ΔF508 mutant is shown in Figure 2.

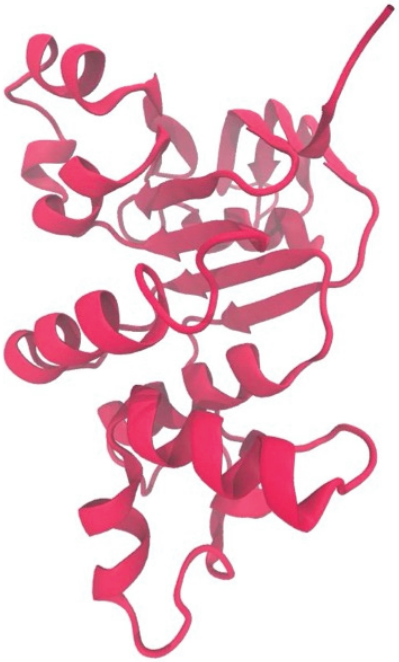

Figure 2: The 3D structure of Phe508del Mutant CFTR modeled using SWISS MODEL tool

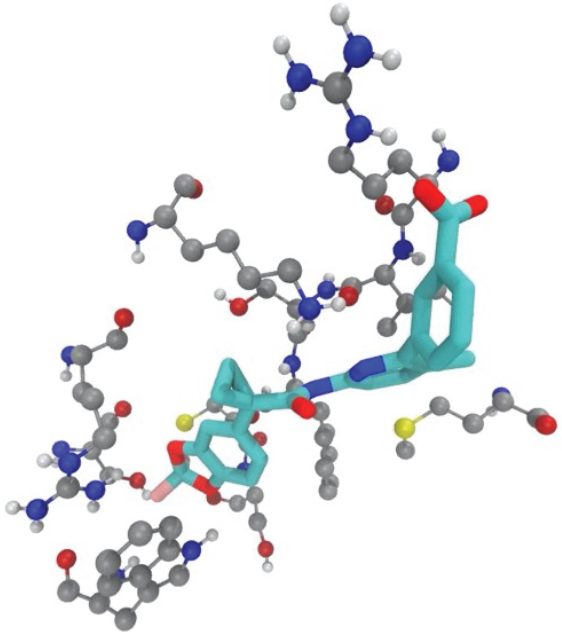

Figure 3: Binding pose of the Lumacaftor within the binding pocket of NBD1 protein

The corrector molecule (lumacaftor) plays a crucial role in the trafficking of the ΔF508 mutant protein to the cell membrane to increase the lung function. Molecular docking was performed to calculate the binding affinity as well as to identify the docked conformation. The binding energy obtained from the docking calculation was found to be -8.2 kcal/mol. The binding pose of lumacaftor is shown in Figure 3. Based on the interactions of lumacaftor with the amino acids of protein, the optimization was carried out to improve the binding affinity.

During lead optimization, the effectiveness of lumacaftor get positively enhanced by adding some functional groups such as methyl group, amine group and, hydroxyl groups (Table 1). The attachment of hydroxyl group to 7th-position of lumacaftor revealed the best binding energy score (-10.0 kcal/mol), while lumacaftor has a binding energy of -8.2 kcal/mol.

The binding pose of Ligand#1 is shown in Fig. 4. The modified derivative was found to have a very similar binding pose.

When the methyl group was at position-1 of lumacaftor, the lowest binding energy -9.2 kcal/mol was observed. In case of amine group, the best binding energy was found when the functional group was attached to the position-1 of the lumacaftor with a binding score of -9.7 kcal/mol. The hydroxyl group showed the best binding affinity -10.0 kcal/mol, compared to all the derivatives when it was attached to position-7 of the lumacaftor molecule. This structure-activity relationship might be very useful for the future design of CFTR correctors.

S.NO	LIGAND	FUNCTIONAL GROUPS	BINDING ENERGY (kcal/mol)
u1	Ligand#1	OH(7)	-10.0
2	Ligand#2	NH3(1)	-9.7
3	Ligand#3	OH(6)	-9.7
4	Ligand#4	CH3(1)	-9.2
5	Ligand#5	OH(3)	-9.2
6	Ligand#6	CH3(3)	-9.1
7	Ligand#7	CH3(4)	-9.1
8	Ligand#8	NH3(4)	-9.1
9	Ligand#9	CH3(5)	-9.0
10	Ligand#10	OH(1)	-8.9
11	Lumacaftor	-	-8.2

Table 1: Binding energy of optimized lumacaftor derivatives

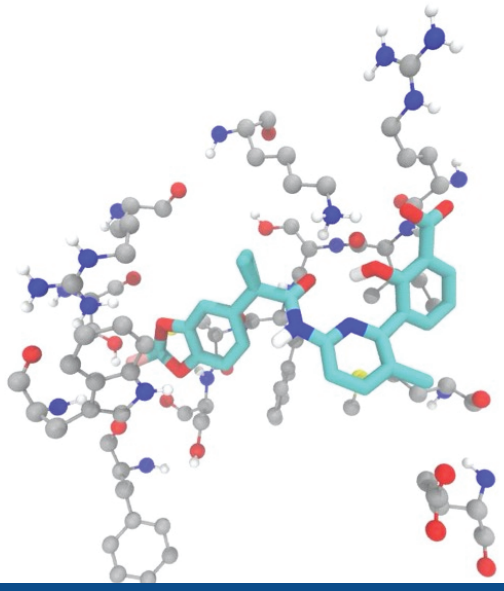

Figure 4: Binding pose of Ligand#1 at the active site of the NBD1 of protein

Conclusion

The mutant CFTR protein loses its structural and functional integrities, leading to cystic fibrosis in the lungs. In the present study, lumacaftor was modified by attaching various functional groups (methyl, amine and, hydroxyl) based on the amino acids found at the active site. From the docking calculations it was found that among the functional groups added, the OH group incorporated at the position-7 of the lumacaftor showed better binding energy -10.0 kcal/mol, compared to lumacaftor(unmodified)interaction with CFTR protein with a binding

energy of -8.2 kcal/mol. This modified derivative might work as a better choice of drug to treat cystic fibrosis (CF).

Conflicts of interest

All the authors declare that they have no conflict of interest

Acknowledgement

Authors thank Prof. N. Srinivasan for his valuable discussion. This work was supported by Chettinad Academy of Research and Education, Kelambakkam, Chennai, Tamilnadu.

References

1. Tsui LC. The spectrum of cystic fibrosis mutations. Trends in Genetics. 1992, 8:392-398.
2. Linsdell P. Functional architecture of the CFTR chloride channel. Molecular membrane biology. 2014, 31:1-6.
3. Goss CH et al., Patient-reported respiratory symptoms in cystic fibrosis. Journal of Cystic Fibrosis. 2009, 8:245-252.
4. Liu F et al. "Molecular structure of the human CFTR ion channel." Cell 2017, 169: 85-95.
5. Veit G et al. "From CFTR biology toward combinatorial pharmacotherapy: expanded classification of cystic fibrosis mutations." Molecular biology of the cell 2016, 27: 424-433.
6. Molinski S et al., "Functional rescue of F508del-CFTR using small molecule correctors." Frontiers in pharmacology 2012, 3:160
7. Edmondson, Claire, and Jane C. Davies. "Current and future treatment options for cystic fibrosis lung disease: latest evidence and clinical implications." Therapeutic advances in chronic disease 2016, 7:170-183.
8. Hudson R et al., "Direct binding of the corrector VX-809 to human CFTR NBD1: evidence of an allosteric coupling between the binding site and the NBD1: CL4 interface." Molecular pharmacology 2017, 92:124-135.
9. Ranganathan S et al., "Early lung disease in infants and preschool children with cystic fibrosis. What have we learned and what should we do about it?" American journal of respiratory and critical care medicine 2017, 195:1567-1575.
10. Schwede T et al., "SWISS-MODEL: an automated protein homology-modeling server." Nucleic acids research 2003, 31:338.
11. Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. Journal of molecular graphics. 1996, 14:33-38.
12. Goodsell DS, Morris GM, Olson AJ. Automated docking of flexible ligands: applications of AutoDock. Journal of molecular recognition. 1996, 9:1-5.
13. Patidar A et al., "Lead Discovery and lead optimization: A useful strategy in molecular modification of lead compound in analog design." International journal of drug design and discovery 2011, 2:458-463.