Computational Analysis of TYRP1+ CAR T-cells Targeting CD19+ and CD28+ Melanoma Cells

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Melanoma is a type of skin cancer originating in the melanocytes and is recognized as one of the most severe and challenging forms of skin cancer to treat. Tyrosinase-related protein 1 (TYRP1) is a crucial protein involved in melanin production and is notably overexpressed in melanoma cells. Recently, TYRP1 has been identified as a prospective target in CAR T-cell therapy, particularly for CD28 receptor binding, to treat patients with rare and cutaneous melanoma subtypes. We hypothesize that the receptor binds to a specific orientation to form a strong interaction. This study aims to elucidate the binding mechanism between CD28+ CAR-T cells and TYRP1-expressing melanoma cells to aid in developing novel therapeutics against melanoma. Initially, molecular docking simulations of CD28 and TYRP1 receptors were conducted. Subsequently, YASARA software was used to perform molecular dynamics simulations of the CD28-TYRP1 complex. The computational analysis revealed strong and stable hook-like interactions between the two receptors, formed at druggable sites as suggested by the P2Rank web server. This investigation into T cell receptor interactions will significantly enhance CAR-T cell therapy's effectiveness for treating cuts- nous and rare melanoma subtypes.

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1. INTRODUCTION

Melanoma, a serious and vigorous form of skin cancer that originates from melanocytes (the cells responsible for the production of melanin, the pigment that colors the skin), is noted to be a dangerous condition that spreads to other parts of the body if not addressed and treated promptly (1,2). Increased exposure to ultraviolet (UV) radiation, whether natural or artificial, increases one's risk of developing melanoma (3). Individuals with fair skin, light hair/eyes, and a family history of melanoma are much more vulnerable to the dangers of melanoma (4). The prevention and mitigation of melanoma involves early detection, thorough treatment, regular skin checks, and other protective measures to restrict UV exposure (5).

In CAR T-cell therapy, the CAR means Chimeric Antigen Receptor and is an

innovative approach to combating cancer in the field of immunotherapy (6). The therapy involves extracting T-cells from an individual's blood, modifying them to express the chimeric antigen receptors (CARs) on their surface, and returning the modified cells to the patient (6). They are designed to sense specific proteins on the surface of cancer cells and attach to them so that the T-cells can destroy the malignant cells (7). This therapy has successfully treated several blood cancers, such as acute lymphoblastic leukemia (all types) (6). Nevertheless, serious adverse effects from CAR T-cell therapy are well-known to exist, including neurotoxicity and cytokine release syndrome (CRS) (8). Overall, this individualized and targeted form of cancer treatment continues to hold great promise with the expansion of CAR T-cell therapy to other cancers like solid tumors (6).

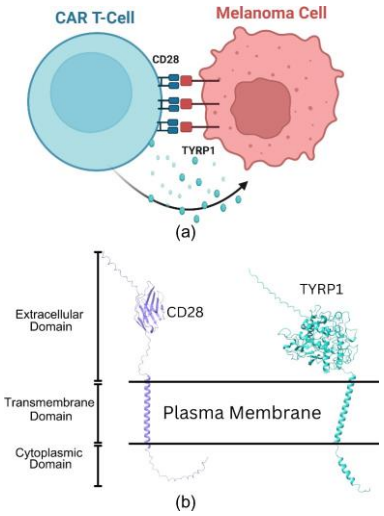

Fig. 1. CAR T-cell in Melanoma. (a) In melanoma, the overexpression of the TYRP1 receptor takes place on the surface of cancer cells. CD28-populated CAR T-cells can be used to detect and kill the cancer cell. (b) CD28 of CAR T-cells and TYRP1 of melanocytes are located on the surface of cells and contain three segments: the extracellular domain, the transmembrane domain, and the cytoplasmic domain.

CD28 is a protein receptor on the surface of T cells and is vital in helping them fully function and remain active (9). It does so by interacting with markers on other immune cells to boost the immune system response, allowing the body to defend against infections (9). CD28 has become a hot topic in autoimmune disease treatment and preventing rejection in organ transplants (9). TYRP1 (Tyrosinase-Related Protein 1) is a protein vital in producing melanin, the natural pigment of the hair, eyes, and skin (10). The protein aids the process of melanin creation by being active in melanocytes, which are the cells responsible for pigment production (10). Defects in the TYRP1 protein can result in pigment-related disorders like albinism, where affected individuals have little to no pigment (10). This makes them more prone to harmful skin conditions with an increased sensitivity to sunlight. A high level of TYRP1 expression has been associated with melanoma progression (11). In addition, these receptors also play a crucial role in tumor growth and progression (11). Finally, these receptors have the potential to escape immune surveillance, thus

making cancer cells more resistant to immune system attacks (12). Since the receptor is essential in melanoma, it could be a potential focus for melanoma-targeted therapies.

A critical computational technique in drug discovery and bioinformatics, molecular docking is to find the favored ligand position when it binds to a protein (receptor) to form a stable complex (13-16). This process involves two main components: scoring functions and search algorithms (17). First, search algorithms, such as Monte Carlo simulations and genetic algorithms, explore all possibilities of the orientation of the ligand within the receptor's binding site (18). Then, scoring functions evaluate the strength of these interactions through the orientation, including forces like van der Waals forces, hydrogen bonds, and electrostatic interactions (18). This approach is essential in drug discovery since potential drug candidates can be identified through their binding affinities to target proteins, effectively fostering new therapeutic designs with precision and efficiency (14,18). In this work, we have used molecular docking simulations to identify the interactions between the CAR T-cell and the melanoma cell receptors. Molecular dynamics (MD) is a method by which the movement of atoms and molecules can be tracked using computer simulations (19,20). MD applies the fundamental laws of physics to help scientists observe how molecules change shape, interact, and engage in various environments (14). This technique is used in biology and chemistry to understand how proteins work, how drugs interact with targets, and how materials behave at the atomic level—such a detailed view of molecular behavior aids in creating new medicines and materials(20). For example, MD can show how a drug molecule fits into a protein's active site or how a material's properties change under different conditions(20). These perspectives are pivotal in designing more effective drugs and creating more advanced materials with specified properties(20).

Recently, Jilani et al. designed TYRP1+ CAR T-cell therapy to target cutaneous and rare melanoma subtypes (21). These CAR T-cells target the CD28 and CD19 receptors overexpressed on the surface of the melanoma cells (21). We hypothesize that the receptor binds to a specific orientation to form a strong interaction. In this work, molecular docking and molecular dynamics simulations were performed to study the mechanism of CD19 and CD28 receptors' interaction with the TYRP receptor. It was found that CD19-TYRP1 and CD28-TYRP1 lock each other and enhance the stability of the complex formed. The molecular docking binding site and the druggable site predicted by the graph neural network agreed to validate these results. The current research work will help design novel CAR T cells against melanoma.

2. RESULTS

It was intended to anticipate protein surface ligand binding locations and use a random forests classifier to assess amino acids as putative ligand binder candidates. Before close-range interactions like hydrophobic and hydrogen bonding occurred, electrostatic interactions helped orient proteins properly. To determine the protein's amino acids and binding site, the graph neural network (GNN) method was utilized. The binding region is displayed in an orange box in Figures 2a, b, and c in yellow-green color. In addition, the areas of complementary charge distributions—where the negatively charged and positively charged regions of two distinct proteins interact—were also considered

by computing the receptors' electrostatic surface potential (ESP) using the ChimeraX software, as shown in Figures 2d, e, and f. The interaction site of the receptors in the black box indicates that the TYRP receptor was mostly negative (red) charged, while CD19 and CD28 were particularly positive (blue). Therefore, charge complementarity also played a significant role in receptor interactions.

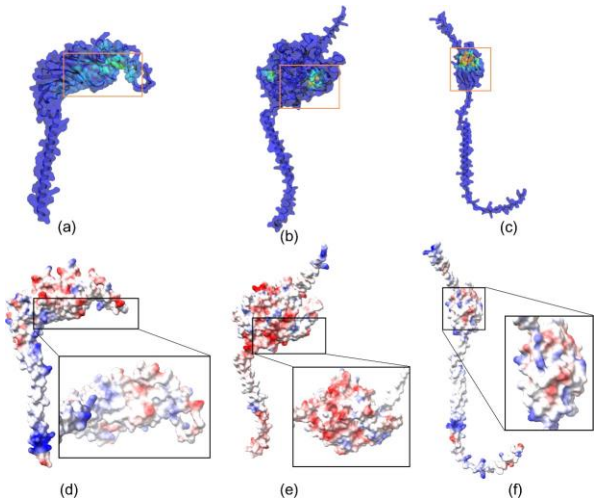

Fig. 2. Nature of the receptors used in this study. (a), (b) and (c) shows the binding site (in range box) predicted by graph neural network of CD19, TYRP, and CD28 receptors, respectively; and (d), (e), and (f) show the electrostatic surface potential (ESP) of the three receptors. The receptor binding site in the block box shows that TYRP is primarily negative (red) charged, while CD19 and CD28 were particularly positive (blue). Therefore, charge complementarity also played a significant role in receptor interactions.

Later, molecular docking simulations were performed using HDOCK software, and the resulting CD19-TYRP and CD28-TYRP complexes are shown in Figure 3. The proteins lock one another for appropriate binding in both scenarios. Visual inspection of the receptor complexes further confirms that the interactions were governed by charge complementarity, as shown in Figure 2. Furthermore, we have discovered that the binding occurs at the protein's drug-gable region, which is predicted by the graph neural network. We think that significant conformational changes are necessary for the receptors to interact because the druggable sites of the protein are located in the lower part of the protein. We assume that the amino acids at the protein's druggable region and the electrostatic surface charge cause these interactions. In addition, we have also computed the number of interactions (hydrogen bonds) formed between the receptors using an in-house script. Based on our script, the CD19-TYRP complex formed 54, and the CD28-TYRP complex framed 41 hydrogen bonds. To understand the binding interactions and ensure the two receptors stay intact, we have also performed molecular docking simulations with GROMACS software. All the simulations were performed on A100 GPU. Both the complexes, CD28-CD80 and CD28-CD86, stay intact during the entire simulations, showing strong interactions between the two proteins. RMSD during the simulation remains below 2.0 Å, and the RMSF does not show significant fluctuations in

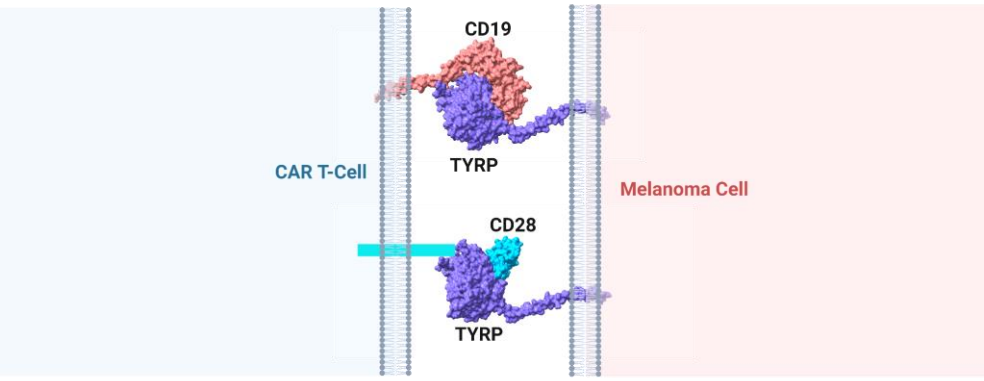

Fig. 3. Docked structure of CD19-TYRP and CD28-TYRP complex. In both cases, the receptors TYRP binds to CD19 and CD28 and locks the protein.

the protein interactions. Finally, binding energy was also computed for both the complexes using PRODIGY software, suggesting that the CD19-TYRP complex has -23.6 kcal/mol while CD28-TYRP has -19.3 kcal/mol.

3. DISCUSSION

Malignant melanoma is a form of skin cancer that has long posed difficulties to the oncology community, with few treatment options available and critical prognoses for patients with advanced illness. However, the recent introduction of innovative therapeutic tactics has changed the prospects of melanoma treatment, an example being chimeric antigen receptor T-cell (CAR T-cell) therapy. Malignant Melanoma has created a daunting situation for patients and professionals in the oncology community. Still, the development of advanced therapeutic approaches, such as CAR T-cell therapy, has progressed ideas and research in the treatment of melanoma.

Many researchers have investigated the potential of CAR T-cell treatment in melanoma, focusing on the frequently expressed gp100 and MART-1 antigens (22). As of 2019, with some patients exhibiting sustained remission and persistent clinical improvements, efforts in investigating CAR T-cell therapy have produced promising results, suggesting that this therapy may play a pivotal role in this tough cancer (23). However, to fully realize its potential, CAR T-cell therapy in solid malignancies (like melanoma) must overcome hindrances (23). The intricate microenvironment of the tumor can present significant challenges to the efficacy of CAR T-cell therapy because of its immunosuppressive nature and physical barriers that prevent penetration by the T-cell (23). In addition, there is still more to be researched in target antigen selection, tumor heterogeneity mitigation, and antigen escape strategy development (23). Despite these setbacks, CAR T-cell

Treatment for melanoma is continually developing, with professionals looking into enhancing T-cell functionality, transport, and persistence within the tumor's environment (23). With a growing understanding of the immunological landscape and the underlying fundamentals behind melanoma, there is increasing hope that CAR T-cell therapy will surface as a transformational treatment approach to this disease (23).

A significant limitation of this study is the quality and accuracy involved, and they heavily rely on the algorithms and models used. Inaccurate models may create false positives. To mitigate and prevent this, we validated our docking by checking the experimentally determined and docked structures. SPR (surface plasmon resonance) or ITC (isothermal calorimetry) should be performed for further validation. These studies will further confirm the binding of the ligands to the protein. Also, a binding analysis requires site-directed mutagenesis and co-crystalline structure to show the amino acids involved in binding. A possible extension of this investigation is to automate the entire pipeline so that we can design and evaluate more peptides in less time. In this research, we have done computational modeling of the CD28-TYRP1 receptor complexes. This research will help in developing CAR T-cells against melanoma.

In this investigation, computational simulations were administered better to understand the CD28-CD80 and CD28-CD86 receptor interaction mechanisms. This study was done through molecular docking and molecular dynamics simulations. Future experimentation should include experimental validations to substantiate these findings further. Nonetheless, the results of this study hold the possibility for designing potential CAR-T cells against cancer. These insights could be fundamental in refining the specificity and efficiency of CAR T-cell therapies, reducing off-target effects, and improving treatment outcomes. Expanding this research to include other co-stimulatory and inhibitory receptors could provide a more comprehensive understanding of T cell modulation in the tumor microenvironment.

4. METHOD

The CD19, CD28, and TYRP proteins were obtained by AlphaFold 3 (24). An advanced version of the AI system developed by DeepMind, AlphaFold 3, is used to predict protein structures accurately. It builds upon the success of AlphaFold 2, further improving its capabilities in understanding protein folding. Using a graph neural network (GNN) called GrASP, the druggable binding site of the protein was predicted (25). The aptamers bind to a positively charged area on the protein because they are highly negatively charged. The potential use of the aptamers is majorly affected by whether the predicted binding site of the protein is positive or negative. Docking simulations were performed using the HDOCK software (26). For each simulation, one of the tertiary structures and the protein were inserted into the software so that it could predict the docking results of each aptamer-protein complex. The preset settings of the software were maintained so that the aptamers would each be docked in a habitat similar to that of the human body. Each aptamer had several possible configurations for their docking simulations, so the configuration with the most outstanding stability was chosen. Visualizing the complexes helps to comprehend the effect of the varying shapes of the aptamers on their binding patterns to the protein. Molecular dynamics is a computer-based technique

that highlights complex systems' dynamics, thermodynamics, and framework by stimulating the actual activities of atoms and molecules over time. This technique entails deciphering Newton's equations of motion for a particle system that communicates within itself. GROMACS software was used to perform molecular dynamics simulations (27). The protein, loaded with TIP3P water molecules, was placed in the protein-ligand complex within a box with a 1 Å surface. To stabilize physiological ion concentrations, sodium (Na+) and chloride (Cl-) ions were added. Cluster analysis was applied to find representative structures.

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