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GLYPICAN 1 LIGANDS IN EXPLORING BILIARY ATRESIA

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ABSTRACT: Biliary atresia (BA), extrahepatic ductopenia, and progressive obliterative cholangiopathy is a childhood disease in which one or more bile ducts are abnormally narrow, blocked, or absent. In animals, plant toxins have been shown to cause biliary atresia. The only effective treatments are operations such as the Kasai procedure and liver transplantation. An association between biliary atresia and deletion of the gene GPC1 has been reported. This study uses computational analysis on Glypican 1, a protein encoded by the GPC1 gene, to obtain ligands that bind to this specific protein. These ligands can be a valuable resource in experimental biliary atresia studies. Results show that ligands bind effectively to Glypican 1. Overall, Glypican 1 ligands form computational stable complexes.

Keywords: biliary atresia, Glypican 1, CAR-T cell therapy, cancer therapy.

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1. INTRODUCTION

The etiology and pathogenic underpinning of the most neonatal severe liver disease, biliary atresia, remains unknown. This disease is an aggressive form of neonatal cholestasis characterized by the destruction and obliteration of the extrahepatic bile ducts [1]. However, there are genetic contributions to the adaptation and response to cholangiopathies and cholestasis. Some studies showed that up-regulation of glypican-3 mRNA expression was observed in biliary atresia livers. Its expression was positively associated with alpha-smooth muscle actin, β-catenin, c-Myc, and cyclin D-1[3]. Most studies show that aductin 3 (ADD3) is frequently involved in biliary atresia

pathogenesis. Although both xpnpep1 and add3a are expressed in the developing zebrafish liver, only knockdown of add3a produced intrahepatic defects and decreased biliary function. Similar results were observed in homozygous add3a mutants—inhibition of Hh signaling rescued biliary defects caused by add3a knockdown [4]. Regarding the GPC1 gene, it was shown by Ke et al. that regarding the two single nucleotide polymorphisms (SNPs), it was found a significantly decreased BA risk associated with rs2292832 (additive model: OR=0.638, 95% CI: 0.467-0.873, P=0.005), and a marginal effect for rs3828336 (heterozygous model: OR=0.564, 95% CI: 0.312-1.020, P=0.058). The haplotype analysis indicated that either Crs2292832-Crs3828336&Trs3828336 or Trs2292832-Trs3828336 conferred a protective effect from BA (OR=0.569, 95% CI=0.414-0.783, P<0.001; OR=0.528, 95% CI: 0.301-0.926, P=0.026). Moreover, the analysis suggested that GPC1 expression via the effect on transcription-factor-binding sites (TFBS) of upstream binding transcription factor (UBTF), as a regulatory DNA variation in Deoxyribonuclease I (DNase I) hypersensitive sites (DHSs). This demonstrates that Common variants of the GPC1 gene were genetically involved in BA risk[5]. As stated before, BA is not a primarily genetic disease, although multiple genes that might increase susceptibility to BA have been identified. Preliminary work via whole-exome sequencing of family trios has identified a variant in the primary cilia protein PKD1L1, suggesting that primary cilia may also play a role in the susceptibility of the extrahepatic bile duct to injury[6]. Overall, there appear to be multiple gene defects associated with BA, but all appear to increase susceptibility or modify the phenotype rather than being primarily responsible for the injury[7]. The lack of an identifiable genetic cause of BA has led to the hypothesis that maternal microchimerism (postzygotic somatic mutation) may be part of the etiology[8,9]. Regarding animal models, the characteristic lesions of BA such as the obstruction of the extrahepatic biliary tree and cholestasis, have been successfully reproduced and investigated in several animal models—such as lamb, calf, zebrafish, and mouse. [10,11]. A group of scientists from the University of Pennsylvania imported a plant species characteristic of that area and used zebrafish bioassays to identify the substance responsible: an isoflavonoid that they named biliatresone[12]. This toxic compound, capable of inducing biliary atresia phenotype, is the basis of the theory that implicates hepatotoxins as etiological agents. Regarding Glypican, it constitutes significant heparan families sulfate proteoglycans. Six glypicans have been identified in mammals called GPC1 through GPC6[13,14,15]. In Drosophila, two glypicans have been identified. These are colled dally (division abnormally delayed) and dally-like. [16]. Glypicans play a vital role in developmental morphogenesis and have been suggested as regulators for the Wnt and Hedgehog cell signaling pathways. They are regulators of fibroblast growth factors and bone morphogenic protein signaling[17]. This study uses computational analysis on Glypican 1 crystallographic structure to find efficient ligands for this protein. The results will enable further experimental studies regarding BA.

2. MATERIALS AND METHODS

To computationally study de Glypican 1 molecule, its crystallographic structure 4ACR[18] was used. The structure was energetically minimized, charges corrected, and protonated at physiological Ph and temperature(Co). Docking studies were performed using AutoDock software package version4.2.6[19]. Binding sites were retrieved from literature using an online binding site search algorithm server[20,21]. The binding site coordinates for docking were as follow x13.52(Å),y-31.17(Å)z76.24(Å) with a radius of 15 (Å). ChEMBL data-based were used for retrieving the compounds used for screening against 4ACR[22]. All structures were converted to SDF files. Docking energies of the ligand-protein complex were represented. Compounds were ordered after their drug-like properties.

3. RESULTS AND DISCUSSION

Glypican 1 was docked against 3493 ligands. The most favorable energetically compound (kcal/mol) is presented in **Figure1**, together with ligand-receptor interactions.

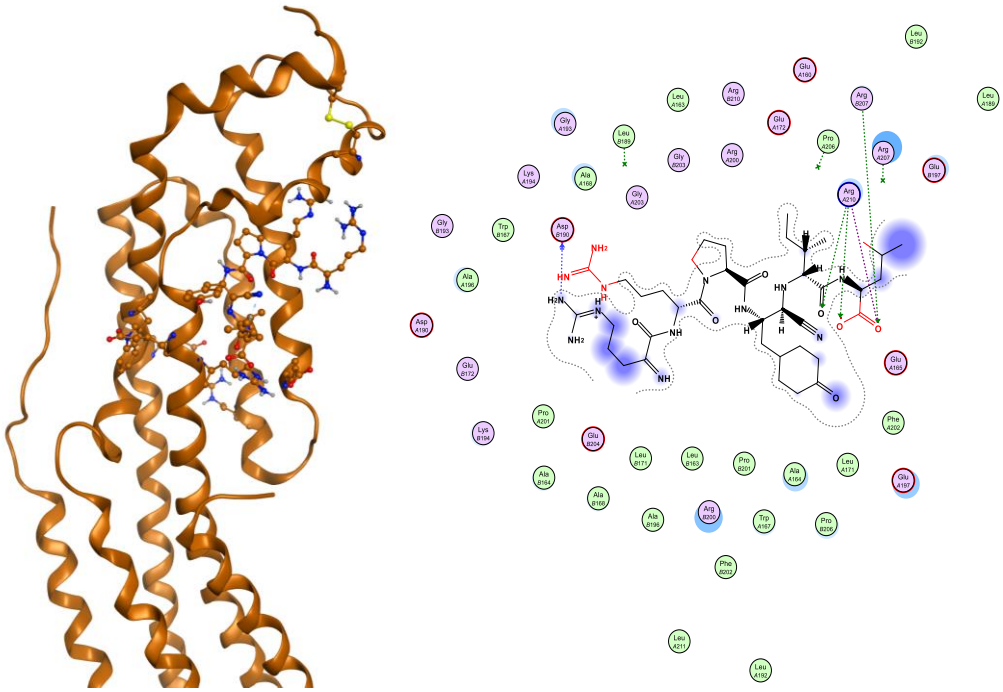


Figure 1: Glypican1 in complex with the most energetically favorable ligand. Ligand receptor interactions are represented. For example, Asp 190 forms a backbone acceptor bond with an amine group, Arg 210 accepts electrons from ligands O atoms, and Arg 207 is a carbonyl group bond.

Table 1: First three most energetically favorable compounds and their interaction energy.

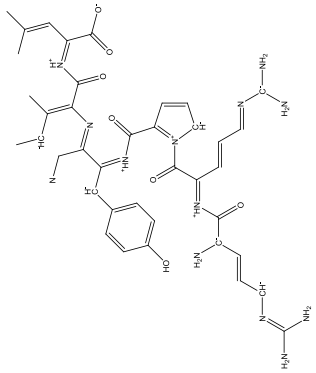
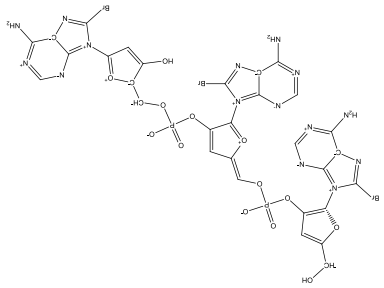
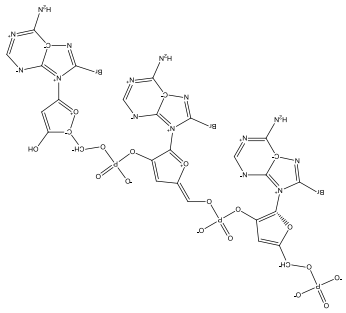
Nr	Compound	Complex energy	Ligand pose energy	Steric energy
1		-251.442	-270.535	-225.606
2		-251.037	-252.804	-176.581
3		-235.329	-244.384	-158.201

Table1 Compounds with the lowest complex energy (kcal/mol).

In **Figure 2**, all energy population is represented.

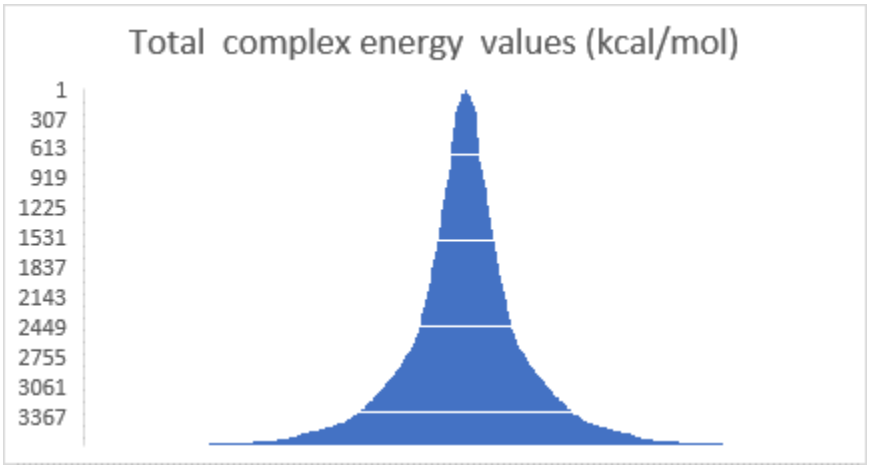


Figure 2: Energy population for all data sets (3493 compounds).

The affinity of the ligand for a specific receptor is crucial for effectively binding the ligand to the desired target. The highest possible affinity from a protein towards the ligand, or target molecule, can be observed when the protein has a perfect mirror image of the shape of the target surface and a charge distribution that perfectly complements the target surface[23]. In addition, the equilibrium dissociation constant Kd gives the affinity between protein and ligand. The dissociation constant is defined as the ratio between the product of the ligand and receptor molecular concentrations divided by the complex concentration. The lower the Kd value, the higher the protein affinity for the ligand and vice versa. The Kd value is equivalent to the concentration of the ligand at which one-half of the proteins contain bound ligand[24] Affinity is also influenced by the properties of the solution, like pH, temperature, and salt concentration, which may affect the stable state of the proteins and ligands and hence their interaction and by the presence of other macromolecules causes macromolecular crowding[25]. Structural biology has been highlighted as part of drug discovery programs in the pharmaceutical industry and structural genomics programs. Although several biochemical and or biophysical techniques can study the function of a protein, a molecular understanding of a protein can only be obtained by combining available data with the three-dimensional structure. Furthermore, big data analysis is implied in this process[26]. Lastly, Glypican-1 (GPC-1) is a cell surface heparan sulfate proteoglycan that is critical during normal development. It is overexpressed in a variety of solid tumors. The role of GPC-1 in the TME and on the tumor cell is broad, while GPC-1 regulates signaling by several growth factors, including FGF, HGF, TGF-β, Wnt, and Hedgehog (Hh). Signaling via these pathways promotes tumor growth and invasive and metastatic ability. In this light, Glypican 1 is a crucial gene product that needs further experimental and computational studies[27,28,29,30].

4. CONCLUSION

Ligands can effectively bind to Glypican 1. Developing a Glypican 1 ligand enables experimental studies for further characterization and developments based on this molecule.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

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The authors contributed equally to this manuscript.

CONFLICT OF INTEREST

There are no conflicts of interest.

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