The Investigation of LPA Binding Sites on LPA 1 And LPA 4

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Abstract. Lysophosphatidic acid (LPA) is a class of biologically active lipid molecules with important biological functions. LPA can induce various cellular responses through six LPA receptors (LPA1-6) on cell membranes to achieve its biological functions. This study utilized computer simulation tools such as AutoDock Vina and RosettaFold to investigate the binding sites of LPA1 and LPA4 to LPA molecules. Based on these results, this study could provide new insights for the subsequent development of extracellular antagonists and agonists, and facilitate drug formation for the treatment of cancer invasion.

Keywords: LPA receptor 1, LPA receptor 4, Molecular docking, AutoDock Vina.

1. Introduction

Cancer is the leading cause of death worldwide. Nearly 10 million people are affected in 2020 [1]. There is extensive research into the mechanisms of cancer development, diagnostic methods, and treatments. However, most of the research and treatments focus on primary cancers, secondary cancers or tumors [2], rather than cancers that have metastasized and spread. Cancer initially starts in one place, but it can spread or metastasize to other parts of the body. Metastatic cancer is difficult to treat and is the main reason cause of death [3]. Therefore, this study investigated cell signaling pathways and receptors associated with cell migration and hoped to provide new insights for drug development for the treatment of metastatic cancer. Among the various signaling pathways of cell membrane receptors, the signaling pathway by Lysophosphatidic acid molecules is a promising target for further studies.

Lysophosphatidic acid (LPA) is a bioactive lipid consisting of a phosphate group, a glycerol backbone and a single acyl chain.[4] LPA can induce various cellular responses through six G-coupled protein receptors (GPCRs) called LPA1-6.[5] GPCRs are multi-pass integral membrane proteins that consist of 7 transmembrane helices, with the N-terminal domain outside and the C-terminal domain inside. Upon binding ligands, the external part of the GPCRs transmits signals across the membrane through conformational changes. Each LPA receptor has its unique function. For example, LPA1 mediates LPA-induced regulation of astrocyte proliferation, spreading, survival, and morphology and adhesion of Schwann cells (SCs) [6]. LPA4 is involved in stress fiber formation and neuronal contraction.[7] At the same time, interactions between LPA receptors give rise to different biological functions. In this study, LPA1 and LPA4 were chosen due to their antagonistic functions in cell migration: previous studies have shown that LPA-mediated cell migration via activation of LPA1 receptors is inhibited upon LPA4 activation.[8]

Depending on the site of intermolecular interaction and the type of protein downstream of the pathway, antagonists of LPA receptors 1 or 4 can be screened for clues for further drug development [9]. Previous studies have shown reduced prenatal survival in LPAR4-deficient mutant mouse embryos, which indicates that LPA4 negatively regulates LPA receptor-1-stimulated osteogenesis.[10] To better understand this problem, in addition to avoiding gene editing methods, targeted studies of LPA1 antagonists could be used to address the LPAR4 deficiency, improve the survival of LPAR4 mutant infants during embryonic development, and reduce the ethical issues associated with conventional gene editing. Second, cell migration stimulated by the LPA signaling pathway is also closely associated with the metastasis of some cancer cells.[11] Targeted inhibitors can be used to inhibit metastasis and the spread of cancer cells.

So far, some results have been achieved about LPA receptors 1 and 4 and their signaling pathways, but some questions still need to be addressed. First, the crystal structure of LPA1 was first reported by Jill E. Chrencik's team in 2015 [12], enabling the community to study the interaction between LPA1 and its ligands using computational tools such as molecular docking, a computational program that attempts to predict non-covalent binding between large molecules or to predict that large molecules (receptors) will bind efficiently to small molecules (ligands). However, the spatial structure of LPA4 has not been fully demonstrated [13], and therefore the interaction between LPA4 and its ligand cannot be predicted directly, which is a huge obstacle to investigating the cause of the antagonistic mechanism of LPA1 and LPA4 receptors. Second, it is clear from the evolutionary mapping of LPA family receptors [14] (Figure 1) that although LPA1 and 4 receptors belong to the LPA family because they both bind to LPA molecules, these two receptors are divided into two branches that are far apart because of the difference in their amino acid sequence which then leads to the difference in their structure. Although the three-dimensional structures of the two receptors are very different, the reason why both receptors can interact with LPA molecules is still unknown.

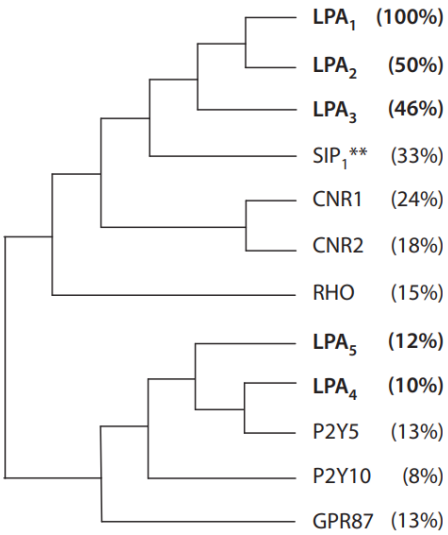

Figure 1. Phylogenetic relationships among LPA receptors 1-5 are shown in bold. Assume the similarity of LPA1 to itself is 100%. Then, the similarity between LPA1 and LPA4 is only 10% which shows they are distinctly different.[15]

Two experiments were designed to solve the above pending issues. Firstly, to solve the problem of the unknown structure of LPA4 which leads to hindered functional studies, this study used computer simulation software to predict the spatial conformation of LPA4 regarding some existing spatial conformations in academia, and sequence comparison of amino acid sequences of LPA1 and LPA4, based on some known structures. It was demonstrated that LPA1 and LPA4 have different structures and antagonistic functions. Secondly, molecular docking was used to predict the binding conformation and binding affinity between LPA and LPA receptors (1 and 4) to explain how LPA receptors (1 and 4) and LPA molecules interact with each other despite their large structural differences between two receptors.

2. Material and Methods

2.1. Experiment 1: Sequence and structure

2.1.1 Sequence Alignment

The overall procedure was done using SnapGene, PyMol [16], Notepad, and AutoDock Vina [17]. The amino acid sequences of LPA1 and LPA4 were obtained from Uniprot and the sequence

comparison was performed using SnapGene software to obtain the identity, similarity and difference.2.1.2 Sub heading.

2.1.2 LPA4 structure prediction

Since the 3D structure of the LPA4 receptor has not been reported yet, RosettaFold protein structure prediction software was applied to predict the 3D structure of the LPA4 receptor [18]. The predicted structure of LPA4 was obtained based on the amino acid sequence of LPA4 from Uniprot and by giving an analogous structure (LPA1 structure) into the program. Finally, the LPA4 structure was visualized on PyMol.

2.1.3 Structure Alignment

The structure data of LPA receptor 1 and LPA molecule was derived from the Protein Data Bank (PDB: 7TD2) [19]. Because the molecule represented in 7TD2 was a complex involving both G-proteins, LPA molecule, and LPA receptor 1, Pymol and Notepad was used to purify LPA molecule and receptor 1. The PDB file of LPA 1 was treated by remaining the essential ATOM 23-322 (LPA 1) and deleting other ATOMs. Likewise, the file of the LPA molecule was treated by remaining all the HETATM and deleting all the ATOMs. Since molecular docking required files in pdbqt format, AutoDock Tools was used to transfer the pdb file into the pdbqt file. During this process, water molecules were deleted, and hydrogen atoms were added with the polar-only restriction.

2.2. Experiment 2: LPA binding site

In this study, AutoDockTools was used to handle the docking simulation of LPA molecules with LPA receptors. A docking box with 12 × 12 × 18 points and a grid spacing of 1Å was set up. The position of the docking box of the LPA1 receptor in the 3D space was x-axis: 82.255, y-axis: 58.188, z-axis: 127.086. The position and the size of the box were chosen with reference to the structure of 7TD2 because there was an original LPA molecule included in the 7TD2 file. The position of the docking box of the LPA4 receptor and the LPA molecule in the 3D space was x-axis: 9.484, y-axis: 1.526, z-axis: - 9.468. Since the docking of LPA and LPA molecules was a rigid receptor and flexible ligand docking, a simple comparison of the complementarity between the binding sites was not possible. Therefore, a quantitative calculation of the energy was performed. Since the number of rotatable bonds in the LPA molecule was 22 out of 32, the three-dimensional geometry of the LPA molecule was numerous. For simplicity, the number of modes was set to 10, so that only 10 conformations were eventually displayed instead of showing all possibilities. In addition, the exhaustiveness parameter, which controls the number of runs, was set to 8. Since each run was executed in parallel, an appropriate value of exhaustiveness could help reduce systematic errors. The optimal binding configuration was selected based on the lowest Gibbs binding free energy.

3. Results

3.1. Experiment 1: Sequence and structure

3.1.1 Sequence alignment

The amino acid sequences of LPA1 receptor and LPA4 receptor were analyzed by the comparison of 332 key amino acids, and it was found that among the 332 amino acids, the identical amino acids of the two receptor amino acid sequences were 70, with 21.08% identity; the similar amino acids were 133, with 40.06% similarity; and the completely different amino acids were 61, with 18.37% difference (Figure 2a). The comparison of these two receptor sequences is consistent with the results of the previous chemical and taxonomic studies: the LPA1 receptor is not very similar to the LPA4 receptor sequence, so the two receptors are not next to each other in the classification and study of evolution.

3.1.2 Structure Alignment

The structure of the LPA1 receptor has been relatively well studied, so this study applied the three-dimensional structure of the LPA1 receptor demonstrated by X-ray crystal diffraction directly from the PDB bank for molecular studies. Since there is still no officially reported 3D structure of the LPA4 receptor, (Figure 2b) this study first predicted the structure of the receptor by computer simulation software. Rosetta Fold, a computer simulation tool, was used in this study to predict the 3D protein structure of the LPA4 receptor. Visualization by PYMOL software revealed that these two receptors are similar in general shape. Despite this, there are still some subtle structural differences between the two receptors. For example, the structures of the C- and N-terminal ends of the LPA receptors showed large differences.

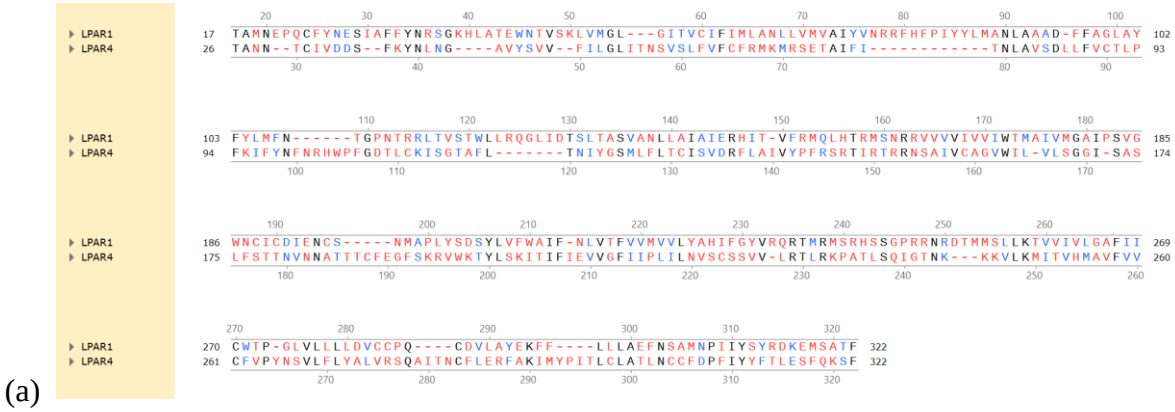

Figure 2. (a) The alignment of amino acid sequences of LPA receptor 1 and LPA receptor 4. (b) The alignment of the three-dimensional structure of LPA receptor 1 and LPA receptor 4. The pale cyan helix refers to LPA receptor 4, whose structure is predicted by using Rosetta Fold, while the white helix refers to LPA receptor 1.

3.2. Experiment 2: LPA binding site

3.2.1 Analysis of affinity

The interaction affinity of LPA1 receptor and LPA molecule and LPA4 receptor with LPA molecule was derived by molecular docking using the Autodock (Table 1). Docking LPA molecules into the models of these two receptors revealed that the LPA1 receptor has an overall higher affinity for the LPA molecule at the binding site, compared to the Rosetta Fold model of the LPA4 receptor, which has a lower affinity for interaction with the LPA molecule.

In order to prevent chance conditions from affecting the experiment, five sets of replicate tests were performed for each group in this study. As shown in the figure, this is the data statistics for each group of ten models, for a total of five groups. Generally speaking, an affinity of -7.0 or less kcal/mol

can prove that the protein binds more strongly to the ligand, so I performed further analysis for all models with an affinity of -7.0 or less kcal/mol. In order to get the best-predicted model, this study used PYMOL to predict the molecular structure and compared the structure with the LPA molecule in the LPA receptor complex and selected the model with the most similar conformation by comparing the phospholipid head position and bond deflection. Finally, Model 3-1 in LPA4 and Model 4-2 in LPA1 were selected, and the value of Kd was calculated using the Gibbs free energy formula. According to the derivation principle of the formula, the smaller the value of Kd, the less the ligand and protein dissociate, so the stronger the affinity.

In Table 2, affinity refers to the affinity of the model's LPA molecule with its corresponding receptor. The larger the absolute value of this affinity, the stronger the interaction force. rmsd refers to the distance between a set of fixed points in 3D space. In this case, it refers to the distance between that model and the first model (the best model) for which the program is run once. Ten models are run for each COMMAND procedure, and the molecular docking procedure is run five times for each receptor, yielding a total of 50 molecular docking models. Based on molecular orientation, structure and affinity magnitude, the best model is selected for further analysis.

Table 1. The affinity data results of the interactive simulations of LPA4 receptor and LPA1 receptor on Autodock Vina.

LPA receptor 4_Rosetta

	Mode	Affinity (kcal/mol)	Distance from best mode rmsd l.b	Distance from best mode rmsd u.b	ΔGbinding (kcal/mol)
1	1	-7.1	0	0	-7.1
	2	-7.0	1.544	2.806	-7.0
	3	-6.9	1.612	3.509	-6.9
	4	-6.9	1.379	2.855	-6.9
	5	-6.8	2.02	3.43	-6.8
	6	-6.8	1.197	2.158	-6.8
	7	-6.7	2.171	3.655	-6.7
	8	-6.2	5.071	9.457	-6.2
	9	-6.0	1.944	3.939	-6.0
	10	-5.5	1.762	3.555	-5.5
2	1	-6.9	0	0	-6.9
	2	-6.8	1.825	3.687	-6.8
	3	-6.5	1.874	3.013	-6.5
	4	-6.4	2.005	3.883	-6.4
	5	-6.2	4.868	9.926	-6.2
	6	-5.8	1.167	2.656	-5.8
	7	-4.3	5.085	10.04	-4.3
	8	-3.6	5.33	9.834	-3.6
	9	-3.2	3.578	5.841	-3.2
	10	-3.0	3.898	9.319	-3.0
3	1	-7.2	0	0	-7.2
	2	-6.8	4.868	9.635	-6.8
	3	-6.7	1.633	3.449	-6.7
	4	-6.7	1.423	2.885	-6.7
	5	-6.5	4.771	9.432	-6.5
	6	-6.4	4.951	9.28	-6.4
	7	-6.3	1.367	2.324	-6.3
	8	-6.0	1.677	3.116	-6.0
	9	-5.7	5.079	9.195	-5.7
	10	-5.1	2.249	4.445	-5.1

LPA receptor 1

	Mode	Affinity (kcal/mol)	Distance from best mode rmsd l.b	Distance from best mode rmsd u.b	ΔGbinding (kcal/mol)
1	1	-7.0	0	0	-7.0
	2	-7.0	4.261	7.092	-7.0
	3	-6.7	3.084	7.075	-6.7
	4	-6.6	3.156	6.76	-6.6
	5	-6.6	3.414	7.195	-6.6
	6	-6.5	2.027	2.544	-6.5
	7	-6.5	3.093	6.16	-6.5
	8	-6.4	2.883	5.638	-6.4
	9	-6.4	4.32	7.144	-6.4
	10	-6.2	3.126	5.532	-6.2
2	1	-7.0	0	0	-7.0
	2	-6.9	1.595	2.496	-6.9
	3	-6.8	1.288	3.749	-6.8
	4	-6.7	1.391	2.477	-6.7
	5	-6.6	1.969	3.693	-6.6
	6	-6.5	3.34	5.661	-6.5
	7	-6.3	2.676	5.37	-6.3
	8	-6.3	2.841	3.916	-6.3
	9	-6.3	1.559	3.798	-6.3
	10	-6.2	1.539	2.348	-6.2
3	1	-7.2	0	0	-7.2
	2	-7.1	1.819	4.166	-7.1
	3	-7.1	1.493	4.044	-7.1
	4	-7.1	1.262	2.014	-7.1
	5	-7.0	1.769	2.547	-7.0
	6	-6.9	3.964	7.331	-6.9
	7	-6.9	1.85	3.13	-6.9
	8	-6.8	2.199	5.177	-6.8
	9	-6.8	2.381	4.997	-6.8
	10	-6.8	2.545	3.368	-6.8

	Mode	Affinity (kcal/mol)	Distance from best mode rmsd l.b	Distance from best mode rmsd u.b	ΔGbinding (kcal/mol)
4	1	-7.0	0	0	-7.0
	2	-6.8	1.433	2.508	-6.8
	3	-6.3	1.587	3.461	-6.3
	4	-6.2	4.749	10.128	-6.2
	5	-6.1	4.76	9.577	-6.1
	6	-5.8	2.015	4.258	-5.8
	7	-5.7	2.385	5.316	-5.7
	8	-5.6	2.545	4.896	-5.6
	9	-5.6	4.666	9.514	-5.6
	10	-5.2	2.705	4.504	-5.2
5	1	-7.1	0	0	-7.1
	2	-7.0	2.734	5.609	-7.0
	3	-6.6	1.596	2.784	-6.6
	4	-6.2	1.685	3.539	-6.2
	5	-6.2	5.266	10.308	-6.2
	6	-5.9	5.682	10.111	-5.9
	7	-5.8	5.378	10.129	-5.8
	8	-5.5	2.354	4.724	-5.5
	9	-5.3	5.8885	10.332	-5.3
	10	-4.4	2.357	5.103	-4.4

	Mode	Affinity (kcal/mol)	Distance from best mode rmsd l.b	Distance from best mode rmsd u.b	ΔGbinding (kcal/mol)
4	1	-7.5	0	0	-7.5
	2	-7.4	1.316	4.411	-7.4
	3	-7.3	1.407	2.34	-7.3
	4	-7.2	1.309	3.236	-7.2
	5	-7.2	1.319	1.807	-7.2
	6	-7.1	2.195	5.028	-7.1
	7	-7.0	1.573	2.152	-7.0
	8	-7.0	1.704	3.991	-7.0
	9	-7.0	1.733	2.538	-7.0
	10	-6.8	2.077	2.659	-6.8
5	1	-7.2	0	0	-7.2
	2	-6.9	1.782	3.65	-6.9
	3	-6.7	1.465	2.609	-6.7
	4	-6.7	1.714	2.805	-6.7
	5	-6.6	2.192	4.873	-6.6
	6	-6.6	1.609	2.808	-6.6
	7	-6.4	2.17	3.903	-6.4
	8	-6.4	1.418	2.234	-6.4
	9	-6.3	1.732	3.292	-6.3
	10	-6.3	1.371	2.087	-6.3

3.2.2 Analysis of docking pocket

The binding pocket structure of the LPA molecule and LPA receptors are generated by PYMOL.

Officially, the seven amino acid residue sequences (Try-24, Lys-39, Thr-109, Gly-110, Arg-124, Gln-125 and Lys-294) exist in different structures of the transmembrane receptor, among which tyrosine and lysine are located in the 34th and 39th positions of the amino acid sequence of LPA receptor 1, respectively, and both exist in the extracellular Topological domain of the first transmembrane region. Threonine and glycine are located at 109 and 110 throughout the protein sequence, respectively, and exist in the extracellular Topological domain between the second and third transmembrane. Arginine and glutathione are located at positions 124 and 125, and are present in the third transmembrane domain. Finally, lysine, located at the 294th position of the amino acid sequence of LPA receptor 1, is present in the extracellular Topological domain between the sixth and seventh transmembrane (Figure 3).

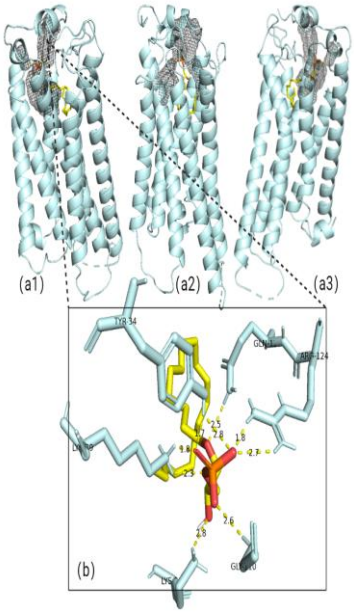

Figure 3. The interaction of the LPA molecule with the LPA1 receptor in the 7TD2 file in PDB, microscopically analyzing the official model given for the docking of the LPA1 receptor with the LPA molecule. (a) The binding pocket of the LPA molecule officially with LPA receptor 1 is shown from three different perspectives. (b) Officially, residues in LPA receptor 1 involve polar interaction with the atom in the LPA molecule. The seven amino acids which have polar interaction with LPA receptor 1 are Try-24, Lys-39, Thr-109, Gly-110, Arg-124, Gln-125 and Lys-294. Most of them are interacting with the phospholipid complex (shown in orange and red) of the LPA receptor 1.

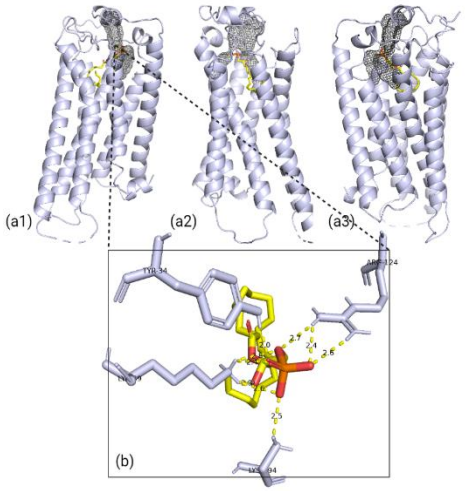

Figure 4. The binding site of the LPA1 receptor to its model 4-2. (a) The binding pocket of the LPA molecule predicted by model 4-2 with LPA receptor 1 is shown from three different angles. This is shown by PYMOL software, and the binding pocket can be seen in the form of mesh. (b) Amplified binding site. More microscopic amino acid residues are shown. The amino acid sequences on LPA receptor 1, which has polar interaction with the LPA molecule, are marked on the graph.

In the predicted results of the LPA1 receptor model 4-2, four amino acids belonging to the LPA1 receptor play a key role in the interaction with the LPA molecule. These four amino acids are: tyrosine at position thirty-four, lysine at position thirty-nine, arginine at position 124 and lysine at position two hundred and ninety-four. Tyrosine-34 and Lysine-39 are both located at the extracellular topological domain which links the N-terminal of the first transmembrane domain; Arginine-124

locates at the third transmembrane helix; Lysine-294 locates at the extracellular topological domain which links the N-terminal of the seventh transmembrane helix [20] (Figure 4).

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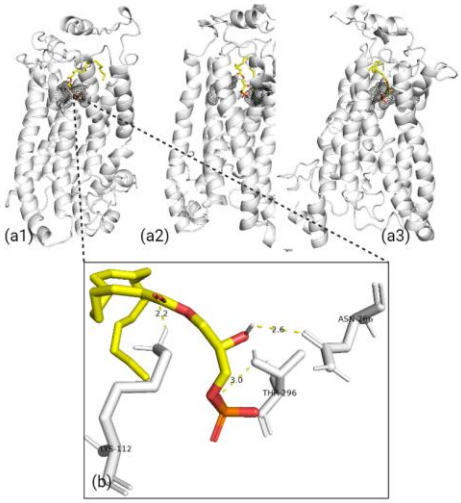

Figure 5. The binding site of the LPA4 receptor to its model 3-1. (a) The binding pocket of the LPA molecule predicted by model 3-1 with LPA receptor 4 is shown from three different angles. This is shown by PYMOL software, and the binding pocket can be seen in the form of mesh. (b) Amplified binding site. More microscopic amino acid residues are shown. The amino acid sequences on LPA receptor 4, which has polar interaction with the LPA molecule, are marked on the graph.

Based on the predicted model 3-1, three amino acids of LPA receptor 4 were found important during the interaction: Lysine at position 112, Threonine at position 296 and Asparagine at position 266. Lysine-112 is the last amino acid of the extracellular topological domain which links with the third transmembrane domain; Threonine-296 locates at the seventh transmembrane domain; Asparagine-266 locates at the sixth transmembrane domain [22] (Figure 5).

4. Discussion

4.1. Experiment 1: Sequence and structure

To better understand the signaling of LPA molecules through LPA receptors, it is very important to study the structure of LPA receptors. Although the LPA 1 receptor has already been studied for many years, there are few studies on LPA4 so far, and there is no LPA4 receptor structure supported by experimental results on Protein Data Bank. Therefore, this study has significance in the prediction of LPA receptor 4 structure.

In this study, RosettaFold which is widely used in structural biology was taken to predict the structure of LPA 4. RosettaFold is a computational tool that uses deep-learning-based algorithms rapidly and precisely to predict the protein 3D structure.[23] Using software to simulate the protein structure beyond the experimental method is innovative. However, there are still limitations to investigate the protein folding pattern, a 50-year-old grand challenge in biology [24], in this study. First of all, because RosettaFold is a computational method to predict protein structure, there must be a certain amount of error. Furthermore, experimental accuracy in protein structure has to be refined

via molecular dynamics simulations. [25] In the future, the prediction of the structure of LPA receptors can be verified via X-ray crystallography, NMR or cryo-EM.

4.2. Experiment 2: LPA binding site

The prediction of the 3D structure of the LPA receptor in this study provides the structural basis for the molecular docking of the LPA molecule with the LPA receptor. This provides the groundwork and new ideas for future studies of novel antagonists and agonists. However, the molecular docking in this study still has limitations. In ligand-receptor interactions, it often leads to conformational change. Changes in the receptor structure led to changes in its function and finally to signal generation.

However, the AutoDock Vina and Tools used in this study are based on rigid protein and flexible ligand docking for docking and do not take into account the conformation change of the receptors. This may lead to imprecise prediction of the binding site on the receptor. Experimental accuracy in protein structure can be refined via molecular dynamics simulations. [26] In addition, current CPU used in this study was not sufficient for the molecular dynamic verification. In future research, if advanced computer equipment is available, the molecular dynamics simulation can be performed. In any case, the prediction of either the structure of the LPA receptors or the interaction between the LPA molecule and the LPA receptors will be more convincing if X-ray crystallography, NMR or cryo-EM can yield observational results.

These binding sites obtained by molecular docking can provide ideas for future studies of receptor agonists and inhibitors. Agonists and inhibitors can be used for drug development.

5. Conclusion

In this study, we predicted the receptor structure of LPA4 and discovered the binding sites of LPA molecules to LPA receptors 1 and 4 by using RosettaFold and AutoDock Vina. These binding sites and proteins could be used as directions for drug development for the treatment of cancer metastasis.

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