Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Structure-based identification of liensinine as a natural allosteric SHP2 inhibitor with anti-proliferative activity in HepG2 cells

Beom Su Seo, Dong Oh Moon*

Department of Biology Education, Daegu University, 201, Daegudae-ro, Gyeongsan-si, Gyeongsangbuk-do 38453, Republic of Korea

Article Info

Article history:

Received: July 27, 2025
Accepted: November 11, 2025
Published: December 31, 2025

Use your device to scan and read the article online

Abstract

SHP2, encoded by the PTPN11 gene, is a non-receptor tyrosine phosphatase that plays a key role in oncogenic Ras/MAPK signaling. Aberrant SHP2 activity contributes to the progression of various cancers, including liver cancer. In this study, we used an AI-based virtual screening platform (HyperLab) to evaluate 127 natural compounds for SHP2 allosteric inhibition. Liensinine, a bisbenzylisoquinoline alkaloid from Nelumbo nucifera, was identified as a top candidate with strong predicted binding to the SHP2 allosteric tunnel site. This tunnel-shaped pocket is located at the interface between the N-SH2, C-SH2, and PTP domains, where allosteric inhibitors stabilize SHP2 in its closed, inactive conformation by preventing domain rearrangement. Docking analyses using HyperLab and CB-Dock2 consistently supported its interaction with key regulatory residues. Biochemical assays confirmed that Liensinine inhibits SHP2 phosphatase activity in a dose-dependent manner, with an IC_{50} of ~5.2 μM. In HepG2 cells, Liensinine reduced cell viability to approximately 70% at 20 μM and 50% at 50 μM, indicating a concentration-dependent cytotoxic effect. Additionally, RNA-seq data analysis revealed upregulated PTPN11 expression in hepatocellular carcinoma tissues compared to normal liver. These quantitative findings strengthen the experimental evidence for Liensinine's inhibitory potential. Together, these findings suggest that Liensinine may serve as a natural SHP2 allosteric inhibitor with anticancer potential.

Keywords: SHP2, PTPN11, Liensinine, hepatocellular carcinoma, allosteric inhibitor, MAPK pathway, natural product, molecular docking

1. Introduction

Since the identification of tyrosine phosphorylation as a pivotal regulator of cancer-related signaling pathways, the proteins responsible for maintaining cellular phosphotyrosine levels have emerged as key targets for therapeutic development [1]. Among these, the non-receptor protein tyrosine phosphatase encoded by the PTPN11 gene, commonly known as SHP2, plays a crucial role in mediating signals from growth factor receptors to downstream pathways, particularly the RAS/RAF/ERK cascade [2-4]. This signaling integration places SHP2 at the core of processes involved in cell growth, differentiation, and oncogenic transformation. Aberrant SHP2 activity, including its overexpression or activating mutations, has been implicated in the progression of numerous cancers. These include hematological malignancies such as leukemia, as well as solid tumors like pancreatic ductal adenocarcinoma (PDAC), gastric cancer, ovarian cancer, melanoma, hepatocellular carcinoma (HCC), non-small cell lung cancer (NSCLC), and prostate cancer [5-9].

Early attempts to develop SHP2 inhibitors primarily focused on active site inhibitors, such as ATP-competitive compounds [10]. However, these inhibitors faced significant challenges, including poor drug-like characteristics [11-13]. They exhibited off-target effects on other phosphatases and kinases, and their ionizable phosphotyrosine mimetics resulted in low membrane permeability, limiting their therapeutic potential.

The advent of allosteric inhibitors marked a breakthrough in SHP2-targeted therapy. In 2016, the first class of allosteric inhibitors targeting the tunnel site of SHP2 was introduced, with SHP099 emerging as a prominent lead compound [14]. These inhibitors work by stabilizing SHP2 in its autoinhibited conformation, effectively blocking its enzymatic and scaffolding activities [15-17]. This mechanism not only ensures high selectivity and potency against SHP2 but also minimizes off-target effects, making it a promising alternative to traditional active site inhibitors. Beyond SHP099, the development of other allosteric inhibitors has expanded, incorporating diverse structural modifications to enhance efficacy. For example, compounds like SHP389 and SHP394 introduced variations in the central pyrazine core to improve binding affinity and stability [18, 19]. These advancements have led to more effective inhibition of SHP2 activity in preclinical models. Furthermore, dual allosteric inhibition strategies targeting

* Corresponding author.
E-mail address: domoon@daegu.ac.kr (D.O. Moon).
Doi: http://dx.doi.org/10.14715/cmb/2025.71.12.5

additional druggable sites on SHP2 have been explored, offering the potential for greater therapeutic impact. Several allosteric SHP2 inhibitors, including derivatives of SHP099 such as TNO155 and RMC-4630, are currently undergoing clinical evaluation [20, 21]. These compounds have shown promise in suppressing tumor growth and improving outcomes in cancers driven by RTK/RAS/MAPK signaling. For instance, in a phase I clinical trial, RMC-4630 demonstrated a disease control rate of 71% in patients with KRASG12C non-small cell lung cancer.

In parallel with synthetic drug development, natural products have garnered increasing attention as promising scaffolds for SHP2 inhibition due to their structural diversity, inherent bioactivity, and generally favorable safety profiles. Unlike many synthetic inhibitors, natural compounds often possess complex ring systems and stereochemistry that can facilitate high-affinity binding to allosteric or cryptic sites on target proteins. Motivated by these advantages, the present study employed HyperLab (https://hyperlab.hits.ai/) [22-24], molecular docking platform, to evaluate the SHP2-binding potential of 127 natural products previously reported to exhibit anticancer activity. This virtual screening approach focused on identifying compounds with high predicted affinity for the SHP2 allosteric tunnel site. Among the screened candidates, Liensinine emerged as the top-ranking compound, exhibiting the most favorable docking score. This result highlights Liensinine as a promising lead molecule for further development as a natural product–based SHP2 inhibitor.

2. Materials and Methods

2.1. Inhibitor Binding Pocket Determination for SHP2

The binding pocket of SHP2 was identified using the Protein Data Bank (PDB) entry 5EHR. This structure represents the X-ray diffraction solution of SHP2 in complex with SHP099, the first discovered allosteric inhibitor targeting the tunnel allosteric site. This site, located at the interface between the C-SH2 and PTP domains, includes critical residues such as Glu250, Arg111, Phe113, Thr219, and Gln506 [14]. The tunnel allosteric site plays a key role in stabilizing SHP2's autoinhibited conformation, effectively blocking its enzymatic and scaffolding functions by locking the protein in a closed, inactive state and preventing substrate access to the catalytic domain. Based on previous studies and its functional importance, the tunnel allosteric site targeted by SHP099 was selected as the binding pocket for analysis in HyperLab. As shown in Figure 1, the minimum distance between the ligand and the binding pocket, determined through HyperLab, was calculated to be 2.79 Å. This distance represents the shortest physical interaction between the ligand and the binding site, serving as a critical indicator of molecular interaction strength. Such a short distance typically supports strong interactions like hydrogen bonding, ionic interactions, or van der Waals forces, all of which contribute to the high binding efficiency and selectivity of the ligand.

2.2. Structure-Based Screening of SHP2 Inhibitors

A total of 127 natural compounds with reported anticancer activity were selected based on previous studies [25, 26]. These compounds were not pre-identified as SHP2 inhibitors but were broadly chosen for their known anticancer potential to explore new SHP2-targeting candidates. The SDF files for each compound were retrieved from the PubChem database (https://pubmed.ncbi.nlm.nih.gov). The crystal structure of SHP2 in its autoinhibited conformation (PDB ID: 5EHR) was downloaded from the RCSB Protein Data Bank and prepared for docking analysis. Molecular docking was performed using HyperLab (https://hyperlab.hits.ai/), an AI-based virtual screening platform, to evaluate the binding affinity of the selected compounds to the SHP2' tunnel allosteric site. To validate the docking results, an additional structure-based screening was carried out using CB-Dock2 (http://183.56.231.194:8001/cb-dock2/php), which automatically identifies potential binding cavities and performs blind docking. The consistency of high-affinity binding predictions across both platforms supported the reliability of the selected hit compound, Liensinine. The chemical structure of Liensinine ($C_{37}H_{42}N_2O_6$) was obtained from the PubChem database (CID: 73206). For in vitro assays, Liensinine (≥98% purity, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in DMSO and diluted to the indicated concentrations with cell culture medium or assay buffer.

2.3. SHP2 Phosphatase Activity Assay

The phosphatase activity of SHP2 was evaluated using the SHP2 Full-Length Assay Kit (BPS Bioscience, Cat# 79330) following the manufacturer's instructions. Briefly, recombinant full-length human SHP2 enzyme (0.2 ng/µl) was preincubated with 0.5 µM SHP2 activating peptide and 5 mM DTT in 1× assay buffer. The reaction mixture (25 µl per well) was prepared in a 96-well black low-binding plate and incubated at room temperature for 1 hour to allow enzyme activation. Following preincubation, DiFMUP (6,8-difluoro-4-methylumbelliferyl phosphate) was added to a final concentration of 10 µM in a 25 µl substrate solution, resulting in a total reaction volume of 50 µl per well. Fluorescence intensity was measured every 18 seconds for up to 5 minutes using a Spark plate reader (Tecan) with excitation at 360 nm and emission at 460 nm. All samples were measured in triplicate, and background fluorescence (blank wells without enzyme) was subtracted from all readings. To assess inhibitor effects, the known

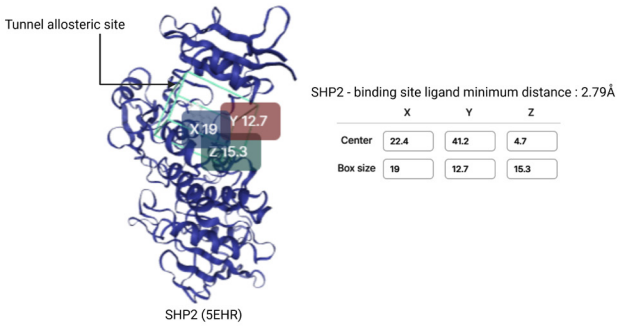

Fig. 1. Definition of SHP2 Allosteric Tunnel Site for SHP099 Binding Using HyperLab Analysis. The structure of SHP2 (PDB ID: 5EHR) was analyzed to define the binding site of inhibitors. Using HyperLab, the binding site was specified as the tunnel allosteric site, located at the interface of the C-SH2 and PTP domains. The binding site was defined with precise spatial coordinates for docking studies: center coordinates of X = 22.4, Y = 41.2, and Z = 4.7, and box dimensions of X = 19, Y = 12.7, and Z = 15.3. These parameters accurately encompass the key amino acid residues within the tunnel allosteric pocket. The minimum distance between the binding site and the ligand was calculated to be 2.79 Å, reflecting strong and specific interactions, essential for the inhibitor's high affinity and selectivity.

SHP2 allosteric inhibitor SHP099 was used as a positive control at concentrations ranging from 0 to 100 nM. The Liensinine was tested in a dose-dependent manner from 0 to 10 μM.

2.4. Cell Line Selection and Culture

Based on differential gene expression analysis using the TNMplot database (https://tnmplot.com/analysis/), hepatocellular carcinoma (HCC) was selected as the target cancer type due to its significantly elevated expression of PTPN11 (SHP2) in tumor samples compared to normal liver tissue. TNMplot integrates RNA-seq data from TCGA, GTEx, and GEO repositories to enable comparative analysis of gene expression between tumor and normal tissues. When available, TNMplot utilizes pre-defined matched tumor–normal sample pairs provided within these datasets, ensuring reliable comparison of differential gene expression. Among several liver cancer cell lines, HepG2 cells were chosen for in vitro assays owing to their well-characterized phenotype and consistent SHP2 expression. HepG2 cells were obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 2 mM L-glutamine. Cells were cultured in a humidified incubator at 37 °C with 5% CO_2, and the culture medium was replaced every 2–3 days. For all experiments, cells were used during the exponential growth phase and seeded at a density of 2×10^4 cells/mL.

2.5. Cell Viability Assay (MTT Assay)

Cell viability was assessed using the MTT assay to evaluate the cytotoxic effect of Liensinine on HepG2 cells. HepG2 cells were seeded in 96-well plates at a density of 2×10^4 cells per well in 200 μL of complete DMEM medium. Cells were treated with Liensinine at concentrations ranging from 0 to 100 μM and incubated at 37 °C with 5% CO_2 for 24 hours. Following incubation, 10 μL of MTT solution (5 mg/mL in PBS) was added to each well and the plates were further incubated for 4 hours. After incubation, 100 μL of DMSO was added to solubilize the formazan crystals formed by metabolically active cells. Absorbance was measured at 570 nm using a microplate reader (e.g., Spark, Tecan). Cell viability was calculated as a percentage relative to untreated control wells. MTT assays were performed in triplicate wells and repeated in at least three independent biological experiments to ensure reproducibility.

2.6. Statistical Analysis

All data are presented as mean ± standard deviation (SD) from at least three independent experiments. The normality of data distribution was verified before applying parametric statistics. Statistical significance between two groups was determined using an unpaired two-tailed Student's t-test. Statistical analyses were performed using GraphPad Prism, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Screening of SHP2 Inhibitors Using HyperLab

From the virtual screening of 127 anticancer natural compounds against the SHP2 allosteric tunnel site using the HyperLab docking platform, Liensinine exhibited a binding score of −6.0 kcal/mol, indicating a moderately strong predicted interaction with SHP2 (Supplementary Data 1). While this affinity was lower than that of the positive control SHP099 (−10.3 kcal/mol), Liensinine ranked among the higher-scoring natural compounds. Considering both its docking performance and favorable physicochemical properties, Liensinine was selected as a lead candidate for further biological validation.

3.2. Predicted Binding Mode of Liensinine at the SHP2 Allosteric Tunnel Site

As shown in Figure 2A, molecular docking of Liensinine with SHP2 using the HyperLab platform revealed that the compound is positioned within the well-characterized allosteric tunnel site, which includes key regulatory residues such as Glu250, Arg111, Phe113, and Thr219. The binding mode showed that Liensinine forms hydrogen bonds with His114 and Glu249, engages in π–π stacking with Phe113, and establishes electrostatic interactions with Arg111 and Glu249. These interactions anchor Liensinine within the N-SH2/PTP interface, a site critical for maintaining the autoinhibited conformation of SHP2. The overall interaction pattern closely resembles that of known

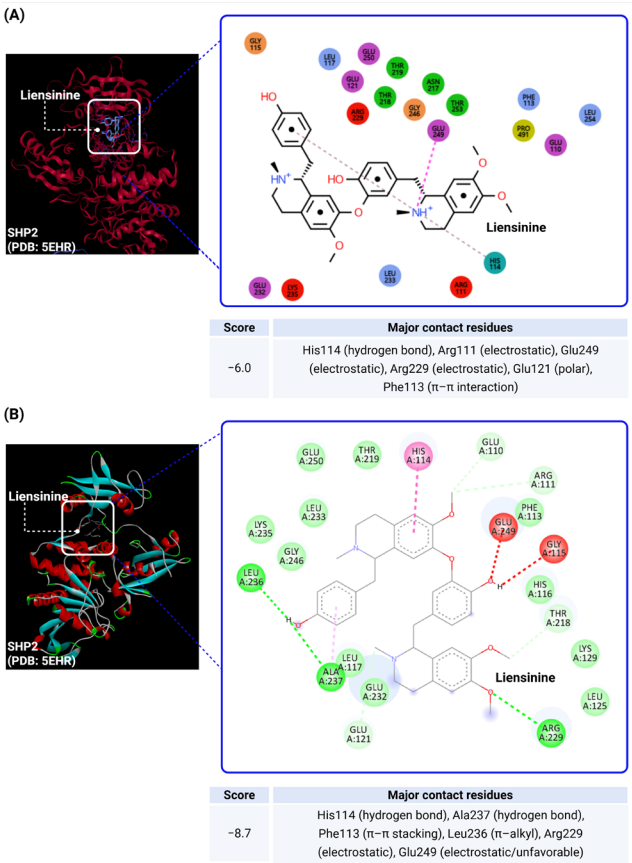

Fig. 2. Predicted binding modes of Liensinine to the SHP2 allosteric tunnel site using HyperLab and CB-Dock2. (A) Molecular docking of Liensinine with SHP2 (PDB ID: 5EHR) using the HyperLab platform predicted a binding pose within the well-characterized allosteric tunnel site, involving key residues such as His114, Arg111, Glu249, Arg229, Glu121, and Phe113. (B) Docking analysis using CB-Dock2 also positioned Liensinine within the same tunnel region, though oriented slightly toward the entrance. Major interactions included His114, Ala237, Phe113, Leu236, Arg229, and Glu249. Both models demonstrate that Liensinine engages critical regulatory residues in the SHP2 allosteric site, supporting its potential as a natural allosteric inhibitor.

SHP2 allosteric inhibitors such as SHP099.

As shown in Figure 2B, an independent docking analysis was performed using CB-Dock2 to validate this binding mode, which identified a similar binding pose of Liensinine within the same tunnel pocket. In this model, Liensinine maintained key interactions with His114 and Glu249, and additionally formed hydrophobic and electrostatic contacts with Ala237, Leu236, Arg229, and Glu232—residues located near the entrance region of the tunnel site. Although the orientation differed slightly from the HyperLab prediction, both results consistently support the conclusion that Liensinine binds within the SHP2 allosteric tunnel, stabilizing interactions with residues critical for autoinhibition.

These findings suggest that Liensinine has the potential to function as a natural SHP2 allosteric inhibitor by targeting the tunnel site that regulates SHP2 activity.

3.3. Liensinine Inhibits SHP2 Phosphatase Activity in a Dose-Dependent Manner

To evaluate the inhibitory effect of Liensinine on SHP2 activity, a fluorometric phosphatase assay was performed using increasing concentrations of Liensinine (0–10 μM). As shown in Figure 3, Liensinine suppressed SHP2 activity in a dose-dependent manner. While treatment with 2 μM did not result in a statistically significant reduction, significant inhibition was observed from 4 μM onward ($p < 0.05$). At 4, 6, 8, and 10 μM, SHP2 activity was reduced to approximately 76%, 44%, 35%, and 28%, respectively, relative to the untreated control. To estimate potency, the percent activity data were fitted to a sigmoidal dose–response curve, yielding a predicted IC_{50} value of approximately 5.2 μM. This result suggests that Liensinine can act as a moderate inhibitor of SHP2 phosphatase activity in vitro.

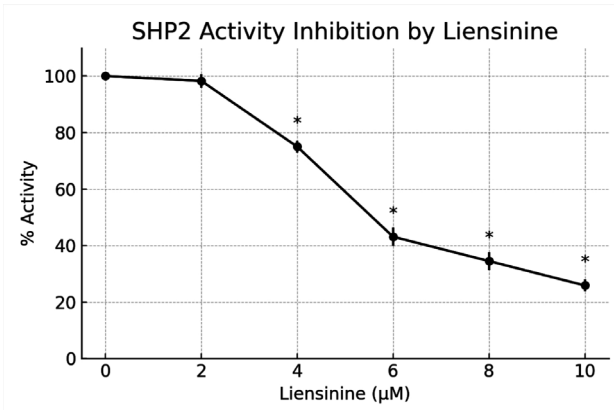

Fig. 3. Dose-dependent inhibition of SHP2 phosphatase activity by Liensinine. Recombinant full-length human SHP2 (0.2 ng/μL) was incubated with an activating peptide and treated with increasing concentrations of Liensinine (0, 2, 4, 6, 8, and 10 μM) in a 96-well black plate using the SHP2 Full-Length Assay Kit (BPS Bioscience, Cat# 79330). Enzymatic activity was measured based on fluorescence generated from DiFMUP (10 μM) dephosphorylation, with excitation at 360 nm and emission at 460 nm. All reactions were performed in triplicate. Fluorescence values were normalized to the untreated control (0 μM) and expressed as percent activity. Data are presented as mean ± SD (n = 3). Statistical significance was determined using unpaired two-tailed Student's t-tests, and $p < 0.05$ was considered significant ($p < 0.05$ vs. control).

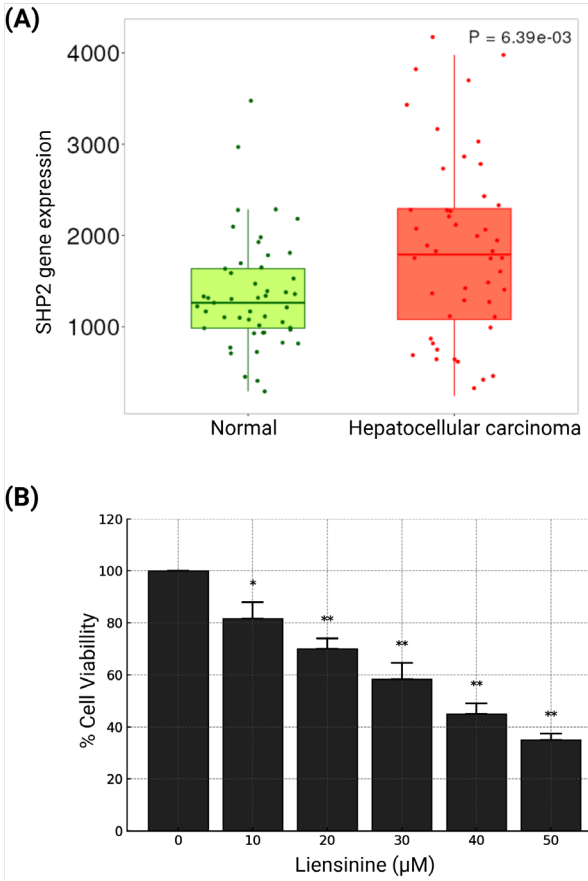

Fig. 4. SHP2 (PTPN11) is upregulated in hepatocellular carcinoma and suppressed by Liensinine treatment. (A) Differential expression analysis of PTPN11 in hepatocellular carcinoma (HCC) using RNA-seq data from the TNMplot database. Box plot compares SHP2 (PTPN11) expression between paired tumor and adjacent normal liver tissues (n = 50 each). Median expression in tumor samples was 1.42-fold higher than in normal tissues ($p = 6.39 \times 10^{-3}$; Mann–Whitney U test). (B) Effect of Liensinine on HepG2 cell viability. Cells were treated with Liensinine (0–50 μM) for 24 hours, and cell viability was measured by MTT assay. Data are presented as mean ± SD from three independent experiments. Statistical significance was determined using unpaired two-tailed Student's t-test versus control (0 μM): $p < 0.05$ (*), $p < 0.001$ (**).

3.4. SHP2 is upregulated in liver cancer and inhibited by Liensinine.

To investigate the role of SHP2 in liver cancer, SHP2 gene expression was analyzed using RNA-seq data from paired tumor and adjacent normal liver tissues via the TNMplot database. As shown in Figure 4A, PTPN11 expression was elevated in hepatocellular carcinoma tissues compared to normal liver tissues, with a 1.42-fold increase in median expression. These results suggest that SHP2 may be functionally involved in hepatocellular tumorigenesis. To assess whether Liensinine affects cell viability in liver cancer, HepG2 cells were treated with increasing concentrations of Liensinine (0–50 μM), and an MTT assay was performed. As shown in Figure 4B, Liensinine reduced cell viability in a dose-dependent manner. A modest decrease was observed at 10 μM, while concentrations of 20 μM and above resulted in a more pronounced reduction in viability. At the highest dose (50 μM), cell viability was reduced to approximately 35% of control levels. These findings indicate that Liensinine exerts a potent anti-proliferative effect on hepatocellular carcinoma cells.

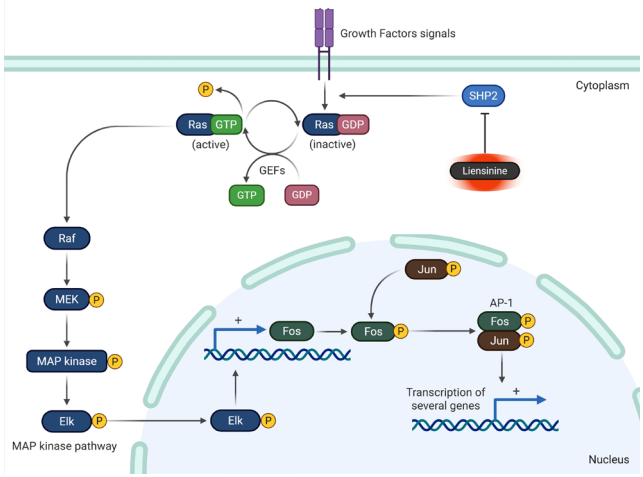

Fig. 5. Proposed mechanism of Liensinine-mediated inhibition of the SHP2–Ras/MAPK signaling pathway in hepatocellular carcinoma cells. Liensinine inhibits the oncogenic Ras/MAPK signaling cascade by targeting SHP2, a critical phosphatase that facilitates Ras activation downstream of growth factor receptors. Under normal conditions, SHP2 promotes the conversion of Ras from its inactive GDP-bound form to the active GTP-bound state via regulation of guanine nucleotide exchange factors (GEFs), such as the GAB1–GRB2–SOS1 complex. Activated Ras initiates a phosphorylation cascade through Raf, MEK, and MAP kinase, ultimately leading to the activation of nuclear transcription factors such as Elk and AP-1 (Fos/Jun complex), which regulate the transcription of proliferation-related genes. Liensinine binds to the allosteric tunnel site of SHP2, stabilizing its autoinhibited conformation and suppressing phosphatase activity. As a result, Ras activation is impaired, downstream MAPK signaling is attenuated, and nuclear gene transcription is reduced. This mechanism contributes to the anti-proliferative effect of Liensinine observed in hepatocellular carcinoma cells.

4. Discussion

Liensinine, a bioactive alkaloid derived from the green embryo of mature Nelumbonaceae seeds [27], has been traditionally recognized for its cardiovascular benefits, including anti-arrhythmic properties [28], smooth muscle relaxation, blood pressure reduction, and vasodilation [29]. More recently, this natural compound has gained attention for its emerging anti-cancer potential, with studies demonstrating its inhibitory effects across multiple cancer types, such as gastric [30], gallbladder [31], colorectal [32], and breast cancers [33]. In breast cancer models, liensinine was shown to enhance chemosensitivity via DNM1L-dependent mitochondrial fission [34]. Additionally, its role in promoting apoptosis and inducing G2/M cell cycle arrest in gallbladder cancer has been linked to modulation of the PI3K/AKT signaling pathway [31].

SHP2 is a key regulator of the Ras/MAPK signaling pathway, which is essential for controlling cell proliferation, differentiation, and survival [2, 35, 36]. Acting downstream of receptor tyrosine kinases (RTKs) such as EGFR, FGFR, and PDGFR, SHP2 facilitates the activation of Ras, a critical GTPase that transitions between its inactive GDP-bound state and active GTP-bound state [2, 36-38]. By dephosphorylating inhibitory tyrosine residues, including pY32 on Ras, SHP2 stabilizes the GTP-bound active form of Ras, thereby enhancing its activity [3, 39]. Additionally, SHP2 dephosphorylates GTPase-activating proteins (GAPs) such as RasA, reducing their ability to inactivate Ras [9]. SHP2 also interacts with the GAB1-GRB2-SOS1 complex, promoting the guanine nucleotide exchange factor (GEF) activity of SOS1, which facilitates the conversion of GDP-bound Ras to its active GTP-bound form [40]. This sustained Ras activation drives downstream signaling through the RAF/MEK/ERK phosphorylation cascade, linking extracellular growth signals to nuclear transcription factors that regulate key cellular processes such as proliferation and survival.

In this study, a structure-based virtual screening of 127 anticancer natural compounds identified Liensinine as a top-scoring molecule with strong predicted binding affinity for the SHP2 allosteric tunnel site. Molecular docking results from two independent platforms, HyperLab and CB-Dock2, consistently indicated that Liensinine binds to the autoinhibitory tunnel region of SHP2, engaging in critical interactions with residues such as Glu249, Arg111, and His114. These findings were further validated by biochemical assays, which demonstrated that Liensinine inhibits SHP2 phosphatase activity in a dose-dependent manner with a predicted IC_{50} of approximately 5.2 μM. Moreover, Liensinine significantly reduced the viability of HepG2 liver cancer cells, suggesting a functional anti-proliferative effect that may be mediated by SHP2 inhibition.

The lower docking score of Liensinine (−6.0 kcal/mol) compared with SHP099 (−10.3 kcal/mol) suggests fewer or weaker non-covalent interactions within the SHP2 allosteric tunnel. SHP099 achieves high affinity mainly through hydrophobic and hydrogen-bond contacts with Phe113, Arg111, and Glu250, whereas Liensinine's bulky bisbenzylisoquinoline scaffold may experience steric hindrance, limiting binding energy. Despite its moderate affinity, Liensinine remains a promising natural scaffold for optimization to improve SHP2-binding efficacy.

Liensinine inhibited SHP2 phosphatase activity with an IC50 of ~5.2 μM, indicating moderate potency compared with potent allosteric inhibitors such as SHP099 (0.071 μM) and RMC-4550 (1.55 nM). Although its micromolar potency is insufficient for direct clinical application, Liensinine provides a framework for developing refined derivatives targeting SHP2.

Moreover, at relatively high concentrations (20–50 μM), Liensinine reduced HepG2 cell viability, suggesting possible off-target or additional mechanisms beyond SHP2 inhibition. Further biochemical and cellular studies will be necessary to confirm the specificity and therapeutic potential of Liensinine.

Docking poses were selected based on the top-ranked binding energy and interaction consistency across CB-Dock2 and HyperLab (PIGNet2) platforms. CB-Dock2 integrates both structure-based cavity detection and template-based docking using FitDock, providing high-confidence poses with RMSD < 2.0 Å to known ligand–protein complexes [41]. In contrast, HyperLab employs a physics-informed graph neural network (PIGNet2) that evaluates intermolecular interactions by summing van der Waals, hydrogen bond, hydrophobic, and metal terms rather than using empirical scoring functions [42]. Slight differences in ligand orientation between the two platforms were observed, particularly around the SHP2 allosteric tunnel entrance. These discrepancies are attributed to distinct energy landscapes: CB-Dock2 optimizes poses based on spatial cavity fit, whereas HyperLab prioritizes energetically favorable contact patterns derived from learned inte-

raction potentials. Despite these variations, both methods consistently predicted Liensinine within the allosteric tunnel region (involving residues Phe113, Arg111, and Glu250), supporting the reliability of the predicted binding mode. Such cross-validation enhances confidence in the docking interpretation by combining empirical and AI-driven scoring principles.

Although Liensinine exhibited moderate SHP2 inhibitory activity in vitro, its metabolic stability and potential biotransformation in cellular environments were not evaluated in this study. Previous reports indicate that bisbenzylisoquinoline alkaloids undergo hepatic metabolism, which may influence intracellular concentration and efficacy. Therefore, further studies assessing Liensinine's metabolic stability, cellular uptake, and pharmacokinetic properties will be necessary to fully elucidate its therapeutic potential. In addition, systematic evaluation of its toxicity and safety profile will be required to assess its suitability for clinical translation.

Collectively, these results position Liensinine as a promising lead compound for the development of natural product–based SHP2 inhibitors targeting allosteric regulatory mechanisms. By stabilizing SHP2 in its inactive conformation, Liensinine may effectively suppress oncogenic Ras/MAPK signaling, offering therapeutic potential against SHP2-driven malignancies such as hepatocellular carcinoma. A schematic summary of the proposed mechanism is illustrated in Figure 5, where Liensinine inhibits SHP2, thereby blocking Ras activation and downstream phosphorylation events in the MAPK cascade. This ultimately leads to reduced nuclear transcriptional activity mediated by AP-1 and other transcription factors, contributing to the observed anti-proliferative effects. Future studies will focus on in vivo efficacy, selectivity profiling, and structural optimization of Liensinine derivatives to further enhance SHP2 inhibitory potency and pharmacokinetic properties.

5. Conclusion

This study identified Liensinine as a natural allosteric inhibitor of SHP2 through structure-based virtual screening. Docking analyses revealed that Liensinine binds to the SHP2 allosteric tunnel site, forming stable interactions with key regulatory residues. Biochemical assays confirmed that Liensinine inhibits SHP2 phosphatase activity in a dose-dependent manner with an IC_{50} of approximately 5.2 μM. Moreover, Liensinine significantly reduced the viability of HepG2 liver cancer cells, suggesting that its anti-proliferative effect is associated with SHP2 inhibition. These findings indicate that Liensinine may serve as a promising natural compound for developing SHP2-targeted therapeutics against hepatocellular carcinoma.

Funding

This research was supported by the Daegu University Future Scholars Research Grant, 2025.

Conflicts of Interest

The author declares no conflicts of interest.

References

1. Agazie YM, Hayman MJ (2003) Molecular mechanism for a role of SHP2 in epidermal growth factor receptor signaling. Mol Cell Biol 23: 7875–7886 doi: 10.1128/MCB.23.21.7875-7886.2003
2. Araki T, Nawa H, Neel BG (2003) Tyrosyl phosphorylation of Shp2 is required for normal ERK activation in response to some but not all growth factors. J Biol Chem 278: 41677–41684 doi: 10.1074/jbc.M306461200
3. Bagdanoff JT, Chen Z, Acker M, Chen YN, Chan H, Dore M, Firestone B, Fodor M, Fortanet J, Hentemann M, Kato M, Koenig R, LaBonte LR, Liu S, Mohseni M, Ntaganda R, Sarver P, Smith T, Sendzik M, Stams T, Spence S, Towler C, Wang H, Wang P, Williams SL, LaMarche MJ (2019) Optimization of fused bicyclic allosteric SHP2 inhibitors. J Med Chem 62: 1781–1792 doi: 10.1021/acs.jmedchem.8b01725
4. Bunda S, Burrell K, Heir P, Zeng L, Alamsahebpour A, Kano Y, Raught B, Zhang ZY, Zadeh G, Ohh M (2015) Inhibition of SHP2 mediated dephosphorylation of Ras suppresses oncogenesis. Nat Commun 6: 8859 doi: 10.1038/ncomms9859
5. Chen S, Li X, Wu J, Li J, Xiao M, Yang Y, Liu Z, Cheng Y (2021) Plumula Nelumbinis: A review of traditional uses, phytochemistry, pharmacology, pharmacokinetics and safety. J Ethnopharmacol 266: 113429 doi: 10.1016/j.jep.2020.113429
6. Chen YN, LaMarche MJ, Chan HM, Fekkes P, Garcia Fortanet J, Acker MG, Antonakos B, Chen CH, Chen Z, Cooke VG, Dobson JR, Deng Z, Fei F, Firestone B, Fodor M, Fridrich C, Gao H, Grunenfelder D, Hao HX, Jacob J, Ho S, Hsiao K, Kang ZB, Karki R, Kato M, Larrow J, La Bonte LR, Lenoir F, Liu G, Liu S, Majumdar D, Meyer MJ, Palermo M, Perez L, Pu M, Price E, Quinn C, Shakya S, Shultz MD, Slisz J, Venkatesan K, Wang P, Warmuth M, Williams S, Yang G, Yuan J, Zhang JH, Zhu P, Ramsey T, Keen NJ, Sellers WR, Stams T, Fortin PD (2016) Allosteric inhibition of SHP2 phosphatase inhibits cancers driven by receptor tyrosine kinases. Nature 535: 148–152 doi: 10.1038/nature18621
7. Dance M, Montagner A, Salles JP, Yart A, Raynal P (2008) The molecular functions of Shp2 in the Ras mitogen activated protein kinase ERK1 2 pathway. Cell Signal 20: 453–459 doi: 10.1016/j.cellsig.2007.10.002
8. Dong S, Li FQ, Zhang Q, Lv KZ, Yang HL, Gao Y, Yu JR (2012) Expression and clinical significance of SHP2 in gastric cancer. J Int Med Res 40: 2083–2089 doi: 10.1177/030006051204000605
9. Elimam H, Alhamshry NAA, Hatawsh A, Elfar N, Moussa R, Radwan AF, Abd Elmawla MA, Elkashlan AM, Zaki MB, Abdel Reheim MA, Mohammed OA, Doghish AS (2024) Natural products and long noncoding RNA signatures in gallbladder cancer: a review focuses on pathogenesis, diagnosis, and drug resistance. Naunyn Schmiedebergs Arch Pharmacol 397: 9549–9571 doi: 10.1007/s00210-024-03279-1
10. Grosskopf S, Eckert C, Arkona C, Radetzki S, Bohm K, Heinemann U, Wolber G, von Kries JP, Birchmeier W, Rademann J (2015) Selective inhibitors of the protein tyrosine phosphatase SHP2 block cellular motility and growth of cancer cells in vitro and in vivo. ChemMedChem 10: 815–826 doi: 10.1002/cmdc.201500015
11. Guo Z, Duan Y, Sun K, Zheng T, Liu J, Xu S, Xu J (2024) Advances in SHP2 tunnel allosteric inhibitors and bifunctional molecules. Eur J Med Chem 275: 116579 doi: 10.1016/j.ejmech.2024.116579
12. Han T, Xiang DM, Sun W, Liu N, Sun HL, Wen W, Shen WF, Wang RY, Chen C, Wang X, Cheng Z, Li HY, Wu MC, Cong WM, Feng GS, Ding J, Wang HY (2015) PTPN11 Shp2 overexpression enhances liver cancer progression and predicts poor prognosis of patients. J Hepatol 63: 651–660 doi: 10.1016/j.jhep.2015.03.036
13. Hill KS, Roberts ER, Wang X, Marin E, Park TD, Son S, Ren Y, Fang B, Yoder S, Kim S, Wan L, Sarnaik AA, Koomen JM, Messina JL, Teer JK, Kim Y, Wu J, Chalfant CE, Kim M (2019) PTPN11 plays oncogenic roles and is a therapeutic target for BRAF wild type melanomas. Mol Cancer Res 17: 583–593 doi:

10.1158/1541-7786.MCR-18-0777
14. Hu Z, Li J, Gao Q, Wei S, Yang B (2017) SHP2 overexpression enhances the invasion and metastasis of ovarian cancer in vitro and in vivo. Onco Targets Ther 10: 3881–3891 doi: 10.2147/OTT.S138833
15. Kang EJ, Lee SK, Park KK, Son SH, Kim KR, Chung WY (2017) Liensinine and nuciferine bioactive components of Nelumbo nucifera inhibit the growth of breast cancer cells and breast cancer associated bone loss. Evid Based Complement Alternat Med 2017: 1583185 doi: 10.1155/2017/1583185
16. Kerr DL, Haderk F, Bivona TG (2021) Allosteric SHP2 inhibitors in cancer: targeting the intersection of RAS resistance and the immune microenvironment. Curr Opin Chem Biol 62: 1–12 doi: 10.1016/j.cbpa.2020.11.007
17. LaMarche MJ, Acker M, Argintaru A, Bauer D, Boisclair J, Chan H, Chen CH, Chen YN, Chen Z, Deng Z, Dore M, Dunstan D, Fan J, Fekkes P, Firestone B, Fodor M, Garcia Fortanet J, Fortin PD, Fridrich C, Giraldes J, Glick M, Grunenfelder D, Hao HX, Hentemann M, Ho S, Jouk A, Kang ZB, Karki R, Kato M, Keen N, Koenig R, LaBonte LR, Larrow J, Liu G, Liu S, Majumdar D, Mathieu S, Meyer MJ, Mohseni M, Ntaganda R, Palermo M, Perez L, Pu M, Ramsey T, Reilly J, Sarver P, Sellers WR, Sendzik M, Shultz MD, Slisz J, Slocum K, Smith T, Spence S, Stams T, Straub C, Tamez V Jr, Toure BB, Towler C, Wang P, Wang H, Williams SL, Yang F, Yu B, Zhang JH, Zhu S (2020) Identification of TNO155 an allosteric SHP2 inhibitor for the treatment of cancer. J Med Chem 63: 13578–13594 doi: 10.1021/acs.jmedchem.0c01170
18. Li J, Reed SA, Johnson SE (2009) Hepatocyte growth factor signals through SHP2 to regulate primary mouse myoblast proliferation. Exp Cell Res 315: 2284–2292 doi: 10.1016/j.yexcr.2009.04.011
19. Lim J, Hwang SY, Moon S, Kim S, Kim WY (2019) Scaffold based molecular design with a graph generative model. Chem Sci 11: 1153–1164 doi: 10.1039/C9SC04503A
20. Liotti F, Kumar N, Prevete N, Marotta M, Sorriento D, Ierano C, Ronchi A, Marino FZ, Moretti S, Colella R, Puxeddu E, Paladino S, Kano Y, Ohh M, Scala S, Melillo RM (2021) PD 1 blockade delays tumor growth by inhibiting an intrinsic SHP2 Ras MAPK signalling in thyroid cancer cells. J Exp Clin Cancer Res 40: 22 doi: 10.1186/s13046-020-01818-1
21. Liu Y, Yang X, Gan J, Chen S, Xiao ZX, Cao Y (2022) CB Dock2: improved protein ligand blind docking by integrating cavity detection docking and homologous template fitting. Nucleic Acids Res 50: W159–W164 doi: 10.1093/nar/gkac394
22. Matozaki T, Murata Y, Saito Y, Okazawa H, Ohnishi H (2009) Protein tyrosine phosphatase SHP 2: a proto oncogene product that promotes Ras activation. Cancer Sci 100: 1786–1793 doi: 10.1111/j.1349-7006.2009.01257.x
23. Moon S, Zhung W, Yang S, Lim J, Kim WY (2022) PIGNet: a physics informed deep learning model toward generalized drug target interaction predictions. Chem Sci 13: 3661–3673 doi: 10.1039/D1SC06946B
24. Mullard A (2018) Phosphatases start shedding their stigma of undruggability. Nat Rev Drug Discov 17: 847–849 doi: 10.1038/nrd.2018.201
25. Naeem A, Hu P, Yang M, Zhang J, Liu Y, Zhu W, Zheng Q (2022) Natural products as anticancer agents: current status and future perspectives. Molecules 27: 8367 doi: 10.3390/molecules27238367
26. Neel BG, Gu H, Pao L (2003) The Shp ing news: SH2 domain containing tyrosine phosphatases in cell signaling. Trends Biochem Sci 28: 284–293 doi: 10.1016/S0968-0004(03)00091-4
27. Nichols RJ, Haderk F, Stahlhut C, Schulze CJ, Hemmati G, Wildes D, Tzitzilonis C, Mordec K, Marquez A, Romero J, Hsieh T, Zaman A, Olivas V, McCoach C, Blakely CM, Wang Z, Kiss G, Koltun ES, Gill AL, Singh M, Goldsmith MA, Smith JAM, Bivona TG (2018) RAS nucleotide cycling underlies the SHP2 phosphatase dependence of mutant BRAF NF1 and RAS driven cancers. Nat Cell Biol 20: 1064–1073 doi: 10.1038/s41556-018-0169-1
28. Petrocchi A, Ciammaichella A (2024) A patent review of SHP2 allosteric inhibitors 2018 to present. Expert Opin Ther Pat 34: 383–396 doi: 10.1080/13543776.2024.2365410
29. Sarver P, Acker M, Bagdanoff JT, Chen Z, Chen YN, Chan H, Firestone B, Fodor M, Fortanet J, Hao H, Hentemann M, Kato M, Koenig R, LaBonte LR, Liu G, Liu S, Liu C, McNeill E, Mohseni M, Sendzik M, Stams T, Spence S, Tamez V, Tichkule R, Towler C, Wang H, Wang P, Williams SL, Yu B, LaMarche MJ (2019) 6 Amino 3 methylpyrimidinones as potent selective and orally efficacious SHP2 inhibitors. J Med Chem 62: 1793–1802 doi: 10.1021/acs.jmedchem.8b01726
30. Schmidt MF, Groves MR, Rademann J (2011) Dynamic substrate enhancement for the identification of specific second site binding fragments targeting a set of protein tyrosine phosphatases. Chembiochem 12: 2640–2646 doi: 10.1002/cbic.201100414
31. Seo S, Lim J, Kim WY (2023) Molecular generative model via retrosynthetically prepared chemical building block assembly. Adv Sci 10: e2206674 doi: 10.1002/advs.202206674
32. Shen Y, Bian R, Li Y, Gao Y, Liu Y, Xu Y, Song X, Zhang Y (2019) Liensinine induces gallbladder cancer apoptosis and G2 M arrest by inhibiting ZFX induced PI3K AKT pathway. Acta Biochim Biophys Sin Shanghai 51: 607–614 doi: 10.1093/abbs/gmz041
33. Tsutsumi R, Ran H, Neel BG (2018) Off target inhibition by active site targeting SHP2 inhibitors. FEBS Open Bio 8: 1405–1411 doi: 10.1002/2211-5463.12493
34. Wang JL, Nong Y, Jing MX (1992) Effects of liensinine on haemodynamics in rats and the physiologic properties of isolated rabbit atria. Yao Xue Xue Bao 27: 881–885
35. Wang Y, Li YJ, Huang XH, Zheng CC, Yin XF, Li B, He QY (2018) Liensinine perchlorate inhibits colorectal cancer tumorigenesis by inducing mitochondrial dysfunction and apoptosis. Food Funct 9: 5536–5546 doi: 10.1039/C8FO01137K
36. Welsh CL, Allen S, Madan LK (2023) Setting sail: maneuvering SHP2 activity and its effects in cancer. Adv Cancer Res 160: 17–60 doi: 10.1016/bs.acr.2023.03.003
37. Wu J, Zhang H, Zhao G, Wang R (2021) Allosteric inhibitors of SHP2: an updated patent review 2015 to 2020. Curr Med Chem 28: 3825–3842 doi: 10.2174/1568011817666200928114851
38. Yang JH, Yu K, Si XK, Li S, Cao YJ, Li W, Zhang JX (2019) Liensinine inhibited gastric cancer cell growth through ROS generation and the PI3K AKT pathway. J Cancer 10: 6431–6438 doi: 10.7150/jca.32691
39. Zhang H, Wang X, Guo Y, Liu X, Zhao X, Teka T, Lv C, Han L, Huang Y, Pan G (2021) Thirteen bisbenzylisoquinoline alkaloids in five Chinese medicinal plants: botany traditional uses phytochemistry pharmacokinetic and toxicity studies. J Ethnopharmacol 268: 113566 doi: 10.1016/j.jep.2020.113566
40. Zhang K, Zhao H, Ji Z, Zhang C, Zhou P, Wang L, Chen Q, Wang J, Zhang P, Chen Z, Zhu HH, Gao WQ (2016) Shp2 promotes metastasis of prostate cancer by attenuating the PAR3 PAR6 aPKC polarity protein complex and enhancing epithelial to mesenchymal transition. Oncogene 35: 1271–1282 doi: 10.1038/onc.2015.184
41. Zhou J, Li G, Zheng Y, Shen HM, Hu X, Ming QL, Huang C, Li P, Gao N (2015) A novel autophagy mitophagy inhibitor liensinine sensitizes breast cancer cells to chemotherapy through DNM1L mediated mitochondrial fission. Autophagy 11: 1259–1279 doi:

10.1080/15548627.2015.1056970
42. Seo S, Lim J, Kim WY (2023) Molecular generative model via retrosynthetically prepared chemical building block assembly. Adv Sci 10: 2206674 doi: 10.1002/advs.202206674