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Discovery of Small Molecules for Cancer Immunotherapy Based on Targeting PD-1

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ABSTRACT

As the fatal nature of cancer, developing new treatment methods is very crucial. There are already several branches of cancer treatment, however, this research focused mainly on immunotherapy, which developed immune checkpoints that stop the performance of PD-1, a protein that controls immune tolerance in the tumor microenvironment. The process of the experiment started by using several ways to find possible binding sites; then identifying potential compounds that could bind to the site well, and lastly to validate that the compounds are suitable for drug usage.

KEYWORDS: *Immunotherapy, PD-1, immune checkpoint, drug discovery, Cancer, binding*

INTRODUCTION

During the past decades, cancer has increased severely, and it is urgent to develop an effective treatment. Overall, the incident rates have increased by 0.8% per year since 1975. However, the mortality rates caused by cancer have decreased by more than 50% from 1970 to 2019. Among children, the rate decreased from 6.3% to 1.8%, and for adolescents, the morality rate decreased from 7.2% to 2.8%.[1] By definition, cancer is a group of diseases for typical human cells, however, characterized by uncontrollable growth. The growth of cells causes collateral damage to the human body, eventually leading to death if not treated. Cancer can also be defined as a malignant tumor that spread un purposely. However, not all tumors are cancer. A benign tumor is relatively temper and treatments are not required depending on the situation. [2, 3, 4]

The causes of cancer still remain unclear in many aspects. Typically, the growth of cancer causes metabolic changes as a result of a more suitable microenvironment.

Cancer can be treated in several ways, including chemotherapy, surgery, radiation therapy, hormonal therapy, targeted therapy such as immunotherapy, etc. Surgery is the only way to remove the malignant tumor in some cases, and also generally the primary way to remove a benign tumor. During this surgical process, other healthy cells should not be damaged. [5] Immunotherapy, mainly focusing on the purpose of boosting the immune system to identify and control cancer cells, has the limitation that cancer cells tend to hide from the immune system and thus create difficulties in immunotherapy. Current clinic trials modify immunotherapy in two aspects. From one aspect, vaccines are used to inject genes inside and combine with targeted cancer cells. The immune system thus recognizes the cancer cells by presenting them with highly antigenic immunostimulatory cellular debris, eventually, the patient's immune system attacks cancer cells. Other aspects focus on the immune system itself. An alteration will be made directly to the immune system, making it highly sensitive to the cancer cells, and eventually leading to the unmasking of cancer cells and activating the immune system. [6]

Among immunotherapy, the development of immune checkpoint inhibitors seems to be promis- ing in recent years. For a T-cell to be activated, it has to be attached to certain cells called antigen-presenting cells (APC). After the activation of T-cells, immune checkpoints play a role as the set of inhibitory pathways that immune cells possess to regulate and control the durability of the immune response while maintaining self-tolerance. [7] Immune checkpoint inhibitors, on the other hand, suppress the immune checkpoints, prevent them from attaching and therefore promote the activation of T-cells. Recent studies focused mainly on PD-1/PD-L1 and CTLA-4 inhibitors, which showed promising results. For example, Ipilimumab, which targets cytotoxic T- lymphocyte antigen-4 (CTLA-4), is the first approved immune checkpoint inhibitor. It prevents the inhibition of T-cells. [8]

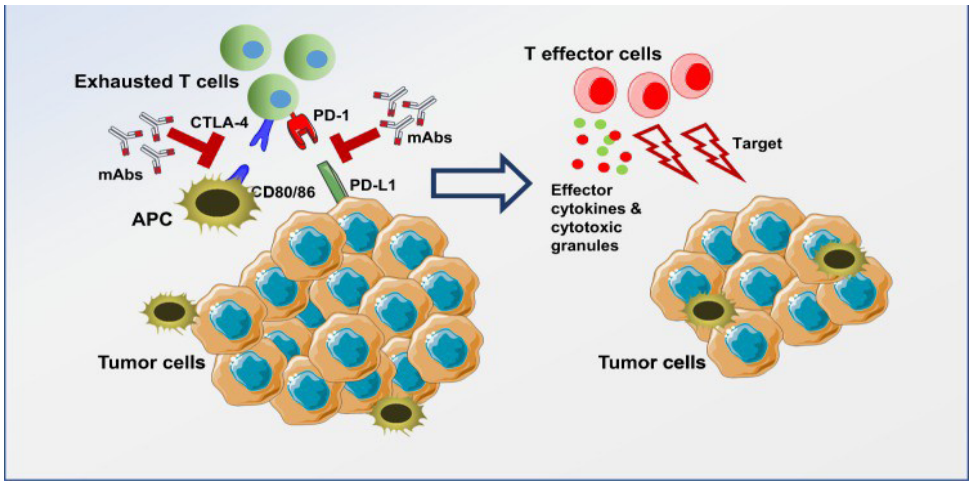


Figure 1. Immune checkpoints such as PD-1/PD-L1 and CTLA-4 are necessary checkpoints in order for the immune system to be activated and function correctly.

PD-1, along with its ligands PD-L1 and PD-L2, control the induction and maintenance of immune tolerance within the tumor microenvironment. They inhabit T cell activation, proliferation, survival, and cytotoxic secretion within the cancer cells. [9] In normal cases, the presence of PD-1 and PD-L1 automatically prohibits inflammation and overreacted immune systems. How- ever, with the presence of tumor cells, PD-1 and PD-L1 will behave abnormally and inhibits the host's antitumor immunity. Therefore, there is a necessity to develop anti-PD-1/PD-L1 antibodies, which is the main focus of the following content. [10]

METHODOLOGY

Analyzing Binding Sites

Geometric Methods

First of all, the binding site of PD-1 has been analyzed from the geometric aspects. The data was extracted from an online protein modeling server and used what is called DoGSiteScorer. DoGSiteScorer uses a difference in the Gaussian filter to detect potential binding pockets of PD-1. [11] The result will be analyzed based on the graph and the form.

As shown in the geometric structure of the protein (Shown in figure2), there are four potential binding pockets. P-0 has the greatest volume of 238.78 $Å^3$ (Shown in table1), however not the highest drug ability score (0.55) (Shown in table1). In this model the highest drug score is 0.62, which is P-1 with a volume of 209.79 $Å^3$. P-2 has a volume of 153.92 $Å^3$, and drug score of 0.33. P-3 has a volume of 127.74 $Å^3$ and a drug score of 0.29. (The data are shown in table1) Based on the table the relatively efficient binding pockets by geometric analyzation is P-1 site, and thus further research can focus on developing ligands targeting P-1 site.

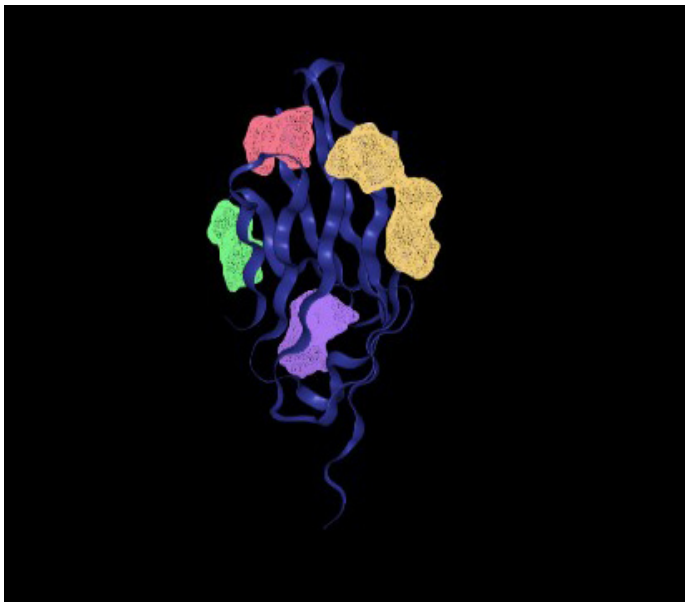


Figure 2. Binding sites on PD-1 protein.

Table 1. Scores of each binding site based on Geometric Ways

Name	Color	Volume Å^3	Surface Å^2	Drug Score
P _0	Yellow	238.78	593.75	0.55
P _1	Purple	209.79	319.78	0.62
P _2	Green	153.92	326.88	0.33
P _3	Red	127.74	329.38	0.29

Energy-Based Methods

Another method of finding binding sites of PD-1 is to use the energetic-based method which analyzes the resulting base on interaction energy. I used the FT site, and after I input the code 3RRQ (the code representing PD-1 protein) the result is shown below.

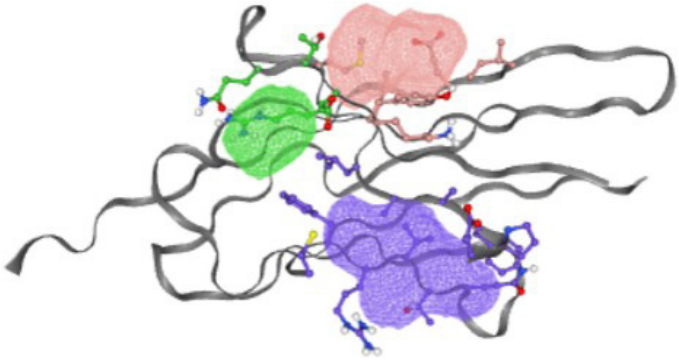


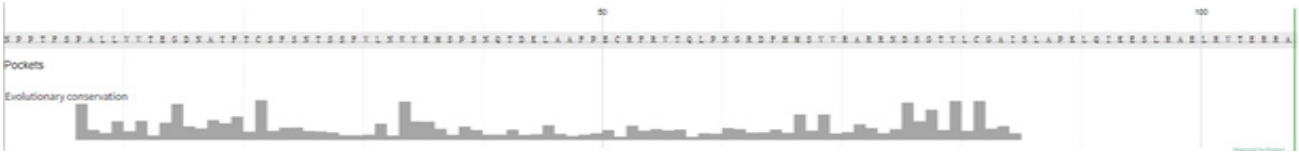
Figure 3. Binding sites based on the reaction energy

As shown in the graph, there are three binding sites in total, one of the potential binding pockets in the result of the geometric method seems to be proven invalid. The binding sites are colored in the following order: pink, green and purple. The purple one appears to have the greatest volume, with the pink one following and green having the smallest volume. However, the evolutionary conservation is not shown clearly in this result. [12, 13, 14]

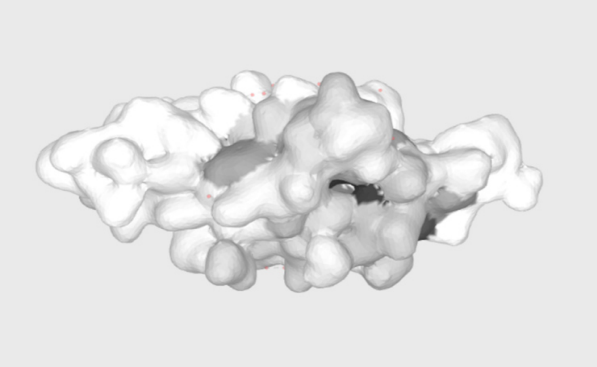
Machine Learning Methods

The affinity of the binding site is then analyzed from a web-based application that will predict the binding site of protein and ligands based on the machine learning method. Based on the computed data and structural visualization, there are several binding sites that demonstrated relatively high scores on the surface of the protein.

As shown in the picture above, the darker the surface color of the molecule the higher score of the pocket. Evolutionary conservation named CYS A 54 possessed the highest score of approximately 4.176 based on each residue. Other sites including TYR A 121 and CYS A 123 also reach a high score and are thus valuable for further evaluation of drug-binding sites. [15]



(a)Scores of each evolutionary conservation



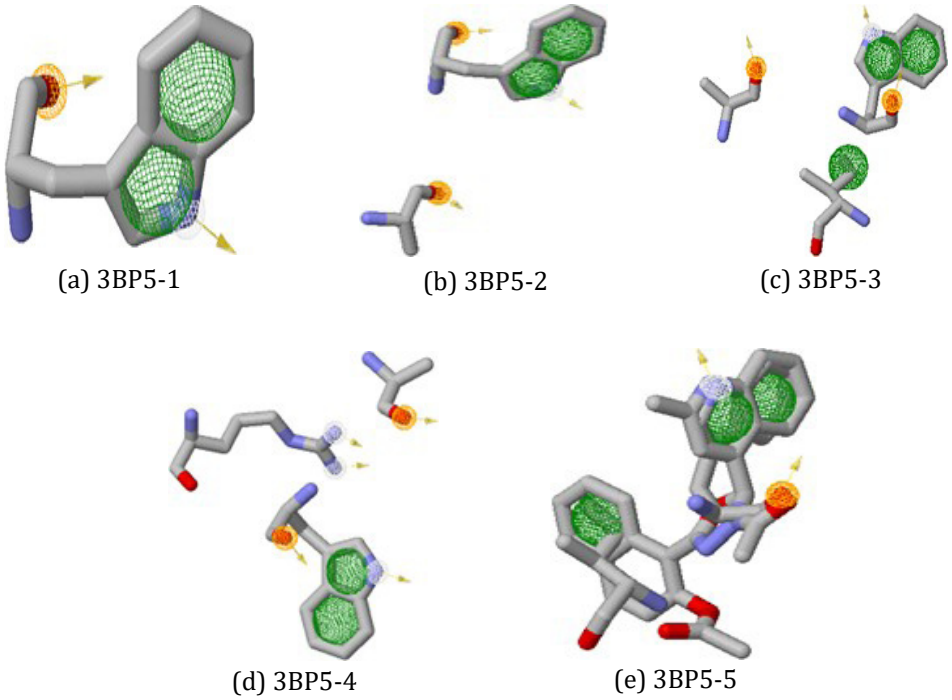
(b)Evolutionary conservations shown on the PD-1 protein.

Figure 4. Binding sites based on machine learning methods.

ANALYZING POTENTIAL CHEMICALS

Identifying Potential Chemicals

The next process focuses on virtual screening, which is a tool for monitoring the process of finding the compound that bind to the protein using a computer. Pocket Query was used to make the pharmacophore of the protein. First, we entered the PDB code 3BP5, and then I used the data of the first five results from chain B. Each result is sent to ZINC Pharmer to Perform a pharmacophore. After selecting a certain amount of pharmacophore classes three chemicals were extracted from each result.



(a) 3BP5-1 (b) 3BP5-2 (c) 3BP5-3

(d) 3BP5-4 (e) 3BP5-5

Figure 5. Graphs of each Pharmacophore

The chemicals above are shown following the order of the five resulting groups 1-5. Each set of the three chemicals is from the same group. The score of RMSD represents the result of the suitability of the overlay. The lower of RMSD is the more desirable the chemical is. Based on the result above, the number of RMSD as well as the mass of the chemicals are all very similar within a single group, yet the values vary greatly between groups. ZINC87085967 and ZINC94739262 seem to have the lowest RMSD score. Further analysis will be performed. [16]

Filtering Chemicals

Based on the previous results, there are fifteen compounds selected as potential binding ligands of PD-1. The following experiments focused on testing the efficiency of each compound when apply to the PD-1. I used SwissDock to complete the process, and enterd each compound name with PD-1.[17] I estimated the lowest ΔG for each compound. Lower ΔG represents a more efficient reaction.

As shown on the graph, compound ZINC87085967 appears to have the lowest ΔG of -8.37. Most of the other compounds have ΔG around 6 to 8. Compound ZINC93236075 also has a ΔG of 8.01. Both ZINC87085967 and ZINC93236075 could be considered valuable compounds for further evaluation.

Table 2. Chemicals extracted by ZINCPharmer

(a) Results of the Pharmocaphore

Results	Ch	Sz	Dist	Score
3BP5-1	B	1	0	0.947059
3BP5-2	B	2	8.1914	0.919713
3BP5-3	B	2	8.1914	0.888907
3BP5-4	B	3	10.0846	0.885257
3BP5-5	B	2	6.6422	0.882636

(b) Chemicals Infromation

Name	RMSD	Mass
ZINC94739262	0.063	291
ZINC94739498	0.126	293
ZINC01636716	0.131	289
ZINC90486834	0.439	370
ZINC95459217	0.439	384
ZINC93236075	0.468	301
ZINC12502613	0.223	479
ZINC12502596	0.223	479
ZINC12502634	0.223	519
ZINC74746522	0.027	302
ZINC87085967	0.034	361
ZINC79361341	0.054	339
ZINC21941365	0.303	440
ZINC21941370	0.307	382
ZINC13086034	0.376	458

Table 3. Energy required for each compound to bind the protein

Compound	Estimated ΔG (kcal/mol)	Compound	Estimated ΔG (kcal/mol)
ZINC94739262	-6.12	ZINC94739498	-7.73
ZINC01636716	-7.03	ZINC90486834	-7.63
ZINC95459217	-7.07	ZINC93236075	-8.01
ZINC12502613	-7.28	ZINC12502596	-7.54
ZINC12502634	-7.83	ZINC74746522	-7.15
ZINC87085967	-8.37	ZINC79361341	-7.03
ZINC21941365	-7.10	ZINC21941370	-6.78
ZINC13086034	-7.12		

The next step is mainly focusing on identifying the drug properties of the leading compounds for future practical usage in drugs. For a drug to have good absorption and permeation, the drug properties must follow the Lipinski's rule. Lipinski's rule contains:

1. No more than 5 hydrogen bond donors

2. Calculated LogP (ClogP) no more than 5

3. Molecular mass less than 500 Daltons

4. No more than 10 hydrogen bond acceptors

If the selected compound fulfilled all the requirements, they are preferred to be compounds of practical drugs. I first used a website and entered the chemical ID above, getting the SMILES ID of the chemicals. [18] I used Swiss ADME then, after entering the SMILES ID the information on the chemicals related to the drug properties is shown. The superior chemicals are selected in this step.

Table 4. Selected chemicals based on Lipinski's Rule

Compound	Number of H-bond Donors	CLogP	Molecular Mass (Daltons)	H-bond Acceptors
ZINC87085967	3	2.90	361.46	4
ZINC93236075	1	1.31	301.36	6
ZINC12502634	4	2.41	519.40	4

The data of the experiment are listed above. Based on the graph, all of three compounds suit the first requirements of no more than 5 hydrogen bond donors. Their cLogP value is all below 5, and they have no more than 10 H-bond acceptors. However, ZINC12502634 has a molar mass greater than 500. As a result, the only qualified compound would be ZINC87085967 and ZINC93236075 for potential drugs.

CONCLUSION

In summary, two compounds were identified that can be used as drugs aiming to cure cancer. The whole experiment is separated into several step-in order to identify the compounds and then used certain methods to testify to their ability to be used as drugs. These compounds mainly serve as the inhibitors of PD-1, which can prevent PD-1 from disguising cancer cells and thus hide from the human immune system. The resulting compounds are ZINC93236075 and ZINC87085967. Both are suitable for binding with PD-1 and are effective for human uses. This experiment can be perceived as a new branch of cancer treatment, and the resulting compounds can be further testified for the possibility to use as actual drugs.

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