Development of STimulator of Interferon Genes Agonists in Silico

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Abstract. STimulator of Interferon Genes (STING) is now considered as a promising target for tumour immunotherapy. In normal cells, STING is able to activate the generation of Type I interferon (IFN) and in turn can induce the activity of T cells, but in cancer cells, the expression of STING is inhibited due to the hypomethylation of its promoter. Cyclic dinucleotides were taken as the agonists to trigger the cGAS/STING pathway in cancer cells. However, this type of agonist is hard to be administrated to patients with tumour, and thus the discovery of STING agonists focuses on the development of small molecular drugs. In developing small molecular drugs for target proteins, computer-aided drug discovery (CADD) is an important tool. The utilize of this tool can reduce waste of time and budget which are consumed in the development of ligands with traditional methods. In this research, Schrödinger, a type of CADD software, was utilized for virtual screening agonists for activating STING effectively. There were four ligands obtained after virtual screening the small molecule database, and their interaction with target protein was analysed and compared.

1. Introduction
Immunotherapy is one of the hottest research topics regarding tumour suppression. STimulator of Interferon Genes (STING) has become a promising target of anti-tumour therapy. STING is a transmembrane protein which could be expressed in various types of tissues, and it is mainly found in the endoplasmic reticulum (ER)[1]. Accordingly, STING-Cyclic GMP-AMP synthase (cGAS) pathway plays an important role in activating innate antitumor immune signalling[2]. In normal cells, the activated cGAS by aberrant cytosolic DNA is able to generate the mammalian 2’,3’cGAMP, leading to activate STING protein. Subsequently, the activation of STING induces the generation of Type I interferon (IFN) and other pro-inflammatory cytokines, contributing to promote immune response in turn[3]. However, in various cancer cells, the expression of STING and cGAS is inhibited following the hypomethylation of their promoters. As a result, tumour cells have adapted ways to escape from immune surveillance, which are always observed in the colon cancer cells and melanoma cells regularly[4, 5].

Several pharmaceutical companies and research institutes pay much attention to the STING protein, hoping to find a promising STING agonist as the agent for corresponding various cancers. Currently, the strategy of drug design focuses on modifying cyclic dinucleotides and obtained that are mimic of endogenous STING ligands cGAMP, such as ADU-S100[6]. Although these kinds of agonists have shown a level of potential efficacy in pre-clinical trials, the mode of administration limits the potential application, as it could only be injected intratumorally due to its low metabolic stability. Besides this, the mimic of endogenous STING ligands works through patients with solid tumour so far[7, 8]. To
overcome these challenges, Gajewski and Higgs suggested that non-nucleotide small molecule maybe a great choice as STING agonists in their recently work[9], since small molecules have good stability and they can be administered either by oral or by injection in animal models, which shows desirable anti-tumour activity. Therefore, the small molecule STING agonist is an attractive way to target STING-cGAS signalling pathway and has the potential to optimize treatment modalities and effects.

For rapid screening of optimal small molecules as STING agonists, computer-aided drug discovery (CADD), an effective tool for lead compounds identification and drug research, will be applied in this study. Since novel drug development is extremely complex, time-consuming and costly, usually taking more than a decade and billions of dollars, which would be saved by the usage of CADD in the process of drug discovery. Generally, the main role of in silico methods in drug discovery is to screen better compounds that have desired biological properties in vitro and in vivo. Moreover, the computational analysis provides necessary guidance for the experiment, thus this analysis method is able to reduce the number of candidate small molecules which are need to be evaluated experimentally[10]. Zhong and Li applied CADD for identifying STING agonists within drug library and successfully obtained three candidates which were tested in the animal models in the following experiments[11]. Thus, CADD is a great analysis tool used in this research to dock small molecules with STING.

This study will explore several small molecular drugs which can bind to STING protein. Schrödinger, a CADD software, will be utilized to virtual screen ligand database and select optimal compound candidates. Moreover, Schrödinger will score the compounds, according to the interaction between compounds and target protein. Four top scored compounds will be selected and described in this research.

2. Materials and methods

2.1. Preparation of protein and ligands

Three-dimensional (3D) X-ray crystal structure of STING protein was obtained from protein data bank (PDB) website. This protein structure was pre-treated by Protein Prepared Wizard[12] with default parameter in Schrödinger Suit 2017-1 (Schrödinger, LLC, NY, USA) which mainly including protonation, missing side-chains, missing loops, counterions/random small molecules/waters and bonding/ionisation/tautomerisation. Subsequently, the active of this protein structure was identified for grid generation in the Receptor Grid Generation model. In this step, the receptor binding site was identified by selecting ligand molecule, this position was set as the centroid of the enclosing box, and other parameters were default. Meantime, the ligands from SPECS database were prepared by the Ligprep suite of Schrödinger Suit 2017-1 (Schrödinger, LLC, NY, USA), using default settings in this step at pH= 7.0 ±2.0[13].

2.2. Ligand docking

After finishing the preparation of protein and ligands, the prepared molecules were passed through Ligand Docking in Schrödinger Suit 2017-1 (Schrödinger, LLC, NY, USA). In detail, the parameter settings were as follows: after docking keep 10% of best compounds in the process of the high throughput virtual screening and reserve 1% of best compounds after standard precision (SP) and extra precision (XP) ligand docking[12]. The default settings were used in all settings whose changes are not mentioned here.

3. Results and discussions

Molecular docking is to locate the translation, rotation and conformation of a given ligand which maximizes interactions of that ligand with the protein. The simulated pairing of ligands and proteins could explore the optimal ligand structure for binding to the proteins with known three-dimensional crystal structures and binding pockets. In this simulated docking process which is based on protein structure, tens of thousands of possible ligands are collected from compound library, and the results of this binding conformation are evaluated by several metrics including the docking score[14]. Herein, Schrödinger[15], a CADD software, was utilized to give the investigation of interactions between
small molecules and STING protein. The ligand-protein binding is a vital step in the docking process. Understanding the molecular interactions between small molecules and proteins is of great value in designing and optimizing the treatment of small molecules of target proteins. The interactions between them usually include hydrogen bond which is a stable link, π cation and Van der Waals’ force that is the most unstable connection, etc[16].

Table 1. Top 4 small molecules selected from virtual screening

| Compounds | Structure | Docking score (kcal/mol) |
|-----------|-----------|--------------------------|
| C1        | ![Structure of C1](image1) | -9.742                   |
| C2        | ![Structure of C2](image2) | -9.668                   |
| C3        | ![Structure of C3](image3) | -9.513                   |
| C4        | ![Structure of C4](image4) | -9.187                   |

After finishing the preparation of protein and ligands, the prepared molecules were passed through ligand docking in Schrödinger. In detail, the parameter settings were as follows: keep 10% of best compounds in the process of the high throughput virtual screening and reserve 1% of best compounds after standard precision (SP) and extra precision (XP) ligand docking. As shown in Table 1, most of the unsuitable ligands were filtered out and top 4 satisfied ligands named C1-C4 were selected to analyse the interaction between the receptor and ligands.
Figure 1. The prediction of binding patterns of C1 within the active site pocket of STING. Compounds in green stick refers to the refined ligand pose. Grey sticks represent amino acid residues of STING. Green line of dashes refers to good link, yellow line of dashes meant hydrogen bond and orange dotted line indicates the connection between two molecules is bad.

The understanding of binding modes of potent inhibitors with the targets was beneficial to further chemical modifications. Figure 1 shows that C1 could generate multiple intermolecular interactions in the binding site of STING protein. In detail, C1 interacted with STING through five hydrogen bonds between the head and the hinge region (THR263, THR267, SER162, ARG238). Noteworthy, SER162 was able to form two hydrogen bonds with hydroxyl group and carboxamido group at the head of C1. Additionally, ARG238 formed π cation supramolecular interactions with the triazinane at the centre of the compound structure. Overall, C1 can combine structurally with STING, as they create intermolecular connections in many parts.

Figure 2. The prediction of binding patterns of C2 to STING.

C2 was docked with the catalytic cleft of STING. As Figure 2 shown, the molecular interactions between C2 and target proteins were almost consistent with the interactions between C1 and protein. The only difference is that the THR267 residue was hydrogen bonded to C1, but this residue did not interact with the C2. This results the molecular connection between the targeted protein and C2 is firm.
Figure 3. Binding patterns of compound C3 as predicted by Glide docking simulation.

C3 was docked with the bind site of STING for the prediction of binding modes between these two molecules. Figure 3 illustrates the connection between C3 and STING protein. Triazinane at the center of this compound formed π cation interaction with ARG238, which was different from C1 and C2. In addition, THR263 and SER162 interacted with the compound by forming the hydrogen bond. It should be noted that the two groups linked to two different amino acids were located on both sides of pyridine. Therefore, C3 has ability to form stronger molecular interaction with STING.

Figure 4. Binding patterns of compounds C4 as predicted by Glide docking simulation.

As for C4 it formed three hydrogen bonds between the 2-methylenehydrazine-1-carboxamide group in the tail and GLY166 and ARG238. As shown in Figure 4, the hydroxyl group located in the middle of the compound bonded with SER162 from two chains by hydrogen bonds. Additionally, at the head, GLY166 from other chain formed the hydrogen bond which fix the head’s conformation. To summarize, these findings agreed well with our preceding inductive conclusion that top 4 compounds were privileged structures in the activity target STING protein.

4. Conclusion

STING is a potential target in tumour immunotherapy. It is one of the most important elements in activating immune system in normal cells, while its function is inhibited in cancer cells. Thus, the activation of STING is a pivotal step in tumour immunotherapy. What is needed to do is finding out small molecular drugs, which can activate STING protein effectively. Furthermore, this compound could replace modified cyclic dinucleotides that are applying in clinic but bringing pain to patients. Nevertheless, there were only a few studies to seek for diverse chemical series of STING agonists and
to evaluate them in simulational models. With the purpose to discover novel series of agonists of STING protein, CADD was applied in this research. CADD allows researchers to use computers to quickly screen suitable small molecules, which greatly shortens the test time and budget. It could screen compounds with promising biological properties that can be better bond to target proteins in vivo and in vitro. In addition, computer analysis has ability to provide data support for experiment as well.

In this research, a CADD software, Schrödinger, was utilized in preparing STING protein obtained from PDB and ligand which downloaded from SPECS database. Multi-step virtual screening was conducted to process the ligand docking. Final, top four proper ligands, interacting with STING with higher docking score than other compounds, were selected. Through analyzing and comparing the interaction between these four compounds with STING protein, they all could be perfectly chimeric in STING binding pocket and form strong and stable connections with STING. In the further research, these compounds will be tested about their binding stability and pharmacological effects with laboratory experiments.

5. Reference

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