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Network Pharmacology Identifies AKT1, SRC, and STAT3 as Therapeutic Targets of Tempeh-Derived Peptides in Breast Cancer

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Abstract

Breast cancer remains a major cause of mortality among women, particularly the aggressive subtypes HER2-positive and triple-negative breast cancer (TNBC). Fermented foods such as tempeh contain bioactive peptides with potential therapeutic properties, including anticancer activity, yet their molecular mechanisms in cancer remain unclear. This study aimed to investigate the potential of tempeh-derived peptides as anti-breast cancer agents using a network pharmacology approach integrated with molecular docking. Tempeh peptides were collected from previously published literature. Target genes of tempeh-derived peptides were predicted and compared with breast cancer–associated genes to identify overlapping candidates. These were analyzed through protein–protein interaction networks and subjected to functional and pathway enrichment to uncover key molecular mechanisms. The results showed that tempeh-derived peptides are closely linked to key oncogenic pathways, including PI3K-Akt, ErbB, MAPK, JAK-STAT, and general cancer signaling. Protein–protein interaction network analysis highlighted AKT1, SRC, STAT3, and PIK3CA as central hub proteins with well-established roles in regulating proliferation, migration, angiogenesis, and survival. AKT1 is strongly connected to HER2-driven signaling, SRC is involved in both HER2+ and therapy-resistant TNBC, STAT3 is critically implicated in TNBC biology, and PIK3CA functions as a pivotal upstream regulator of AKT1, underscoring their therapeutic significance. Molecular docking confirmed strong binding affinities of peptides such as Trp-Met-Phe-Asp-Trp, Pro-Phe-Tyr-Phe, and Trp-Met-Gly-Pro-Tyr to these hubs, suggesting disruption of phosphorylation-dependent activation and downstream oncogenic cascades. These findings support the potential of tempeh-derived peptides as multi-target modulators in aggressive breast cancer subtypes and highlight the need for experimental validation to advance their therapeutic application.

1. Introduction

Breast cancer remains one of the most prevalent and lethal malignancies among women worldwide, with an increasing burden particularly associated with aggressive subtypes such as HER2-positive and triple-negative breast cancer (TNBC) [1, 2]. Despite advances in targeted therapies and immunotherapy, treatment resistance and systemic toxicity remain significant challenges. Consequently, there is growing interest in identifying safe, affordable, and multi-targeted therapeutic agents,

particularly those derived from natural or dietary sources [3].

Fermented foods have gained recognition for their ability to generate bioactive compounds, including peptides, with diverse health-promoting properties [4]. Tempeh, a traditional Indonesian fermented soybean product, is a notable example. The fermentation process, primarily mediated by *Rhizopus* species, results in the breakdown of soybean proteins into short-chain bioactive peptides with potential antioxidant, anti-inflammatory, immunomodulatory, and anticancer activities [5]. Several studies have reported that tempeh-derived peptides and isoflavone metabolites can modulate oxidative stress, inhibit inflammatory mediators, and induce apoptosis in cancer cell models, although most findings are limited to preliminary in vitro observations [6, 7]. These early reports suggest that tempeh may serve as a source of functional peptides with therapeutic relevance, but systematic mechanistic studies, particularly in breast cancer, are lacking.

Importantly, conventional breast cancer therapies often encounter resistance due to compensatory signaling and are associated with dose-limiting toxicities [8]. Bioactive peptides from dietary sources, including tempeh, may offer advantages in this regard, as they are typically low-molecular-weight, biodegradable, and capable of simultaneously modulating multiple targets, potentially overcoming pathway redundancy while minimizing systemic toxicity [9]. However, despite this theoretical promise, the direct link between tempeh peptides and breast cancer biology remains underexplored, and no large-scale pharmacological characterization has yet been performed.

Network pharmacology offers a holistic framework for uncovering complex interactions between bioactive compounds, protein targets, and disease-related pathways [10]. By integrating computational target prediction, protein–protein interaction (PPI) networks, and pathway enrichment analyses, this approach allows the identification of potential therapeutic mechanisms at a systems level [11, 12]. Unlike traditional experimental approaches, which can be resource-intensive and time-consuming when screening multiple compounds and targets, network pharmacology enables high-throughput, systems-level predictions that prioritize the most promising targets for validation [13]. This makes it particularly valuable in the early stages of natural product research, where peptide libraries from complex matrices such as tempeh require rational prioritization before moving into experimental assays. Such methods have been successfully applied to natural product research, but their application to tempeh-derived peptides in the context of breast cancer is still lacking.

Given that cancer progression is driven by dysregulation of multiple interconnected pathways, bioactive peptides capable of modulating these pathways hold significant promise for multi-target anticancer strategies. This study investigated the potential of tempeh-derived peptides against breast cancer using a network pharmacology approach complemented by molecular docking, aiming to identify key signaling pathways and hub proteins influenced by these peptides and to provide mechanistic insights into their therapeutic relevance.

2. Materials and Methods

A list of bioactive peptides used in this study was identified from tempeh, based on evidence obtained from published scientific literature [6, 7]. The molecular structures of these peptides were retrieved in the SMILES (Simplified Molecular Input Line Entry System) format from PubChem, providing a standardized input for subsequent computational analysis. An integrative bioinformatics framework was employed to predict and validate molecular targets associated with breast cancer. SwissTargetPrediction (http://www.swisstargetprediction.ch) and the Similarity Ensemble Approach (SEA) (https://sea.bkslab.org/) were used to predict the protein targets of the peptides. Predictions were restricted to *Homo sapiens* as the target organism, and only high-probability interactions (probability score > 0.7) were considered to ensure confidence in the results. Lower-scoring predictions were excluded to minimize false positives before subsequent analyses. Gene expression data and its association with breast cancer were obtained from GEPIA (http://gepia.cancer-pku.cn/), the Open Targets Platform (https://platform.opentargets.org/), DisGeNET (https://www.disgenet.org/), and the Comparative Toxicogenomics Database (CTD) (https://ctdbase.org/).

Network pharmacology analysis was then used to examine the interplay between peptide-associated proteins and breast cancer–related targets. Predicted targets were mapped into the STRING database (https://string-db.org/) to generate a protein–protein interaction (PPI) network, which was visualized in Cytoscape (https://cytoscape.org/). A confidence score threshold of ≥ 0.90 was applied in STRING to ensure only high-confidence interactions were included, as recommended in the STRING documentation. Topological features of the network were evaluated using Degree, Betweenness Centrality, and Closeness Centrality to identify hubs with the highest regulatory potential. These metrics were selected because they

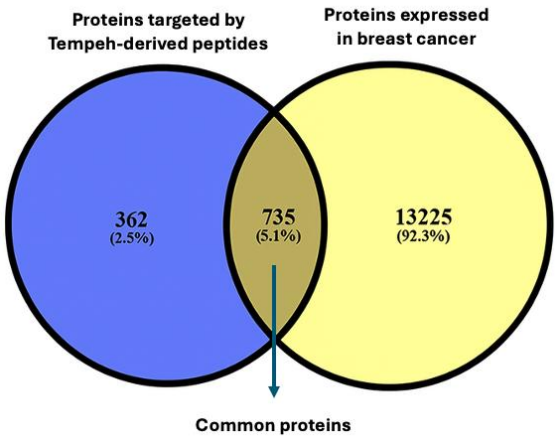

Figure 1. Venn diagram illustrating the intersection between predicted protein targets of tempeh-derived bioactive peptides (n = 1,097) and breast cancer–associated proteins (n = 13,960). A total of 735 overlapping proteins were identified, representing potential molecular targets for therapeutic intervention in breast cancer.

capture connectivity (Degree), control over information flow (Betweenness), and efficiency of communication across the network (Closeness), providing a balanced assessment of node importance.

To further refine the identification of critical targets, a Skyline Query was performed [9]. This method involves multidimensional filtering based on the dominance of proteins across the three topological metrics. Proteins not dominated in any dimension (nondominated points) were retained as key therapeutic targets.

Pathway enrichment analysis was subsequently conducted using the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases to characterize the biological functions and signaling pathways modulated by the target proteins. Only pathways with an adjusted p-value < 0.05 were considered significant to minimize false-positive enrichment. This analysis provided mechanistic insight into how tempeh-derived peptides may regulate critical molecular pathways involved in breast cancer progression. Functional enrichment analysis was further supported using ShinyGO (http://bioinformatics.sdstate.edu/go/). Molecular docking was conducted using GNINA (https://gnina.github.io/) following previously described procedures [14].

3. Results and Discussion

3.1. Literature-Based Compilation of Bioactive Peptides in Tempeh

A total of 62 bioactive peptides were compiled from tempeh based on previously published studies. These peptides are naturally occurring compounds generated through the enzymatic degradation of soybean proteins during the fermentation process. The reported peptides have been associated with various potential biological activities, supporting their relevance for further computational and pharmacological analysis [6, 7].

3.2. Overlapping Targets Between Tempeh Peptides and Breast Cancer-Associated Proteins

A total of 1,097 proteins were identified as predicted targets of tempeh-derived bioactive peptides. Separately, 13,960 proteins were found to be associated with breast cancer based on bioinformatics databases and recent scientific literature. An intersection analysis between the peptide target proteins and breast cancer–related proteins revealed 735 overlapping proteins (Figure 1). These overlapping proteins highlight a substantial molecular connection between tempeh peptides and breast cancer and were therefore considered to hold high therapeutic relevance. Accordingly, these 735 proteins were selected as the basis for subsequent network pharmacology analyses aimed at elucidating the mechanisms through which tempeh peptides may exert anticancer effects.

3.3. Key Oncogenic Pathways and Shared Molecular Targets in Breast Cancer

High-confidence PPI network analysis (confidence score ≥ 0.90) identified key molecular interactions among the 735 overlapping proteins. KEGG pathway enrichment using STRING revealed five significantly associated signaling pathways: PI3K-Akt, ErbB, MAPK, JAK-STAT, and Pathways in Cancer (Table 1). These pathways are well established for their pivotal roles in breast cancer progression and therapeutic resistance, particularly in aggressive subtypes such as triple-negative breast cancer (TNBC) and HER2-positive breast cancer [15]. Although HER2-targeted therapies have improved outcomes in HER2-positive patients, dysregulation of these pathways continues to pose therapeutic challenges and contributes to disease aggressiveness and recurrence [16].

Individually, each pathway plays a distinct role in oncogenic signaling. The PI3K-Akt pathway regulates cell survival, proliferation, and angiogenesis, and is frequently activated through PIK3CA mutations in TNBC and HER2-positive tumors [17]. The ErbB pathway, driven by HER2 and its dimerization partners, activates PI3K-Akt and MAPK cascades, promoting tumor growth and resistance [18]. The MAPK pathway is involved in cell cycle progression and invasion and may be activated through alternative mechanisms in TNBC, including KRAS or BRAF mutations [19, 20]. The JAK-STAT pathway, particularly STAT3 signaling, supports anti-apoptotic gene expression

Table 1. Top five enriched signaling pathways identified through KEGG pathway analysis of overlapping target proteins using the STRING database. These pathways are significantly associated with breast cancer pathogenesis and are particularly relevant to HER2-positive and triple-negative breast cancer (TNBC) subtypes.

Breast Cancer Signaling Pathways	Proteins
PI3K-Akt signaling pathway	AKT1, AKT2, AKT3, BAD, BCL2, BCL2L1, BRCA1, CASP9, CCND1, CCNE1, CCNE2, CDK2, CDK2, CDK4, CHRM1, CHRM2, CSF1R, EGFR, EPHA2, EPOR, FGFR2, FLT3, FLT4, GRB2, GSK3B, HSP90AA1, HSP90AB1, IGFR1, IKBKB, IL2, IL2RA, INSR, ITGA2, ITGA2B, ITGA3, ITGA4, ITGA5, ITGAV, ITGB1, ITGB3, ITGB5, ITGB6, ITGB7, JAK1, JAK2, JAK3, KDR, KIT, LPAR1, LPAR3, MAPK1, MAPK3, MDM2, MET, MTOR, NOS3, NTRK1, NTRK2, PDGFRA, PDGFRB, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PIK3R2, PIK3R3, PPP2CA, PRKCA, PRLR, PTK2, RPS6KB1, SYK, TLR2
MAPK signaling pathway	AKT1, AKT2, AKT3, CACNA1H, CASP3, CDC25B, CSF1R, DUSP6, EGFR, EPHA2, ERBB2, FGFR2, FLT3, FLT4, FOS, GRB2, IGF1R, IKBKB, IL1B, INSR, IRAK4, JUN, KDR, MAP2K7, MAPK1, MAPK3, MAPK8, MAPK9, MAPK14, MAPKAPK2, MET, NTRK1, NTRK2, PAK1, PDGFRA, PDGFRB, PLA2G4A, PPP5C, PRKCA, PRKCB, STK3, TNF
ErbB signaling pathway	ABL1, AKT1, AKT2, AKT3, BAD, EGFR, ERBB2, GRB2, GSK3B, JUN, MAP2K7, MAPK1, MAPK3, MAPK8, MAPK9, MTOR, PAK1, PIK3CA, PIK3CB, PIK3CD, PIK3R2, PIK3R3, PRKCA, PRKCB, PTK2, RPS6KB1, SRC
JAK-STAT signaling pathway	AKT1, AKT2, AKT3, BCL2, BCL2L1, CCND1, EGFR, EP300, EPOR, GRB2, IL2, IL2RA, JAK1, JAK2, JAK3, MTOR, PDGFRA, PDGFRB, PIK3CA, PIK3CB, PIK3CD, PIK3R2, PIK3R3, PRLR, PTPN2, STAT3, STAT6
Pathways in cancer	ABL1, ADCY5, AGTR1, AKT1, AKT2, AKT3, ALK, AR, BAD, BCL2, BCL2L1, BDKRB1, BIRC2, BIRC3, BIRC7, CASP3, CASP7, CASP8, CASP9, CCNA1, CCNA2, CCND1, CCNE1, CCNE2, CDK2, CDK4, CDK6, CSF1R, CTBP2, CTNNB1, CXCR4, EGFR, EGLN1, EGLN2, EGLN3, EDNRB, ENDRA, EP300, EPOR, ERBB2, ESR1, ESR2, F2, FGFR2, FLT3, FLT4, FOS, GNAI1, GNAI2, GNAI3, GNAQ, GRB2, GSK3B, GSTO1, GSTP1, HDAC1, HDAC2, HSP90AA1, HSP90AB1, IGF1R, IKBKB, IL2, IL2RA, ITGA2, ITGA2B, ITGA3, ITGAV, ITGB1, JAK1, JAK2, JAK3, JUN, KEAP1, KIT, KLK3, LPAR1, LPAR3, MAPK1, MAPK3, MAPK8, MAPK9, MDM2, MET, MMP1, MMP2, MMP9, MTOR, NCOA1, NOS2, NTRK1, PDGFRA, PDGFRB, PIK3CA, PIK3CB, PIK3CD, PIK3R2, PIK3R3, PPARG, PRKCA, PRKCB, PTGER1, PTGER2, PTGER3, PTGER4, PTGS2, PTK2, RET, ROCK2, RPS6KB1, STAT3, STAT6, WNT3, XIAP

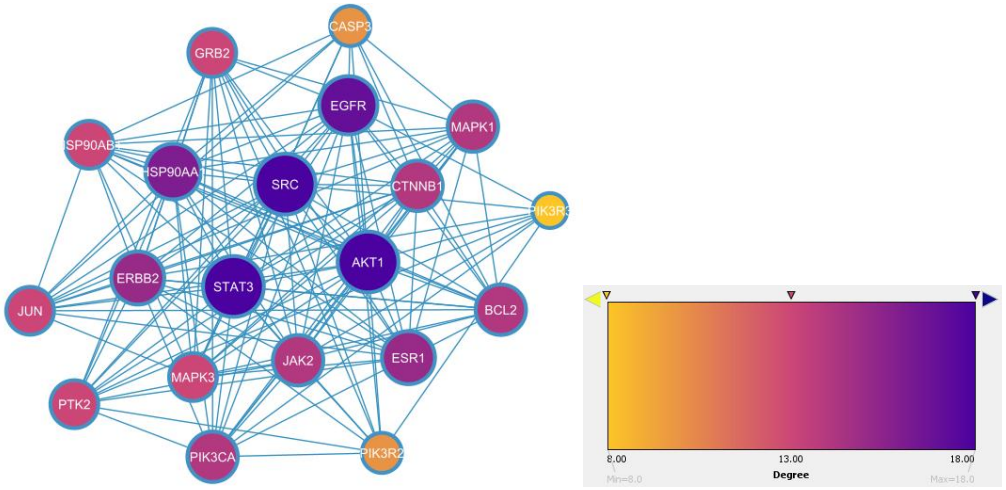

Figure 2. Protein–protein interaction (PPI) network of 147 target proteins, consisting of 1,413 interaction edges. Node size and color intensity represent degree centrality, where darker nodes indicate higher connectivity (degree). Proteins such as AKT1, SRC, and STAT3 exhibited the highest degree values, appearing as the most prominent nodes in the network. These top hub proteins are involved in five key signaling pathways—PI3K-Akt, MAPK, ErbB, JAK-STAT, and Pathways in Cancer—which are critically implicated in the pathogenesis of aggressive breast cancer subtypes, including HER2-positive and triple-negative breast cancer (TNBC).

and cancer stem cell maintenance [21], making it a promising therapeutic target in TNBC.The broad Pathways in Cancer module integrates these signaling axes, highlighting the interconnected nature of breast cancer pathogenesis. Importantly, five proteins (AKT1, AKT2, AKT3, EGFR, and GRB2) were shared across all five pathways, suggesting their role as central regulatory nodes and potential multi-target candidates for therapeutic intervention in aggressive breast cancer.

3.4. Network Topology Analysis and Identification of Key Therapeutic Targets

Following KEGG enrichment, the protein–protein interaction (PPI) network revealed 147 nodes and 1,413 edges. To facilitate interpretation, the top 20 proteins ranked by degree were prioritized (Figure 2), as these highly connected hubs are more likely to regulate critical biological processes. Visualization of the network

Table 2. Topological parameters of key proteins in the breast cancer PPI network. AKT1, SRC, and STAT3 exhibited the highest degree (18) and betweenness centrality (0.950), confirming their roles as central hub proteins. Their high closeness centrality further supports their function as rapid signal transducers in major oncogenic pathways, including PI3K-Akt, MAPK/ErbB, and JAK-STAT.

Target Protein	Degree	Betweenness Centrality	Closeness Centrality	Pathways
AKT1	18	0,950	0,042	PI3K-Akt, MAPK, ErbB, Pathways in cancer
SRC	18	0,950	0.034	PI3K-Akt, MAPK, ErbB, JAK-STAT, Pathways in cancer
STAT3	18	0.950	0.033	JAK-STAT, Pathways in cancer
EGFR	17	0.905	0.029	PI3K-Akt, MAPK, ErbB, JAK-STAT, Pathways in cancer
HSP90AA1	16	0.864	0.023	PI3K-Akt, MAPK, ErbB, JAK-STAT, Pathways in cancer
ERBB2	15	0.826	0.018	PI3K-Akt, MAPK, ErbB, JAK-STAT, Pathways in cancer
ESR1	14	0.792	0.013	JAK-STAT, Pathways in cancer
CTNNB1	14	0.792	0.012	PI3K-Akt, Pathways in cancer
MAPK1	14	0.792	0.011	PI3K-Akt, MAPK, ErbB, Pathways in cancer
BCL2	14	0.792	0.009	PI3K-Akt, Pathways in cancer
PTK2	13	0.760	0.008	PI3K-Akt, MAPK, ErbB, Pathways in cancer
GRB2	13	0.760	0.008	PI3K-Akt, MAPK, ErbB, JAK-STAT, Pathways in cancer
MAPK3	13	0.760	0.008	PI3K-Akt, MAPK, ErbB, Pathways in cancer
JUN	13	0.760	0.005	PI3K-Akt, MAPK, ErbB, JAK-STAT, Pathways in cancer
HSP90AB1	13	0.760	0.005	PI3K-Akt, MAPK, ErbB, JAK-STAT, Pathways in cancer
PIK3R2	10	0.679	0.002	PI3K-Akt, MAPK, ErbB, Pathways in cancer
CASP3	10	0.679	0.001	PI3K-Akt, Pathways in cancer
PIK3R3	8	0.633	0.000	PI3K-Akt, MAPK, ErbB, Pathways in cancer

revealed that these proteins are integrated into five major breast cancer–related pathways (PI3K-Akt, MAPK, ErbB, JAK-STAT, and Pathways in Cancer), which collectively govern proliferation, apoptosis resistance, angiogenesis, and immune modulation [22, 23]. The prominence of these pathways underscores their relevance to aggressive breast cancer subtypes, particularly HER2-positive and TNBC [24].

Topological analysis identified AKT1, SRC, and STAT3 as the most central proteins, each with the highest degree (18) and betweenness centrality (0.950), indicating strong influence within the network (Table 2). Their high closeness centrality further supports their roles as rapid signal transducers. Respectively, AKT1 regulates cell survival (PI3K-Akt) [25], SRC promotes invasion (MAPK/ErbB) [26], and STAT3 drives anti-apoptotic gene expression (JAK-STAT) [27], all contributing to breast cancer progression.

Other proteins such as EGFR, ERBB2, PIK3CA, MAPK1, and GRB2 were involved in key signaling pathways [28], but ranked lower in topological priority. Focusing on AKT1, SRC, and STAT3 aligns with multi-target therapeutic strategies aimed at disrupting central nodes in oncogenic signaling. Their interaction with tempeh-derived peptides may effectively suppress tumor-promoting pathways in breast cancer.

3.5. Identification of Key Targets Using Skyline Query

Following the topological network analysis, the Skyline Query was applied to further illustrate the strategic position of each protein within the network. This approach integrates three topological parameters (Degree, Betweenness Centrality, and Closeness Centrality) into a three-dimensional representation, enabling a clearer depiction of protein distribution and connectivity hierarchy [11]. In this framework, proteins identified numerically can also be evaluated in terms of their spatial relevance and regulatory significance, particularly within HER2-positive and TNBC subtypes. The skyline visualization highlighted the central importance of AKT1, SRC, and STAT3, which clustered within the skyline frontier, a region comprising nodes with strong performance in at least one parameter and not fully dominated by others (Figure 3).

3.6. Pathway Enrichment Analysis

KEGG pathway enrichment revealed the top 20 pathways associated with the predicted protein targets. As shown in Figure 4, three of the five principal pathways, namely PI3K-Akt signaling, ErbB signaling, and Pathways in Cancer, were directly confirmed. The other two, MAPK signaling and JAK-STAT signaling, although not listed explicitly, are likely represented within broader cancer-related categories such as breast cancer, endometrial cancer, and EGFR tyrosine kinase inhibitor resistance, all

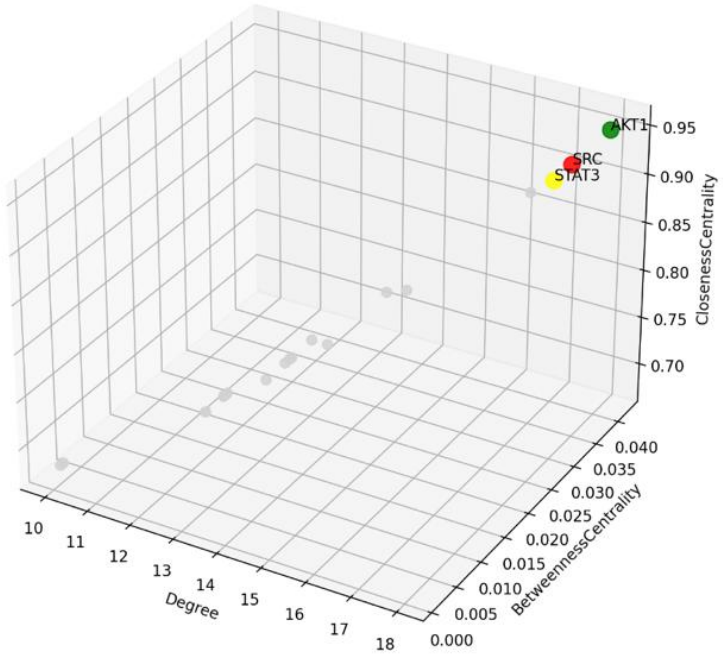

Figure 3. Skyline Query visualization of the protein–protein interaction (PPI) network. The three-dimensional plot integrates Degree, Betweenness Centrality, and Closeness Centrality to depict protein distribution and connectivity hierarchy. AKT1, SRC, and STAT3 are located within the skyline frontier, representing nodes with strong performance in at least one parameter and not fully dominated by others, underscoring their roles as central hubs in breast cancer signaling.

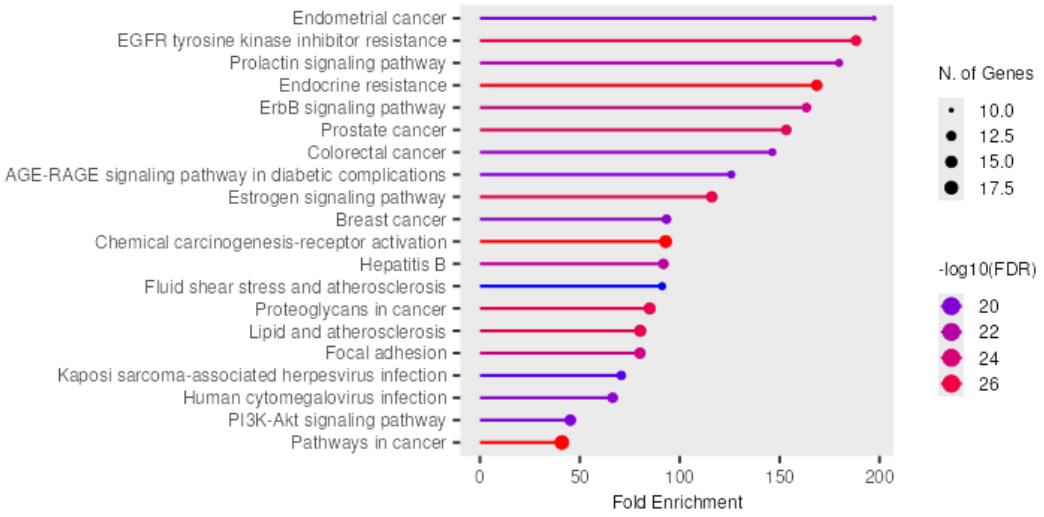

Figure 4. KEGG pathway enrichment analysis of predicted target proteins. The top 20 enriched pathways are displayed, with three principal pathways (PI3K-Akt signaling, ErbB signaling, and Pathways in Cancer) directly confirmed. MAPK signaling and JAK-STAT signaling, although not listed explicitly, are likely represented within broader cancer-related categories such as Breast cancer, Endometrial cancer, and EGFR tyrosine kinase inhibitor resistance.

of which are closely linked to cell proliferation, survival, and therapeutic resistance [29].

These results indicate that tempeh-derived peptides have the potential to act as multi-target agents involved in cancer therapy, cancer-associated coagulation, and immune modulation. Such mechanisms are particularly relevant to aggressive breast cancer subtypes including HER2-positive and TNBC. Hub proteins such as AKT1, SRC, and STAT3 were consistently enriched, emphasizing their central roles in maintaining proliferative and survival signaling in breast cancer [30].

Activation of receptor tyrosine kinases such as HER2, EGFR, and MET initiates downstream signaling through the PI3K-Akt pathway, culminating in AKT1 phosphorylation. Activated AKT1 represents a pivotal node that stimulates the mTOR pathway, thereby

promoting protein synthesis via S6K activation and transcriptional regulation. This cascade enhances tumor proliferation and survival while suppressing apoptosis [31]. In addition, mTOR signaling contributes to the transcriptional activation of PD-L1, enabling immune evasion through PD-1/PD-L1 interaction with immune cells [32]. Tempeh-derived peptides, including Trp-Met-Phe-Asp-Trp, Val-Thr-Met-Thr, Asn-Arg-Asn-Gly-Leu, Arg-Ile-Tyr, Asp-Tyr, and Gly-Phe, were predicted to interact with AKT1, with several showing the potential to interfere with its phosphorylation. Such interactions could attenuate downstream activation of mTOR signaling and reduce PD-L1 expression, thereby limiting proliferative and immune-evasive mechanisms in breast cancer cells. This mechanism is consistent with study that indicating that AKT is one of most critical molecules in the Apoptin signaling pathway, where its activation reduces BCL-2 expression, and induce apoptosis in breast cancer cells [33].

Although the MAPK pathway was not explicitly listed in gen-set enrichment, it remains central in breast cancer biology. Activation of the Ras–BRAF–MEK–ERK axis leads to expression of transcription factors such as MYC and HIF-1α, which promote proliferation, hypoxia adaptation, and angiogenesis [34]. The hub protein SRC, identified in this study, acts as a non-receptor tyrosine kinase that enhances MAPK signaling by activating EGFR and integrins, accelerating downstream ERK and mTOR activation. Peptides such as Pro-Phe-Tyr-Phe, Asp-Ala-Gly-Pro-Tyr-Gly-Pro-Ile, Val-Thr-Met-Thr, Asn-Arg-Asn-Gly-Leu, Gly-Phe, and Glu-Phe were predicted to interact with SRC, suggesting a potential mechanism for reducing focal adhesion formation, impairing MAPK/ERK signaling, and thereby suppressing tumor cell migration and invasion. Such effects are supported by experimental findings showing that fermented soybean peptides decrease SRC phosphorylation, disrupt integrin signaling, and limit breast cancer cell motility.

The ErbB signaling pathway integrates HER2 activation with downstream PI3K-Akt and MAPK cascades. Upon activation, HER2 recruits adaptor proteins that stimulate both PI3K and SRC, thereby reinforcing proliferative and anti-apoptotic signaling pathways [35]. In HER2-positive breast cancer, hyperactivation of the HER2–SRC–AKT1 axis has been recognized as a key driver of tumor aggressiveness and therapeutic resistance [36]. Peptides predicted to interact with PIK3CA and AKT1 may attenuate HER2-mediated PI3K-Akt signaling, whereas targeting SRC could suppress ERK phosphorylation downstream of EGFR. Together, these interactions may suppress proliferative and metastatic signaling in HER2-driven breast cancer.

The JAK-STAT pathway, particularly STAT3, also emerged as a key regulatory axis. Cytokines including IL-6 and IFN-γ activate receptor-associated kinases that phosphorylate STAT3, enabling dimerization and nuclear translocation. Activated STAT3 upregulates pro-survival genes (BCL-2, Cyclin D1) and immunosuppressive molecules such as PD-L1 (26). Peptides including Pro-Phe-Tyr-Phe, Ala-Phe, Thr-Tyr, and Glu-Phe were predicted to disrupt STAT3 phosphorylation or dimerization, thereby inhibiting transcription of angiogenic (VEGF), anti-apoptotic (BCL-XL), and proliferative (Cyclin D1) genes. Such inhibition has been reported for peptides that interfere with the STAT3 SH2 domain and reduce VEGF expression [36]. Importantly, STAT3 signaling is functionally interconnected with the PI3K-Akt pathway through PIK3CA activation, creating a feed-forward loop that amplifies tumor cell survival and immune evasion. Thus, modulation of both STAT3 and PIK3CA by tempeh-derived peptides may provide synergistic suppression of oncogenic signaling in breast cancer.

The integration of these findings is reflected in Pathways in Cancer, where PI3K-Akt, MAPK, ErbB, and JAK-STAT signaling converge. PIK3CA, together with AKT1, SRC, and STAT3, functions as an interconnected hub system coordinating proliferative, angiogenic, invasive, and immune-evasive processes. Their convergence explains the aggressive clinical behavior of HER2-positive and TNBC tumors and the limited efficacy of single-target therapies. By acting on multiple hubs simultaneously, tempeh-derived peptides—including Val-Thr-Met-Thr, Asp-Tyr, Gln-Ala-Phe, Ser-Leu-Cys-Phe, Pro-Phe-Tyr-Phe, Ala-Phe, Thr-Tyr, Glu-Phe, Trp-Met-Gly-Pro-Tyr, and Arg-Ile-Tyr—may suppress redundant signaling and achieve more effective inhibition of breast cancer progression.

This multitarget potential aligns with the principles of network pharmacology, which emphasizes that complex diseases such as cancer require interventions acting on multiple proteins and pathways rather than single targets [37, 38]. By simultaneously modulating PIK3CA, AKT1, SRC, and STAT3, these peptides may attenuate proliferation, invasion, angiogenesis, and immune evasion, highlighting their promise as natural candidates for therapeutic development in aggressive breast cancer subtypes.

3.7. Molecular Docking Analysis

Molecular docking was applied to assess the stability and specificity of interactions between tempeh-derived peptides and key oncogenic proteins. Lower (more negative) binding affinities reflect stronger and more stable interactions [39], indicating potential inhibitory effects on central regulators such as SRC, AKT1, and

Table 3. Molecular docking results of tempeh-derived peptides against the hub proteins SRC, AKT1, and PIK3CA using GNINA. Binding affinity values (kcal/mol) represent the predicted interaction strength, where lower (more negative) scores indicate stronger and more stable binding. Highlighted peptides with the most favorable affinities include Pro-Phe-Tyr-Phe for SRC (–9.27 kcal/mol), Trp-Met-Phe-Asp-Trp for AKT1 (–11.16 kcal/mol), and Trp-Met-Gly-Pro-Tyr for PIK3CA (–9.74 kcal/mol).

Target Protein	Peptides	Affinity (Kcal/mol)	CNN Affinity	Intramol (Kcal/mol)
SRC	Pro-Phe-Tyr-Phe	-9.27	2.820	23.20
SRC	Asp-Ala-Gly-Pro-Tyr-Gly-Pro-Ile	-8.31	3.946	47.33
SRC	Val-Thr-Met-Thr	-8.07	4.914	2.18
SRC	Asn-Arg-Asn-Gly-Leu	-5.21	4.078	0.64
SRC	Gly-Phe	-5.04	3.929	-0.53
SRC	Glu-phe	-4.80	3.397	0.68
AKT1	Trp-Met-Phe-Asp-Trp	-11.16	3.844	31.23
AKT1	Val-Thr-Met-Thr	-8.76	7.220	-2.16
AKT1	Asn-Arg-Asn-Gly-Leu	-6.60	4.757	-2.79
AKT1	Arg-Ile-Tyr	-5.81	4.693	-1.51
AKT1	Asp-Tyr	-4.36	3.489	0.37
AKT1	Gly-Phe	-3.86	2.911	-0.47
PIK3CA	Trp-Met-Gly-Pro-Tyr	-9.74	4.864	27.00
PIK3CA	Val-Thr-Met-Thr	-7.84	6.720	1.34
PIK3CA	Val-Thr-Met-Thr	-7.84	6.720	1.34
PIK3CA	Leu-Asp-Gln-Thr-Pro	-7.35	5.134	-1.23
PIK3CA	Asn-Arg-Asn-Gly-Leu	-6.75	6.501	-0.61
PIK3CA	Gly-Phe	-5.85	4.448	0.05

STAT3. The docking results (Table 3) confirmed that several peptides exhibited strong affinities toward these hubs, consistent with their proposed roles as multi-target inhibitors. Although STAT3 emerged as a hub in the network analysis, docking results showed weak binding (data not shown), which is likely attributable to the structural characteristics of its SH2 domain that generally requires phosphotyrosine-mimetic motifs for effective inhibition. In contrast, PIK3CA demonstrated consistently favorable interactions and functions as a critical upstream regulator of AKT1 within the PI3K-Akt pathway, thereby representing a more biologically and structurally relevant target.

The SRC kinase showed the most favorable binding with the tetrapeptide Pro-Phe-Tyr-Phe (–9.27 kcal/mol), followed by Asp-Ala-Gly-Pro-Tyr-Gly-Pro-Ile (–8.31 kcal/mol) and Val-Thr-Met-Thr (–8.07 kcal/mol). These interactions suggest interference with MAPK/ERK signaling and cytoskeletal reorganization, thereby limiting migratory and invasive potential. In the case of AKT1, the pentapeptide Trp-Met-Phe-Asp-Trp exhibited the strongest binding (–11.16 kcal/mol), while Val-Thr-Met-Thr (–8.76 kcal/mol), Asn-Arg-Asn-Gly-Leu (–6.60 kcal/mol), and Arg-Ile-Tyr (–5.81 kcal/mol) also showed favorable affinities. These peptides may impair phosphorylation-dependent AKT1 activation, with downstream suppression of mTOR signaling and reduced PD-L1 expression, mechanisms directly linked to cell proliferation and immune evasion [40].

Docking with PIK3CA revealed Trp-Met-Gly-Pro-Tyr as the top candidate (–9.74 kcal/mol), followed by Val-Thr-Met-Thr (–7.84 kcal/mol) and Leu-Asp-Gln-Thr-Pro (–7.35 kcal/mol). These interactions point to a potential inhibition of PI3K catalytic activity, thereby reducing AKT1 activation and dampening proliferative signaling. The findings indicate that Trp-Met-Phe-Asp-Trp (AKT1, –11.16 kcal/mol), Pro-Phe-Tyr-Phe (SRC, –9.27 kcal/mol), and Trp-Met-Gly-Pro-Tyr (PIK3CA, –9.74 kcal/mol) are the most promising candidates. Their consistently strong binding affinities highlight the potential of tempeh-derived peptides to function as multi-target modulators capable of attenuating proliferative, survival, and migratory signaling in breast cancer.

In the docking results, several peptides demonstrated favorable binding affinities to key oncogenic proteins, supporting their potential as modulators of critical signaling pathways. Nevertheless, it is important to acknowledge pharmacokinetic challenges that may limit the therapeutic application of dietary peptides. These include potential issues with gastrointestinal stability, susceptibility to enzymatic degradation, and limited cell permeability, all of which could reduce their bioavailability. While these factors may constrain direct translational potential, fermentation-derived peptides remain attractive leads, particularly as scaffolds for

optimization or as part of functional food-based strategies where cumulative dietary exposure may confer measurable biological effects.

4. Conclusions

This study suggests that tempeh-derived peptides have the potential to act as multi-target modulators in breast cancer by interacting with key signaling hubs such as AKT1, SRC, STAT3, and PIK3CA Pathway enrichment connected these proteins to PI3K-Akt, ErbB, MAPK, JAK-STAT, and other cancer-related pathways involved in proliferation, survival, migration, and immune evasion. Molecular docking indicated strong binding of peptides including Trp-Met-Phe-Asp-Trp, Pro-Phe-Tyr-Phe, and Trp-Met-Gly-Pro-Tyr, consistent with potential interference in phosphorylation-dependent signaling. AKT1 appears linked to HER2-driven oncogenic signaling, SRC is associated with both HER2+ and therapy-resistant TNBC, while STAT3 plays a central role in TNBC biology, highlighting their relevance as potential targets. While these results provide mechanistic hypotheses, experimental validation is required to confirm the biological activity and therapeutic potential of tempeh-derived peptides in aggressive breast cancer subtypes.

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