A Hub Signaling Pathway of Antimicrobial-Antifungal-Anticancer Peptides Axis With Cationic Residue Amino Acids on N, C- Terminals Under 500 Dalton Rule Via Network Pharmacology

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Research Article

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Abstract

Background: Short cationic peptides (SCPs) with therapeutic efficacy of Antimicrobial peptides (AMPs), Antifungal peptides (AFPs), and Anticancer peptides (ACPs) are known as enhancement of host defense system. Here, we investigated the uppermost peptide(s), hub signaling pathway(s), and its associated target(s) through network pharmacology.

Method: Firstly, we selected SCPs with positive amino acid residues on N-, C- terminals under 500 Dalton via RStudio. Secondly, EMBOSS pepstats, PASTA 2.0 and Aggrescan were used to remove non- AMPs, after that, ADAM, dbAMP, DBAASP $_{v3.0}$, and MLAMP were utilized for AMPs selection. AMPs-targets were identified from both SEA and STP databases. The overlapping targets between the bacteria-responsive targets (TTD and OMIM) and AMPs-targets were visualized by VENNY 2.1. Thirdly, AFPs were filtered through Antifp tool, and TTD and OMIM selected fungal responsive targets. The overlapping targets between AFPs-targets and fungal-responsive targets were visualized by VENNY 2.1. Fourthly, the overlapping targets between cancer-related targets (TTD and OMIM) and fungal-responsive targets were visualized by VENNY 2.1. Fifthly, signaling pathway analysis of overlapping targets was performed via RStudio. Finally, molecular docking study (MDS) was carried out to discover the most potent peptides on a hub signaling pathway.

Results: A total of 1,833 SCPs were identified, and AMPs, AFPs, and ACPs filtration suggested that 197 peptides-30 targets, 81 peptides-6 targets, and 59 peptides-4 targets are connected, respectively. The AMPs-AFPs-ACPs axis indicated that 27 peptides-2 targets are associated. Each hub signaling pathway for enhancement of host defense system was "Inactivation of Rap1 signaling pathway on AMPs", "Activation of Notch signaling pathway on AMPs-AFPs axis", and "Inactivation of HIF-1 signaling pathway on AMPs-AFPs-ACPs axis". The most potent peptides were assessed via MDS; finally, HPIK on STAT3, HVTK on NOS2 manifested the HIF-1 signaling pathway's highest affinity. Furthermore, the two peptides have better affinity scores than standard selective inhibitors (Stattic, 1400W).

Conclusion: Overall, the most potent SCPs for the host defense system were HPIK on STAT3 and HVTK on NOS2, which might inactivate the HIF-1 signaling pathway.

Introduction

Since the emergence of insulin application in the 1920s, peptide therapeutics have been revealed as highly selective, safe, efficacious, and well-tolerated pharmaceutical agents [1]. Peptides are intrinsic signaling molecules, possess both biochemical and therapeutical attribution, and nearly more than 60 peptides are being used (FDA approved) worldwide as a clinical medication [2]. Peptides' critical properties as potential drug candidates are their high potency on target disease, specificity on a target protein, and minimal toxicity [3]. Certainly, peptides provide potential therapeutic intervention by binding to particular cell surface receptors which stimulate intracellular effects. Given such unique and excellent characteristics, peptide drugs can be used as novel therapies or replacement therapies [4].

Bio-researchers have recently recognized the attractive pharmacological profile of short cationic peptides having significant antibacterial, antifungal, anticancer, and even immunomodulatory activities [5–7]. A report demonstrated that peptides with cation residues (Lysine, Arginine, Histidine) have more significant antimicrobial efficacy than peptides without cation residues [8]. Another study showed that short cationic peptides (SCPs; below six residues) expose better potency than longer peptides. Additionally, SCPs can be synthesized readily by following solid-phase peptide synthesis method [9,10]. A pivotal property of cell-penetrating peptides (CPPs) is their cationic residues, facilitating permeability into the cell membrane [11]. Short peptides with cationic residues (Lysine, Arginine, Histidine) exist essentially in living organisms to function as antimicrobial activity [12]. In animals, antimicrobial peptides (AMPs) are often produced that act as natural innate barriers and elevate immune response to combat microbial infection [13–15]. Interestingly, AMPs have tremendous therapeutic potential to function as antifungal peptides (AFPs) by suppressing the fungal growth such as *Candida* conidia and hyphae [16,17]. It implies that AMPs play essential roles in boosting the immune system against fungal attack and hence, they are considered new biopharmaceuticals to fight or treat fungal infections. Recent studies have supported that cationic peptides act as immune modulators, recognizing signal molecules like lipopolysaccharide secreted by bacterial or fungal molecules [18,19].

Evidence also suggests that AMPs demonstrate the antitumor activity by stimulating human cancer cells [20]. The constructed AMPs have positive amino acid residues that can bind effectively with negatively charged cancer cells components [21]. A study proves that AMPs can potentially disrupt the cancer cell membrane due to the strong electrostatic attraction present between positively charged AMPs and negatively charged molecule "phosphatidylserine" on cancer cells' plasma membranes [22]. Another report supports that AMPs activate the host immune defense system, working as anticancer peptides (ACPs) [23]. Despite these advantages, peptides have some intrinsic weaknesses, such as high molecular weight, degradability, and low permeability [24]. However, these limitations can be resolved through traditional design of biotherapeutic peptides that are more suitable for use as convenient therapeutics. Multifunctional and useful cell-penetrating peptides offer more therapeutics and diagnostic merit, leading to the development of future medicines with improved target delivery, efficacy, and pharmacokinetic properties. From these points of view, we used diverse multiple putative AMPs (or) AFPs prediction tools to identify potential therapeutic of SCPs. The final peptides of ACPs were selected via public databases and thus completed AMPs-AFPs-ACPs axis on SCPs.

In this study, we performed network pharmacology (NP) concept to achieve the AMPs-AFPs-ACPs axis. NP is a collective, systemic, and holistic approach to investigate the relation of molecule(s) and target (s), find the optimal molecule(s) on target protein(s), and provide a crucial hint for identifying the mechanism of a potential lead molecule(s) [25–27]. Moreover, Zhang B. et al. described that NP accelerates the decoding TCM (Traditional Chinese Medicine) from an empirical-based therapy to an evidence-based therapy system, which improves modern drug discovery strategies [28].

In our study, network pharmacology-based analysis was utilized to investigate triple therapeutic feasibility (AMPs-AFPs-ACPs axis) of SCPs. Firstly, SCPs (N, and C- terminal cationic groups; ≤ 500 Dalton) were selected via RStudio analysis. Secondly, the physicochemical propensity of selected SCPs was identified via AMPs screening platform, and a hub signaling pathway of AMPs between AMPs-related targets and host-responsive targets were analyzed. Thirdly, the AFPs screening platform was used to find AFPs from selected AMPs, and a hub signaling pathway of AMPs-AFPs axis was identified between AFPs-related targets and host-responsive targets. Fourthly, AMPs-AFPs-ACPs axis was constructed by retrieving cancer-related targets from public databases. Fifthly, SCPs accepted by AMPs-AFPs-ACPs axis and targets on a hub signaling pathway were subjected to perform MDS. Finally, we found (via network pharmacology) a hub signaling of SCPs which might assume to strengthen the host defense system. Figure 1 shows the overall workflow.

Materials And Methods

Selection of peptides via RStudio

The standard peptides were selected with positive amino acids (Lysine, Arginine, Histidine) on both terminals (N-terminal, C-terminal) or less than 500 Dalton. The selection method of these species was based on RStudio.

AMP evaluation and prediction

The selected peptides were assessed for AMP evaluation utilizing *in silico* analysis. Firstly, EMBOSS Pepstats (https://www.ebi.ac.uk/Tools/seqstats/emboss_pepstats/) were used to identify the physicochemical properties of peptides. Secondly, aggregation of peptides was filtered with rigor on both PASTA 2.0 (https://protein.bio.unipd.it/) and AGGRESCAN (http://bioinf.uab.es/aggrescan/) [56]. Subsequently, final AMP were selected by ADAM (http://bioinformatics.cs.ntou.edu.tw/adam/svm_tool.html), dbAMP(http://140.138.77.240/~dbamp/), $DBAASP_{v3.0}$ (https://dbaasp.org/prediction/general), and MLAMP (http://www.jci-bioinfo.cn/MLAMP) [57–60].

AFP evaluation and prediction

The final AMPs' sequences with FASTA format were input to Antifp database (https://webs.iiitd.edu.in/raghava/antifp/predict3.php) [61]. The final AFPs were selected by the classifier of AntipDS1_binary_model1, AntipDS1_binary_model2, and AntipDS1_binary_model3.

Conversion of (peptide) sequences into SMILES format

The sequences of the final selected AMPs and AFPs were converted to SMILES format through Dendrimer Builder (https://dendrimerbuilder.gdb.tools/) [62].

Identification of peptide-target networks and microbial related targets on database

Based on SMILES (format), target targets related to selected peptides were extracted from both SEA (http://sea.bkslab.org/) [63] and STP (http://www.swisstargetprediction.ch/) [64] with "*Homo Sapiens*" setting. The overlapping targets in the peptide(s)-target(s) networks between SEA and STP were identified by VENNY 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/). The bacterial responsive targets on human were obtained with "bacterial/germ/bacilli" from both TTD (http://db.idrblab.net/ttd/) [65] and OMIM (https://www.omim.org/) [66] databases. After that, the overlapping targets between peptide(s)-target (s) and bacterial responsive targets were identified by VENNY 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/).

Bubble plot of signaling pathway analysis of overlapping targets between peptide-targets and bacterial responsive targets network

The final overlapping targets (bacterial responsive targets on the human) networks ware visualized by STRING (https://string-db.org/) [67]. A bubble plot of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway based on the final overlapping targets was constructed by RStudio.

Identification of peptide-targets network and fungal related targets on database

Based on SMILES, targets associated with selected peptides were identified via both SEA (http://sea.bkslab.org/) and STP (http://www.swisstargetprediction.ch/) with "*Homo Sapiens*" setting. The overlapping targets in peptide-target network between SEA and STP were identified by VENNY 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/). The fungal targets associated with a human were obtained from both TTD (http://db.idrblab.net/ttd/) and OMIM (https://www.omim.org/), entering as "fungal". The overlapping targets between peptide-target targets and fungal related targets were identified by VENNY 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/).

Bubble plot of signaling pathway analysis of overlapping targets between peptide-targets and fungal responsive targets network

The final overlapping targets (fungal responsive targets on the human) construction was visualized by STRING (https://string-db.org/). A bubble plot of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway based on the final overlapping targets was constructed by RStudio.

Identification of peptide-target targets network and cancer-related targets on database

Based on SMILES, targets associated with selected peptides were identified via both SEA (http://sea.bkslab.org/) and STP (http://www.swisstargetprediction.ch/) with "*Homo Sapiens*" setting. The cancer-related targets on human were obtained with "cancer/tumor/neoplasia/carcinoma" from TTD (http://db.idrblab.net/ttd/) and OMIM (https://www.omim.org/). The overlapping targets between peptide-targets and cancer-related targets were identified by VENNY 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/).

Bubble plot of signaling pathway analysis of overlapping targets between peptide-targets and cancer-related targets

The final overlapping targets (cancer-related targets on the human) construction was visualized by STRING (https://string-db.org/). RStudio constructed a bubble plot of KEGG pathway based on the final overlapping targets.

Preparation for docking of peptide molecules

The peptide molecules were converted into SMILES format from Dendrimer builder. The converted SMILES were again converted into .pdb format using Open Babel (http://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html) [68]. Finally, the converted .pdb peptide was converted into .pdbqt format through Autodock.

Preparation for docking of target proteins and positive controls to compare with final peptides

Two target proteins of cancer i.e., STAT3 (.pdb ID: 6TLC), NOS2 (.pdb ID: 4NOS) identified from STRING were converted into .pdbqt format (https://www.rcsb.org/) from .pdb format in order to test the affinity of ligands via Autodock (http://autodock.scripps.edu/) [69]. Subsequently, two positive controls i.e., stattic (Pubchem ID: 2779853) for STAT3 and 1400W (Pubchem ID: 1433) for NOS2, were converted into.pdb format from .sdf format to upload on Pymol, and each of two positive controls was converted again into .pdbqt format to measure affinity through Autodock.

Peptide- target proteins docking test

The final peptides were docked on target proteins, processing autodock4 by setting-up 4 energy range and 8 exhaustiveness as default to obtain 10 different poses of ligand molecules [70]. The 2D binding interactions were constructed through LigPlot+ v.2.2 (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/) [71].

Results

SCPs under 500 Dalton rule

The number of 1,833 peptides with two sufficient conditions (positive N, C- terminals amino acid residues, under 500 Dalton) was selected by RStudio analysis. Table 1 displayed the amount (Da) of each amino acid. The selected peptides were enlisted. (Supplementary Table S1).

Physicochemical refinement for AMPs

The 1,833 peptides were input in EMBOSS Pepstats (https://www.ebi.ac.uk/Tools/seqstats/emboss_pepstats/) on Charge > 0 or 8 ≤ Isoelectric Point ≤ 12 [29]. Secondly, PASTA 2.0 (adjusted to "zero") (https://protein.bio.unipd.it/) was utilized to predict the peptide

aggregation propensity [30]. Thirdly, peptide aggregation was checked by AGGRESCAN (Na4VSS ≥ -40, Na4VSS ≤ 60) (http://bioinf.uab.es/aggrescan/) which was based upon aggregation propensity *in vitro*. Among 1,833 peptides, the number of 236 peptides were selected (Supplementary Table S2). Fourthly, the 236 peptide sequences were input to four platforms including ADAM (http://bioinformatics.cs.ntou.edu.tw/adam/svm_tool.html), dbAMP (http://140.138.77.240/~dbamp/), $DBAASP_{v3.0}$ (https://dbaasp.org/prediction/general), and MLAMP (http://www.jci-bioinfo.cn/MLAMP) to discover AMPs. Finally, from the four databases, 197 out of 236 peptides were obtained as suitable for AMPs (Supplementary Table S3).

AMPs-targets identification

The number of 197 peptides sequences were converted into SMILE format via Dendrimer Builder (https://dendrimerbuilder.gdb.tools/). The SMILE format of peptide sequences was input to SEA (http://sea.bkslab.org/) and STP (http://www.swisstargetprediction.ch/) databases with "*Homo Sapiens"* setting. Figure 2A showed that the number of 375 and 355 targets associated with the 197 peptides were identified by SEA and STP, respectively (Supplementary Table S4). The number of 242 overlapping targets was also identified from the two databases (Supplementary Table S5). Finally, Figure 2B and Table 2 displayed that the number of 30 targets overlapped between the number of 959 AMPs-targets (extracted from TTD and OMIM databases) (Supplementary Table S6) and overlapping 242 targets were selected.

Signaling pathways responsive to bacterial infection on human

Figure 3A exhibited that 13 out of overlapping 30 targets were notably enriched in 11 signaling pathways via KEGG pathway enrichment analysis. Table 3A showed that the detailed description of the 11 signaling was enlisted. Figure 3B displayed that the 13 targets were associated with the number of 197 peptides, and the constructed peptide-targets networks manifested 210 nodes and 1,011 edges. Figure 3C showed that peptide-targets network analysis via overlapping 30 targets was constructed by STRING, which indicated 30 nodes and 68 edges. Among 11 signaling pathways, inactivation of Rap1 signaling pathway was identified as a hub signaling pathway through bubble plot. Figure 3D exhibited that among 11 signaling pathways, the Rap1 signaling pathway's targets were SRC, FPR1, and ITGB1, which was constructed with 158 nodes (3 targets, 155 peptides) and 216 edges on a size map. Among the 3 targets (SRC, FPR1, and ITGB1), ITGB1 connected to 117 peptides was on the highest degree of value. It implies that ITGB1 played a vital role in Rap1 signaling pathways in host defense systems against bacterial infection.

Physicochemical refinement for AFPs

The number of 197 peptides (AMPs) were input into AntipDS1_binary_model1, AntipDS1_binary_model2, and AntipDS1_binary_model3 in antifungal peptide screening platform. Thereby, the number of 91 peptides was accepted as AFPs which were defined as AMPs and AFPs with dual-efficacy for enhancement of host defense system (Supplementary Table S7).

AFPs-targets identification

The number of 91 peptides sequences was converted to SMILE format via Dendrimer Builder (https://dendrimerbuilder.gdb.tools/). The SMILE format of peptide sequences was input to SEA (http://sea.bkslab.org/) and STP (http://www.swisstargetprediction.ch/) with "*Homo Sapiens*" setting. The number of 357 and 330 targets were identified from SEA and STP, respectively (Supplementary Table S8). Figure 4A displayed that the number of 218 overlapping targets was selected from the two databases. (Supplementary Table S9). Figure 4B showed that the number of 6 overlapping targets (TPSAB1, PSEN1, PSEN2, DPP4, STAT3, and NOS2) was identified between the number of AFPs- targets (245 targets from TTD and OMIM databases) (Supplementary Table S10) and overlapping 218 targets.

Signaling pathways responsive to fungal infection on human

Figure 5A showed that 6 targets (TPSAB1, PSEN1, PSEN2, DPP4, STAT3, and NOS2) were connected to 3 signaling pathways via KEGG pathway enrichment analysis. Table 3B showed the detailed description of the 3 signaling. The 6 targets (TPSAB1, PSEN1, PSEN2, DPP4, STAT3, and NOS2) were related to the number of 81 peptides (Supplementary Table S11). Figure 5B exhibited the constructed network exposed 87 nodes (81 peptides, 6 targets) and 1,011 edges. Figure 5C displayed that peptide- targets networking analysis via overlapping 6 targets (TPSAB1, PSEN1, PSEN2, DPP4, STAT3, and NOS2) was constructed by STRING, which indicated 6 nodes and 2 edges. Among 3 signaling pathways, activation of Notch signaling pathway was identified as a hub signaling pathway through bubble plot. Figure 5D showed that Notch signaling pathway's targets were both PSEN1 and PSEN2, and their peptides- targets network was constructed on a size map (34 nodes and 45 edges). Among the 4 targets, PSEN1 and PSEN2 were connected to 9 peptides (KLCK, KCLK, KALK, KVLK, KLGGK, KAFK, KFGK, KFSK, and KSFK) which might have more efficacy than any other AFPs. Besides, it implies that both PSEN1 and PSEN2 played an essential role in the Notch signaling pathway, in aspects of the host defense system against fungal infection on AMPs-AFPs axis.

Cancer-related targets and ACPs-targets identification

TTD and OMIM selected the number of 4,247 cancer-related targets (Supplementary Table S12). Figure 6A exhibited that the number of 4 out of 6 AFP-responsive targets was overlapped with the 4,247 cancer-related targets. Figure 6B showed that 2 targets (STAT3 and NOS2) were targeted to only HIF-1 signaling pathway via KEGG pathway enrichment analysis. Table 3C showed the detailed description of the signaling. Figure 6C exhibited that the 2 targets (STAT3 and NOS2) were related to the number of 27 peptides, and the constructed networks revealed 29 nodes (27 peptides, 2 targets) and 27 edges. Figure 6D showed that peptide- targets networking analysis via overlapping 4 targets (PSEN1, DPP4, STAT3, NOS2) was constructed by STRING (6 nodes and 2 edges). Figure 6E exhibited that only two targets (STAT3 and NOS2) are related directly to HIF-1 signaling pathway. Both STAT3 and NOS2 targets were directly associated with HIF-1 signaling pathway, which played a crucial role in defending the cancer attack. The HIF-1 signaling pathway was connected particularly to all AMPs-AFPs-ACPs axis.

MDS on HIF-1 signaling pathway for host defense system

The ultimate signaling pathway, HIF-1 signaling pathway, was connected to STAT3 (.pdb ID: 6TLC) and NOS2 (.pdb ID: 4NOS): The number of 8 peptides (KPIK, KPVK, KVPK, HPIK, KAFK, KFGK, KSFK, and KFSK) were targeted to STAT3 target, additionally, the number of 19 peptides (RVVK, HMCK, KMCH, HVTK, KCMH, KIIK, KVIK, KILK, KVLK, KALK, KIVK, KIGK, KAIGK, KIAGK, KAGVK, KAGIK, KAGLK, KIGGK, and KVGGK) were targeted to NOS2 target. Table 4 displayed the physicochemical properties of the 27 peptides. The number of 8 peptides was targeted to STAT3 (.pdb ID: 6TLC) and their priorities are as follows: HPIK (-7.3 kcal/mol); KAFK (-7.1 kcal/mol); KPIK (-7.0 kcal/mol); KPVK (-6.8 kcal/mol); KVPK (-6.8 kcal/mol); KFGK (-6.8 kcal/mol); KSFK (-6.7 kcal/mol); and KFSK (-6.4 kcal/mol). Figure 7A showed that “HPIK” peptide was the strongest affinity on STAT3 (.pdb ID: 6TLC) in HIF-1 signaling pathway among 8 peptides. Table 5 displayed its detailed information. Likewise, 19 peptides was targeted to NOS2 (.pdb ID: 4NOS), their priorities are as follows: HVTK (-6.6 kcal/mol); KILK (-6.4 kcal/mol); KAGVK (-6.1 kcal/mol); KIGGK (-6.0 kcal/mol); KAGLK (-5.8 kcal/mol); KAIGK (-5.6 kcal/mol); HMCK (-5.5 kcal/mol); KIAGK (-5.5 kcal/mol); KVIK (-5.5 kcal/mol); KALK (-5.5 kcal/mol); RVVK (-5.4 kcal/mol); KIIK (-5.4 kcal/mol); KIVK (-5.4 kcal/mol); KMCH (-5.3 kcal/mol); KVGGK (-5.3 kcal/mol); KCMH (-5.1 kcal/mol); KVLK (-5.1 kcal/mol); and KAIGK (-5.0 kcal/mol). Figure 7B showed that “HVTK” peptide was the strongest affinity on NOS2 (.pdb ID: 4NOS) in HIF-1 signaling pathway among 19 peptides. Table 6 showed its detailed information. This result showed that the uppermost promising peptides to strengthen immune system against cancer were “HPIK” on STAT3 (.pdb ID: 6TLC) and “HVTK” on NOS2 (.pdb ID: 4NOS).

MDS of positive controls on HIF-1 signaling pathway

The greatest affinity peptide on STAT3 (.pdb ID: 6TLC) was “HPIK” (-7.3 kcal/mol). A representative inhibitor of STAT3 is stattic (PubChem ID: 2779853), which interrupts the tumor cell growth by inhibiting lymphoma activity [31]. Thus, MDS of stattic (PubChem ID: 2779853) was selected to compare with "HPIK". Consequently, the docking score of stattic (PubChem ID: 2779853) was -6.1 kcal/mol. The "HPIK" affinity on STAT3 (.pdb ID: 6TLC) was better than stattic (PubChem ID: 2779853). The higher affinity peptide on NOS2 (.pdb ID: 4NOS) was “HVTK” (-6.6 kcal/mol). A selective inhibitor of NOS2 is 1400W (PubChem ID: 1433), which could inhibit U87MG cells (brain tumor cell) [32]. Hence, MDS of 1400W (PubChem ID: 1433) was carried out to compare with "HVTK"; subsequently, the docking score of 1400W (PubChem ID: 1433) was -5.2 kcal/mol.

Discussion

The SCPs were selected with two rigor criteria: ≤ 500 Dalton and N-, C-terminal cationic amino acid residues. The number of 1,833 SCPs was identified, and consecutively, 197 peptides (AMPs), 91 peptides (AMPs-AFPs axis), and 59 peptides (AMPs-AFPs-ACPs axis) were selected. The associated SCPs with signaling pathways are as followed: 197 peptides-13 targets (AMPs), 81 peptides-6 targets (AMPs-AFPs axis), and 27 peptides-4 targets (AMPs-AFPs-ACPs axis). It was reported that SCPs have functioned as

antimicrobial agents and host defense adjuvants [33]. A study suggested that TLR4 is an upregulated representative target in keratitis of bacterial infection, whereas SOD2 is an upregulated representative target in keratitis of fungal infection from Differential Expressed Genes (DEGs) [34]. It entails that host responses against bacterial and fungal attack might induce significant differences in the immune system. Hence, we regarded it as an independent perturbation of the bacterial and fungal infection. A study indicated that AMPs could bind with negatively charged ions (phosphatidylserine) on the cancer cell membrane and trigger the host defense system [35]. Thus, we performed the analysis of AMPs-AFPs-ACPs axis to investigate potential SCPs for the host immune system.

AMPs-targets network showed that the therapeutic efficacy of host defense system was directly associated with 30 s. The result of the KEGG pathway analysis of 30 targets indicated that 11 signaling pathways were connected to 13 out of 30 targets, suggesting that these signaling pathways were directly related to bacterial infection responses in the human immune system.

The description of the 11 signaling pathways with bacterial infection were briefly discussed as follows: Relaxin signaling pathway: Relaxin prevents inflammatory cytokine-induced by endotoxin in THP-1 (human monocytic cell line), which specializes the immune cells in the period of preterm birth [36]. Glucagon signaling pathway: Glucagon alleviates inflammatory responses of the airway, due to association with the reduction of eosinophils and T lymphocytes by inhibiting TCD4+ cell proliferation [37,38]. Prolactin signaling pathway: Prolactin accelerates secretion of proinflammatory cytokines in peripheral immune cells, modulating the level of responses against pathogens [39,40]. Estrogen signaling pathway: Estrogen increases in the level of expression of AMPs in the host, thereby interrupting bacterial proliferation [41]. Additionally, estrogen-stimulated the expression level of cell-cell junction proteins, thereby intensifying the epithelial rigidity and prohibiting unnecessary loss of outer cells during infection [42]. TNF signaling pathway: Tumor Necrosis Factor (TNF) can induce the recruitment of inflammatory cells and control the mechanism of antimicrobial activities [43]. It implies that TNF can work as a buffer element for immunopotentiation. IL-17 signaling pathway: The knock-out groups of IL-17 are highly susceptible to *K. pneumonia* infection than IL-17 expression groups [44]. AMPK signaling pathway: Activation of AMPK improves host defense system against bacterial infection. Moreover, AMPK is associated with the innate and adaptive immune system [45]. FoxO signaling pathway: FoxO1 protein is expressed by a bacterial infection, strengthening the epithelial barrier of host cells and induces the recruitment of Tregs (Regulatory T Cells) to activate antibacterial defense [46]. HIF-1 signaling pathway: HIF-1α activation in the hypoxic condition recruits inflammatory-associated cells such as macrophages, neutrophils, and dendritic cells as well as induces offensive cytokine production under bacterial infection [47]. HIF-1 inhibition can be a good strategy to relieve inflammation level induced by the bacterial attack in aspects of the host immune system. Rap1 signaling pathway: The inactivation of Rap1 in lymphocytes is a representative treatment against inflammatory disorders [48]. On AMPs signaling pathways, the key mechanism might inhibit the Rap1 signaling pathway selected based on the rich factor.

AMPs-AFPs axis-target networks showed that the therapeutic efficacy of host defense system was directly associated with 6 targets. The result of the KEGG pathway analysis of 6 targets were connected to 3 signaling pathways. Neurotrophin signaling pathway: Inflammation signals in microglial cells induce the secretion of neurotrophin that function as mediators of pain [49,50]. It implies that the neurotrophin signaling pathway's inactivation might modulate inflammatory-related proteins' expression level, thereby resolving host defense-induced inflammation. HIF-1 signaling pathway: The deletion of hypoxia-regulated targets are resistant to fungal infection; more importantly, the low-oxygen condition makes fungal virulence attenuate in murine models [51]. Thus, inactivation of HIF-1 might interrupt the fungal penetration and host immune system. Notch signaling pathway: Notch system plays important roles in Th1 and Th2 cell differentiation, and Notch-mediated immune responses are related to T cell development [52]. It supports that the activation of Notch signaling pathway contributes to enhancing the host defense system. On AMPs-AFPs axis signaling pathways, a key signaling pathway is to activate the Notch signaling pathway which was identified based on the rich factor

AMPs-AFPs-ACPs axis-target networks exhibited that the therapeutic efficacy of host defense system was directly associated with 4 targets. The result of the KEGG pathway analysis on 4 targets was connected to 1 signaling pathway. HIF-1 signaling pathway: HIF-1 overexpression contributes to tumor growth, angiogenesis, and metastasis. However, the overexpression is caused by an oxygen-depleted condition in tumor cells [53,54]. Furthermore, hypoxia makes severe conditions under resistance to cancer therapy such as radiation and medication, increasing tumor survival [55]. It suggests that inactivation of a HIF-1 signaling pathway is an optimal strategy for cancer therapy. This work has been focused on immunomodulatory activities of SCPs, which may improve immune defenses and provide key therapeutic agents from large-scale peptides. We have performed the MDT to select promising peptide candidate(s) on the HIF-1 signaling pathway, and hence the standard molecules (static and 1400w) were compared with them. Moreover, we have suggested a hub signaling pathway (HIF-1 signaling pathway), two key SCPs (HPIK and HVTK), and two key targets (STAT3 and NOS2). This analysis collectively suggested an overlapping signaling pathway "HIF-1 signaling pathway" on AMPs, AMPs-AFPs axis, and AMPs-AFPs-ACPs axis. Therefore, the inactivation of HIF-1 signaling pathway using two selected peptides is a feasible treatment strategy for enhancing the host defense system.

Conclusion

The uppermost SCPs of AMPs-AFPs-ACPs axis for immunopotentiation were firstly investigated through network pharmacology. The number of 1,833 SCPs was funneled sequentially through peptide screening platform, thereby, the number of 197 SCPs (AMPs), and 91 SCPs (AMPs-AFPs axis) were obtained. The number of 27 SCPs (AMPs-AFPs-ACPs axis) was obtained as final promising peptides through cancer-related targets analysis. The 27 SCPs (AMPs-AFPs-ACPs axis) were connected to only the HIF-1 signaling pathway with HPIK-STAT3 and HVTK-NOS2. This analysis provides the network of two SCPs, two targets, and one signaling pathway for the host defense system. Consequently, the key findings on AMPs-AFPs-ACPs axis could be a promising therapeutic strategy for cellular protection against immune disorders.

Abbreviations

ACPs: Anticancer peptides;

AFPs: Antifungal peptides;

AMPs: Antimicrobial peptides;

AMPK: AMP-activated protein kinase;

DEGs: Differential Expressed Genes;

FDA: Food and drug administration;

FoxO: Forkhead box O;

HIF-1: Hypoxia Inducible Factor 1;

KEGG: Kyoto Encyclopedia of Genes and Genomes;

MDS: Molecular Docking Study;

Rap1: Repressor Activator Protein;

SCPs: Short cationic peptides;

SEA: Similarity Ensemble Approach;

SMILES: Simplified Molecular Input Line Entry System;

STP: SwissTargetPrediction;

TCM: Traditional Chinese Medicine;

TNF: Tumor Necrosis Factor;

Tregs: Regulatory T cells

Declarations

Data availability

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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Author contributions

K.K.O.: Conceptualization, Methodology, Formal analysis, Visualization, Investigation, Software, Data Curation, Writing—Original Draft. K.K.O. and M.A.: Investigation, Data Curation. M.A.: Validation, Writing—Review & Editing. D.H.C.: Supervision, Project administration.

Competing interests

The authors declare no competing interests.

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Tables

Table 1. The amount (Da) of 20 amino acids.

Amino acid	Residue mass (DA)
A	71.08
R	156.2
N	114.11
D	115.09
C	103.14
Q	128.14
E	129.12
G	57.06
H	137.15
I	113.17
L	113.17
K	128.18
M	131.21
F	147.18
P	97.12
S	87.08
T	101.11
W	186.21
Y	163.18
V	99.14

A: Alanine; R: Arginine; N: Asparagine; D: Aspartic acid; C: Cysteine; Q: Glutamine; E: Glutamic acid; G: Glycine; H: Histidine; I: Isoleucine; L: Leucine; K: Lysine; M: Methionine; F: Phenylalanine; P: Proline; S: Serine; T: Threonine; W: Tryptophan; Y: Tyrosine; V: Valine

Table 2. The number of 30 targets overlapped between 959 AMPs-targets and overlapping 242 targets.

No.	Targets	No.	Targets
1	ACE	16	CA2
2	ECE1	17	ITGB1
3	EDNRA	18	GLO1
4	MMP3	19	MC1R
5	SIRT1	20	OPRM1
6	SIRT2	21	PPARG
7	TPP2	22	PYGL
8	UBE2I	23	SRC
9	CASP1	24	PLAU
10	FPR1	25	ELANE
11	MMP9	26	STAT3
12	PDYN	27	NOS2
13	MMP12	28	GLUL
14	SIRT3	29	DHFR
15	PDF	30	ITGA5

Table 3(A). Targets in 11 signaling pathways enrichment related to AMPs.

KEGG ID	Description	Targets	False discovery rate
hsa04917	Prolactin signaling pathway	SRC,STAT3	0.0283
hsa04926	Relaxin signaling pathway	SRC,NOS2,MMP9	0.0093
hsa04915	Estrogen signaling pathway	SRC,OPRM1,MMP9	0.0093
hsa04657	IL-17 signaling pathway	MMP3,MMP9	0.0359
hsa04064	NF-kappa B signaling pathway	PLAU,UBE2I	0.0359
hsa04066	HIF-1 signaling pathway	STAT3,NOS2	0.0359
hsa04922	Glucagon signaling pathway	SIRT1,PYGL	0.0360
hsa04668	TNF signaling pathway	MMP3,MMP9	0.0389
hsa04152	AMPK signaling pathway	SIRT1,PPARG	0.0448
hsa04068	FoxO signaling pathway	STAT3,SIRT1	0.0496
hsa04015	Rap1 signaling pathway	SRC,ITGB1,FPR1	0.0243

Table 3(B). Targets in 3 signaling pathways enrichment related to AMPs-AFPs axis.

KEGG ID	Description	Targets	False discovery rate
hsa04330	Notch signaling pathway	PSEN1,PSEN2	0.0044
hsa04066	HIF-1 signaling pathway	NOS2,STAT3	0.0088
hsa04722	Neurotrophin signaling pathway	PSEN1,PSEN2	0.0088

Table 3(C). Targets in 1 signaling pathway enrichment related to AMPs-AFPs-ACPs axis.

KEGG ID	Description	Targets	False discovery rate
hsa04066	HIF-1 signaling pathway	NOS2,STAT3	0.0071

Table 4. The physicochemical properties of final 27 peptides on AMPs-AFPs-ACPs axis.

No.	Peptide sequence	Residue mass	Targets	Charge	Isoelectric point	Aggregation propensity
		(Da)		(> 0)	(8 ≤; ≥ 12)	(Na4VSS ≥ -40; Na4VSS ≤ 60)
1	KPIK	466.65	NOS2	2	10.8	-34.6
2	KPVK	452.62	NOS2	2	10.8	-39.1
3	KVPK	452.62	NOS2	2	10.8	-39.1
4	HPIK	475.62	NOS2	1.5	9.2	-36.6
5	KAFK	474.62	NOS2	2	10.8	-30.0
6	KFGK	460.60	NOS2	2	10.8	-40.0
7	KSFK	490.62	NOS2	2	10.8	-35.1
8	KFSK	490.62	NOS2	2	10.8	-35.1
9	RVVK	482.66	STAT3	2	11.7	-6.7
10	HMCK	499.68	STAT3	1.5	8.0	-36.1
11	KMCH	499.68	STAT3	1.5	8.0	-36.1
12	HVTK	465.58	STAT3	1.5	9.2	-37.7
13	KCMH	499.68	STAT3	1.5	8.0	-36.1
14	KIIK	482.70	STAT3	2	10.8	8.6
15	KVIK	468.67	STAT3	2	10.8	4.0
16	KILK	482.70	STAT3	2	10.8	-0.3
17	KVLK	468.67	STAT3	2	10.8	-4.8
18	KALK	440.61	STAT3	2	10.8	-37.4
19	KIVK	468.67	STAT3	2	10.8	4.0
20	KIGK	426.59	STAT3	2	10.8	-38.6
21	KAIGK	497.57	STAT3	2	10.8	-19.0
22	KIAGK	497.57	STAT3	2	10.8	-19.0
23	KAGVK	483.64	STAT3	2	10.8	-23.6
24	KAGIK	497.67	STAT3	2	10.8	-19.0
25	KAGLK	497.67	STAT3	2	10.8	-27.8
26	KIGGK	483.65	STAT3	2	10.8	-29.0

27	KVGGK	469.62	STAT3	2	10.8	-33.5

Table 5. Binding energy and interactions of potential active SCPs and standard molecule (stattic) on STAT3 (PDB ID: 6TLC).

		Hydrogen Bond Interactions	Hydrophobic Interactions
Peptide sequence	**Binding energy(kcal/mol)**	**Amino Acid Residue**	**Amino Acid Residue**
HPIK	-7.3	Gln361,Tyr446	Gln448,Glu444,Leu358,
			Glu357,His447,Gln448,
KAFK	-7.1	Lys363,Thr443,Tyr446,	Gln448,Glu357,His447,
		Glu357,Gly449,Gln448,	Val445,Glu444
		Gln361	
KPIK	-7.0	Gln361,Tyr446,Gln361	Glu444,Val445,Gly449,
			Gln448,Glu357,Gln448,
			Leu362
KPVK	-6.8	Glu444,Tyr446,Gln361,	Gln448,Glu357,Leu358,
		Tyr446	Gly449,Val445
KVPK	-6.8	Gln361	His447, Glu357,Gln448,
			Tyr446,Gly449,Val445
KFGK	-6.8	Glu306,Arg278,Lys282,	Ile309,Tyr360,Lys283,
		Gln361,Gln448	Gln279,Glu286,Leu362,
			Lys363,Leu450,Gly449,
			Val310
KSFK	-6.7	Lys363,Gly449,Gln448,	Glu444,His447,Tyr446,
		Tyr446,Gln361,Thr443	His447,Glu357,Gln448,
			Val445,Glu357,Leu358,
KFSK	-6.4	Tyr446,Gly449,Gln361,	Gln448,Glu444,Val445,
		Gln448	His447,Glu357,His447,
			Glu357
		Hydrogen Bond Interactions	**Hydrophobic Interactions**
Compound (PubChem ID)	**Binding energy(kcal/mol)**	**Amino Acid Residue**	**Amino Acid Residue**
Stattic (2779853)	-6.1	Gly449,Tyr446,Gln361	Gln448,Tyr446,Glu357,

His447

Table 6. Binding energy and interactions of potential active SCPs and standard molecule (1400W) on NOS2 (PDB ID: 4NOS).

		Hydrogen Bond Interactions	Hydrophobic Interactions
Peptide sequence	**Binding energy(kcal/mol)**	**Amino Acid Residue**	**Amino Acid Residue**
HVTK	-6.6	Gln149,Ser486,Ser453	Asn148,Glu145,Lys103,
			Leu485,An196,Arg195,
			Gln192,Arg454,Ser153,
			Gly152
KILK	-6.4	Ser486,Gln149,Glu145	Lys105,ALa104,Leu485,
			Lys103,Gln192,Gly152,
			Leu100,Ser153,Pro273
KAGVK	-6.1	Ser153,Gln192,Asn196,	Gly152,Arg195,Gln149,
		Arg454,Ser453	Ser486,Leu485,Glu145,
			Lys103,Leu100
KIGGK	-6.0	Glu450,Arg454,Asn196,	Ser453,Trp206,Leu100,
		Lys103,Gln192,Arg195	Leu485,Ser486,Gly152,
			Gln149,Ser153
KAGLK	-5.8	Gln149,Gln192,Asn148,	Arg454,Arg195,Gly275,
		Glu145	Asp274,Pro273,Gly152,
			Ser486,Lys103,Phe188,
			Leu485,Leu100
KAIGK	-5.6	Arg454,Ser486,Gln192	Gln149,Lys103,Leu485,
			Leu100,Glu145,Asn148,
			Gly152
HMCK	-5.5	Gln192,Ser486,Glu245	Ser153,Gly152,Arg195,
		Asn196,Arg454	Lys103,Leu485,Gln149,
			Leu100,Ser453
KIAGK	-5.5	Glu145,Gln192,Arg195	Lys103,Leu100,Leu485,
			Arg454,Gln149,Asn148
KVIK	-5.5	Glu145,Asn196,Gln192,	Ser486,Leu485,Gln149,

		Arg454	Asn148,Pro273,Lys103
KALK	-5.5	Ser486,Gln149,Asp274	Leu100,Gly152,Pro273,
			Asn148,Glu145,Lys103,
			Leu485
RVVK	-5.4	Gln192	Ser153,Arg454,Gln149,
			Gly152,Asp274,Asn148,
			Lys103,Leu485,Leu100
KIIK	-5.4	Lys103,Gln192,Arg195	Leu485,Gln149,Leu100,
			Ser453,Ser153,Gly152,
			Glu145,Ser486
KIVK	-5.4	Pro273,An148,Glu145	Ser486,Lys103,Leu485,
			Asn196,Leu100,Arg454,
			Gln149,Gly152
KMCH	-5.3	Arg195,Gln192,Ser486,	Ser153,Asp274,Asn148,
		Gln149,Lys103	Gly275,Pro273,Glu145,
			Leu100,Arg454
KVGGK	-5.3	Thr121,Arg86,Thr126	Trp90,Glu479,Ile119,
			Val85,Arg83,His84,
			Leu116,Thr109,Pro122,
			Lys123
KIGK	-5.3	Gly152,Lys103,Glu145,	Asn148,Leu485,Ser486,
		Asn196,Arg454	Leu100,Gln149,Ser453
			Ser153
KCMH	-5.1	Lys103,Gln149,Gln192	Ser453,Gly152,Leu100,
			Leu485,Ser486,Ser153
KVLK	-5.1	Ile277,Asn390,Gly279	Arg278,Ser276,Leu344,

			Arg301,Ile391,Pro281,
			Tyr389,Arg388
KAGIK	-5.0	Asn196	Arg454,Arg195,Ser153,
			Leu100,Lys103,Ser486,
			Leu485,Glu145,Asn148,
			Gln149,Gly152,Gln192
		Hydrogen Bond Interactions	**Hydrophobic Interactions**
Compound (PubChem ID)	**Binding energy(kcal/mol)**	**Amino Acid Residue**	**Amino Acid Residue**
1400W(1433)	-5.2	Gln97	Gly455,Arg452,Tyr451,
			Met94,Gln448,Thr95,
			Phe96

Figures

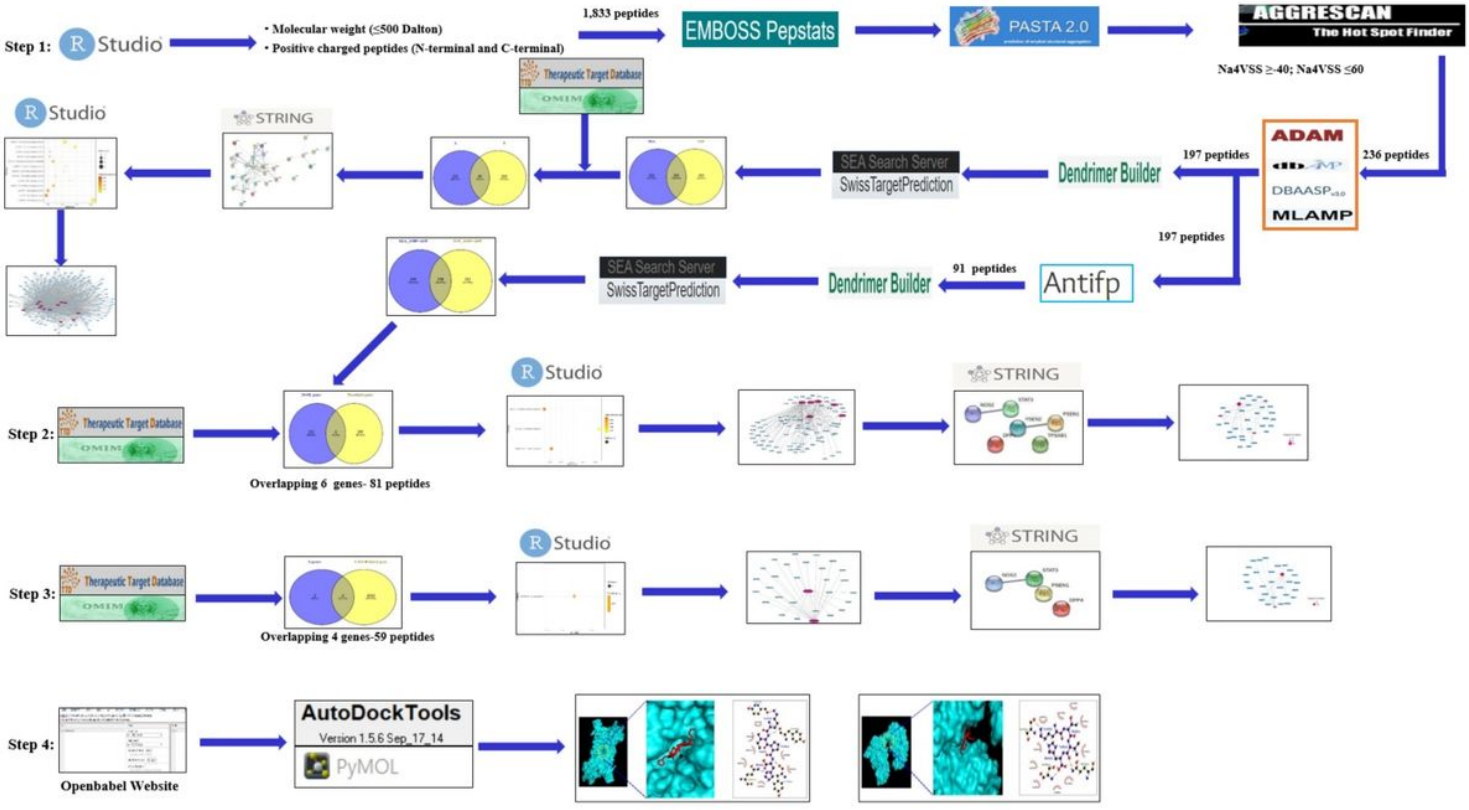


Figure 1

Workflow of AMPs-AFPs-ACPs axis analysis on network pharmacology.

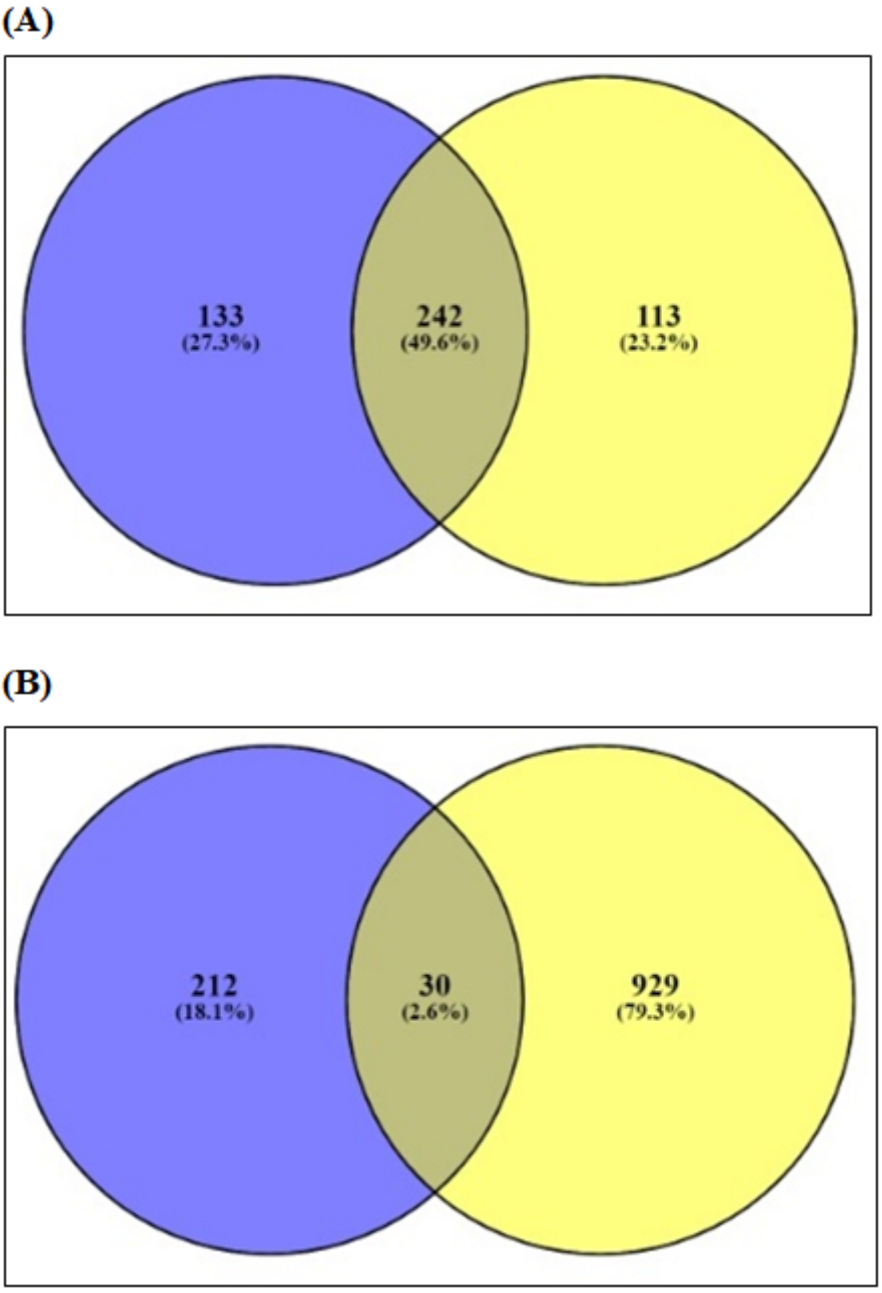


Figure 2

(A) The number of 242 overlapping targeted targets from SEA (375 targets) and STP (355 targets) on AMPs- targets. (B) The number of 30 overlapping targets between the number of 242 overlapping targets and 959 bacterial respond targets.

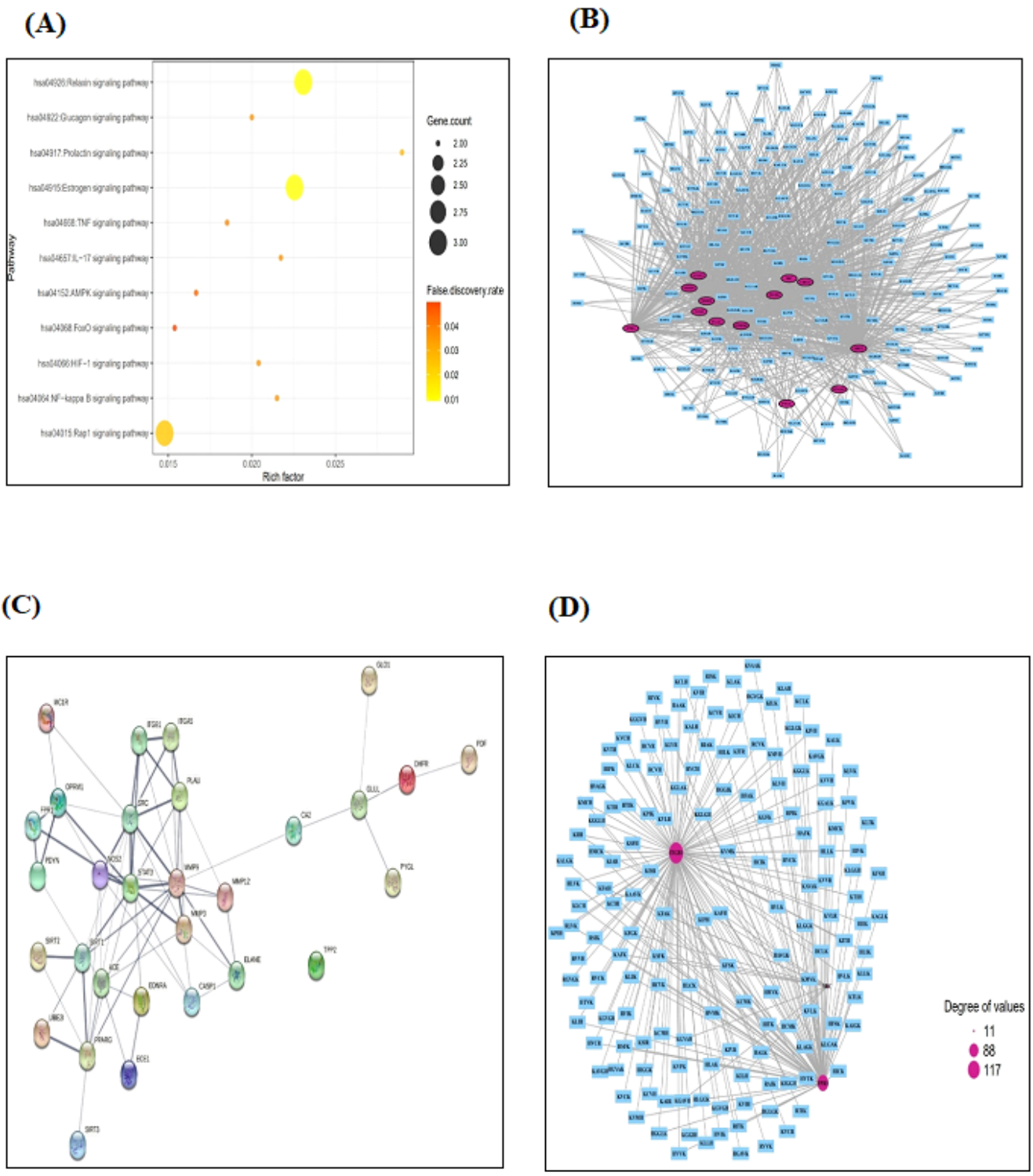
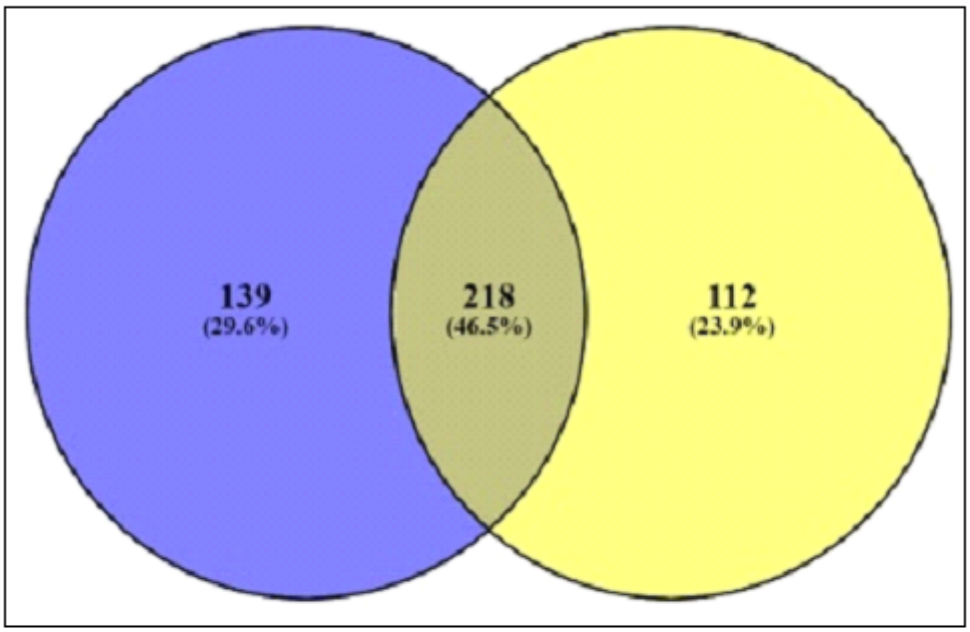


Figure 3

(A) The number of 11 signaling pathways on AMPs. (B) Networks of 11 signaling pathways on 210 nodes (197 Peptides, 13 Targets) and 1011 edges. (C) Protein-protein networks of 30 targets responded to bacterial infection (D) Size map of Rap1 signaling pathway on SRC, FRC1, and ITGB1 targets (158 nodes and 216 edges).

(A)



(B)

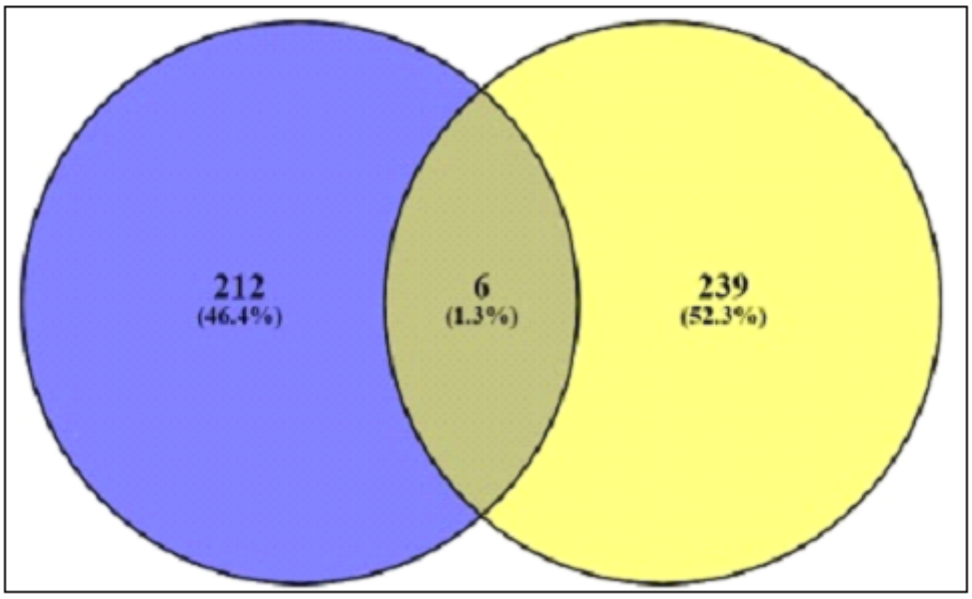


Figure 4

(A) The overlapping 218 targets identified by SEA (357 targets) and STP (330 targets) on AFPs-targets. (B) The number of 6 overlapping targets between the number of 218 overlapping targets and 245 fungal-related targets.

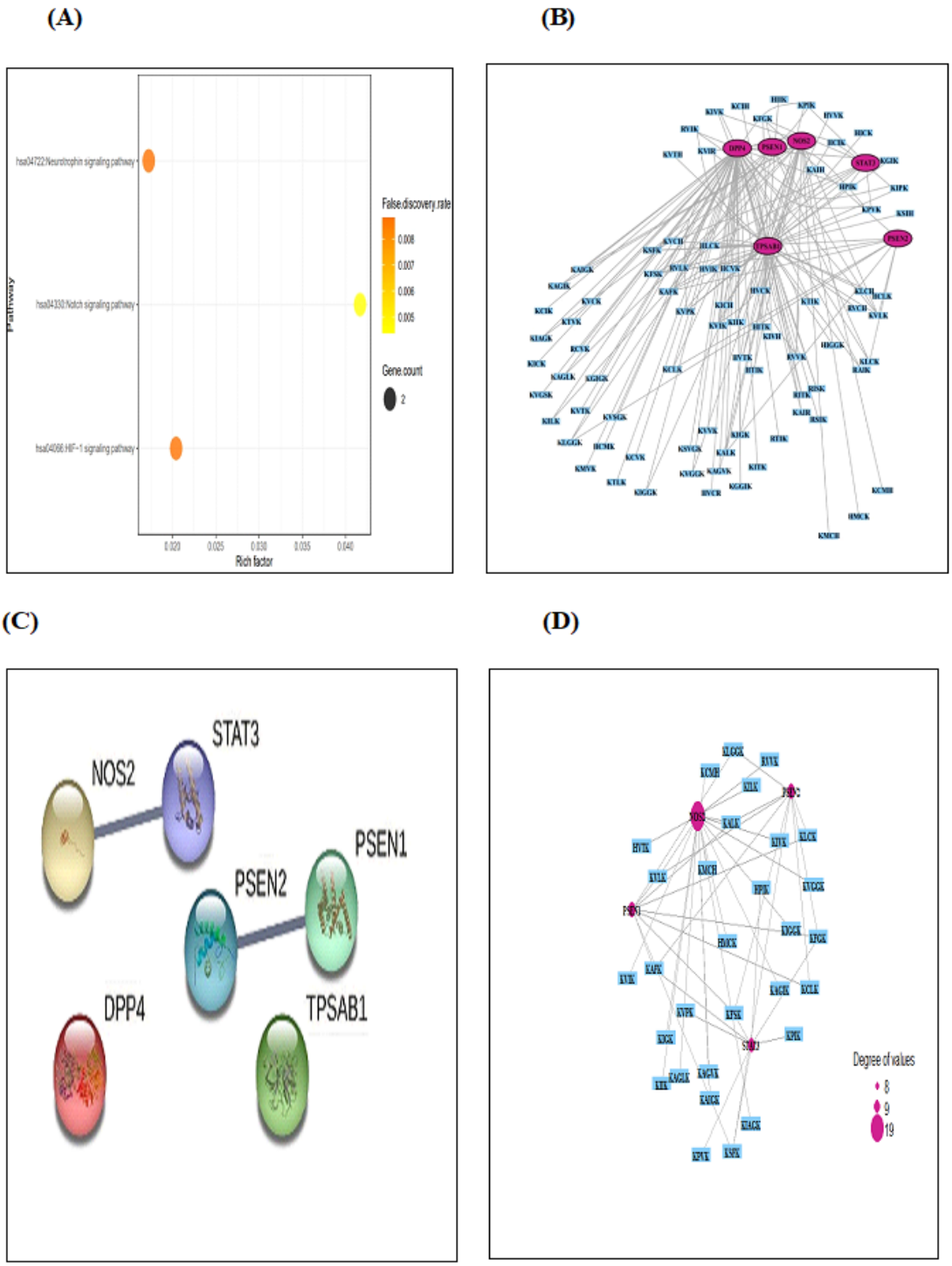


Figure 5

(A) The number of 3 signaling pathways on AMPs-AFPs axis (B) Networks of 3 signaling pathways on 87 nodes (81 peptides and 6 targets) and 150 edges. (C) Protein-protein networks of AMPs-AFPs (6 targets) (D) Size map of Notch signaling pathways on NOS2, STAT3, PSEN1, and PSEN2 (34 nodes and 45 edges).

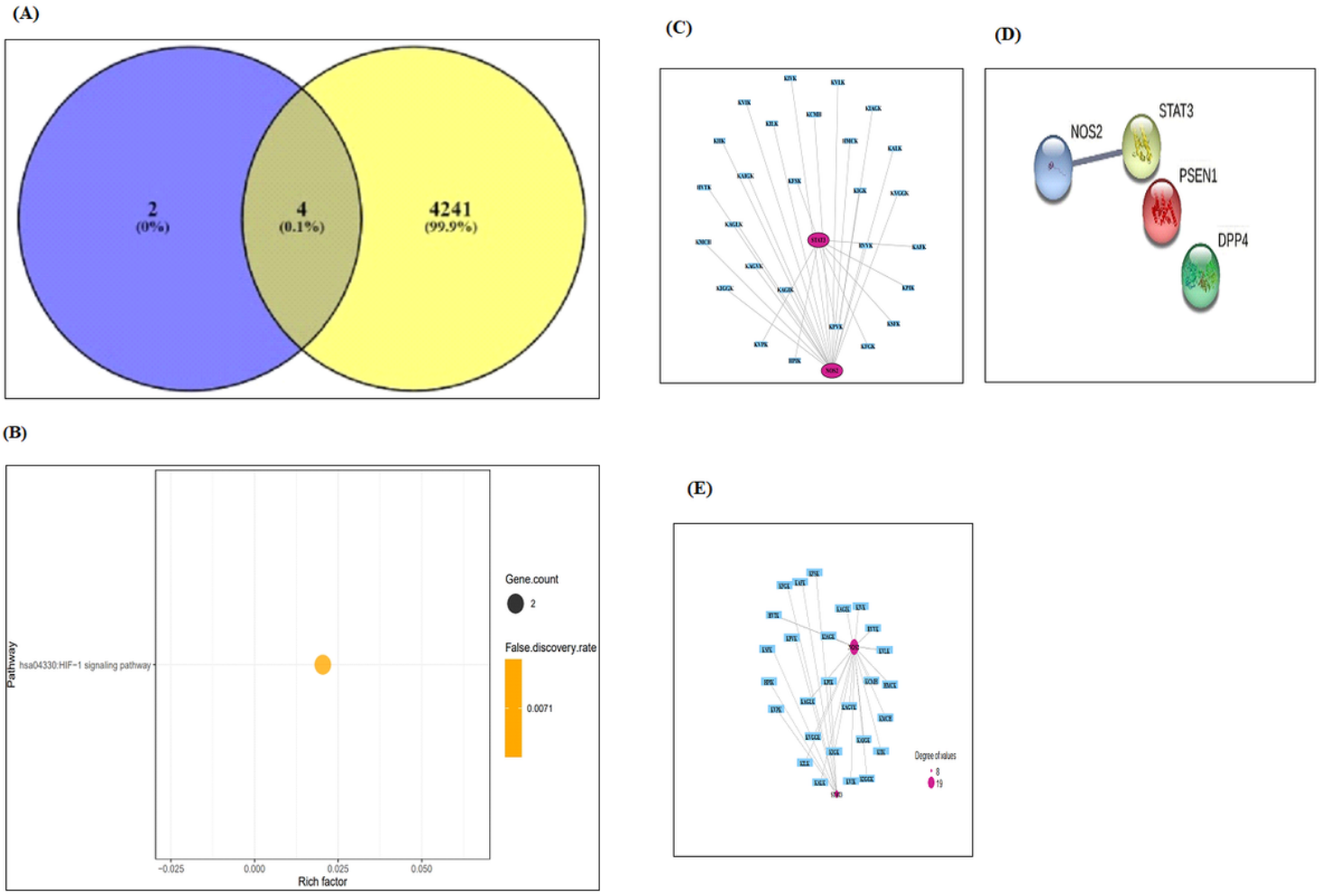


Figure 6

(A) Overlapping targets (4 targets) between AMPs-AFPs axis (6 targets) and cancer- related targets (4,245 targets). (B) The number of 1 signaling pathway on AMPs-AFPs-ACPs axis (C) Networks of HIF-1 signaling pathway on 29 nodes (27 peptides and 2 targets) and 27 edges. (D) Protein-protein networks of AMPs-AFPs-ACPs axis (4 targets) (E) Size map of HIF-1 signaling pathway on NOS2 and STAT3 (29 nodes and 27 edges).

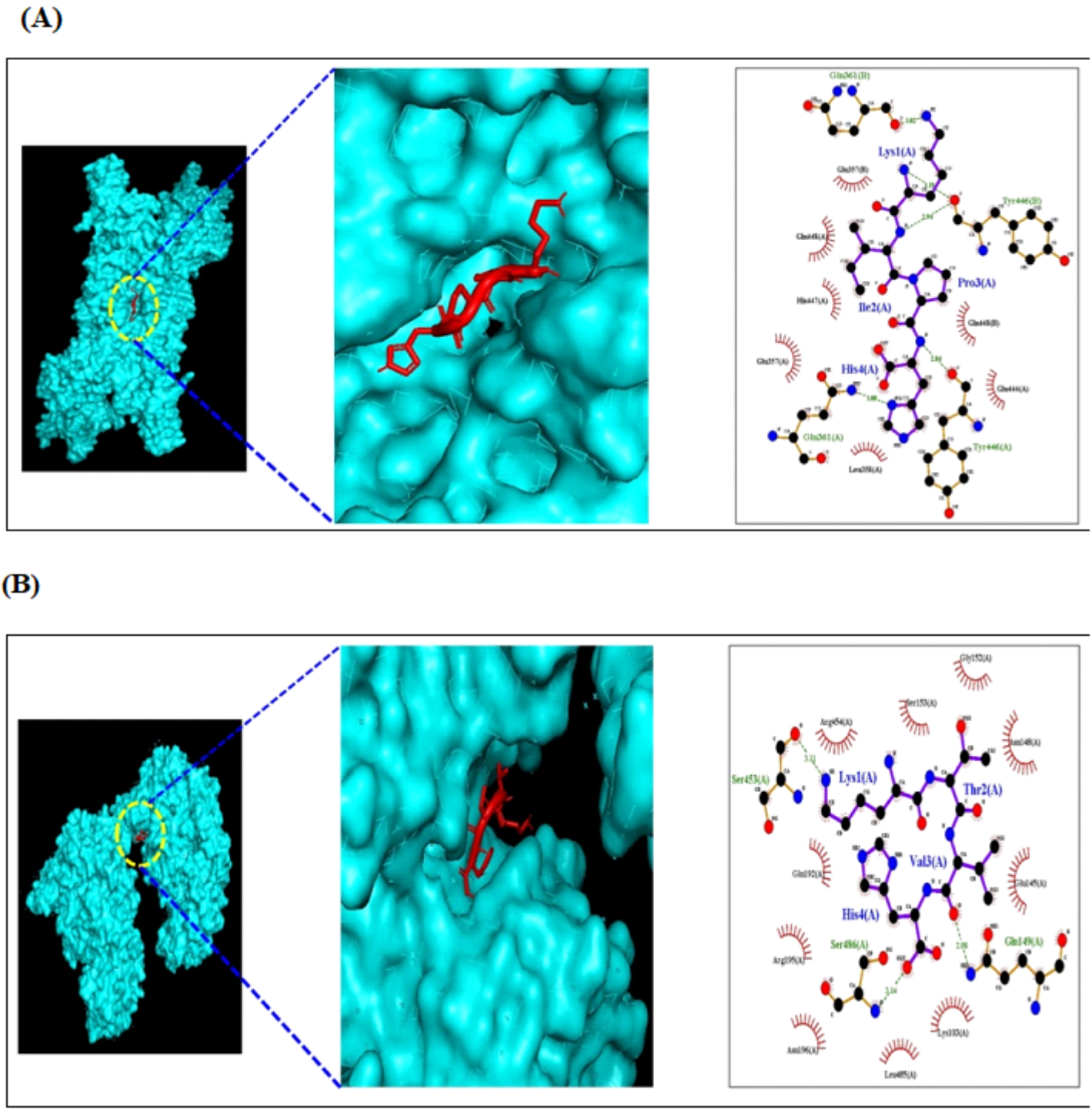


Figure 7

Molecular docking interaction between best docked SCPs and targets. (A) HPIK on STAT3 (PDB ID: 6TLC) (B) HVTK on NOS2 (PDB ID: 4NOS).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1.pdf
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