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In silico study of active anticancer peptides from soybean (*Glycine max* (L.) Merr.) as therapeutic agents in hepatocellular carcinoma

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ABSTRACT

Introduction: Soybean-derived peptides have emerged as promising therapeutic agents in oncology due to their bioactivity, low toxicity, and biocompatibility. This study aimed to conduct a comparative analysis to assess whether soybean-derived anticancer peptides could serve as therapeutic agents in hepatocellular carcinoma (HCC).
Methods: The peptide structures were predicted using UCSF ChimeraX, while the preparation of target proteins and peptides was performed using BIOVIA Discovery Studio Visualizer. The interactions between the peptides and the SALL4-NuRD, VEGF, and GPC3 proteins were analyzed through molecular docking studies.
Results: Docking results revealed that the peptide WMLPSYSPY exhibited superior binding affinity (-237.813) compared to other peptides. Alanine scanning assays demonstrated that residues Tyr6 and Tyr9 played crucial roles in peptide interactions with SALL4-NuRD and VEGF, while Trp1 and Tyr6 were crucial for peptide interaction with GPC3.
Conclusion: Predictive characteristics of the WMLPSYSPY peptide suggest its potential as a therapeutic agent for HCC, albeit with low stability and uptake. Further in vitro and in vivo studies are warranted, alongside structural modifications to enhance its pharmacological properties.

Implication for health policy/practice/research/medical education:
Soybean-derived anticancer peptides show potential as alternative therapeutic agents for hepatocellular carcinoma (HCC) due to their lower toxicity and strong interactions with target proteins. This finding may contribute to the development of new food-derived therapeutic options for safer and more effective cancer treatments.
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Introduction

Hepatocellular carcinoma (HCC) stands among the deadliest cancers globally. In 2020, liver cancer claimed the second-highest number of lives worldwide, with 830,000 fatalities, trailing only lung cancer, which recorded 1.8 million deaths (1). HCC is the predominant form of liver cancer, accounting for 80%-90% of cases worldwide (2,3). The etiology of HCC includes liver pathologies such as cirrhosis and hepatitis C/B infections, lifestyle factors like obesity, diabetes, and excessive alcohol consumption (3).

HCC progression is influenced by various proteins. The SALL4 protein, known for its role in maintaining cell pluripotency during the embryonic phase, is frequently detected in HCC cells, despite becoming inactive as cells mature (4). Studies by Yin et al indicate that HCC patients with heightened SALL4 expression have poorer prognoses than those without SALL4 expression. SALL4 forms complexes with nucleosome remodeling and deacetylase (NuRD), inhibiting the activation of the PTEN gene, which is crucial for tumor suppression (5). The VEGF

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protein is also abundant in HCC cells, and its expression levels influence tumor size. VEGF plays a pivotal role in angiogenesis when its ligands (VEGF-A, VEGF-C, or VEGF-D) bind to VEGF receptors (6). Additionally, GPC3 is highly expressed in HCC cells, promoting HCC proliferation by forming complexes with Wnt (7). These proteins play significant roles in HCC development and are potential therapeutic targets.

According to the Barcelona Clinic Liver Cancer (BCLC) guidelines for HCC treatment, therapeutic drugs are only administered in intermediate and advanced stages (2). Some drugs used include atezolizumab + bevacizumab, sorafenib, lenvatinib, and regorafenib. However, these drugs may cause side effects such as fatigue, diarrhea, hypertension, elevated aspartate transaminase levels, and hand-foot skin reactions (8).

Given the numerous side effects of HCC therapeutic drugs, further research is needed to identify new agents with fewer adverse effects in patients. One class of substances that has garnered significant research attention is active peptides. Active peptides are amino acid chains with lengths ranging from 2 to 20 residues, whose biological activities are determined by the arrangement of their amino acid residues, such as anti-inflammatory, antihypertensive, and antimicrobial properties (9). Several studies have been conducted to investigate the potential of active peptides as the basis for anticancer drugs (10,11). One reason why active peptides are extensively studied is their superior sensitivity compared to small-molecule-based drugs (12). This heightened sensitivity ensures that peptides do not bind to unintended targets, resulting in low toxicity and reduced side effects for users (13).

A frequently studied plant-based source of active peptides is soybean. Soybeans [*Glycine max* (L.) Merr.] contain approximately 40% protein content (14). The high protein content in soybeans provides a plentiful supply of active peptides, as these peptides are derived from protein chains that undergo enzymatic or fermentation hydrolysis to become active and potential for anti-HCC (15). Although some soybean-derived peptides have been studied for general anticancer activity, their specific application against HCC is still limited. To our knowledge, this is the first study to perform a comparative in silico analysis of multiple soybean peptides targeting HCC-related proteins, highlighting its novelty in both scope and approach. In this study, a comparative analysis was conducted among six active anticancer peptides from soybean plants that have been studied in vitro (16-18) against active peptides previously tested for their ability to inhibit HCC development through their interactions with specific HCC target proteins, including SALL4-NuRD (19), VEGF (20), and GPC3 (21). These three active peptides were used as controls in the experiment. This study used a multi-target approach, in which peptides capable of binding two or more target proteins are expected to be more effective in treating diseases (22).

Materials and Methods

Peptide selection and target protein

A set of soybean-derived peptides with reported anticancer activity was selected from the Database of Food-derived Bioactive Peptides (DFBP) (http://www.cqudfbp.net/) (23). Only peptides that had previously been validated through in vitro or in vivo studies were included to ensure biological relevance and to minimize the selection of peptides with unknown anticancer potential. Control peptides with known anticancer properties were included as references to benchmark docking performance. The peptide sequences used in this study are listed in Table 1, along with the corresponding references. The 3D crystal structures of three target proteins relevant to HCC—SALL4-NuRD complex (PDB ID: 5xwr), VEGF (PDB ID: 1vpf), and GPC3 (PDB ID: 7zaw)—were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/) (24) (Table 1).

Peptide structure prediction and preparation

The 3D structures of the peptides were predicted using AlphaFold, which was integrated in UCSF ChimeraX (27). AlphaFold employs deep learning algorithms trained on structural data from the PDB to predict accurate peptide conformations (28). The predicted peptide structures were saved in .pdb format. Both peptides and protein targets were prepared using BIOVIA Discovery Studio Visualizer (v21.1.0.20298). This preparation included removal of water molecules, addition of hydrogen atoms, and optimization of geometry to ensure compatibility with the docking platform.

Molecular docking

Molecular docking simulations were performed using the HPEPDOCK web server (29), which was optimized for peptide–protein docking. Active sites of the target proteins were first predicted using PrankWeb (30). The

Table 1. The active peptide sequences and control used in this study

Test peptide from soybean	
Peptide	**Reference**
IVPK	(16)
LVPK	(16)
WMLPSYSPY	(17)
FEITPEKNPQ	(18)
IETWNPNNKP	(18)
VFDGEL	(18)
Control peptide	
Peptide	**Reference**
RRKFAKFQWI	(19)
QKRKRKKSRYKS	(25)
VAQQAANVAATLK	(26)

residues entering the active site of each target protein can be seen in Table S1 (Supplementary file 1). Subsequently, the residues obtained from PrankWeb are entered into the HPEPDOCK web tool (29) to initiate the docking process. All structures were uploaded in .pdb format, and default parameters were used unless otherwise specified. To compare performance, control peptides known to inhibit each protein target were docked alongside the test peptides. Docking results were evaluated based on binding affinity scores, structural alignment, and interaction profiles. Peptides showing superior or comparable binding affinity to the control peptides were selected for further analysis.

Visualization and interaction analysis

Docked complexes were visualized in 3D using PyMOL (v2.5.4, Schrödinger, LLC) and in 2D using LigPlot+ v2.2.8 (31). Interaction sites, hydrogen bonding, and hydrophobic interactions between peptides and target proteins were analyzed and compared to those of the control peptides.

Root mean square deviation (RMSD) analysis

Structural similarity between test peptides and control peptides in the context of their binding sites was evaluated using RMSD values, calculated via PyMOL. Lower RMSD values indicate a similar mode of binding, suggesting a comparable functional effect.

Computational alanine scanning

To identify key residues involved in the peptide–protein interactions, computational alanine scanning was performed using the BUDE Alanine Scan server (32). Docked complexes were submitted in .pdb format. Residues with the highest positive ΔΔG values were considered most critical for interaction stability and specificity.

Peptide property characterization

Peptide characterization testing included molecular weight, isoelectric point, extinction coefficient, estimated half-life, instability index, and GRAVY. These parameters were analyzed by uploading the peptide sequence to the Expasy ProtParam web tool (https://web.expasy.org/protparam/) (33,34). Bioactivity testing was performed by uploading the peptide sequence to the PeptideRanker web tool (http://distilldeep.ucd.ie/PeptideRanker/) (35). Peptide toxicity testing was conducted by uploading the peptide sequence to the ToxinPred web tool (https://webs.iiitd.edu.in/raghava/toxinpred/) (36). Peptide allergenicity testing was performed by uploading the peptide sequence to the AllerTop v.2.0 web tool (https://www.ddg-pharmfac.net/AllerTOP/) (37). The peptide sequence was uploaded to the MLCPP 2.0 web tool (https://balalab-skku.org/mlcpp2/) (38) to assess its cell penetration ability and uptake efficiency.

Results

Docking results, RMSD, and visualization

The docking results with HPEPDOCK indicate that the peptide WMLPSYPSY exhibits a superior binding affinity compared to the control peptides for each target protein (Table 2). Peptide WMLPSYSPY exhibits stronger binding affinities (-237.813, -216.15, and -190.267) for SALL4-NuRD, VEGF, and GPC3, respectively. These values are superior to those of the respective control peptides for each target protein, namely RRKFAKFQWI for SALL4-NuRD (-200.026), QKRKRKKSRYKS for VEGF (-174.220), and VAQQAANVAATLK for GPC3 (-139.155). Apart from WMLPSYSPY, peptides FEITPEKNPQ (-157.180) and IETWNPNNKP (-174.887) also exhibit lower (more negative) binding affinity values than the GPC3 control, indicating stronger interactions. However, the approach in this study is multi-target, and thus the peptide with the most potential for HCC therapy based on docking results is WMLPSYSPY, as it can bind effectively to all three target proteins.

Table 2. Docking results between the test peptides and target proteins

Peptide	Docking score (kcal/mol)		
	SALL4-NuRD	VEGF	GPC3
IVPK	-123.186	-92.734	-101.153
LVPK	-120.776	-95.992	-102.794
WMLPSYSPY	-237.813	-216.150	-190.267
FEITPEKNPQ	-158.124	-136.080	-157.180
IETWNPNNKP	-185.040	-146.668	-174.887
VFDGEL	-139.123	-113.698	-131.546
RRKFAKFQWI (SALL4-NuRD)	-200.026	-	-
QKRKRKKSRYKS (VEGF)	-	-174.220	-
VAQQAANVAATLK (GPC3)	-	-	-139.155

RMSD measurement results between the peptide WMLPSYSPY and the control peptides for each target protein showed values of 0.856 Å, 2.886 Å, and 0.250 Å for the SALL4-NuRD, VEGF, and GPC3 control peptides, respectively (Table 3). These results indicate that the structure of the WMLPSYSPY peptide exhibits conformational similarity to each control peptide, as it falls above the threshold < 2.0 Å or between 2.0 and 3.0 Å.

The 2D visualization results depicted interactions between the WMLPSYSPY peptide and SALL4-NuRD, VEGF, and GPC3 target proteins through hydrogen and hydrophobic bonds (Figures 1a-3b; Supplementary file 1, Table S2). Residues Trp1, Tyr6, Pro8, and Tyr9 could form hydrogen bonds with residues from SALL4-NuRD. Residues Ser5, Tyr6, and Tyr9 could form hydrogen bonds with VEGF. Only residue Ser5 could form a hydrogen bond with GPC3.

Computational alanine scanning results

The results of computational alanine scanning indicated that residues Trp6 and Tyr9 had the highest ΔΔG values compared to other residues when interacting with the SALL4-NuRD and VEGF proteins, suggesting that these residues might play a crucial role in peptide-protein interactions. In the interaction between the WMLPSYSPY residues and GPC3, the residues Trp1 and Tyr6 exhibited the highest ΔΔG values, indicating their significant role in maintaining the stability of the peptide-protein interaction (Table 4).

Peptide characterization results

The peptide characterization results indicated that the WMLPSYSPY peptide was acidic. The extinction coefficient of the peptide was 8480 M-1 cm-1. The WMLPSYSPY peptide was prone to instability within cells and under certain temperature conditions, and it exhibited hydrophilic properties. Bioactivity testing suggested that the peptide could exert effects on cells, tissues, and organisms. Toxicity and allergenicity testing indicated that the WMLPSYSPY peptide was non-toxic and non-allergenic. Results from MLCPP 2.0 testing indicated that the WMLPSYSPY peptide fell into the class of peptides capable of penetrating cells, although with low uptake efficiency (Table 5).

Discussion

This study was conducted to compare the potential of soybean-derived anticancer peptides previously investigated in vitro by other studies for their ability to inhibit the progression of HCC through molecular interactions with key HCC-associated proteins, namely SALL4-NuRD, VEGF, and GPC3 (16-19). The three control peptides were selected based on the published experimental evidence demonstrating their strong binding affinity to SALL4, VEGF, and GPC3, respectively. Their selection was informed by reported IC_{50} values and prior application as reference inhibitors in related therapeutic studies (16-19). Using molecular docking simulations, we evaluated the binding affinity and interaction profiles of these peptides with the target proteins. The aim of this study was to provide insights into the therapeutic potential

Table 3. RMSD measurement between the WMLPSYSPY peptide and the control peptides

Control peptide	RMSD (Å)
RRKFAKFQWI (SALL4-NuRD)	0.856
QKRKRKKSRYKS (VEGF)	2.886
VAQQAANVAATLK (GPC3)	0.250

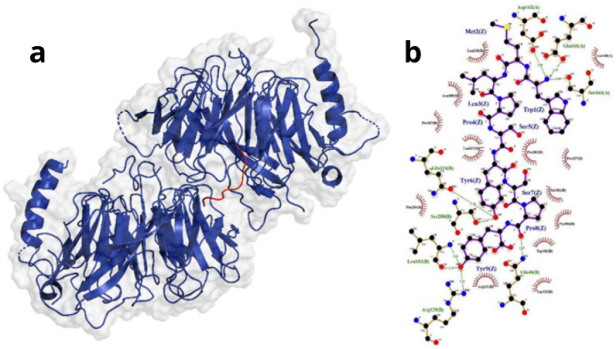

Figure 1. 3D (a) and 2D (b) visualization of the docking results of the WMLPSYSPY peptide with SALL4-NuRD.

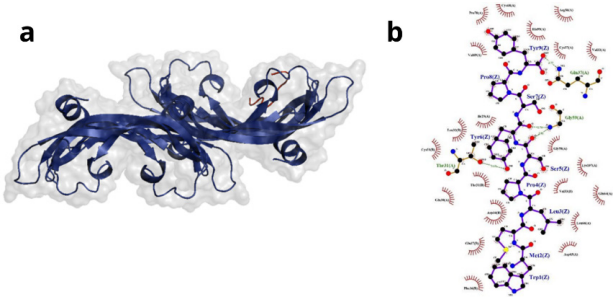

Figure 2. 3D visualization (a) and 2D (b) visualization of the docking results of the WMLPSYSPY peptide with VEGF.

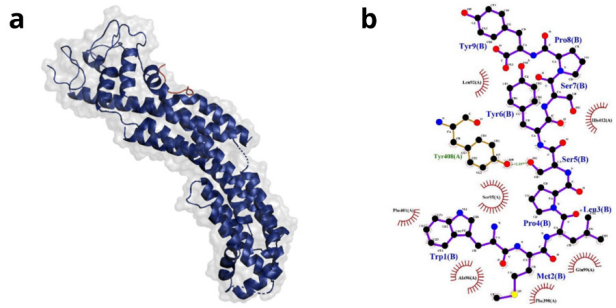

Figure 3. 3D visualization (a) and 2D (b) visualization of the docking results of the WMLPSYSPY peptide with GPC3.

Table 4. Computational alanine scanning results between the WMLPSYSPY peptide and the SALL4-NuRD, VEGF, and GPC3 target proteins

Residues	Amino acid	ΔΔG (kJ/mol)		
		SALL4-NuRD	VEGF	GPC3
1	Trp	3.6	9.2	19.2
2	Met	4.2	-15.7	2.8
3	Leu	1.7	4.8	3.1
4	Pro	4.7	1.7	0
5	Ser	1.2	0.6	-4.3
6	Tyr	12.2	21.4	11.9
7	Ser	0.1	0.3	-
8	Pro	8.3	3.6	2.9
9	Tyr	17.8	14.3	-

Table 5. Physicochemical and bioinformatic characteristics of the WMLPSYSPY peptide based on *in silico* analysis

Peptide characterization	Predicted value
Peptide	WMLPSYSPY
Number of amino acids	9
Molecular weight (Da)	1143.32
Theoretical pI	5.52
Extinction coefficient (M^{-1} cm^{-1})	8480
Estimated half-life	2.8 h (mammalian reticulocytes, in vitro)
	3 min (yeast, in vivo)
	2 min (*Escherichia coli*, in vivo)
Instability index	126.82
Aliphatic index	43.33
(GRAVY)	-0.289
Bioactive score	0.653399
Toxicity	Non-toxin
Allergenicity	Probable non-allergen
Class of CPPs	Cell-penetrating peptide
Uptake efficiency	Low

GRAVY: Grand average of hydropathicity; CPP, cell-penetrating peptides.

of food-derived peptides for HCC treatment. These bioactive peptides may serve as alternative therapeutic agents to conventional drugs, which are often associated with significant adverse effects.

Current pharmacological treatments for HCC such as atezolizumab + bevacizumab, sorafenib, lenvatinib, and regorafenib, are known to cause side effects, including fatigue, diarrhea, and hypertension (8). A systematic review by Griffiths et al also found that the side effects of small-molecule tyrosine kinase inhibitor (TKI) HCC therapy drugs, such as sorafenib, lenvatinib, and regorafenib, were greater than those of immune checkpoint inhibitor (ICI) therapy drugs (46% compared to 28%) (39). Moreover, the toxicity observed after therapy was between TKIs (21%) and ICIs (28%). These findings underscore the urgent need for novel therapeutic options with improved safety profiles. Bioactive peptides represent a promising alternative due to their high specificity to target molecules, which may result in lower toxicity and fewer off-target effects compared to existing HCC treatments.

The active peptide samples used in this study consisted of 6 active peptides (16-18) and 3 active peptides used as controls (19,20,26) for each target protein. The limited number of soybean-derived peptides analyzed in this study reflects the scarcity of available and validated anticancer peptides in publicly accessible peptide databases. In this work, we exclusively selected peptides that had been experimentally confirmed to exhibit anticancer activity and that were listed in the DFBP. Research by Kim et al showed that peptides with the sequence WMLPSYSPY from defatted soybeans exhibit anticancer properties by inducing cytotoxicity in P388D1 cell strains, macrophage monocytes in rats involved in tumor development (17). Research by Chen et al demonstrated that peptides with the sequences IVPK and LVPK from black soybeans possess anticancer properties in HepG2 (liver), MCF-7 (lung), and HeLa (cervical) cell strains by inducing cytotoxic and apoptotic effects (16). Research by Wang et al (2008) indicated that peptides with the sequences FEITPEKNPQ, IETWNPNNKP, and VFDGEL from soybeans can inhibit topoisomerase II protein activity (18). Research by Liu et al showed that the peptide FFW (RRKFAKFQWI) can disrupt the SALL4-NuRD complex to exhibit anticancer properties by increasing Ca^{2+} levels to trigger apoptosis, enhancing cell adhesion to suppress tumor movement, and activating tumor-suppressing genes (19). Research by Wang et al demonstrated that the VEGF125-136 peptide (QKRKRKKSRYKS) can serve as a VEGFR protein inhibitor (20). Research by Wu et al showed that the M27-29 peptide derived from Musa domestica larvae possesses anticancer properties by targeting the GPC3 protein (26).

The results of molecular docking between the 6 active peptides indicate that the peptide WMLPSYSPY exhibits better binding affinity to the SALL4-NuRD, VEGF, and GPC3 proteins compared to the control peptides (Table 2). These findings suggest that the WMLPSYSPY peptide has the potential to bind to the target proteins more effectively than the control peptides. To assess the similarity of WMLPSYSPY with the control peptides, RMSD testing was conducted to determine whether the peptide adopts conformations close to those of the control peptides, thereby potentially exerting similar effects. The RMSD test results show that the WMLPSYSPY peptide has RMSD values above the threshold of < 2.0 Å or between 2.0 and 3.0 Å (Table 3). These results indicate that the conformation of WMLPSYSPY when binding to SALL4-NuRD, VEGF, and GPC3 is similar to that of the

control peptides.

3D and 2D visualizations demonstrate that the WMLPSYSPY peptide binds to each target protein through hydrogen and hydrophobic bonds (Figures 1-3). Hydrogen bonds play a role in maintaining the stability of the protein-peptide complex, thereby potentially exerting inhibitory effects (40), and the same applies to the hydrophobic bonds between the peptide and protein (41).

Tyr6 contributed significantly to binding in silico based on ΔΔG values (Table 4). Residues with positive ΔΔG values contribute to peptide-protein stability (32). However, this result should be interpreted cautiously, as computational alanine scanning does not fully reflect the dynamic and complex nature of protein interactions in vivo. Unlike SALL4-NuRD and VEGF, where Tyr9 also plays an essential role in forming interactions with the peptide, Trp1 of the peptide is crucial in its interaction with GPC3. Tyr9 of the peptide does not interact with any residues when interacting with GPC3, similar to Ser7. Upon closer examination, the residues most involved in interactions with the protein have several hydrophobic bonds with residues from the target protein (Supplementary file 1, Table S2).

Analysis of peptide characteristics revealed molecular weight, isoelectric point, extinction coefficient, estimated half-life, instability index, aliphatic index, GRAVY, bioactivity, toxicity, allergenicity, and the peptide's ability to penetrate cells. The molecular weight of the WMLPSYSPY peptide was predicted to be 1143.32 Da. This molecular weight falls within the range of peptide drugs approved by the FDA, with chain lengths of 2-10 amino acids (above 1100 Da) (42). The isoelectric point (pI) represents the pH at which a protein has no net charge (43). WMLPSYSPY was predicted to be acidic (pI=5.52) and stable under physiological conditions. Its moderate hydrophobicity may favor interaction with cell membranes, potentially enhancing uptake in tumor cells. The extinction coefficient indicates the amount of light absorbed by a protein, which can be predicted based on the amino acid residue components (34). The prediction results show that the peptide has an extinction coefficient value of 8480 M-1 cm-1, which is quite high due to the presence of one tryptophan residue and two tyrosine residues in the WMLPSYSPY peptide. Similar results were also found in the study by Kaur et al (43).

The estimated half-life predicts the duration required for half the amount of protein to degrade after entering the cell. The estimated half-life of the WMLPSYSPY peptide in mammalian reticulocyte cells, yeast, and E. coli bacteria is 2.8 hours, 3 minutes, and 2 minutes, respectively. These results indicate that the peptide has a relatively short half-life. The presence of large amino acid residues such as phenylalanine, tyrosine, arginine, and tryptophan can facilitate protease binding to the peptide. Small amino acid residues such as serine, alanine, glycine, and threonine also play a role in maintaining the bond between the protease and the peptide (44). The presence of serine, tyrosine, and tryptophan residues, constituting 55% of the peptide, results in its short half-life.

The instability index predicts the stability of the peptide, with values below 40 predicted to be stable and values above 40 predicted to be unstable (34). The instability index of the WMLPSYSPY peptide is 126.82, indicating that the peptide is likely unstable when tested in vitro and in vivo. The aliphatic index predicts the stability of the protein at a certain temperature based on the presence of aliphatic groups such as valine, isoleucine, alanine, and leucine (34). The predicted aliphatic index value of 43.33 indicates that the peptide is prone to degradation at certain temperatures. The GRAVY value is calculated by dividing the total hydrophobicity values of all amino acids in the peptide by the number of residues (34). A more negative GRAVY value indicates that the peptide is hydrophilic, and vice versa (43). The GRAVY value of -0.289 indicates that the peptide is hydrophilic, allowing it to bind well with cell tissues.

Bioactivity testing was conducted to determine whether the peptide is bioactive and can affect cells, tissues, and living organisms. Testing using PeptideRanker will yield values between 0-1, where values above 0.5 indicate bioactivity (35). The WMLPSYSPY peptide showed a value of 0.653399, indicating bioactivity. Toxicity and allergenicity testing were conducted to classify the peptide as toxic or non-toxic and allergenic or non-allergenic. Prediction results with ToxinPred and AllerTop v.2.0 indicate that the WMLPSYSPY peptide is non-toxic and non-allergenic. This is consistent with the characteristics of peptides, which tend to have low toxicity (13). Predictions of whether the WMLPSYSPY peptide can penetrate cells through the MLCPP 2.0 web indicate that the peptide can penetrate cells but has low uptake efficiency into cells.

The predicted characteristics of the WMLPSYSPY peptide in silico suggest its potential as a therapeutic agent for HCC. However, challenges such as low structural stability, limited cellular uptake, and pharmacokinetic limitations must be addressed to ensure its clinical applicability. Several strategies can be employed to enhance peptide performance, including amino acid modification, conjugation with cell-penetrating peptides (CPPs), nanoparticle-based delivery, and structural cyclization. For instance, substituting L-amino acids with D-amino acids can improve stability, as D-amino acids are less susceptible to protease degradation (45). Although WMLPSYSPY shows predicted cell-penetrating ability, pairing it with CPPs may further enhance uptake and stability (45). Additionally, encapsulation in nanoparticles can protect peptides from enzymatic degradation and biological barriers such as the intestinal or mucosal layers, thereby increasing systemic absorption (46).

A particularly promising approach is peptide cyclization,

which provides a more rigid conformation that enhances target specificity while reducing proteolytic susceptibility. Ji et al demonstrated that cyclic peptides exhibit superior binding affinity and resistance to degradation compared to their linear counterparts. A classic example is the conversion of somatostatin, a linear 14-amino acid hormone, into octreotide, an 8-amino acid cyclic analog with significantly improved stability and half-life (47). Applying a similar cyclization strategy to WMLPSYSPY could further improve its pharmacological potential.

Furthermore, the clinical feasibility and pharmacokinetics of peptide-based drugs cannot be easily assessed using conventional small-molecule drug criteria. Peptides often violate Lipinski's Rule of Five, particularly in terms of molecular weight and polarity. Despite this, several peptide-based drugs such as alisporivir (for hepatitis C) and desmopressin (for hemophilia) have achieved clinical success, largely due to their low toxicity and high target selectivity (42). Therefore, applying traditional drug-likeness rules may be inadequate for peptides. Comprehensive in vitro and in vivo evaluations are essential to assess their stability, bioavailability, and therapeutic safety under physiological conditions, ensuring their viability as clinical candidates.

Limitation of the study

This study was conducted using a computational approach to predict the therapeutic potential of soybean-derived anticancer peptides against HCC. While the peptide sequences used in this study were obtained from previously published and experimentally validated research, our analysis focused solely on molecular docking and in silico evaluations. As such, the predicted interactions and binding affinities, although informative, may not fully reflect biological activity in vivo due to limitations in peptide stability, bioavailability, and pharmacokinetics. Furthermore, this study did not include experimental validation such as in vitro cytotoxicity assays or in vivo efficacy studies.

Conclusion

A comparative study between six soy-derived anticancer peptides and known anticancer peptides targeting SALL4-NuRD, VEGF, and GPC3 proteins revealed that WMLPSYSPY exhibited the most favorable binding characteristics, suggesting its potential as a promising lead candidate for further preclinical evaluation in HCC therapy. RMSD testing of the WMLPSYSPY peptide also showed conformational similarity to the control peptides. Further laboratory-scale research on the peptide is needed, as in silico predictions indicate low uptake and stability. Several methods that can be utilized include modifying the amino acid structure and pairing the peptide with drug delivery systems (CPPs and nanoparticles). Future work should include experimental validation through cytotoxicity assays, apoptosis analysis, and cell migration assays to determine the peptide's functional impact in HCC models.

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Conflict of interests

The authors have no conflicts of interest to declare.

Ethical considerations

Duplication and plagiarism were assessed and verified using Turnitin software.

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Supplementary files

Supplementary file 1 contains Tables S1 and S2.

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