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Protein-protein Docking Studies of Estrogen Receptor Alpha and TRIM56 Interaction for Breast Cancer Drug Screening

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Abstract

Breast cancer is the highest mortality cause in women with cancer. Protein-protein docking for target-based screening is an effective approach in breast cancer drug discovery via estrogen receptor (ER) signaling. TRIM56, an E3 ubiquitin protein ligase, can bind to and stabilize ER alpha. Thus, drug screening that can inhibit or weaken the interaction between ER alpha and TRIM56 is promising to obtain novel yet specific breast cancer drugs. In this study, we performed protein-protein docking studies for ER alpha and TRIM56 interaction and virtual screening for FDA-approved drugs from the ZINC database against ER alpha and TRIM56 complex protein model structure. We utilized Cluspro 2.0, PyRx 0.8, and Pymol 2.4.1 to conduct protein-protein docking, virtual screening, and model structure visualization. PIP and PLIP software were also applied to analyze the amino acid residue between proteins or protein-ligands. Based on the protein-protein docking, ER alpha and TRIM56 established interaction. Utilizing this complex protein as a macromolecule in the virtual screen of 1071 molecules of FDA-approved drugs, we obtain the top five lowest binding energy molecules *i.e.*, dutasteride, dihydroergotamine, nilotinib, ergotamine, and bromocriptine. In addition, the energy binding affinity between ER alpha-dutasteride complex with TRIM56 was weakened in the presence of dutasteride. In conclusion, protein-protein docking between ER alpha-TRIM56 was able to select FDA-approved drugs that could bind to the complex, and dutasteride binding to ER alpha-TRIM56 complex weakened the interaction.

Keywords: *protein-protein docking, estrogen receptor alpha, TRIM56, breast cancer, ubiquitin.*

INTRODUCTION

Breast cancer incidence reached 14.1 million, and cancer mortality peaked at 8.2 million in 2012 worldwide. Still, breast cancer is the highest mortality in women with cancer (Bray, *et al*., 2018). The estrogen receptor is the prominent target for cancer treatment dan prevention, primarily breast, prostate, colon, and ovarian cancer (Nilsson &

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Gustafsson, 2011). Estrogen receptor isoform alpha (ER alpha) and beta (ER beta) are distributed in the various human tissue in which more than 70% of breast cancer cases are ER alpha positive (Tecalco-Cruz, *et al*., 2018). A high resistance rate toward many chemotherapeutic agents for breast cancer including tamoxifen, stimulates the need to discover the specific breast cancer drug targeting estrogen receptors (Chang, 2012; Viedma-RodríGuez, *et al*., 2014).

Protein-protein docking is an *silico* approaches for structure-based design for drug discovery targeting protein-protein interaction (PPI). Protein-protein docking resulted three-dimensional structure of two interacting proteins complex and provides the well-defined interface regions, so-called 'hot spots' (Kaczor, *et al*., 2018; Porter, *al*., 2019). These hot spots are plays role in most of the complex binding energy and can be targeted for PPI modulator (Kaczor, *et al*., 2018). Thus, the protein complex from protein-protein docking can be applied for drug screening to obtain these modulators. Drug screening using target-based utilize chemoinformatic is a prominent approach in drug discovery research (Dallakyan & Olson, 2015). This approach enables rational drug design targeting specific proteins, which is more effective drug discovery process compares to screening thousands of plant extract samples (Vakser, 2014). Novel drug discovery for anti-breast cancer targeting ER alpha which applies computational tools, allows more specific breast cancer drug discovery to run effectively. ER is a prominent target for drug development in preventing and treating breast, prostate, colon, and uterine cancer (Nilsson & ustafsson, 2011).

In ER signaling, E3 ubiquitin ligase protein TRIM56 acts as a factor regulator, binding to ER alpha (Tecalco-Cruz, *et al*., 2018). TRIM56 binds to and stabilizes ER alpha, where low level of TRIM56 protein expression causes the inhibition of breast cancer cell growth (Xue, *et al*., 2019). Novel drug screening that can inhibit or weaken the interaction between ER alpha and TRIM56 is potential to obtain novel yet specific breast cancer drug.

In this study, we performed protein-protein docking studies to simulate and analyze the interaction between ER alpha and TRIM56. The best model with the lowest energy score was then utilized for virtual screening of FDA-approved drugs available in the ZINC database. This computational analysis provides a prediction platform to obtain the inhibitor of ER alpha-TRIM56 interaction, which weakens their energy binding.

METHODS

Proteins and Ligands Preparation

The crystal structu of TRIM56 (PDB ID: 5JW7) and ER alpha (PDB ID: 1A52) were retrieved from Protein Data Bank (https://www.rcsb.org/). Ligand associated in the structure (Zn and SOP peptide in 5JW7; EST and Au in 1A52) and water were all separated from the proteins. Addition of polar hydrogen atoms and Kollman charges to the proteins were then performed using PyMol software and ed the structure as PDB files to be uploaded in ClusPro 2.0 (Kozakov, *et al*., 2017) for protein-protein docking.

The compounds about 1615 FDA-approved drugs were downloaded from ZINC database (Sterling & win, 2015). Their energy form was minimized and converted to pdbqt format by Open Babel in PyRx 0.8. Among 1615 FDA-approved drugs, as much as 1071 drugs were successfully minimized.

Protein-protein Docking of Estrogen Receptor (ER) Alpha and TRIM56

Protein-protein interacti simulation between ER alpha and TRIM56 was performed using ClusPro 2.0 (https://cluspro.bu.edu/publications.php) (Kozakov, *et al*., 2017). Protein-protein docking using ClusPro is a three-step computation: 1) rigid-body docking using billions of global

protein conformation databases, 2) clustering 1000 structures with the lowest binding energy based on root-mean-square deviation (RMSD) to get a representation of model cluster, 3) sorting the chosen structure based on the minimum energy.

The 3D structures for the input in ClusPro 2.0 were PDB ID: 5JW7 for TRIM56 and 1A52 for ER alpha. The ER alpha 3D structure was an ER alpha ligand-binding domain (LBD). Four cluster models were obtained from the protein-protein docking based on the algorithm score: balanced, electrostatic-favoured, hydrophobic-favoured and Van der Waals + electrostatic. Cluster model, which showed the lowest energy based on "balanced" coefficient was chosen to be visualized and analyzed using PyMol 2.4.1 software.

Validation on the protein-protein docking result was performed by plotting the pdb file format of the best cluster model obtained from ClusPro 2.0 in the Ramacandran Plot Server (https://zlab.umassmed.edu/bu/rama/index.pl). The analysis of interacted amino acid residues was then performed after the best cluster model interaction between ER alpha and TRIM56 being obtained. The protein interface analysis was utilized PIC web server online (http://pic.mbu.iisc.ernet.in/cgi/submit_job.cgi).

Virtual Screening for Compounds to Estrogen Receptor (ER) Alpha and TRIM56 Complex

Virtual screening to discover novel drugs that interact with ER alpha and TRIM56 was conducted in 1615 FDA-approved drugs retrieved from the ZINC database (Sterling & Irwin, 2015). The virtual screening was performed using PyRx 0.8 (https://pyrx.sourceforge.io/) (Dallakyan & Olson, 2015). Using AutoDock Vina assembled in PyRx 0.8, all the 1615 FDA-approved drugs were subjected for docking against the predicted active site of ER alpha and TRIM56. This active site prediction utilized web server online FTMap (https://ftmap.bu.edu/login.php) to locate protein hot spots (Kozakov, *et al*., 2015). Energy optimization of the 1615 FDA-approved drugs molecule using default energy minimization parameters set by Open Babel built in PyRx 0.8 tools (uff force field and conjugate gradients for optimization algorithm with 'total number of steps'; 'number of steps for update'; and 'stop if energy difference is less than number' were set on 200; 1; and 0.1, respectively) resulted in 1071 molecules (Dallakyan & Olson, 2015). These 1071 molecules were then automatically converted its molecule file format into pdbqt format, thus can be docked to ER alpha and TRIM56 complex. The center was set to (X: 92.6582, Y: 13.1986, and Z: 88.9864), and the grid box was set to maximum (X: 62.9523, Y: 83.4567, and Z: 72.7673) with the exhaustiveness equal to 8.

Visualization of the ligand conformation toward ER alpha-TRIM56 complex utilized software PyMol 2.4.1. (https://pymol.org/2/). Docking calculation resulted in the binding energy, in which the PyRx 0.8, automatically showed the lowest energy for each compound with the standard limit of 2 Å.

RESULTS

Protein-protein Docking Simulation Showed the Interaction between Estrogen Receptor (ER) Alpha and TRIM56

Simulation of protein-protein 3D interaction between ER alpha (PDB ID: 1A52) and TRIM56 (PDB ID:5JW7) applied web server online Cluspro 2.0. The downloaded 3D structure of ER alpha and TRIM56 structure were cleaned from its native ligand, water, and compounds/metals associated with the deposited 3D structure prior their use for docking.

The best model score for ER alpha and TRIM56 protein-protein interaction from thirty best models derived from the Cluspro 2.0 docking results is summarized in Table 1 (Supplementary files S1. for all 30 models). These model scores were categorized based on the cluster size, which shows the number of neighbors within radius 9 Å

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Table 1. Best model score of ER alpha and TRIM56 protein complex.

Cluster	Members	Representative	Weighted Score
0	62	Center	-850.3
		Lowest Energy	-976.7

C-α RMSD. Model 0 (cluster 0) is the model which has the most neighbors. Model 0 of ER alpha and TRIM56 visualization were then performed using PyMol to analyze the interaction between two proteins. This model was then used for virtual screening in PyRx tools.

Model 0 for ER alpha and TRIM56 complex visualization is displayed in Figure 1, as well as the amino acid residues involved in the interaction. Protein interface analysis using PIC web server online (http://pic.mbu.iisc.ernet.in/cgi/submit_job.cgi) showed the interaction existed between ER alpha and TRIM56. This protein-protein docking validation by Ramacandran plotting showed that the interaction between ER alpha and TRIM56 based on this model 0 obtained from ClusPro 2.0 is highly preferred (92.5%). Hydrophobic interactions within 5 Å were established between Valine 422 (V422) in ER alpha and Threonine 235 (T235) of TRIM56. Ionic interaction also occurred between Glutamic Acid 339 (E339) of ER alpha with Arginine 203 (R203) and Lysine 232 (K232) of TRIM56, and Glutamic Acid 419 (E419) of ER alpha with Lysine 232 (K232) of TRIM56. The detail of the interaction between these two proteins is displayed in Table 2. The information of protein-protein interface interaction is useful for further analysis on small molecule docking toward ER alpha and TRIM56 protein complex.

Dutasteride Binds to Estrogen Receptor (ER) Alpha and TRIM56 Complex and Weakens the Binding Affinity

Model structure of ER alpha and TRIM56 interaction with the best model score from protein-protein docking by Cluspro 2.0 was used for virtual screening in PyRx 0.8 tools. The model protein complex of ER alpha-TRIM56 as macromolecule was used to screen 1615 FDA-approved drugs in the ZINC database as ligands.

The maximum grid box was set to perform docking of 1071 molecules, which has been

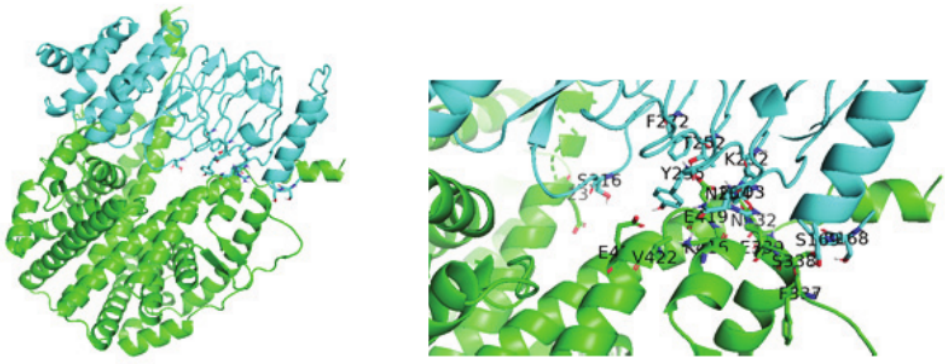

Figure 1. Visualization of 3D structure of ER alpha and TRIM56 protein complex. The cartoon version of ER alpha structure is shown in green and TRIM56 is in tosca with all structures displayed (A) or zoomed in 12Å with amino acid residues involved in the interaction are displayed.

Table 2. Protein-protein interface interaction between ER alpha and TRIM56 model structure.

Types	ER alpha		with	TRIM56	
	Position	Residue		Position	Residue
Hydrophobic Interaction (within 5 Å)					
	422	Valine		235	Tyrosine
Hydrogen Bonds Main Chain – Side Chain					
	338	Serine		168	Serine
	337	Phenylalanine		169	Serine
	416	Lysine		203	Arginine
Side Chain - Side Chain					
	338	Serine		168	Serine
	416	Lysine		204	Asparagine
	339	Glutamic Acid		203	Arginine
	339	Glutamic Acid		203	Arginine
	339	Glutamic Acid		203	Arginine
	339	Glutamic Acid		232	Lysine
	339	Glutamic Acid		232	Lysine
	419	Glutamic Acid		232	Lysine
	423	Glutamic Acid		235	Tyrosine
	423	Glutamic Acid		235	Tyrosine
	419	Glutamic Acid		252	Threonine
	523	Glutamic Acid		316	Serine
Ionic Interactions (Within 6 Å)					
	339	Glutamic Acid		203	Arginine
	339	Glutamic Acid		232	Lysine
	419	Glutamic Acid		232	Lysine
Cation-Pi Interactions (Within 6 Å)					
	531	Lysine		272	Phenylalanine

optimized its energy toward ER alpha and TRIM56 complex protein model. The docking of these 1071 molecules resulted energy binding scores ranging from -2.4 to -11.5 kcal/mol. The top five lowest were dutasteride (ZINC000003932831) (-11.5 kcal/mol), dihydroergotamine (ZINC000003978005) (-11.3 kcal/mol), nilotinib (ZINC000006716957), ergotamine (ZINC000052955754), and bromocriptine (ZINC000053683151) showed the same energy binding affinity *i.e* -10.9 kcal/mol (Supplementary Table 2.). Interestingly, dutasteride, widely used for benign prostatic hyperplasia (BPH) symptoms treatment in men with enlarged prostate, is the FDA-approved drug that showed the highest affinity to the ER alpha-TRIM56 complex (Figure 2).

Amino acid residue analysis from the interaction established by dutasteride (ZINC000003932831) and ER alpha-TRIM56 protein complex using PLIP tools (Salentin, 2015) is shown in Figure 3 and Table 4. There are amino acids residue in which they initially were interacted to establish affinity between ER alpha and TRIM56, which eventually interacted with dutasteride after docking simulation. Phenylalanine 272 (PHE272) residue of TRIM56, which originally interacted with Lysine 531 (LYS531) of ER alpha, showed to establish hydrophobic interaction with dutasteride in the halogen ring. Glutamic Acid 423 (GLU423) residue of ER alpha was also shown to have hydrophobic interaction with dutasteride in the androstane heterocyclic, replaced the interaction

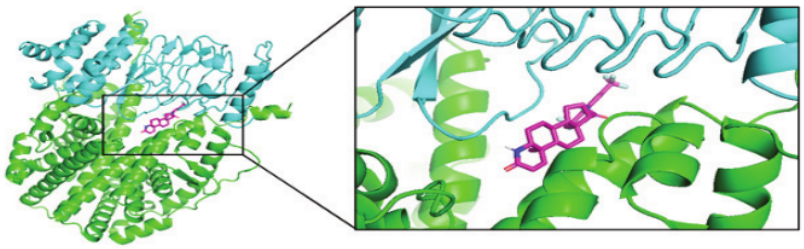

Figure 2. Three-dimensional structure of model ER alpha (green) and TRIM56 (tosca) complex and docked with ligand dutasteride (ZINC000003932831) in magenta.

with Tyrosine 235 (TYR235) of TRIM56. The protein-protein docking applied Cluspro 2.0 was then also proceeded to confirm the model score of ERalpha-TRIM56 when dutasteride is docked into the binding pocket. ER alpha-dutasteride complex was used as the receptor, and TRIM56 as the ligand resulted in higher model score dan lesser number of cluster members than the model score of ER alpha-TRIM56 without dutasteride (Supplementary Table S3).

DISCUSSION

ER is prominent specific target for preventing and treating breast cancer (Rodriguez-Gonzalez, *et al*., 2008). Tamoxifen is well known chemotherapeutic agent for breast cancer which regulating ER alpha (Xue, *et al*., 2019). Along with the higher incidence of tamoxifen resistance in decades, the investigation on finding molecule targeting ER alpha was extensively performed (Chang, 2012; Viedma-RodríGuez, *et al*., 2014). Study in PROTACS development, successfully designed the molecules which can recruits the Von-Hippel-Lindau (VHL) E3 ubiquitin complex to target and degrade ER alpha so that the breast cancer cell growth was inhibited (Rodriguez-Gonzalez, *et al*., 2008).

ER signaling is regulated by ubiquitin proteasome system (Kondakova, *et al*., 2020). Well studied and established knowledge for ER ubiquitination is the BRCA1/BARD1 and MDM2

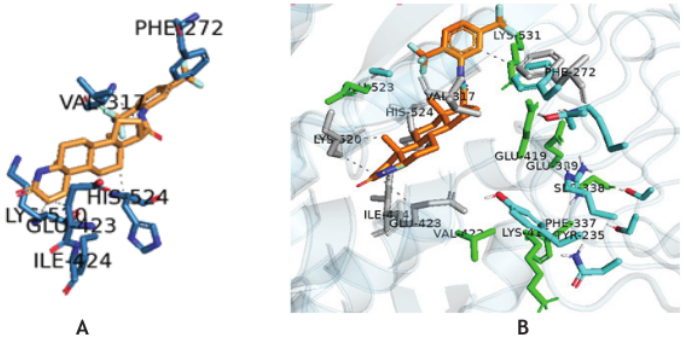

Figure 3. The 3D structure of amino acid residue of ER alpha-TRIM56 complex (blue) which involves in the interaction with dutasteride (yellow). (A). Structure overlay of ER alpha and TRIM56 complex before (ER alpha in green, TRIM56 in tosca) and after (ER alpha and TRIM56 in grey) dutasteride was docked in the binding pocket (B).

Table 4. Amino acid residue in ER alpha-TRIM56 complex protein interaction with dutasteride.

Interaction Type	Residue number	Residue Type	Protein Unit	Distance (Å)
Hydrophobic Interaction	272	Phenylalanine	TRIM56	3.62
	317	Valine	TRIM56	2.50
	317	Valine	TRIM56	3.40
	423	Glutamic Acid	ER alpha	3.65
	424	Isoleucine	ER alpha	3.59
	520	Lysine	ER alpha	3.65
	520	Lysine	ER alpha	3.38
	524	Histidine	ER alpha	3.56
Halogen Binding	317	Valine	TRIM56	2.90

which were ubiquitinated ER alpha and stimulate ER alpha degradation, thus suppressed the development of breast and ovarian tumor (Duong, *et al.*, 2007; Eakin, *et al.*, 2007). SMURF1, an E3 ubiquitin ligase also found to control ubiquitination of ER alpha and increases its stability (Yang, *et al.*, 2018). Another potential mechanism of ER alpha-ubiquitin regulation is based on the study that revealed the interaction between ER alpha and TRIM56. TRIM56 is an E3 ubiquitin ligase which is highly expressed in the innate immune system (Shen, *et al.*, 2012). TRIM56 binds to and stabilize ER alpha, unlike the common ubiquitin ligase that degrade it (Tecalco-Cruz, *et al.*, 2018; Xue, *et al.*, 2019). The binding of TRIM56 to ER alpha increase the breast cancer cell proliferation. Thus, targeting the inhibition of ER alpha and TRIM56 interaction is the promising approach for novel breast cancer drug discovery.

In this computational study, we successfully provide the information that indeed ER alpha established the interaction with TRIM56. This interaction is stabilized with hydrogen bonds and several types of interactions including hydrophobic, ionic, and cation-Pi among the amino acid residues lies in the protein interface (Figure 1 and Table 2). Virtual screening on 1071 optimized molecule of FDA-approved drugs against the ER alpha-TRIM56 complex resulted molecules with the top five lowest binding energy whose indication is not for breast cancer treatment. These top five FDA-approved drugs are dutasteride, dihydroergotamine, nilotinib, ergotamine, and bromocriptine.

Dutasteride, drug used for treating the symptoms of BHP in enlarged prostate male was molecule that showed the highest binding affinity among 1071 molecules toward ER alpha-TRIM56 complex. Indeed, the studies on 5-alpha-reductase inhibitor (including dutasteride) on breast cancer were reported. Treatment of dutasteride decreased MDA-MB 468 and MDA-MB 231 cell viability and increased the sensitivity of the cells toward chemotherapeutic agent (Von Wahlde, *et al.*, 2015). The study on the safety use of dutasteride to treat alopecia in breast cancer patients also revealed that there was no increased estrogen level (Rozner, *et al.*, 2019). However, both studies did not relate the dutasteride role in ER alpha-TRIM56 interaction.

In addition, the other four drugs which showed interaction with ER alpha-TRIM56 complex are known for various treatments. Dihydroergotamine and ergotamine widely used for migraine. Nilotinib is the potential treatment for various type of leukemia including chronic myeloid leukemia (CML). Bromocriptine is known for galactorrhea treatment due to prolactin-associated disorders as well as additional treatment in surgery or radiotherapy for acromegaly, or as single treatment for early Parkinson syndrome or for

additional treatment of levodopa in advanced case with motoric complication and off-label usage for treatment of restless legs and neuroleptic malignant syndrome (https://go.drugbank.com/).

We confirmed based on the computational approach, that the presence of dutasteride weaken the interaction between ER alpha and TRIM56. Thus, extensive investigation *in vitro*, *in vivo*, biochemical and cell biology assays is required to provide stronger evidence to discover novel drug targeting ER alpha and TRIM56 interaction.

CONCLUSION

Protein-protein docking studies provide information on the interaction between ER alpha and TRIM56. FDA-approved drugs: dutasteride, dihydroergotamine, nilotinib, ergotamine and bromocriptine were top-five lowest binding energy molecules which were generated from virtual screening against ER alpha-TRIM56 complex. The binding of dutasteride weakened the binding energy between ER alpha-TRIM56. Proceeding to repurpose dutasteride as anti-breast cancer drug targeting ER alpha-TRIM56 interaction is promising. Thus, extensive validation by *in vitro*, *in vivo*, biochemistry, as well as cell biology assays is crucial to strengthen this computational assay evidence.

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