RESEARCH PAPER

Isorhamnetin-3-O-Neohesperidoside as a Potential Akt Inhibitor for Hepatocellular Carcinoma: A Molecular Docking Study

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ABSTRACT

Hepatocellular carcinoma is one of the deadliest forms of malignancy that has limited therapeutic options with high percentage of mortality. The P13K/AKT is an important mechanism in regulating cell cycle, proliferation and metabolism and frequently altered in hepatocellular carcinoma. The suppression of AKT levels is one of the promising strategies against HCC. The present study aimed to evaluate the compound Isorhamnetin-3-O-neohesperidoside, an important natural flavonoid present in most fruits and berries as a potential AKT inhibitor using molecular docking approaches. Hepatocellular carcinoma–associated proteins were retrieved from the STRING database A protein–protein interaction (PPI) network was constructed and analysed using Cytoscape to identify key hub proteins. Network topological analysis based on node degree and betweenness centrality identified AKT as the major hub protein. Kaplan–Meier survival analysis indicates that increased AKT expression was associated with reduced overall survival in hepatocellular carcinoma patients. Molecular docking was performed using Auto Dock Vina to evaluate the binding affinity between the ligand and the target protein. Docking results demonstrates that there was a strong binding interaction between the ligand and the active site of AKT. The complex was stabilized through conventional hydrogen bonds with residues THR291, TYR272, VAL271, and GLU298, the findings proves that the Isorhamnetin-3-O-neohesperidoside may act as a potential inhibitor of the AKT protein and could serve as a promising lead compound for the development of therapeutic agents against hepatocellular carcinoma.

Keywords: Hepatocellular carcinoma, AKT, molecular docking, Isorhamnetin-3-O-neohesperidoside, protein-protein interaction network.

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1. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common primary liver malignancies and represents a major cause of cancer-related mortality worldwide. The disease often progresses silently during the early stages that leads to late diagnosis and limited therapeutic interventions. Despite advances in surgical and pharmacological treatments, the overall survival rate of HCC patients remains poor due to tumor recurrence, metastasis, and resistance to drugs (**1**). Therefore, identifying key molecular targets involved in HCC progression is essential for the development of more effective therapeutic strategies.

In recent years, natural compounds derived from medicinal plants have gained considerable attention for their potential role in cancer therapy. Many plant-derived secondary metabolites possess diverse pharmacological properties, including antioxidant, anti-inflammatory, and anticancer activities. Among these compounds, flavonoids have been extensively studied because of their ability to modulate several cellular signaling pathways involved in cancer progression (**2**) Isorhamnetin-3-O-

neohesperidoside (IHN) is a naturally occurring flavonoid glycoside predominantly present in an O-methylated form in several medicinal plants such as berries, Oenanthe javanica, and Ginkgo biloba. Previous studies have reported that isorhamnetin derivatives exhibit a wide range of biological activities including antioxidant, neuroprotective, cardioprotective, anti-inflammatory, and anticancer effects (**3**)**.** In addition, the anticancer potential of isorhamnetin-based compounds has been reported in several malignancies including oesophageal and gastric cancers (**4**). However, the molecular mechanisms underlying the potential anticancer activity of isorhamnetin-3-O-neohesperidoside in hepatocellular carcinoma are still not clearly understood.

The progression of hepatocellular carcinoma is associated with the dysregulation of multiple cellular signalling pathways that control cell proliferation, survival, metabolism, and apoptosis. Among these, the PI3K/AKT signalling pathway plays an important role in regulating cell growth and survival. AKT is a serine/threonine protein kinase that acts as a central mediator of several downstream signalling events. Abnormal activation of this pathway has been reported in various cancers, including hepatocellular carcinoma, where it contributes to tumor growth and resistance to apoptosis (**5**). As it plays a major role in regulating multiple cellular processes, proteins involved in the PI3K/AKT pathway are often explored as potential therapeutic targets in cancer research.

With the advancement of computational biology, protein–protein interaction (PPI) network analysis has become an important tool for understanding the complex molecular interactions involved in disease progression. PPI networks help to identify the highly connected proteins, known as hub proteins, which often play crucial regulatory roles in biological systems (**6**). Identifying such hub proteins provides valuable insights into key molecules that may serve as potential targets for therapeutic intervention. Furthermore, survival analysis using clinical datasets can help to evaluate the prognostic significance of these candidate targets by correlating gene expression levels with patient survival outcomes.

In addition to target identification, molecular docking has emerged as an effective computational method for predicting the interaction between small molecules and protein targets. Docking studies provide insights into binding affinity, interaction patterns, and possible inhibitory mechanisms of bioactive compounds. Similar *in silico* drug design approaches have been successfully applied to identify potential inhibitors against various biological targets in previous studies (**7**). Integrating network analysis with docking approaches can therefore provide a comprehensive understanding of potential therapeutic interactions between natural compounds and disease-related proteins.

Therefore, the present study aimed to explore the potential anticancer activity of isorhamnetin-3-O-neohesperidoside against hepatocellular carcinoma using an integrated approach. Hepatocellular carcinoma–associated proteins were first analysed through protein–protein interaction network analysis to identify key hub proteins involved in the disease network. The prognostic relevance of the identified hub protein was further evaluated using Kaplan–Meier survival analysis. Subsequently, molecular docking analysis was performed to investigate the binding interaction between the compound and the selected target protein.

2. METHODOLOGY

2.1 Retrieval of Hepatocellular Carcinoma Associated Proteins

Hepatocellular carcinoma–associated proteins were retrieved using the STRING database integrated with the Disease Ontology resource **(8**). The disease term DOID:684 (hepatocellular carcinoma) was used to identify disease-related proteins. The search was restricted to Homo sapiens, and the resulting protein list was used for further network analysis.

2.2 Construction of the Protein Protein Interaction Network

The retrieved proteins were submitted to the STRING database to construct a protein–protein interaction network. STRING integrates various interaction sources including experimental data, database annotations, co-expression, and text mining to predict functional associations among proteins. The interaction network was generated based on the confidence score and exported for further analysis.

2.3 Network Visualization and Topological Analysis

The PPI network generated from STRING was exported in TSV format and imported into Cytoscape software for visualization and topological analysis. Network parameters such as node degree, betweenness centrality, and clustering coefficient were calculated using the Network Analyzer tool (**9**). Proteins with higher degree and centrality values were considered key nodes in the interaction network. Hub protein identification was performed based on the connectivity of nodes within the network.

2.4 Survival Analysis of Hub Proteins

The prognostic significance of the identified hub protein was evaluated using the Kaplan–Meier Plotter database (**10**). Survival curves were generated to compare overall survival between patient groups with high and low expression levels of the selected gene. The hazard ratio and log-rank p-value were calculated to determine the

statistical significance of gene expression on patient survival.

2.5 Protein Structure Preparation

The three-dimensional structure of the selected target protein (PDB ID: 3O96) was retrieved from the Protein Data Bank (**11**). Prior to docking analysis, the protein structure was prepared by removing water molecules, co-crystallized ligands, and other heteroatoms using molecular visualization tools. The prepared protein structure was then used as the receptor for molecular docking studies.

2.6 Ligand Preparation

The chemical structure of the ligand used in this study was obtained from the PubChem database (**12**). The ligand structure was downloaded in appropriate format and energy minimized prior to docking to ensure stable conformations.

2.7 Molecular Docking Analysis

Molecular docking analysis was performed using AutoDock Vina to evaluate the binding affinity between the ligand and the target protein. Docking simulations were carried out by positioning the ligand within the active site of the receptor protein. The binding energy values were calculated, and the most stable protein–ligand complex was selected based on the lowest binding energy.

2.8 Visualization of Protein Ligand Interactions

The docked complexes were analyzed using Discovery Studio Visualizer BIOVIA (2021) to examine the interactions between the ligand and the protein. Two-dimensional and three-dimensional interaction diagrams were generated to identify hydrogen bonds, van der Waals interactions, π–anion interactions, and other non-covalent interactions involved in ligand binding.

3. RESULTS

Hepatocellular carcinoma-associated proteins were retrieved from the Disease Ontology database integrated in STRING. The disease term DOID:684 (Hepatocellular carcinoma) was selected, which resulted in 17 target proteins (Figure 1). The generated PPI network consisted of 17 nodes and 79 edges, indicating the presence of multiple functional associations among the proteins. The average node degree was 9.29, suggesting that each protein interacted with approximately nine other proteins in the network. The average local clustering coefficient was 0.763, indicating a relatively high level of interconnectivity among the proteins. The expected number of edges was 73, whereas the observed network contained 79 edges, with a PPI enrichment p-value of 0.252. This suggests that the interactions observed among the proteins are close to the expected level for a random network of similar size.

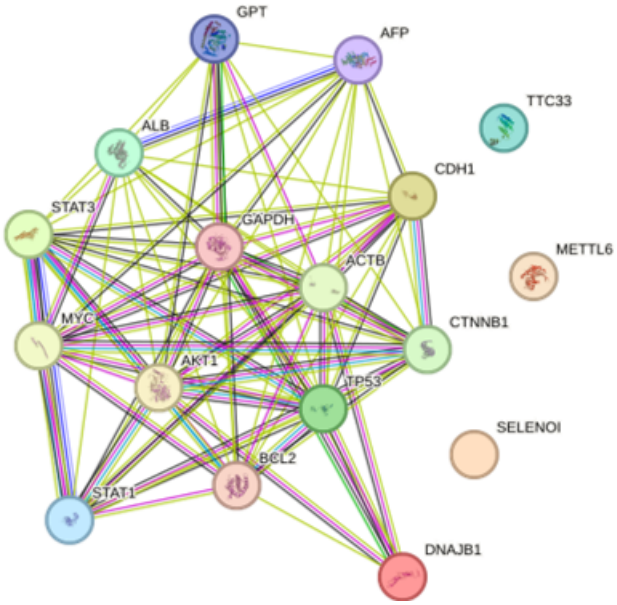

Figure 1: Protein protein interaction network of hepatocellular carcinoma associated target proteins constructed using the STRING database.

3.1 Protein Protein Interaction Network Analysis

The protein protein interaction network was built to examine the interaction among the proteins. The network had 14 nodes and multiple edges that showed how the proteins were linked to each other. The constructed Protein protein interaction network showed that the chosen proteins interacted with each other in a very close way. **(Figure 2)**. The constructed protein–protein interaction network consisted of 14 nodes, representing CTNNB1, ACTB, STAT1, ALB, STAT3, BCL2,

DNAJB1, AFP, GAPDH, MYC, TP53, CDH1, GPT, and AKT. Network topology analysis using node degree and betweenness centrality identified AKT as the hub protein, showing the highest connectivity within the network. **(Figure 3)**

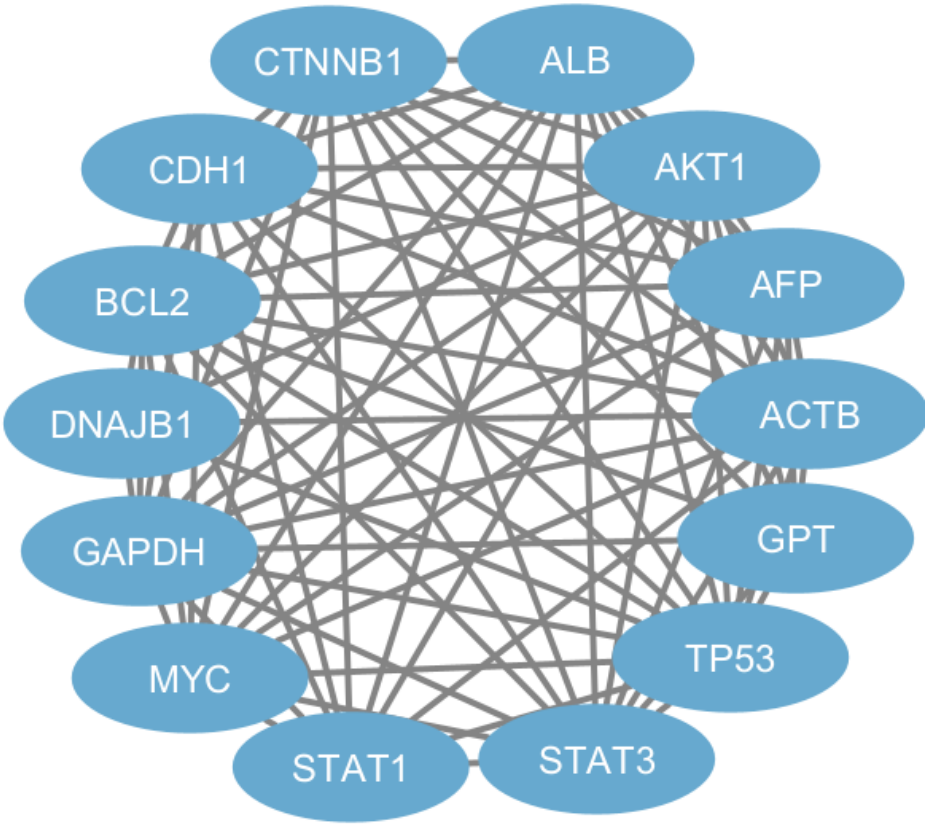

Figure 2 : Protein–protein interaction network of hepatocellular carcinoma–related proteins constructed using Cytoscape.

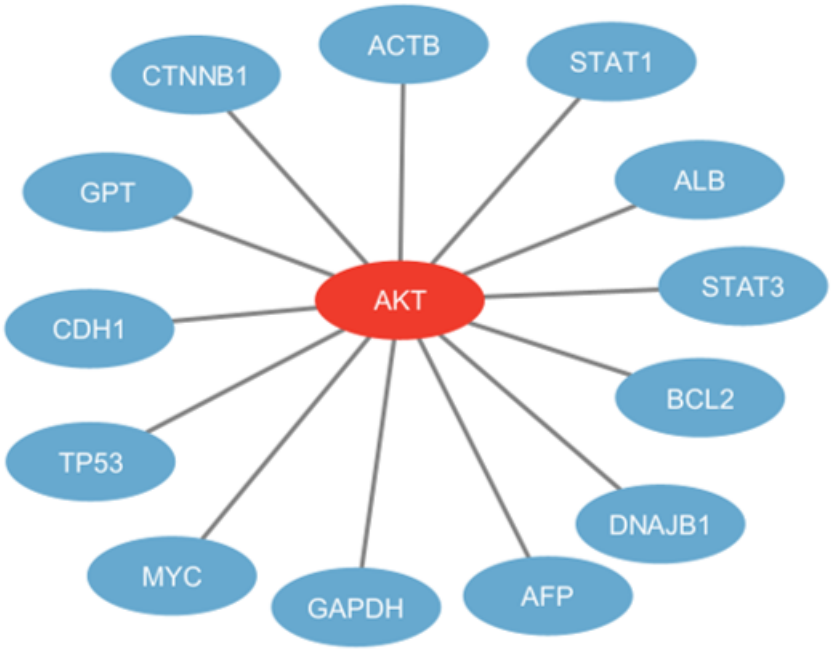

Figure 3: Identification of hub protein from target disease genes interaction networks.

Kaplan–Meier survival analysis showed that patients with high AKT expression exhibited lower overall survival compared with those with low AKT expression (**Figure 4)**. The hazard ratio was 1.4 with a log-rank p value of 0.067, supporting the selection of AKT as a potential target for subsequent molecular docking analysis.

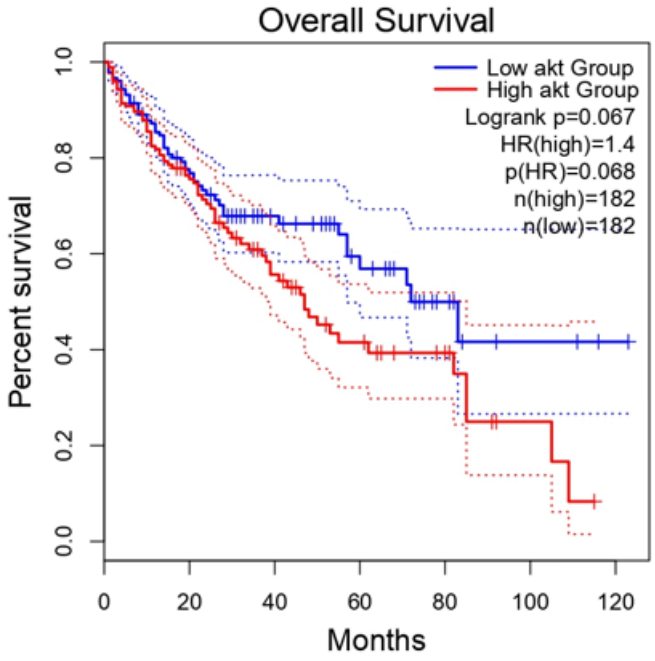

Figure 4: Kaplan Meier overall survival analysis of AKT expression in hepatocellular carcinoma patients.

3.2 Molecular docking

Molecular docking predicts the binding affinity and the structure formed by the protein-ligand complex. The information on the docking scores of the ligands to the proteins is in Table 1. The visualization of the interactions formed by the ligands and proteins is found in (**Figure 5**). The molecular docking analysis revealed that the ligand formed multiple interactions within the active site of the

AKT protein. The 3D and 2D interaction analyses showed the presence of conventional hydrogen bonds with residues such as THR291, TYR272, VAL271, and GLU298. Additional interactions including carbon hydrogen bonds, van der Waals interactions, π–anion interactions with ASP274, and π–alkyl interactions with LEU264 and TYR18 were also observed. These interactions indicate stable binding of the ligand within the active pocket of the AKT protein.

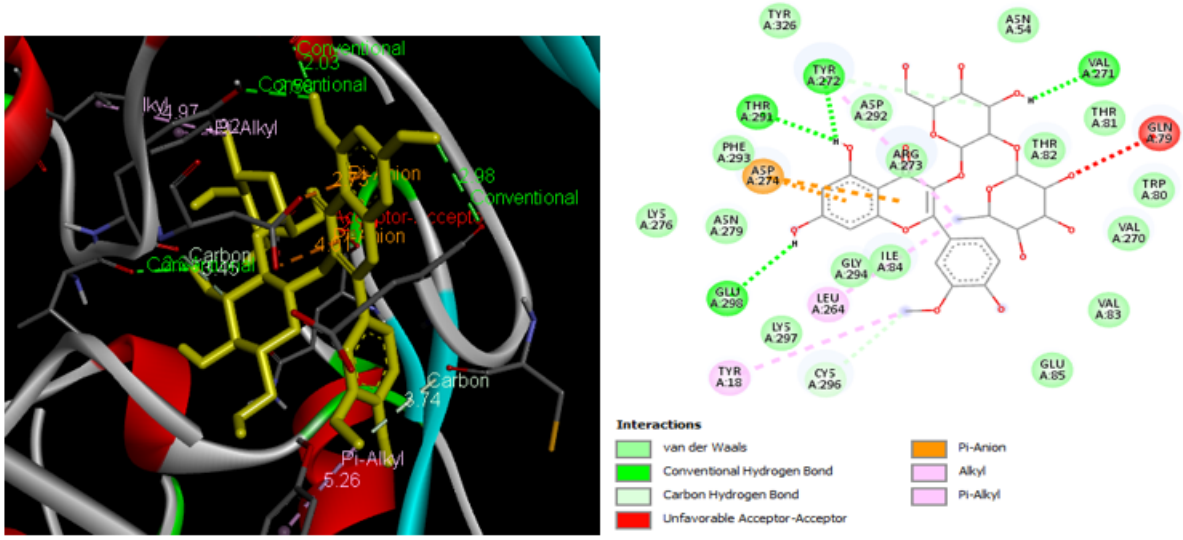

Figure 5 : Three-dimensional and two-dimensional interaction profiles of the ligand docked with the AKT protein.

4. DISCUSSION

Hepatocellular carcinoma is one of the most common and aggressive primary liver cancers and represents a major cause of cancer-related mortality worldwide. The development and progression of HCC are closely associated with dysregulation of several signaling pathways that control cell proliferation, apoptosis, and survival. Identification of key molecular targets involved in these pathways is essential for understanding disease mechanisms and for developing effective therapeutic strategies (**13**)

In the present study, protein protein interaction network analysis was performed to understand the functional association among the selected proteins. The constructed network consisted of 14 nodes, and network topology analysis identified AKT as the hub protein based on node degree and betweenness centrality. Hub proteins often play central roles in biological networks because they regulate multiple signaling pathways and interact with several other proteins. Therefore, identification of hub genes through Protein protein interaction analysis provides important insights into key regulatory molecules involved in disease progression.

Akt is a central serine/threonine kinase that plays a pivotal role in regulating cell survival, proliferation, glucose metabolism, and resistance to apoptosis. The statistically significant reduction in total Akt expression suggests that IHN not only interferes with Akt activation but also downregulates the overall abundance of this oncogenic signaling molecule. This dual inhibition further weakens the survival signaling in hepatocellular carcinoma cells and proves that IHN has a good anti - survival action.

To further evaluate the clinical significance of AKT, Kaplan–Meier survival analysis was performed. The analysis showed that patients with high AKT expression exhibited lower overall survival compared with those with low AKT expression, with a hazard ratio of 1.4 and a log-rank p value of 0.067. Previous studies have also reported that increased AKT expression or activation is associated with poor prognosis in patients with hepatocellular carcinoma, highlighting its potential role as a prognostic biomarker and therapeutic target (**14**).

Since AKT was identified as the hub protein and showed a potential association with patient survival, it was selected as the target for molecular docking analysis. The docking results revealed that the ligand forms several interactions within the active site of the AKT protein, including hydrogen bonds, van der Waals interactions, and hydrophobic interactions with key amino acid residues. These interactions indicate stable binding of the ligand within the active pocket of the protein.

Targeting the PI3K/AKT signaling pathway has been widely explored as a therapeutic approach in hepatocellular carcinoma. Inhibition of AKT signaling has been shown to suppress tumor cell proliferation and promote apoptosis in several cancer models. Therefore, compounds capable of interacting with AKT may have potential as therapeutic agents for the treatment of hepatocellular carcinoma **(15).** A study proves that

stratifin an important integral protein promotes HCC progression by activating AKT signaling through the R56 and R129 binding sites. This discovery opens new avenues for a promising therapeutic strategy for the treatment of HCC. (**16)**

Overall, the integration of PPI network analysis, survival analysis, and molecular docking highlights AKT as a key molecular target in hepatocellular carcinoma and suggests that the identified ligand may act as a potential inhibitor of AKT signaling.

5. CONCLUSION

The current study aims at network pharmacology and molecular docking approach to identify the potential therapeutic targets of hepatocellular carcinoma. and evaluate the inhibitory potential of Isorhamnetin-3-O-neohesperidoside. Protein–protein interaction network analysis revealed AKT as a key hub protein with high connectivity, indicating its central role in hepatocellular carcinoma progression. Survival analysis further supported its clinical significance and also proves that elevated AKT expression was associated with reduced overall survival in patients.

Molecular docking analysis of the protein and ligand show that that Isorhamnetin-3-O-neohesperidoside exhibits strong binding affinity toward the active site of the AKT protein, forming multiple stabilizing interactions including hydrogen bonds and hydrophobic interactions. These findings suggest that the compound has a very good potential as an AKT inhibitor.

To conclude this, the study highlights that the Isorhamnetin-3-O-neohesperidoside is a potential lead molecule for targeting AKT in hepatocellular carcinoma. But this analysis should be confirmed by experimental validation through western blotting to confirm the presence of proteins and to prove the therapeutic efficacy and mechanism of action.

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