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Hyaluronic Acid Nanomaterials in Targeted Drug Delivery for Cancer Therapy

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Abstract

Hyaluronic acid and the CD44 receptor have been the subject of 3D interaction and molecular analysis that has revealed crucial residues, binding specificity, stabilizing relationships, and structural insights within the complex. The analysis has focused on amino acid interactions. It is a crucial field of study with both fundamental and applied consequences because this understanding not only illuminates the molecular mechanisms directing their interaction but also shows promise for future therapeutic approaches. The new methods cover a variety of strategies, such as creating highly focused treatments, combining treatments with other well-known techniques like immunotherapy and chemotherapy, and moving toward customized medicine. Combining state-of-the-art nanotechnology with hyaluronan-based pharmaceuticals could improve lung cancer therapy's precision, bioavailability, and drug delivery. Clinical trials will be essential in proving these medicines' safety and effectiveness so that they may be incorporated into standard cancer treatment. Moreover, investigating immune regulation via hyaluronan may open up new avenues for bolstering the body's defenses against cancer. With a calculated binding energy score of -6.70, the interaction between hyaluronan and the CD44 protein receptor was observed to be remarkably strong and favorable, suggesting a robust connection. This highlights the potential for utilizing hyaluronic nanomaterials to facilitate the targeted delivery of commercially available cancer drugs to specific cancer sites.

Keywords: *anti-cancer properties, molecular docking, CD44 receptor, cancer treatment, computational analysis*

1. Introduction

A significant portion of extracellular matrix is composed of hyaluronic acid (HA), a linear mucopolysaccharide that is mostly composed of glucuronic di-saccharide and N-acetylglucosamine that are alternately repeated. In addition to its N-acetyl group, which can be utilized for additional chemical alterations, HA also contains carboxylic and hydroxyl groups. HA is produced by a wide range of cells, including fibroblasts. Higher water-binding capacity, nontoxicity, biodegradability, cytocompatibility, and nonimmunogenicity are only a few of the superior physiochemical characteristics of HA. Owing to HA's biological properties, there is a lot of interest in creating HA-based nanomaterials for a variety of biomedical uses, such as molecular imaging and drug delivery systems (DDS). It is well recognized that many cancer cells overexpress HA-biding receptors, including RHAMM, LYVE-1 receptors, and CD44 HA-based nanomaterials can protect against the immune response and modulate the immune system due to their inherent anti-inflammatory and anti-immunogenic properties. HA has the potential to be an immunotherapy-based cancer treatment system (Kim et al., 2018). After being generated, the various classes of HA work in concert with the various cutaneous compartments. Here, they interact with certain receptors (such as CD44, hyaluronan-mediated motility receptors [RHAMM], and Lyve-1), leading to a variety of cell responses and biochemical cascades (Laurino et al., 2015) . Numerous studies have

concentrated on ways to target CD44 in an effort to enhance the delivery of drugs and discrimination between healthy and malignant tissue, while lowering residual toxicity and off-target accumulation, ever since it was discovered that the receptors are over-expressed in a variety of solid tumors, including pancreatic, breast, and lung cancer (Underhill , 1992). The CD44 molecule's two ends' conservation may be due to structural requirements for interacting with both the cytoskeleton and HA (Mattheolabakis et al,, 2015). Receptor shedding is one method by which CD44 can be deregulated from the cell surface (Isacke et al., 2002). Epithelial, hematopoietic, and neuronal cells have been shown to express the hyaluronic acid receptor CD44 at low levels, although many tumor cells overexpress it (Huang G and Huang H, 2018). Because of its carboxylic and hydroxyl groups, the HA backbone could be modified to accommodate different therapeutic requirements (Kumar et al., 2019). In certain cancers, there is an increase in the common form of CD44's expression during the carcinogenesis process. Furthermore, non-native CD44 variants may be produced during translation by alternative splicing of the cytoplasmic domains of CD44 (Platt and Szoka Jr, 2008). Using nanoparticles, the different carrier types can be divided into drug delivery systems. This article focused on how several tumor-targeted medication delivery systems based on HA have been developed. These systems comprise HA-drug conjugate tumor-targeted drug delivery systems, HA-targeted drug delivery systems with amphiphilic derivatives of HA, HA-surface-modified tumor-targeted drug delivery systems, and HA-gene drug conjugate tumor-targeted drug delivery systems (Huang and Huang, 2018).

Since the CD44 receptor is endogenously expressed at low levels on a variety of cells, it needs to be activated in normal tissue. On the other hand, tumor-derived cells do not require an activation mechanism because the expressed CD44 receptor's high affinity function is sufficient. In this case, internalization and HA binding can be accomplished without additional steps. These interactions may encourage tumor cell migration since HA levels are higher at the margins of rapidly growing tumors. CD44 can be utilized as a biomarker to target cancer since many tumor cells, especially those of breast and lung cancer, have overexpressed CD44 receptors on their surfaces (Lee, 2020). Their low toxicity and exceptional biocompatibility make them the ideal therapeutic choice for the formation of newly created blood vessels . Several groups report that targeting drug-loaded NP with HA polymer has yielded encouraging results in vitro and in preclinical trials (Yasin, 2022).

2. Materials And Methods

Protein Identification

The work investigates the use of molecular fragment scoring on the protein surface as a computational method to find protein binding sites. The binding sites of the CD44 protein receptor are varied and include both natural and artificial ligands. Although computational characterisation of binding sites is difficult, it is essential to comprehend molecular interactions under different circumstances. The importance of computational tools is emphasized as the paper focuses on recent developments in identifying druggable regions on proteins for drug creation. The study focuses on the human CD44 protein in particular, emphasizing its importance in biological processes by highlighting its interactions with hyaluronic acid and extracellular matrix components. The three-dimensional structure of the protein (PDB ID: 1UUH) is utilized in the study to analyze it, highlighting the significance of comprehending protein-ligand interactions for biotechnological and pharmaceutical applications (Ruppert et al., 1997; Henrich et al., 2010; Vajda and Guarnieri, 2006).

Receptor protein Preparation:

Receptor proteins are essential for interactions with different substances; in this work, a receptor protein's A-chain is separated for analysis. Chain selection is a technique used to simplify complicated protein structures by concentrating on particular domains or subunits that are important to the study. The protein is ready for molecular docking or structural analysis by taking into account only the A-chain, which isolates its interactions from those of other subunits. Structural biology depends on having access to precise 3D protein structures, and the Protein Data Bank (PDB) is used to provide those structures. Refinement yields a refined PDB file suitable for targeted structural analyses by methodically eliminating ligands, heteroatoms, and water molecules. To perform subsequent structural studies reliably and meaningfully, this preliminary purification is necessary.

Molecular docking

Molecular docking is being explored more and more as a lead finding method as the structures of an increasing number of proteins and nucleic acids are known. As more experimentally described protein structures are found, the number of proteins that can be docked against homology-modeled targets rises. The "drug-likeness" and accuracy of docking hits are being investigated as more docking tests are conducted. Through refinement of the pertinent search algorithms and improvement of the scoring

function, the molecular docking technique will develop into a dependable drug-design tool that integrates substantial amounts of biological data. The identified ligand-protein interaction that has the strongest ligand-targeted protein interaction was selected (Shoichet et al., 2002).

The PDB-identifiers for the list of receptors and their ligands are filled in by the algorithm, which also transforms them to AutoDock format (PDBQT) after querying the relevant biological databases. The UniProtKB and Protein Data Bank (PDB), two open-access specialist biological databases, are the sources of the data. The outcome is a tool for preparing data for use in the AutoDock family of docking software (Samarskaya et al., 2018).

For the creation of new drugs as well as the study of protein-ligand interactions, computational docking is frequently used. Usually, the procedure begins with a target whose structure is known, like the crystal structure of a therapeutic enzyme. The bound conformation as well as binding free energy for smaller molecules to the target are then predicted using docking. While single docking studies are helpful in examining the target's function, virtual screening—which involves docking and ranking a sizable library of compounds—may be utilized to find novel inhibitors for therapeutic development. A collection of free, open-source programs called AutoDock is used to virtually screen and computationally dock tiny compounds with macromolecular receptors (Forli et al., 2016).

Preparation of protein as PDBQT files

The docking workspace is now located in the preference folder. Protein that has been prepared has been imported into the Auto Dock 1.5.6 workspace. The first step was calculating the protein's Gasteiger charges after merely adding polar hydrogen atoms. Kollman charges were added thereafter. The PDBQT file format was used to hold the protein. Afterwards, the ligand was included and the root of the torsion tree was chosen. Additionally, the ligand was stored in PDBQT format. To carry out the computational procedure, the ligand and protein were loaded into the computing environment in PDBQT format.

Grid parameters

Assigning the grid parameters, which point the ligand to the required location on the protein, is the most important stage in the molecular docking process. Grid spacing was set to 0.425 Å by default. The values of x = -0.609, y = 1.873, and z = 15.387 were fixed for the center grid box with offset values of -8.743, -36.064, and 6.154. The dimensions of x, y, and z were assigned 84 x 108 x 74 grid points. The corresponding maps contained a total of 1564513 grid points. These characteristics surrounded the protein's whole three-dimensional active site. The output was stored in the grid parameter file (GPF) format by the grid parameter. Table 1 depicts the determination of active site of the ligand for interaction.

Table 1: Determination of Active site of the ligand

S. No	Amino acid	Residue Number
1	Tyrosine	169
2	Isoleucine	168
3	Isoleucine	143
4	Asparagine	39
5	Aspartic acid	23
6	Isoleucine	22
7	Alanine	20
8	Glutamine	21
9	Lysine	38
10	Arginine	162

AUTOGRID and AUTODOCK RUN

The grid log file (GLG) was created when the AutoGrid application was run using the GPF file as input. The grid was then turned on after that. After a flawless AutoGrid run, the output was produced with the docking parameter file (DPF) type using the Lamarckian genetic technique. Once the docking log file (DLG) was ready, the AutoDock runtime as well as DPF files were utilized as input. The generated DLG file included the 10 most important free binding energies as well as the inhibitory constants on each run. The complex having the least binding energy was retained for additional research in PDBQT format following data processing, binding energy sorting, and PDB format description.

3. Results and Discussion

Analysis of protein-ligand interaction

Figure 1 depicts the RMSD with Least binding energy analysis

RMSD TABLE

Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	10	-6.70	0.00	36.32	RANKING
2	1	7	-5.97	0.00	25.89	RANKING
3	1	4	-5.61	0.00	7.88	RANKING
4	1	2	-4.85	0.00	21.15	RANKING
5	1	1	-4.78	0.00	10.45	RANKING
6	1	3	-4.67	0.00	8.55	RANKING
7	1	5	-4.19	0.00	29.44	RANKING
8	1	8	-3.99	0.00	12.12	RANKING
9	1	6	-3.20	0.00	21.70	RANKING
10	1	9	-2.65	0.00	31.45	RANKING

Figure 1: RMSD Table

The RMSD table, which provides information on binding energy, cluster RMSD, reference RMSD, ranking, sub-ranking, and grep pattern for each run, summarizes the results of the molecular docking process. Significantly, a low RMSD value shows that the two molecules are closely aligned in their binding conformation, and a remarkable binding energy score of -6.70 suggests that hyaluronan and the CD44 protein receptor have a strong connection.

Two-dimensional ligand-protein interaction

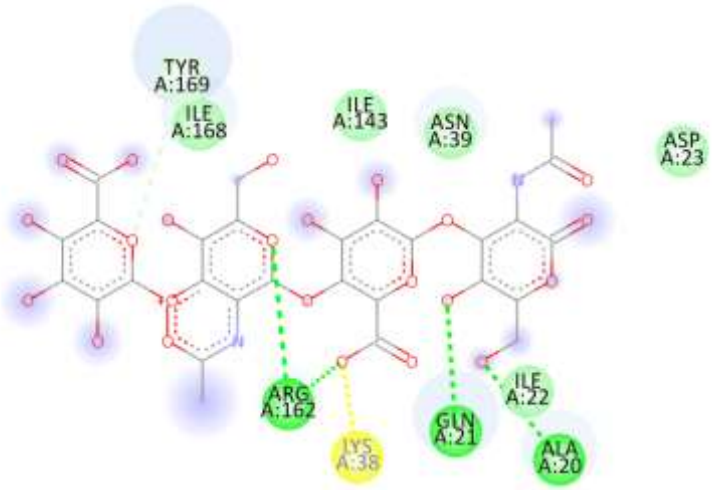

Figure 2 : 2-D interaction image

"Tyrosine" is found at residue 169 in the amino acid sequence, and "isoleucine" is found at residue 168, occurring twice at positions 22 and 143. The positions of "aspartic acid" at residue 23, "asparagine" at residue 39, and "alanine" at residue 20 are identified. "Lysine" is at residue 38 while "glutamine" is at residue 21 farther down. "Arginine" is the last item in the list, found at residue 162. Comprehending the configuration of amino acids is essential to understanding the organization and structure of a protein or peptide sequence.

3-D interaction of CD44-HA complex:

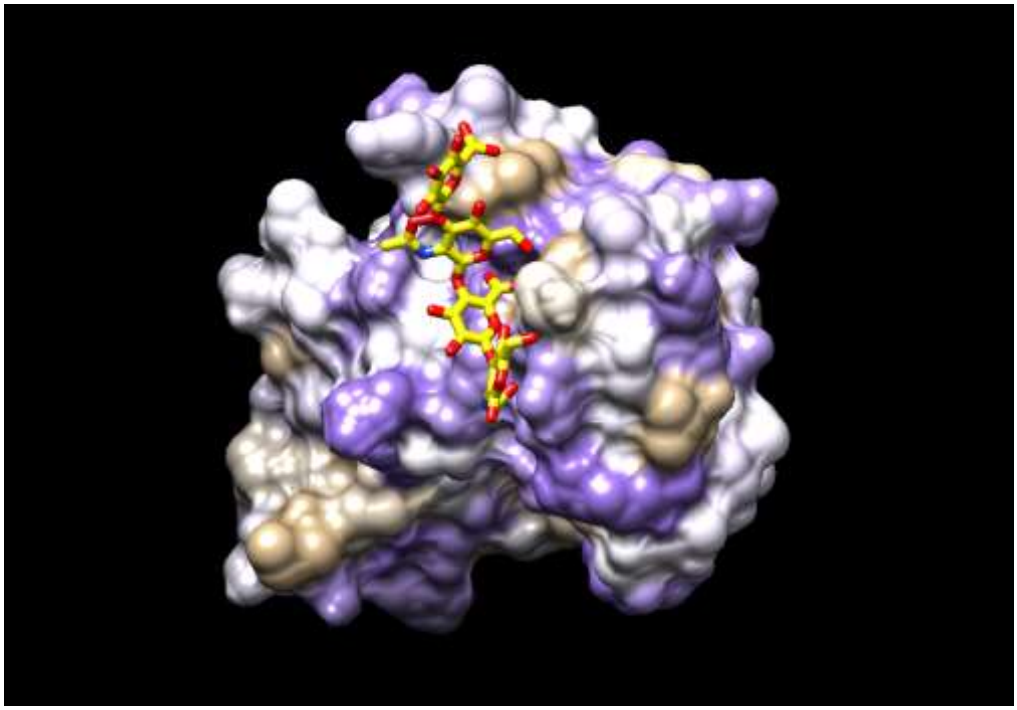

Figure 3: 3-D interaction image

The investigation identified particular amino acid residues in the CD44 receptor that are essential for its interaction with hyaluronic acid. For the entire binding and recognition process, these residues are necessary.

4. Conclusion

Drug delivery systems incorporating hyaluronic acid-based nanoparticles have great potential to improve the accuracy and potency of commercially accessible cancer medications including paclitaxel (PTX) and doxorubicin. Hyaluronan and the CD44 protein receptor have a strong association, as demonstrated by the low binding energy of -6.70. The molecular investigation and three-dimensional interaction between hyaluronic acid and the CD44 receptor have revealed structural insights, binding selectivity, and critical residues. This work, which mainly examined interactions between amino acids, is important for both basic research and possible medical uses. It provides insight into the molecular processes behind their interaction and points to potential directions for future treatment strategies.

References:

1. Kim, J. H., Moon, M. J., Kim, D. Y., Heo, S. H., & Jeong, Y. Y. 2018. Hyaluronic acid-based nanomaterials for cancer therapy. Polymers, 10(10), 1133.
2. Laurino, C., Palmieri, B., & Coacci, A. 2015. Efficacy, safety, and tolerance of a new injection technique for high-and low-molecular-weight hyaluronic acid hybrid complexes. Eplasty, 15.
3. Underhill, C. 1992. CD44: the hyaluronan receptor. Journal of cell science, 103(2), 293-298.
4. Mattheolabakis, G., Milane, L., Singh, A., & Amiji, M. M. 2015. Hyaluronic acid targeting of CD44 for cancer therapy: from receptor biology to nanomedicine. Journal of drug targeting, 23(7-8), 605-618.
5. Isacke, C. M., & Yarwood, H. 2002. The hyaluronan receptor, CD44. The international journal of biochemistry & cell biology, 34(7), 718-721.
6. Huang, G., & Huang, H. 2018. Application of hyaluronic acid as carriers in drug delivery. Drug delivery, 25(1), 766-772.
7. Kumar, R., Singh, M., Meena, J., Singhvi, P., Thiyagarajan, D., Saneja, A., & Panda, A. K. 2019. Hyaluronic acid-dihydroartemisinin conjugate: Synthesis, characterization and in vitro evaluation in lung cancer cells. International journal of biological macromolecules, 133, 495-502.
8. Platt, V. M., & Szoka Jr, F. C. 2008. Anticancer therapeutics: targeting macromolecules and nanocarriers to hyaluronan or CD44, a hyaluronan receptor. Molecular pharmaceutics, 5(4), 474-486.
9. Huang, G., & Huang, H. 2018. Hyaluronic acid-based biopharmaceutical delivery and tumor-targeted drug delivery system. Journal of Controlled Release, 278, 122-126.
10. Lee, S. Y., Kang, M. S., Jeong, W. Y., Han, D. W., & Kim, K. S. 2020. Hyaluronic acid-based theranostic nanomedicines for targeted cancer therapy. Cancers, 12(4), 940.
11. Yasin, A., Ren, Y., Li, J., Sheng, Y., Cao, C., & Zhang, K. 2022. Advances in hyaluronic acid for biomedical applications. Frontiers in Bioengineering and Biotechnology, 10, 910290.
12. Ruppert, J., Welch, W., & Jain, A. N. 1997. Automatic identification and representation of protein binding sites for molecular docking. Protein Science, 6(3), 524-533.
13. Henrich, S., Salo-Ahen, O. M., Huang, B., Rippmann, F. F., Cruciani, G., & Wade, R. C. 2010. Computational approaches to identifying and characterizing protein binding sites for ligand design. Journal of Molecular Recognition: An Interdisciplinary Journal, 23(2), 209-219.
14. Vajda, S., & Guarnieri, F. 2006. Characterization of protein-ligand interaction sites using experimental and computational methods. Current Opinion in Drug Discovery and Development, 9(3), 354.
15. Shoichet, B. K., McGovern, S. L., Wei, B., & Irwin, J. J. 2002. Lead discovery using molecular docking. Current opinion in chemical biology, 6(4), 439-446.
16. Samarskaya, V. O., Kovalenko, A. V., Sroslova, G. A., Postnova, M. V., Shiroky, A. A., & Serov, A. G. 2019,. Molecular docking data preparation tool. In Saratov Fall Meeting 2018: Computations and Data Analysis: from Nanoscale Tools to Brain Functions (Vol. 11067, pp. 176-181). SPIE.
17. Forli, S., Huey, R., Pique, M. E., Sanner, M. F., Goodsell, D. S., & Olson, A. J. 2016. Computational protein–ligand docking and virtual drug screening with the AutoDock suite. Nature protocols, 11(5), 905-919.