

Exploration of Drug-Target Interaction Against Gastric Cancer-Derived Exosomes Using a Predicted GPX4 Protein Structure Based on AlphaFold2

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Abstract: The gastric cancer-derived extracellular vesicle miR-431-5p can induce ferroptosis in gastric cancer cells by downregulating the levels of SLC7A11 and GPX4, thereby inhibiting the occurrence and development of gastric cancer. Currently, drug design based on this mechanism is not yet fully developed. Therefore, focusing on the critical ferroptosis inhibition target GPX4 within this pathway, we utilized emerging AI tools for complete protein prediction and drug design. We used AlphaFold2 to predict the complete protein structure of GPX4, visualized its model using PyMOL, and analyzed its binding sites. Based on the existing drug-binding model, we then performed molecular docking with the predicted GPX4 protein using AutoDock Vina. Compared with existing models, we found that the drug-binding sites differed, which warrants further experimental analysis to explore the reasons behind these differences. This study suggests that using AI tools to assist in drug design is feasible and offers reference value for future drug development.

Keywords: GPX4, gastric cancer, AlphaFold2, exosomes, structure prediction

1. Introduction

Gastric cancer has become one of the most common malignant tumors in the world due to its high incidence and mortality rates, making it the third leading cause of cancer-related deaths. In recent years, although surgery, radiotherapy, chemotherapy, and other treatments have gradually improved the 5-year survival rate for patients with early gastric cancer, surgical treatment often results in large wounds and slow recovery [1]. Moreover, radiotherapy and chemotherapy can lead to side effects, insufficient accuracy, environmental pollution, and reliance on expensive instruments, which diminishes the overall effectiveness of these treatments. Due to the limitations of these primary treatment methods, a significant number of patients still experience poor prognoses and metastasis, resulting in unsatisfactory treatment outcomes [1,2].

Exosomes are a type of extracellular vesicle with a diameter of 40-160 nm, rich in various bioactive substances such as proteins, lipids, and nucleic acids. They are widely present in body fluids such as blood, cerebrospinal fluid, and saliva, playing a crucial role in intercellular information transmission and signal transduction [9,10]. Due to their unique membrane structure, excellent biocompatibility, long circulating half-life, low immunogenicity, and ability to penetrate tissue barriers, exosomes have

become a research hotspot and a potential emerging target for disease diagnosis, treatment, and drug delivery [8]. Tumor cells can secrete exosomes to regulate stromal cells such as macrophages, neutrophils, endothelial cells, and fibroblasts within the tumor microenvironment, inducing the formation of a microenvironment conducive to tumor growth [3,4]. Therefore, some studies suggest that exosomes have the potential to become new targets for tumor diagnosis and treatment [5,6]. In addition, miRNAs are abundant and crucial biomolecules in exosomes, regulating tumor cell development through mechanisms such as signaling pathways, gene expression, energy metabolism, and immunogenicity. These miRNAs exhibit differential expression between tumor patients and healthy individuals and are considered important potential biomarkers for tumor diagnosis and prognosis.

Ferroptosis is a novel programmed cell death mechanism induced by the excessive accumulation of lipid peroxides within cells [11]. Its main biochemical characteristics include reduced GSH levels, decreased GPX4 expression, Fe^{2+} overload, and excessive accumulation of ROS and lipid peroxides [15]. Ferroptosis is regulated by various cellular metabolic pathways, including redox homeostasis, iron metabolism, mitochondrial activity, and the metabolism of amino acids, lipids, and sugars [6,7,12]. In recent years, the regulatory mechanisms of ferroptosis in diseases have gradually been elucidated. The development of engineered exosomes targeting ferroptosis can potentially address the adverse reactions and drug resistance seen in traditional treatment methods, offering promising clinical applications [13].

Glutathione peroxidase 4 (GPX4) is a selenium-containing protein. As research has progressed, more functions of GPX4 have been discovered, such as its involvement in cellular signaling pathways, protection of mitochondrial ATP from oxidative stress, and inhibition of mitochondrial apoptosis [14]. GPX4 uses GSH as a substrate to reduce lipid peroxides to normal phospholipid molecules, making it the most important inhibitor of ferroptosis [16]. The SLC7A11/GPX4 pathway is central to ferroptosis regulation, fully exerting its antioxidant function. SLC7A11 inhibits ferroptosis by promoting GSH synthesis, and research shows that SLC7A11 is often overexpressed or upregulated in tumor cells [17,18]. GPX4, downstream of SLC7A11, activates GSH to prevent the excessive accumulation of intracellular lipid peroxides, thereby inhibiting ferroptosis [19]. Exosomal miR-431-5p can induce ferroptosis in gastric cancer cells by increasing lipid ROS, total iron, and MDA levels, while downregulating SLC7A11 and GPX4 levels. Therefore, by focusing on specific small-molecule interactions within the biological process of ferroptosis, designing small molecules that target GPX4 to inhibit its activity can induce ferroptosis in gastric cancer cells and thereby inhibit cancer progression [20].

In summary, we aim to design specific drugs targeting GPX4, providing new strategies for gastric cancer treatment. The spatial structure of proteins determines their functional and physicochemical characteristics, which play an essential role in the development of targeted drugs. Therefore, accurately predicting protein structures is a key challenge. Current protein analysis methods used domestically and internationally include X-ray diffraction crystallography, NMR nuclear magnetic resonance imaging, and cryo-electron microscopy (Cryo-EM) [21]. However, due to the complexity of protein structures and various environmental factors, these methods often fail to achieve sufficient prediction accuracy, making it difficult to decipher complete protein structures. In recent years, computer modeling techniques have emerged as powerful tools for protein structure analysis. For example, AlphaFold2 offers prediction accuracy at the atomic level, allowing us to accurately predict protein structures for downstream drug design [22].

2. Methods

2.1. Workflow

To obtain the complete GPX4 protein sequence, we searched the UniProt database for GPX4, selected the humanized GPX4, and retrieved the corresponding protein sequence. We then input the protein sequence into AlphaFold2, executing the code to obtain the predicted complete protein structure data. To visualize the obtained protein data for better binding site design, we imported the results into PyMOL for visualization processing. To illustrate the complete drug design process, we used PyMOL to identify amino acid binding sites for existing small-molecule drugs. Subsequently, we performed molecular docking predictions with the predicted GPX4 protein using AutoDock Vina. To demonstrate the reference value of this approach, we compared the small-molecule binding sites in this structure with those of existing drug-binding models using PyMOL.

2.2. Introduction to AlphaFold2

AlphaFold2 uses primary amino acid sequences and comparative sequences of homologs as inputs to directly predict the 3D coordinates of proteins with atomic accuracy. Its operation relies on three main modules: the MSA module, the Evoformer, and the Structure module. The MSA module performs multiple sequence alignment on the input sequence to identify conserved regions, while the Evoformer uses graph networks and multiple sequence comparisons to complete structure prediction. Finally, the Evoformer outputs are converted into the protein's 3D structure by the Structure module. AlphaFold2 offers advantages such as low error rates, ease of operation, and relatively low cost, making it well-suited for protein-folding prediction problems. However, it has limitations, including the inability to analyze protein complexes and predict more specific molecular structures such as miRNAs. Structural analysis relies on certain modules or models, and removing them may lead to significant data losses, necessitating further optimization.

2.3. GPX4 Structure Prediction

We input the GPX4 protein sequence obtained from the UniProt database into AlphaFold2, replaced non-essential amino acids with X, and executed the program to obtain the predicted protein folding structure. The predicted GPX4 protein structure was then visualized using PyMOL software.

2.4. Docking of Inhibitory Small Molecule TMT10 with GPX4 Protein

We imported the GPX4 protein into the working directory, performed dehydration and hydrogenation treatments, and designated it as the receptor protein. We then imported the small molecule TMT10, added hydrogen to it, and detected its torsion center and torsion bonds. A grid box was designed to cover the docking site of the GPX4 protein, and we set the AutoDock Vina parameters to run molecular docking. The display type of the small molecule and GPX4 protein was adjusted based on the docking results to enhance the clarity of the experimental outcomes. PyMOL was used to determine the specific binding location of the small molecule on the GPX4 protein.

3. Results

3.1. Workflow Analysis

Here we mainly present some analysis results. Figure 1 shows the complete structural data of the GPX4 protein predicted using AlphaFold2, primarily reflecting the model's confidence level. To better identify the binding sites, we visualized the protein using PyMOL, rotated the model to generate

Figure 2, and observed the structural details of the protein from different angles. Figure 3 presents the molecular docking results of small-molecule inhibitory drugs with GPX4. Analysis and comparison show that the binding sites differ from those identified by traditional experimental methods. Although not every result is perfect, we aim to present the complete AI-assisted drug design process to provide reference value for readers.

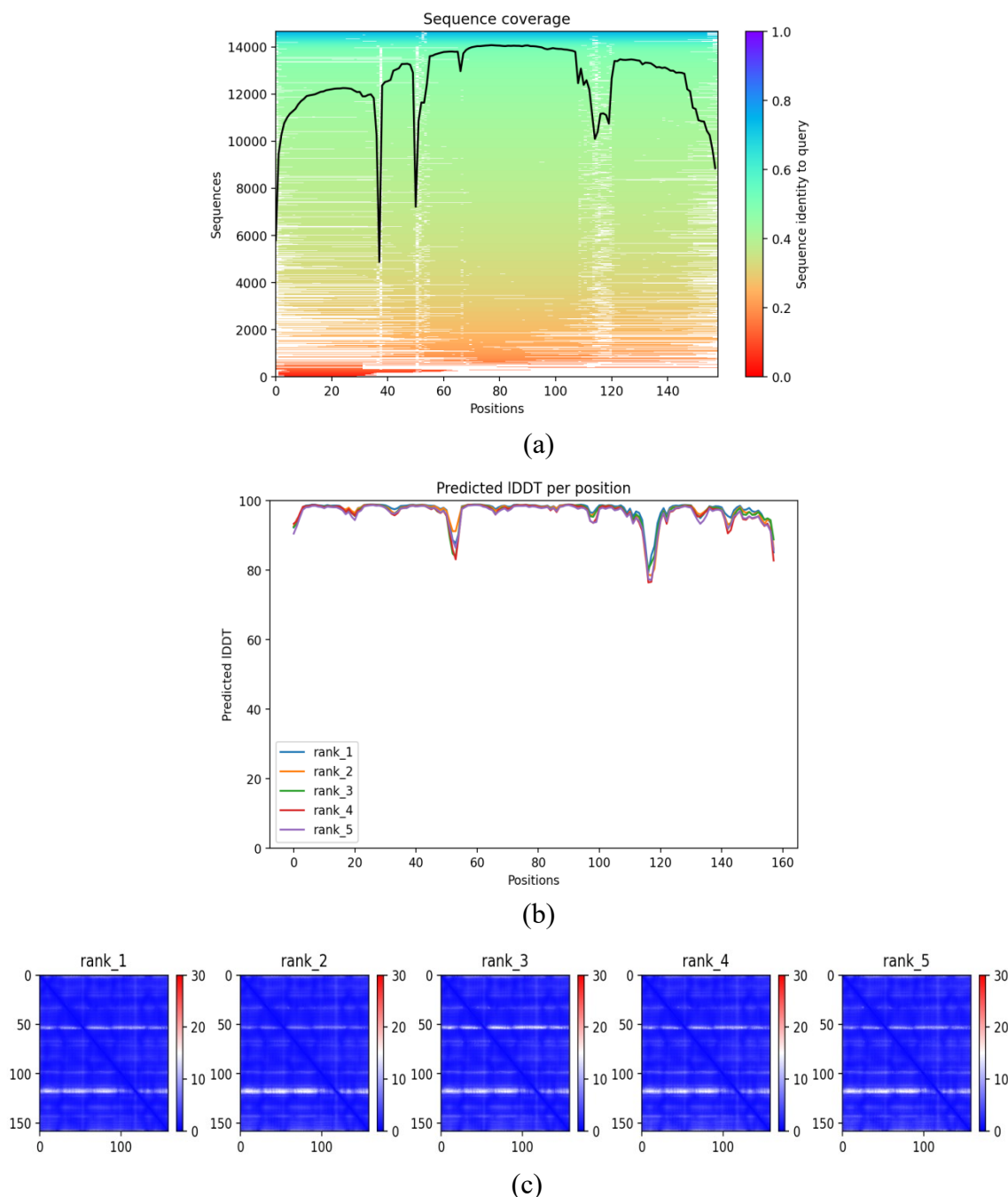


Figure 1: AlphaFold2 Predicted Structure of GPX4. (a) Distribution and consistency curve of the MSA constructed during the inference of the GPX4 sequence using AlphaFold2. (b) Comparison of PLDDT scores for five models predicted by AlphaFold2. (c) Comparison of confidence levels of five models predicted by AlphaFold2.

3.1.1. GPX4 Sequence and Prediction Results

Figure (a) shows the distribution of the MSA constructed by AlphaFold2 during the inference of GPX4 sequences. A total of 14,000 homologous sequences were identified, of which 6,000 had a consistency of over 60% with GPX4 protein sequences, indicating that AlphaFold2 has sufficient credibility in predicting GPX4 structures. The consistency curve shows discrepancies around positions 40-50 and 110-120.

Figure (b) presents a comparison of the PLDDT confidence curves. The five curves overlap or are closely aligned, indicating high consistency among the models. Notable deviations are observed around positions 50 and 110-120. The PLDDT scores correspond well with the trend of the MSA feature matrix shown in Figure (a). The overall confidence level is above 80, indicating high prediction reliability.

Figure (c) illustrates the confidence levels of the five models predicted by AlphaFold2, showing significant deviations around positions 50 and 110-120. The overall confidence levels are high and consistent, reflecting the trend observed in Figure (b).

3.2. Visualization of GPX4 Protein Results Using PyMOL

We used PyMOL to visualize the predicted GPX4 protein structure. Regions with low confidence between positions 50 and 110-120 were marked in red, and the protein surface structure was displayed. Figures (d), (e), and (f) show the protein model from different angles, obtained by rotating the structure. In the visualized protein structure, multiple alpha helices, beta sheets, and loop structures are observed, with clear protrusions and grooves on the protein surface. These protrusions and grooves, compared to smooth surfaces, can serve as potential binding sites for drug design. The overall protein shape is spherical and relatively compact, typically functioning as a binding protein for antibodies. The predicted GPX4 structure can also be analyzed for surface polarity and charge distribution, aiding in the design of drug pockets and other protein analysis tasks. The regions with low confidence, marked in red, are primarily loop structures. Due to the instability of their irregular amino acid sequences and flexible conformations, accurately predicting these regions is challenging. This observation is consistent with the confidence levels shown in Figures (a), (b), and (c). These regions are typically not used for designing binding sites, and their impact on GPX4 binding site design is minimal.

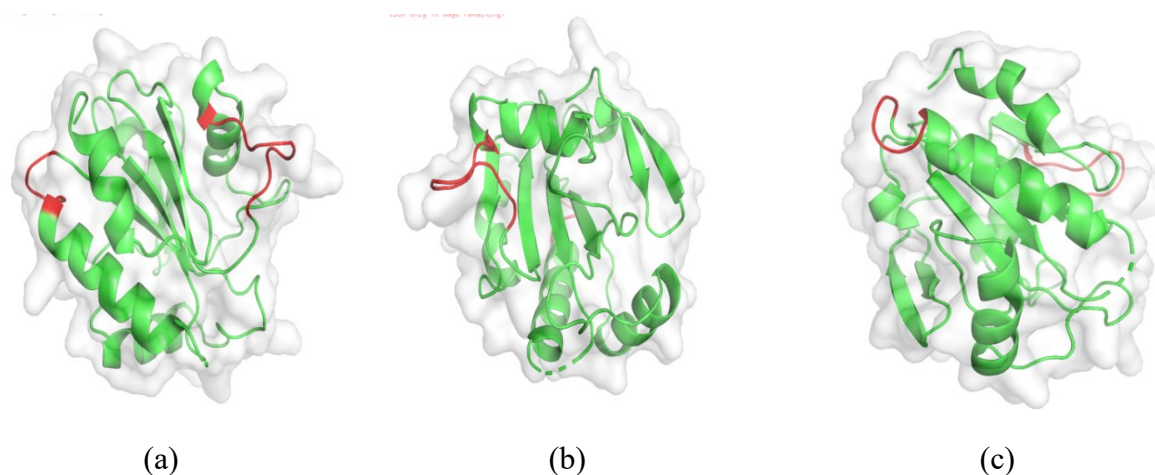


Figure 2: PyMOL Visualization of GPX4 Protein. (a), (b), and (c): Protein visualization models from different perspectives, with low-confidence regions around positions 50 and 110-120 marked in red and high-confidence regions shown in green.

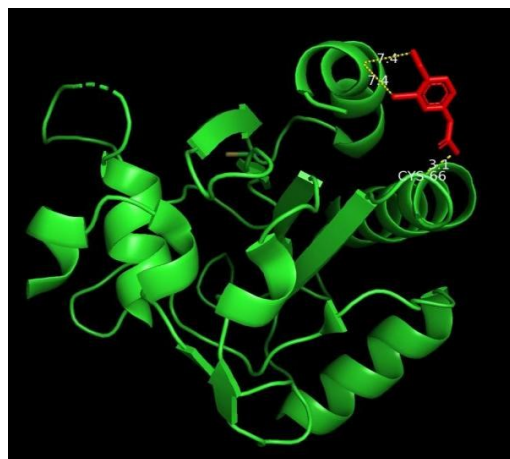
3.3. Docking Results of Inhibitory Small Molecule TMT10 with GPX4

By searching the PDB database, we found ligand co-crystallization structures of GPX4-U46C-R152H and the small molecule TMT10, which are highly similar to GPX4. This allowed us to analyze the binding characteristics and regions of small-molecule ligands, aiding in the design of molecular docking sites and validating the stability of TMT10 binding in the predicted high-precision GPX4 protein model. Figures (a) and (b) show the 7U4J structure, where multiple alpha helices, beta sheets, and loop structures can be observed. Visualization processing indicates that TMT10 binds near the 66th amino acid residue in a loop structure. According to the predicted data in Figure 1, the GPX4 structure has high confidence between positions 60 and 70, providing a useful reference for future molecular docking designs.

TMT10 is a GPX4 activity inhibitor that covalently binds to specific sites on GPX4, inhibiting its expression and inducing ferroptosis in cells. If the stability of this binding can be verified, it could provide new strategies for targeted ferroptosis induction. Due to the limited accuracy of the complex structure obtained using X-ray crystallography (resolution of 1.81 Å), the electron cloud density is unclear, and the atomic structure cannot be precisely observed. Additionally, X-ray methods often produce fragmented protein sequences, reducing overall confidence. Therefore, we performed molecular docking of the high-precision complete GPX4 structure predicted by AlphaFold2 with TMT10 using AutoDock Vina, generating visualization results shown in Figures (c) and (d). The results indicate that TMT10 binds to amino acids at positions 90, 91, and 92 in the complete GPX4 sequence. This binding site differs from that in the 7U4J structure, suggesting potential instability or multiple binding sites for TMT10 on GPX4. Alternatively, this difference may be due to other GPX4 conformations that preferentially bind to different sites. This highlights the importance of combining AI predictions with traditional experimental methods to achieve more accurate drug design outcomes.



(a)



(b)

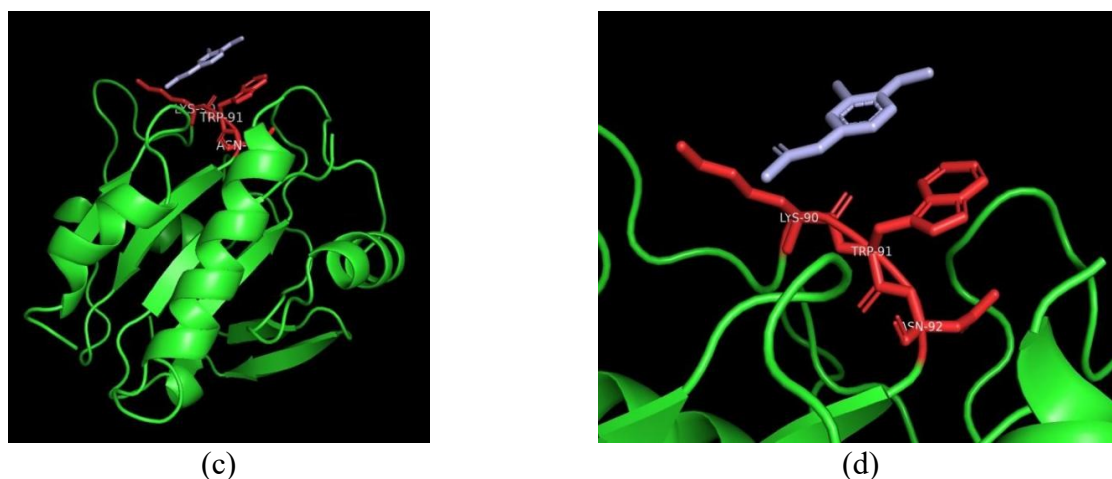


Figure 3: Structural Analysis of 7U4J and Docking Results of TMT10 with GPX4. (a): Crystal structure of human GPX4-U46C-R152H in complex with TMT10 (7U4J). (b): PyMOL analysis showing TMT10 binding sites in 7U4J, with TMT10 marked in red and GPX4-U46C-R152H in green. TMT10 binds near the 66th amino acid residue in the loop structure. (c) and (d): Visualization results of molecular docking between TMT10 and the predicted complete GPX4 sequence. TMT10 binds to amino acids at positions 90, 91, and 92.

4. Conclusion

In this study, we focused on the key ferroptosis signaling pathway, SLC7A11/GPX4, induced by the gastric cancer extracellular vesicle miR-431-5p. Specifically, we examined GPX4, a crucial inhibitor of ferroptosis within this pathway. Using AlphaFold2, we predicted the complete structure of the target protein GPX4. The protein structure was visualized using PyMOL, and molecular docking analysis with existing drugs was performed using AutoDock Vina. This study demonstrates the complete process of drug molecular docking by integrating AI tools for drug docking prediction and analysis based on gastric cancer exosome-related diseases. Comparative analysis of the relevant data indicates that using AI for structure prediction and molecular docking is a feasible and effective approach.

The experimental data predicted by AI may differ from results obtained using X-ray crystallography and other traditional experimental methods. These differences could be attributed to the presence of multiple binding sites during molecular docking or the existence of alternative conformations of the GPX4 protein. The molecule may prefer binding to different sites within the complete protein sequence. This suggests that when designing binding sites, referencing the complete protein structure sequence predicted by AI allows for a more comprehensive analysis. Such an approach can enhance design efficiency, improve drug binding stability, and yield more reliable experimental results. In the future, we aim to collect additional data based on AlphaFold2 to further model various types of biomolecules. By leveraging exosome-related models, we can analyze and simulate processes such as drug delivery, transmembrane transport, and signal transduction. This will help us better understand disease pathogenesis, drug transport pathways in the body, and mechanisms of drug efficacy. Additionally, by incorporating human physiological environments into our analyses, we can optimize molecular docking and drug design based on target proteins, ultimately enhancing our ability to address cancer-related challenges.

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