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Original Article

Mechanistic insights into the anticancer, anti-inflammatory, and antioxidant effects of yellowfin tuna collagen peptides using network pharmacology

Tara S. Kairupan[1,2], Nova H. Kapantow[3*], Trina E. Tallei[4,5*], Nurdjannah J. Niode[1,2], Yulianty Sanggelorang[6], Linda WA. Rotty[7,8], Herlina IS. Wungouw[9], Shirley ES. Kawengian[1,2], Fatimawali Fatimawali[10] and Nur B. Maulydia[11]

[1]Department of Dermatology and Venereology, Faculty of Medicine, Universitas Sam Ratulangi, Manado, Indonesia; [2]Department of Dermatology and Venereology, Faculty of Medicine, Prof. Dr. R. D. Kandou Hospital, Manado, Indonesia; [3]Department of Nutrition, Faculty of Medicine, Universitas Sam Ratulangi, Manado, Indonesia; [4]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sam Ratulangi, Manado, Indonesia; [5]Department of Biology, Faculty of Medicine, Universitas Sam Ratulangi, Manado, Indonesia; [6]Public Health Science Study Program, Faculty of Public Health, Universitas Sam Ratulangi, Manado, Indonesia; [7]Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Universitas Sam Ratulangi, Manado, Indonesia; [8]Department of Internal Medicine, Faculty of Medicine, Prof. Dr. R. D. Kandou Hospital, Manado, Indonesia; [9]Department of Physiology, Faculty of Medicine, Universitas Sam Ratulangi, Manado, Indonesia; [10]Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Universitas Sam Ratulangi, Manado, Indonesia; [11]Graduate School of Mathematics and Applied Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia

*Corresponding authors: novakapantow@unsrat.ac.id (NHK); trina_tallei@unsrat.ac.id (TET)

Abstract

Marine-derived collagen peptides have been acknowledged for their therapeutic potential, especially in cancer therapy and inflammation management. The aim of this study was to investigate the molecular mechanisms that contribute to the anticancer, anti-inflammatory and antioxidant properties of yellowfin tuna collagen peptides (YFTCP) utilizing a network pharmacology approach. The YFTCP was extracted from the bones of yellowfin tuna (*Thunnus albacares*) and subsequently hydrolyzed with trypsin. Seventeen peptides were discovered using liquid chromatography in conjunction with high-resolution mass spectrometry (LC-HRMS). A network pharmacology method was utilized to investigate the interactions between the discovered peptides and their biological targets. Additionally, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed to identify pertinent biological pathways involved in the anticancer, antioxidant, and anti-inflammatory effects of these peptides. GO analysis revealed key associations between YFTCP and critical cancer- and inflammation-related genes encoding proteins such as CCND1, SRC, AKT1, IL-1β, TNF, and PPARG, which exhibited significant interactions. These proteins are essential for the regulation of the cell cycle, the development of tumors, and the response to inflammatory stimuli. The KEGG analysis also revealed that YFTCP was involved in a number of critical pathways, such as endocrine resistance, cancer pathways, Kaposi sarcoma-associated herpesvirus infection, proteoglycans in cancer, and human cytomegalovirus infection. These findings highlight the potential use of YFTCP as a multifaceted therapeutic agent, indicating their role in regulating important biological pathways associated with cancer development and inflammation. This study provides new valuable insights into the pharmacological properties of YFTCP, paving the way for future studies and drug development focused on these bioactive peptides.

Keywords: Yellowfin tuna collagen peptides, network pharmacology, anti-inflammatory effects, anticancer mechanism, KEGG pathway

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Introduction

Collagen peptides have attracted considerable interest in recent years because of their diverse biological effects and potential therapeutic uses [1]. These peptides are derived from various animal sources, such as cow (*Bos taurus*), pig (*Sus scrofa*), sheep (*Ovis aries*), chicken (*Gallus gallus*), duck (*Anas platyrhynchos*), horse (*Equus caballus*), salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), goat (*Capra hircus*), rabbit (*Oryctolagus cuniculus*), and turkey (*Meleagris gallopavo*) [2]. Collagen peptides are particularly recognized for their roles in enhancing skin health, facilitating wound healing, and supporting joint function [2]. Evidence has suggested that bioactive peptides are highly promising for the treatment of cancer and the regulation of inflammation [3]. Consequently, they are significant targets of pharmacological research. Marine-derived collagen, particularly from fish, offers notable benefits compared to mammalian collagen, including lower immunogenicity and enhanced bioavailability [4].

Yellowfin tuna (*Thunnus albacares*), prevalent in tropical and subtropical waters, is acknowledged as a significant source of collagen [5,6]. Additionally, it presents potential as an alternative source of antioxidant peptides, including collagen hydrolysates [7]. The collagen peptides sourced from yellowfin tuna exhibit significant antioxidant properties, as demonstrated by assays such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), which show low half-maximal inhibitory concentration (IC_{50}) values [8,9]. Peptides derived from yellowfin tuna collagen also demonstrate various bioactive properties [1]. However, despite growing interest in marine-based collagen peptides, the molecular pathways underlying the anticancer and anti-inflammatory effects of yellowfin tuna collagen peptides (YFTCP) are not well understood, indicating a promising area for future research.

Understanding the interactions between bioactive peptides and their molecular targets is crucial for identifying their therapeutic potential, especially via in silico network pharmacology. This approach is an innovative methodology that integrates systems biology and pharmacology and is acknowledged as an effective instrument for investigating the complex relationships between biological systems and pharmacological interventions [10]. Identifying essential genes and biological pathways affected by these peptides can elucidate their fundamental mechanisms of action. The aim of this study was to determine the anticancer, antioxidant, and anti-inflammatory characteristics of YFTCP using a network pharmacology approach. In this study, 18 peptides from yellowfin tuna collagen were identified using liquid chromatography-high resolution mass spectrometry (LC-HRMS). Their interactions with key genes associated with cancer and inflammation were explored through enrichment analyses, including gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. This study is expected to uncover the molecular mechanisms driving the therapeutic effects of YFTCP, providing a foundation for future drug development and therapeutic applications.

Methods

Preparation of collagen from tuna fish bones

The collagen extraction technique was adapted from a previously described method, incorporating a modification that replaced the spray dryer with a freeze dryer for the drying process of the collagen extract [11]. The tuna fish bone samples were cleaned of residual dirt, divided into small fragments, and then thoroughly washed. Each raw material (bone) was immersed in a 0.1 M NaOH solution at a 1:10 (w/v) ratio at ambient temperature (32±2°C) for 72 hours to remove non-collagen proteins and lipids, with daily changes of the NaOH solution. The fish bones were subsequently rinsed with distilled water until a neutral pH was attained. The collagen extraction procedure entailed immersing tuna fish bones in a 0.5 M CH_3COOH solution at a 1:10 (w/v) ratio for 72 hours at ambient temperature. The extraction mixture was filtered using muslin cloth to eliminate the residue from the supernatant. The supernatant underwent precipitation through the addition of 0.9 M NaCl for a duration of 24 hours, utilizing the salting-out method [11]. The precipitate was then centrifuged at 8,000 rpm for 30 minutes, followed by dehydration of the collagen using a freeze dryer for 24 hours.

Proteomic sample preparation

A 500 mg dried collagen sample was added to 15 mL of Tris-HCl buffer (pH 8.0) and vortexed for one minute, subsequently subjected to sonication for 60 minutes at 50°C. The solution was subsequently centrifuged at 7,000 × g for six minutes at ambient temperature. A total of 500 µL of the supernatant was combined with 500 µL of cold acetone (-20°C), vortexed for one minute, then centrifuged at 10,000 × g for five minutes at 4°C. Subsequent to the removal of the supernatant, the remaining sample was concentrated utilizing a vacuum concentrator (Eppendorf, Hamburg, Germany) at 60°C for a duration of 30 minutes. The concentrated sample was then resuspended in 500 µL of 50 mM Tris-HCl buffer and 500 µL of 50 mM ammonium bicarbonate. To this, 4 µL of 50 mM dithiothreitol was added, and the mixture was vortexed for one minute, followed by incubation at 75°C for 15 minutes. Subsequently, 9 µL of iodoacetamide was added, and the sample was incubated in the dark for 30 minutes. For digestion, 5 µL of 0.1 mg/mL Pierce Trypsin Protease Mass Spectrometry (MS) grade (Thermo Fisher, Waltham, USA) was added, vortexed for one minute, and incubated at 37°C for 17 hours. Finally, 10 µL of formic acid (Merck, Darmstadt, Germany) was added, and the sample was filtered through a 0.2 µm polyvinylidene fluoride (PVDF) filter, making it ready for LC-MS injection.

Liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis

The prepared samples were analyzed using a Thermo Scientific Vanquish Horizon UHPLC system (Germering, Fürstenfeldbruck, Germany), equipped with a Thermo Scientific Acclaim PepMap 100 C18 analytical column (150 mm length × 1 mm ID × 3 µm particle size). The mobile phase consisted of two solvents: solvent A, MS-grade water with 0.1% formic acid, and solvent B, MS-grade acetonitrile with 0.1% formic acid. The flow rate was established at 75 µL/min, with a total duration of 45 minutes. The gradient was implemented in the following manner: 5% solvent B was initiated for one minute, followed by a linear gradient from 5% to 50% solvent B over a duration of 30 minutes. The system was then maintained at 50% solvent B for two minutes, increased to 90% solvent B for an additional two minutes, and then finally reverted to 5% solvent B for the rest of the analysis. The column temperature was held at 30°C, and the injection volume was set to 5 µL. Mass spectrometry was conducted using an Orbitrap Exploris 240 HRMS (Thermo Scientific, Bremen, Germany). The acquisition mode used was full MS/dd-MS2 with positive polarity. The full MS resolution was set to 120,000 full-width at half maximum (FWHM) with a scan range of 350–1,500 m/z. The maximum injection time was set to 100 milliseconds, with an intensity threshold of 8,000 and charge states of 2–6. The mass tolerance was set to 5 ppm. For dd-MS2, the resolution was set to 15,000 FWHM, with a normalized collision energy of 30 and nitrogen as the collision gas. The ion source used was Optamax NG Heated Electrospray Ionization (H-ESI) (Thermo Fisher Scientific, San Jose, USA) with a spray voltage of 4,000 V in positive mode. The sheath gas was set to 10 arbitrary units (AU), the auxiliary gas to 5 AU, and the sweep gas to 1 AU. The ion transfer tube temperature was maintained at 300°C. Data were analyzed using Proteome Discoverer 2.5 software (Thermo Scientific, San Jose, USA). Sequest HT (Thermo Scientific, San Jose, USA) was used for peptide identification, while the UniProt (https://www.uniprot.org) and NCBI (https://www.ncbi.nlm.nih.gov) databases were employed for protein matching and annotation.

Network pharmacology

Protein target prediction

Peptides identified from the LC-HRMS analysis were subjected to protein target prediction using the publicly Online Mendelian Inheritance in Man (OMIM) database [12]. This resource identified potential protein targets from peptide sequences, which were filtered for relevance to cancer, oxidative stress, and inflammation pathways by integrating OMIM data with prediction confidence scores from databases that incorporate OMIM's curated gene and protein information. A Venn diagram was employed to visualize the protein target prediction [13].

Protein-protein interaction (PPI) network construction

A protein-protein interaction (PPI) network was constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database to visualize the relationships between the predicted targets [14]. The interaction network was analyzed to identify key hub proteins involved in cancer and inflammation processes, based on network topology parameters, including degree, betweenness, and closeness centrality [15]. Degree centrality identified proteins with the most connections, highlighting their importance in network stability. Betweenness centrality measured a protein's role as a bridge in connecting different network regions, which is crucial for signal mediation. Closeness centrality evaluated how efficiently a protein could spread information throughout the network.

Gene ontology (GO) and pathway enrichment analysis

The biological functions and pathways related to the predicted protein targets were investigated using GO and pathway enrichment analyses. This involved tools like STRING and the KEGG database [16,17]. GO analysis categorized the target proteins based on their roles in molecular functions, biological processes, and cellular components [18]. Meanwhile, KEGG pathway enrichment helped identify key signaling pathways, particularly those linked to cancer, inflammation, and other critical biological processes [19]. The significance of enrichment was evaluated using a p-value cutoff of <0.05.

Network visualization and analysis

The resulting PPI networks were visualized and analyzed using Cytoscape 3.8 software (Cytoscape Consortium, San Diego, USA) [20]. Key clusters within the network were identified using the Molecular COmplex Detection (MCODE) algorithm [21], and functional module analysis [22] was conducted to determine the most critical pathways influenced by YFTCP. The final step involved correlating the identified protein targets with the pathways enriched in the GO and KEGG analyses. The aim was to link specific peptides from YFTCP to molecular pathways involved in antioxidant, anticancer, and anti-inflammatory actions. This approach facilitated the identification of key molecular mechanisms potentially modulated by YFTCP, providing mechanistic insights into their therapeutic effects.

Peptide-protein target docking

Peptide and protein target preparation

Peptides identified from the LC-HRMS analysis were subjected to molecular docking studies. The peptide sequences were converted into three-dimensional (3D) structures using Chimera software (UCSF, San Francisco, USA), ensuring the correct protonation states and structural integrity. Energy minimization of the peptide structures was performed using the AMBER force field [23] in Chimera to obtain the most stable conformations for docking. The crystal structures of the identified protein targets were retrieved from the Protein Data Bank (PDB) [24]. Proteins were selected based on their relevance to cancer and inflammation pathways, as identified through network pharmacology analysis. The protein structures were cleaned by removing any water molecules, co-crystallized ligands, and ions.

Skyline query analysis

The Skyline query method (MacCoss Lab, University of Washington, Seattle, USA) [25] was employed to identify central target proteins based on multiple network metrics, including degree, betweenness centrality, closeness centrality, and clustering coefficient. This approach ensures balanced selection by filtering multi-dimensional data and identifying proteins that are not dominated in any specific metric, highlighting their significant roles in the overall network structure and function [26].

Molecular docking analysis

HPEPDOCK was employed to perform peptide-protein docking analysis [27]. The prepared peptide structures were used as ligands, and the cleaned protein structures were set as receptors. For each peptide, docking was conducted using HPEPDOCK's default parameters, with the

flexible peptide docking mode enabled to allow for peptide conformational changes during the binding process. The docking process involved placing the peptide within the predicted binding site of the protein, based on active site predictions. The binding affinity between the peptides and protein targets was evaluated based on the docking score provided by HPEPDOCK, which quantifies the strength of the peptide-protein interactions. The docking poses were ranked according to their binding energies, and the best-ranked poses were selected for further analysis. Visual inspection of the docked complexes was conducted using PDBSum [28] to examine the key interactions, such as hydrogen bonds and hydrophobic interactions. The docked complexes were further analyzed to identify specific amino acid residues involved in the peptide-protein interactions. Key residues at the binding interface were noted, and the types of interactions (e.g., hydrogen bonding, van der Waals forces) were mapped. The stability and relevance of the interactions were assessed based on docking scores and visual inspection of the binding mode. These results were compared with the known active sites and functional domains of the proteins to determine whether the peptides interacted with crucial regions involved in their biological activity. The highest rank was calculated using plotly from R packages (Plotly Technologies Inc., Montreal, Canada) [29].

Results

Collagen-derived peptides from yellowfin tuna bones

A total of 17 peptides were identified by LC-HRMS analysis. The identified peptides contained glycine (G)- and proline (P)-rich sequences, characteristic of collagen-derived peptides. The peptide sequences are presented in **Table 1**. These peptides represented fragments of the collagen protein, reflecting typical motifs found in collagen's triple-helical structure. Many peptides exhibited repeating GP sequences, a hallmark of collagen, suggesting the retention of bioactivity essential for its structural and functional roles. The peptides contained several hydroxyproline-rich sequences, indicative of their origin from collagen, which is vital for maintaining its stability and integrity.

Table 1. Peptides identified from yellowfin tuna collagen via liquid chromatography-high resolution mass spectrometry (LC-HRMS), showcasing glycine (G) and proline (P) rich sequences typical of collagen

Identity	Peptide	Glycine (G) and proline (P) sequences
A	DGEVGAQGPSGPAGPAGER	Yes
B	EGLKGNR	No
C	GAAGEGGKPGER	No
D	GADGAVGKDGPR	Yes
E	GDGGPAGPK	Yes
F	GEPGPAGVVGPAGAR	Yes
G	GETGPAGIAGPAGPAGPR	Yes
H	GFSGLDGAK	No
I	GFTGMQGPPGPSGATGEQGPAGASGPAGPR	Yes
J	GPAGAAGLR	Yes
K	GPAGSAGSAGK	Yes
L	GPSGPQGAR	Yes
M	GSEGPAGAR	Yes
N	QGPAGPGGER	Yes
O	TGAAGAAGAR	Yes
P	TGDRGETGPAGIAGPAGPAGPR	Yes
Q	TGGSCTLDGQVFADR	No

Network pharmacology

Target prediction

The Venn diagram illustrates the target prediction analysis across four categories: anti-inflammatory, antioxidant, anticancer, and general protein targets (**Figure 1**). The analysis identified a substantial number of unique protein targets in each category, with 134 in the anti-inflammatory group, 117 in the antioxidant group, 139 in the anticancer group, and 234 in the

general protein target group. These findings highlight the distinct protein targets associated with each category. Notably, no targets were shared across all four groups, highlighting the specificity of each category. However, 26 targets were shared between antioxidant, anticancer, and general protein target groups, suggesting the presence of multifunctional proteins involved in both antioxidant and anticancer activities. Additionally, one target was shared among anti-inflammatory, antioxidant, and anticancer groups, and one target was shared between anti-inflammatory and general protein targets. Furthermore, two targets were common to both anticancer and general protein targets, while five were shared between antioxidant and general protein targets, and eight were common between anti-inflammatory and general protein targets. Overlaps between pairs of groups included 45 shared targets between anti-inflammatory and antioxidant, six shared targets between anti-inflammatory and anticancer groups, and three shared targets between antioxidant and anticancer groups. Although the number of overlapping targets was relatively small, these suggested that certain targets play roles across multiple functions. Specifically, the 26 shared targets between antioxidant and anticancer categories indicated potential multifunctionality and therapeutic relevance in addressing both antioxidant and anticancer activities. The findings highlight the specificity of protein targets in each category, with overlaps suggesting multifunctional proteins.

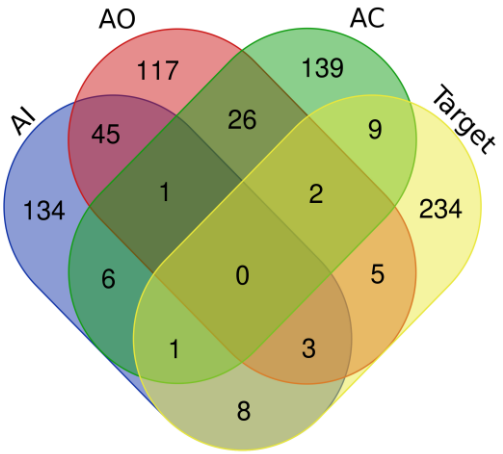

Figure 1. Venn diagram representing target prediction analysis across four categories: anti-inflammatory (AI), antioxidant (AO), anticancer (AC), and target (general protein target). The diagram illustrates both the unique and shared protein targets among the categories, highlighting distinct target groups and overlaps that suggest the presence of potential multifunctional proteins.

Protein-protein interactions

PPIs in anticancer activity

The network analysis of the peptides' anticancer activity is presented in **Table 2**. Proto-oncogene tyrosine-protein kinase (SRC), G1/S-specific cyclin-D1 (CCND1) and RAC-alpha serine/threonine-protein kinase (AKT1) were identified as central proteins with the highest degree (10), highlighting their key roles in oncogenic signaling. SRC also exhibited the highest betweenness centrality (0.194), indicating its pivotal role as a network connector. While mechanistic/mammalian target of rapamycin (mTOR) and heat shock protein 90 alpha family class A member 1 (HSP90AA1) had high degrees (9), their lower betweenness centrality indicated less central, though still important roles. In contrast, matrix metalloproteinase-2 (MMP2), mast/stem cell growth factor receptor (KIT), and thymidylate synthase (TYMS) displayed lower closeness centrality but a clustering coefficient of 1.000, signifying their inclusion in tightly interconnected clusters.

PPI analysis identified key interactions involving MMP2, mTOR, formyl peptide receptor 1 (FPR1), HSP90AA1, SRC, caspase-8 (CASP8), TYMS, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), CCND1, NAD-dependent protein deacetylase sirtuin-1 (SIRT1), AKT1, and KIT, which were associated with the anticancer activity of the selected peptide

(**Figure 2**). The proteins regulate processes such as tumor invasion, cell proliferation, apoptosis, and survival, which are critical for cancer development. The interactions of mTOR, AKT1, PIK3CA, and SRC are crucial within the PI3K/AKT/mTOR signaling pathway, which is a significant regulator of cancer cell growth and metabolism. Additionally, the proteins MMP2 and FPR1 are associated with cancer cell migration and metastasis, while CASP8 and TYMS are implicated in apoptotic processes and DNA synthesis, respectively.

Table 2. Key protein metrics in the anticancer network analysis, showing degree, betweenness centrality, closeness centrality, and clustering coefficient for each protein

Degree*	Betweenness centrality	Closeness centrality	Clustering coefficient	Name	Description
10	0.094	0.917	0.733	CCND1	G1/S-specific cyclin-D1
10	0.194	0.917	0.711	SRC	Proto-oncogene tyrosine-protein kinase
10	0.094	0.917	0.733	AKT1	RAC-alpha serine/threonine-protein kinase
9	0.012	0.846	0.889	mTOR	Mechanistic/mammalian target of rapamycin (a serine/threonine-protein kinase)
9	0.012	0.846	0.889	HSP90AA1	Heat shock protein HSP 90-alpha
8	0.003	0.786	0.964	CASP8	Caspase-8
8	0.003	0.786	0.964	SIRT1	NAD-dependent protein deacetylase sirtuin-1
8	0.006	0.786	0.964	PIK3CA	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform
7	0.000	0.733	1.000	MMP2	Matrix metalloproteinase-2 (also known as 72 kDa type IV collagenase)
6	0.000	0.688	1.000	KIT	Mast/stem cell growth factor receptor (a proto-oncogene tyrosine-protein kinase)
2	0.000	0.524	1.000	TYMS	Thymidylate synthase
1	0.000	0.500	0.000	FPR1	fMet-Leu-Phe receptor

*Degree refers to the number of direct connections or edges a specific node has in a network

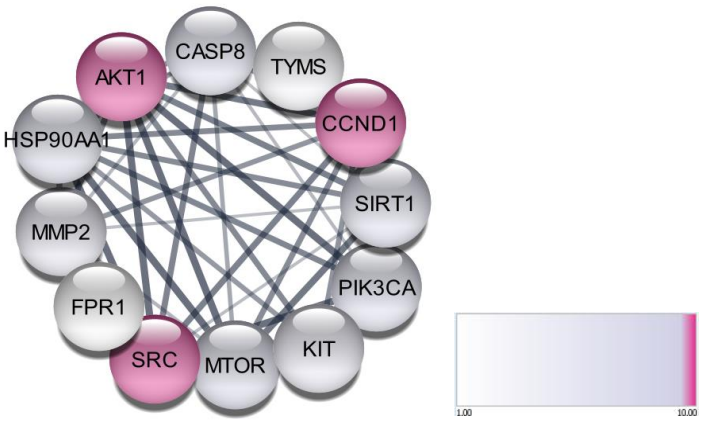

Figure 2. Protein-protein interaction (PPI) analysis of anticancer activity associated with the selected peptides. Key proteins, including matrix metalloproteinase-2 (MMP2), mechanistic/mammalian target of rapamycin (mTOR), proto-oncogene tyrosine-protein kinase (SRC), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), G1/S-specific cyclin-D1 (CCND1), and RAC-alpha serine/threonine-protein kinase (AKT1) are involved in cancer-related pathways, such as the PI3K/AKT/mTOR axis, which regulates cell growth and metabolism. MMP2 and fMet-Leu-Phe receptor (FPR1) are implicated in cell migration and metastasis, while caspase-8 (CASP8) and thymidylate synthase (TYMS) are associated with apoptosis and DNA synthesis.

PPIs in anti-inflammatory activity

The network analysis for anti-inflammatory activity, presented in **Table 3**, revealed that interleukin-1β (IL-1β) and tumor necrosis factor (TNF) were identified as the most central proteins, each exhibiting the highest degree (10), indicating their pivotal roles in inflammatory signaling. Both proteins also had the highest betweenness centrality (0.252), underscoring their critical function as connectors within the network. Caspase-1 (CASP1) followed with a degree of 7, reflecting its essential role in apoptosis and inflammation. Signal transducer and activator of transcription 6 (STAT6), peroxisome proliferator-activated receptor gamma (PPARG), and prostaglandin-endoperoxide synthase 2 (PTGS2) each had a degree of 6 and high clustering coefficients (0.933), suggesting their involvement in tightly interconnected groups regulating inflammation. CCND1, with a degree of 5 and the highest clustering coefficient (1.000), was positioned within a well-connected subnetwork. Cathepsin G (CTSG) had a moderate degree (4) but a relatively high betweenness centrality (0.182), indicating its significant bridging role. P2X purinoceptor 7 (P2RX7), major histocompatibility complex, HLA class II histocompatibility antigen DR β1 (HLA-DRB1), and presenilin-1 (PSEN1) exhibited lower degrees (2–3) but high clustering coefficients (1.000), implying their function in localized interactions. Coagulation factor II receptor-like 1 (F2RL1), with the lowest degree and centrality, appeared less involved in the broader network but may exert specific, localized effects in inflammatory processes.

Table 3. Network analysis of key proteins involved in anti-inflammatory activity, showing degree, betweenness centrality, closeness centrality, and clustering coefficient

Degree*	Betweenness centrality	Closeness centrality	Clustering coefficient	Display name	Description
10	0.252	0.917	0.444	IL-1β	Interleukin-1β
10	0.252	0.917	0.444	TNF	Tumor necrosis factor, membrane form
7	0.067	0.733	0.667	CASP1	Caspase-1
6	0.004	0.647	0.933	STAT6	Signal transducer and activator of transcription 6
6	0.004	0.647	0.933	PPARG	Peroxisome proliferator-activated receptor gamma
6	0.004	0.647	0.933	PTGS2	Prostaglandin G/H synthase 2
5	0.000	0.611	1.000	CCND1	G1/S-specific cyclin-D1
4	0.182	0.611	0.500	CTSG	Cathepsin G
3	0.000	0.550	1.000	P2RX7	P2X purinoceptor 7
2	0.000	0.524	1.000	HLA-DRB1	HLA class II histocompatibility antigen DRB1 β chain
2	0.000	0.524	1.000	PSEN1	Presenilin-1 CTF subunit
1	0.000	0.393	0.000	F2RL1	Proteinase-activated receptor 2, alternate cleaved 1

*Degree refers to the number of direct connections or edges a specific node has in a network

The PPI analysis for anti-inflammatory activity related to the chosen peptide revealed significant interactions among TNF, HLA-DRB1, PPARG, P2RX7, STAT6, PSEN1, F2RL1, CCND1, IL-1β, PTGS2, CTSG, and CASP1 (**Figure 3**). Key proteins, such as IL-1β and TNF, were positioned centrally, reflecting their pivotal roles in the network as highly connected nodes. TNF is a significant cytokine that participates in inflammation, immune regulation, and cell death, whereas IL-1β is essential in mediating immune and inflammatory responses. Both proteins demonstrated significant interactions with other proteins, notably CASP1, associated with apoptosis, and CCND1, which governs the cell cycle, in addition to F2RL1, a protein implicated in inflammation via protease signaling pathways. The network identified closely linked subgroups, such as STAT6, PPARG, and PTGS2, which serve as essential regulators of inflammation. STAT6 is crucial for IL-4/IL-13 signaling, while PPARG contributes to immune regulation and metabolism. PTGS2 is significant for the synthesis of inflammatory prostaglandins. Proteins such as HLA-DRB1, which is involved in antigen presentation, PSEN1, associated with inflammation in neurodegeneration, and P2RX7, which regulates cytokine release, were situated on the network's periphery, suggesting their specialized functions in localized inflammatory interactions. This organization or topology of the interaction network highlighted the significance

of IL-1β and TNF in inflammatory signaling, along with the auxiliary roles of other proteins in regulating the inflammatory response.

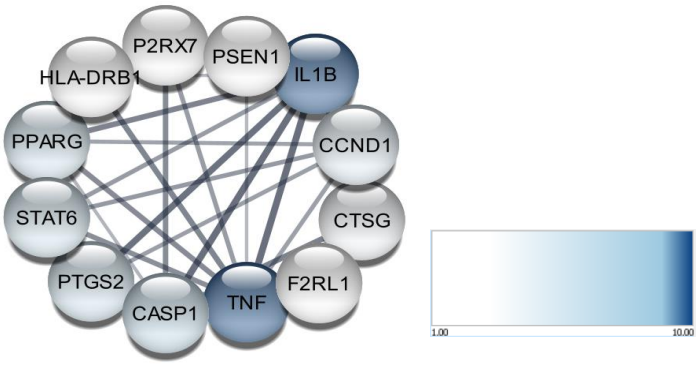

Figure 3. Protein-protein interaction (PPI) network for anti-inflammatory activity. Interleukin-1β (IL-1β) and tumor necrosis factor (TNF) are central proteins with the highest number of connections, underscoring their crucial roles in inflammatory signaling. The network also includes interactions with other significant proteins, such as caspase-1 (CASP1), G1/S-specific cyclin-D1 (CCND1), and proteinase-activated receptor 2, alternate cleaved 1 (F2RL1), while peripheral proteins like HLA class II histocompatibility antigen DRB1 β chain (HLA-DRB1), presenilin-1 CTF subunit (PSEN1), and P2X purinoceptor 7 (P2RX7) are involved in more specialized roles. This visualization emphasizes the central role of IL-1β and TNF in regulating the inflammatory response.

PPIs in antioxidant activity

The network analysis for antioxidant activity (**Table 4**) revealed PPARG as the most central protein with the highest degree (7) and betweenness centrality (0.411), highlighting its critical role in the network. PPARG also showed a relatively low clustering coefficient (0.381), indicating that it serves as a bridge in the network, connecting other proteins. TNF followed with a degree of 6 and moderate betweenness centrality (0.286), reflecting its key involvement in inflammatory responses but with fewer direct interactions than PPARG.

Table 4. Network analysis metrics for key proteins involved in antioxidant activity showing degree, betweenness centrality, closeness centrality, and clustering coefficient. PPARG and TNF as central nodes, exhibiting the highest degrees and betweenness centrality, highlighting their essential roles in regulating antioxidant processes

Degree*	Betweenness centrality	Closeness centrality	Clustering coefficient	Display name	Description
7	0.411	0.889	0.381	PPARG	Peroxisome proliferator-activated receptor gamma
6	0.286	0.800	0.467	TNF	Tumor necrosis factor, membrane form
5	0.056	0.727	0.700	IL-1β	Interleukin-1β
4	0.054	0.667	0.667	KIT	Mast/stem cell growth factor receptor
3	0.000	0.615	1.000	NOS2	Nitric oxide synthase, inducible
3	0.000	0.615	1.000	SIRT3	NAD-dependent protein deacetylase sirtuin-3, mitochondrial
2	0.000	0.533	1.000	PIK3CA	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform
1	0.000	0.500	0.000	EPHX2	Bifunctional epoxide hydrolase 2
1	0.000	0.471	0.000	CASP10	Caspase-10 subunit p23/17

*Degree refers to the number of direct connections or edges a specific node has in a network

IL-1β had a degree of 5 and a higher clustering coefficient (0.700), suggesting it was part of a tightly connected subgroup, likely involved in localized interactions within the network. KIT showed a degree of 4 and a moderate clustering coefficient (0.667), indicating its role in specific signaling pathways related to inflammation and growth factors. Proteins such as nitric oxide synthase 2 (NOS2), Sirtuin 3 (SIRT3), and PIK3CA had lower degrees (2–3) but high clustering

coefficients (1.000), meaning they were part of well-connected, localized clusters, possibly related to specific functional processes. Lastly, epoxide hydrolase 2 (EPHX2) and caspase 10 (CASP10), with the lowest degrees (1) and no betweenness centrality, played peripheral roles in the network, indicating their involvement in specialized or isolated interactions.

The PPI network highlights PPARG's central role in mediating interactions between antioxidant and inflammatory proteins, emphasizing the link between oxidative stress and inflammation in the peptide's activity (**Figure 4**). The network reveals interactions between PPARG and several key antioxidant-related proteins, such as SIRT3, EPHX2, and NOS2. Additionally, the network includes inflammatory proteins such as TNF, IL-1β and KIT. The inclusion of both antioxidant-related proteins and inflammatory proteins underscores the interconnection between oxidative stress and inflammation, suggesting that the peptides may influence pathways that link these two biological processes.

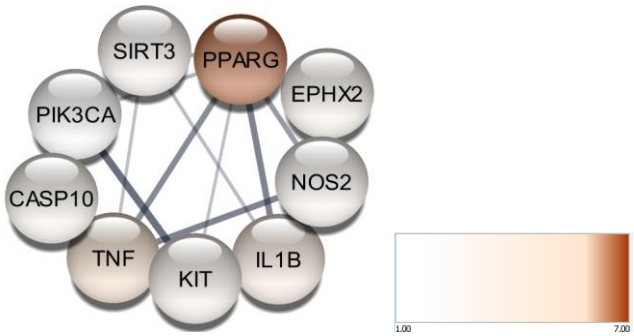

Figure 4. Protein-protein interaction (PPI) network for antioxidant activity related to the chosen peptide. Peroxisome proliferator-activated receptor gamma (PPARG) is the central protein, interacting with key antioxidant-related proteins such as NAD-dependent protein deacetylase sirtuin-3 (SIRT3), bifunctional epoxide hydrolase 2 (EPHX2), and nitric oxide synthase (NOS2). The network also includes inflammatory proteins, such as tumor necrosis factor (TNF), interleukin-1β (IL-1β), and Mast/stem cell growth factor receptor (KIT), highlighting the connection between oxidative stress and inflammation.

Enrichment of GO and KEGG pathway

The enrichment analysis (**Table 5**) identified pathways significantly linked to anticancer, anti-inflammatory, and antioxidant activities, demonstrating critical functions in cancer progression, immune response modulation, and oxidative stress reduction. Among the anticancer pathways, the pathways in cancer had the highest enrichment score (7.754) and a highly significant association (p=1.7e-8), emphasizing its pivotal role in cancer-related mechanisms. Additional significant pathways encompassed endocrine resistance (enrichment score of 8.353; p=4.4e-9), highlighting resistance mechanisms in cancer, as well as Kaposi sarcoma-associated herpesvirus infection and proteoglycans in cancer, both exhibited an enrichment score of 7.220 (p=6.0e-8), indicating the impact of viral oncogenesis on cancer progression.

Table 5. Enrichment analysis of pathways related to anticancer (AC), anti-inflammatory (AI), and antioxidant (AO) activities, highlighting significant involvement in cancer, immune responses, and oxidative stress processes

Pathway	Enrichment score	*p*-value	Count	Class
Pathways in cancer	7.754	1.7e-8	8	AC
Endocrine resistance	8.353	4.4e-9	6	AC
Kaposi sarcoma-associated herpesvirus infection	7.220	6.0e-8	6	AC
Proteoglycans in cancer	7.220	6.0e-8	6	AC
Human cytomegalovirus infection	7.018	9.5e-8	6	AC
C-type lectin receptor signaling pathway	3.921	1.2e-4	4	AI
Necroptosis	3.585	2.6e-4	4	AI
NOD-like receptor signaling pathway	3.585	2.6e-4	4	AI
Human cytomegalovirus infection	3.444	3.6e-4	4	AI
Human papillomavirus infection	3,071	8.5e-4	4	AI
Apoptosis	4.349	4.4e-5	4	AO

Pathway	Enrichment score	*p*-value	Count	Class
TNF signaling pathway	4.349	4.4e-5	4	AO
Chagas disease	4.349	4.4e-5	4	AO
Tuberculosis	4.349	4.4e-5	4	AO
Alzheimer disease	3.310	4.9e-4	4	AO

Human cytomegalovirus infection, with an enrichment score of 7.018 (p=9.5e-8), further underscored the link between viral pathways and cancer. Anti-inflammatory pathways, such as the C-type lectin receptor signaling pathway (enrichment score of 3.920; p=1.2e-4), play a role in immune modulation relevant to inflammation, while necroptosis and the NOD-like receptor signaling pathway (both with enrichment score of 3.585; p=2.6e-4) are involved in immune cell death processes that potentially reduce inflammation. Human cytomegalovirus infection and human papillomavirus infection pathways also appeared in this class, highlighting the influence of viral factors on immune responses.

Key antioxidant pathways included apoptosis and the TNF signaling pathway (both with enrichment score of 4.349; p=4.4e-5), which are critical in addressing oxidative damage, as well as Chagas disease and tuberculosis, with similar enrichment scores and p-values, suggesting cross-talk between oxidative stress and immune defenses. Alzheimer's disease (enrichment score= 3.310; p=4.9e-4) reflects oxidative stress involvement in neurodegeneration, linking this pathway to potential antioxidant effects. Overall, these enriched pathways suggested that targeting them could provide therapeutic benefits in cancer treatment, inflammation reduction, and oxidative stress management.

The pathway enrichment analysis for activities related to anticancer, anti-inflammatory, and antioxidant effects is presented in **Figure 5**. The x-axis displays enrichment scores, with dot sizes indicating the number of proteins in each pathway and color intensity representing the statistical significance (based on -log(p-value)). Anticancer pathways had the highest levels of enrichment and statistical significance, followed by those related to inflammation and antioxidants. This suggests the potential therapeutic value of these pathways in treating cancer, managing inflammation, and addressing diseases linked to oxidative stress.

Molecular docking analysis

Molecular docking analysis of 17 peptides identified by LC-HRMS (Peptide A to Peptide Q) (**Table 1**) was conducted to determine the binding affinities to selected protein targets. Based on the comprehensive Skyline analysis (**Figure 6**), which incorporated network metrics such as degree, closeness centrality, and betweenness centrality, six proteins—AKT, SRC, CCND1, IL-1β, TNF, and mTOR—were selected for their central roles in regulating key biological processes such as anticancer activity, anti-inflammatory responses, and antioxidant mechanisms. These proteins ranked highly in the analysis, highlighting their importance in the network's structure and function, and underscoring their significance in the biological system.

The docking results (**Figure 7**) further showed that some peptides demonstrated strong binding across multiple targets, whereas others, such as Peptide E, exhibited weaker but potentially more selective interactions. Notably, Peptide A displayed the lowest average binding affinity (-170.892 kcal/mol), suggesting it may engage broadly and effectively with the studied proteins, underscoring its potential as a multifunctional therapeutic agent.

The interaction between Peptide A and the TNF-α receptor is presented in **Figure 8**. This interaction was emphasized due to the pivotal role of TNF-α in regulating anticancer, anti-inflammatory, and antioxidant pathways. Peptide A exhibited a strong binding affinity for TNF-α, a key cytokine involved in immune modulation and cancer progression. Although Peptide A also demonstrated significant binding to SRC (-180.556 kcal/mol) and mTOR (-184.556 kcal/mol), the emphasis on TNF-α arose from its extensive involvement in a wide range of critical biological processes. While SRC and mTOR are integral to cancer development, inflammatory responses, and oxidative stress regulation, TNF-α's central role across these pathways makes it an essential target for therapeutic strategies. Therefore, our focus on TNF-α based on its significant relevance within the framework of the study's objectives.

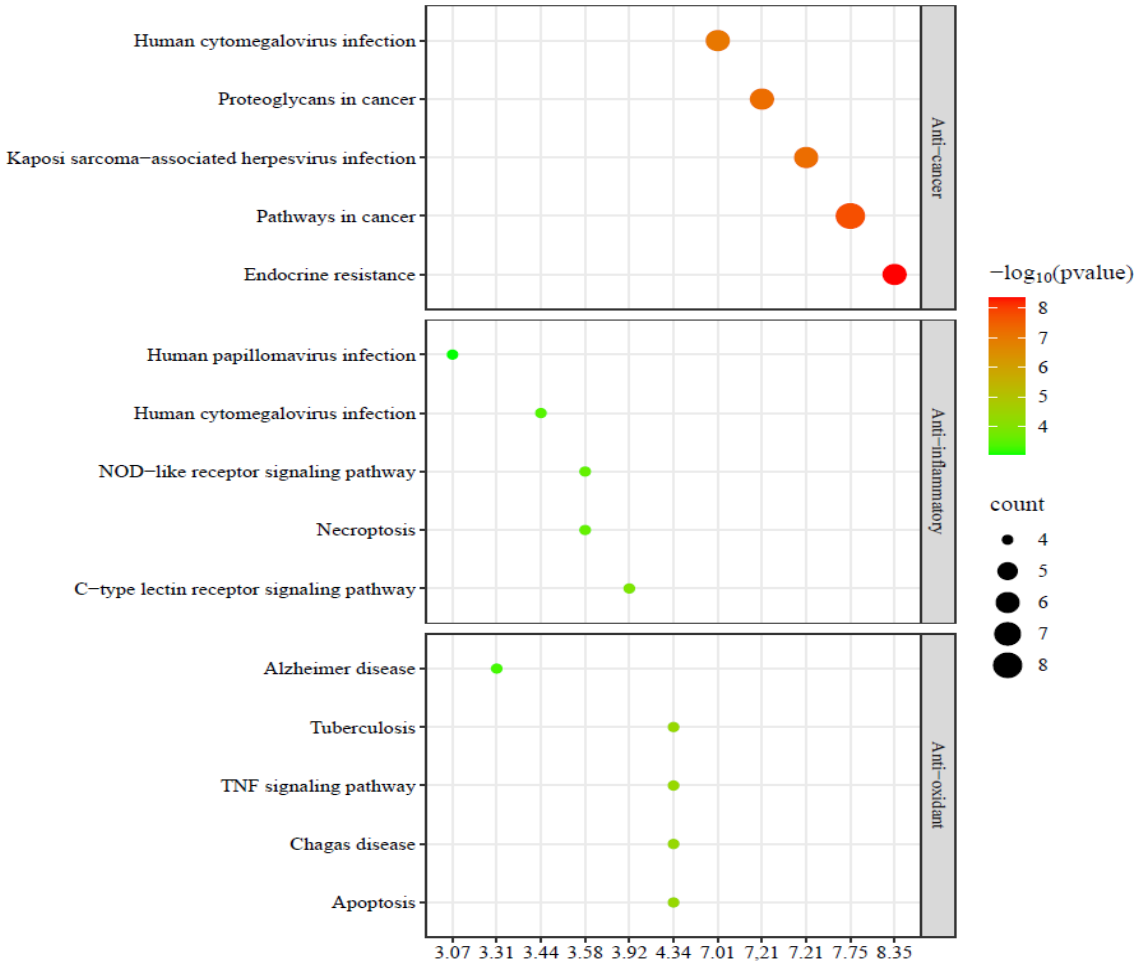

Figure 5. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for anticancer, anti-inflammatory, and antioxidant pathways. Dot sizes indicate protein count and color intensity shows statistical significance (-log(*p*-value)). Anticancer pathways exhibit the strongest enrichment, while anti-inflammatory and antioxidant pathways show moderate enrichment, highlighting their roles in immune response and oxidative stress.

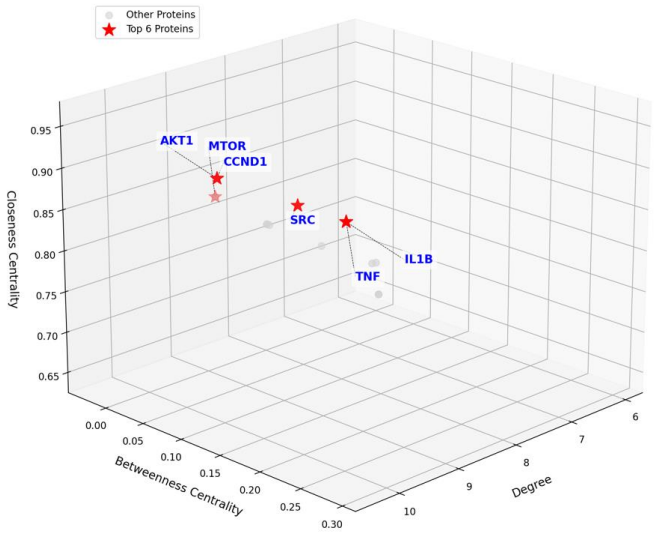

Figure 6. Skyline analysis identifying key proteins (RAC-alpha serine/threonine-protein kinase (AKT1), proto-oncogene tyrosine-protein kinase (SRC), G1/S-specific cyclin-D1 (CCND1), interleukin-1β (IL-1β), tumor necrosis factor (TNF), and mechanistic/mammalian target of rapamycin (mTOR)) involved in anticancer, anti-inflammatory, and antioxidant mechanisms, based on network metrics (degree, closeness centrality, and betweenness centrality).

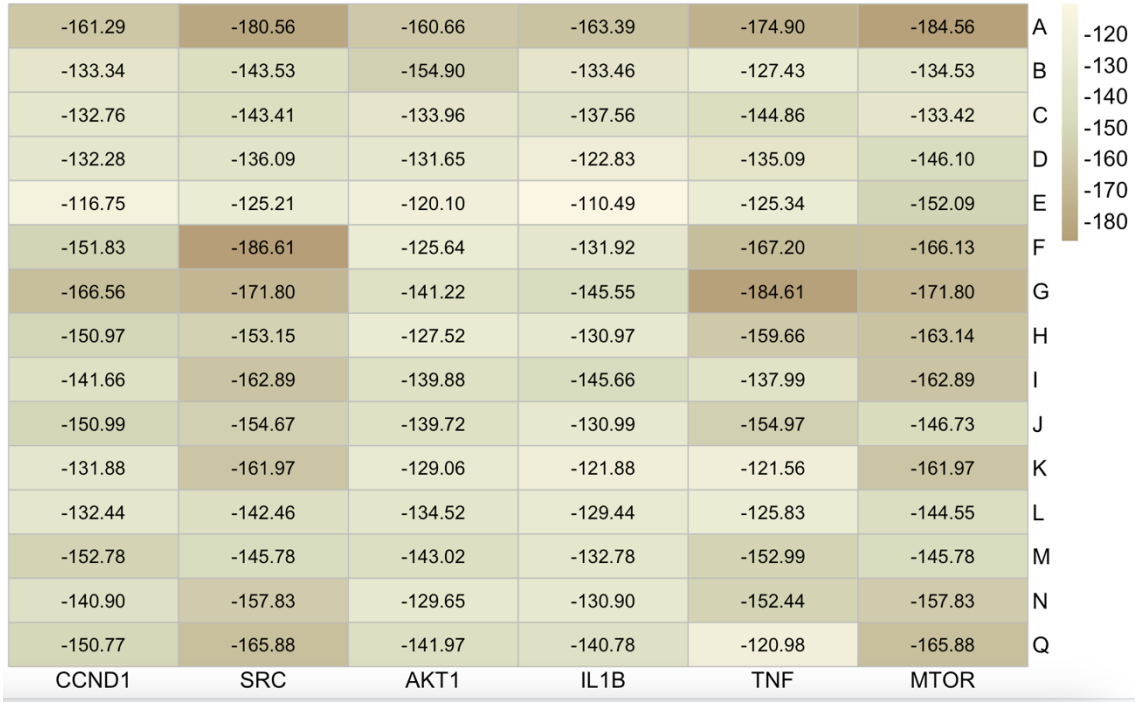

Figure 7. Molecular docking analysis of identified peptide from yellowfin tuna (*Thunnus albacares*) to protein targets within anticancer, anti-inflammatory, and antioxidant. Molecular docking results highlight Peptide A's strong binding affinities to multiple proteins, including mechanistic/mammalian target of rapamycin (mTOR) (-184.556 kcal/mol), tumor necrosis factor (TNF) (-174.897 kcal/mol), and proto-oncogene tyrosine-protein kinase (SRC) (-180.556 kcal/mol). Peptide F binds SRC most strongly (-186.612 kcal/mol), while Peptide G has the highest affinity for G1/S-specific cyclin-D1 (CCND1) (-166.556 kcal/mol). Peptides E and K exhibit weaker binding across all targets, positioning Peptide A as a promising broad-spectrum modulator with target-specific potential for Peptides F and G.

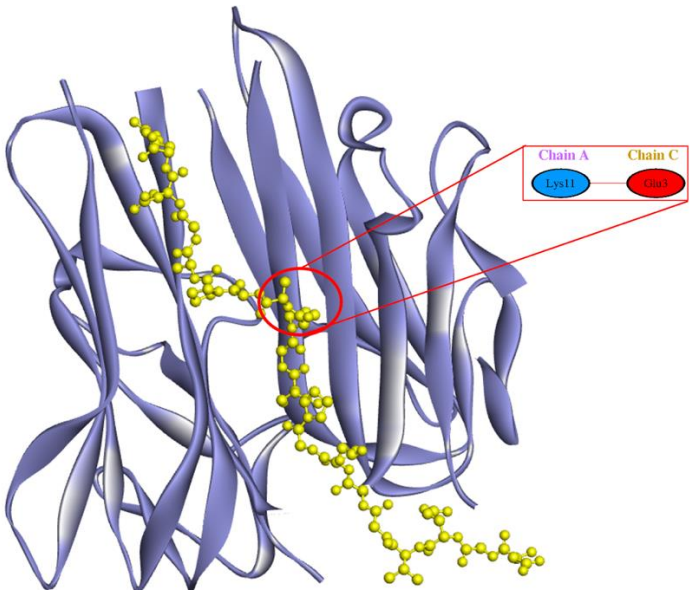

Figure 8. Interaction between Peptide A and tumor necrosis factor-alpha (TNF-α). The 3D structure of TNF-α features β-sheets, with Peptide A depicted as yellow spheres binding at a specific site. Key residues, Lys11 (blue) and Glu3 (red), suggest electrostatic or hydrogen bonding interactions, highlighting the potential mechanism for modulating TNF-α activity in inflammation and immunity.

The 3D structure of TNF-α features β-sheets and other secondary structural elements, while Peptide A is represented as a chain of yellow spheres binding to a specific site on TNF-α. A closer look at the interaction site revealed two important amino acid residues: Lys11 in chain A and Glu3

in chain C. The positions of these residues suggested that the binding between Peptide A and TNF-α was likely driven by electrostatic or hydrogen bonding interactions. The positively charged Lys11 and negatively charged Glu3 might form complementary bonds, providing insight into how Peptide A binds to TNF-α and potentially modulates its activity in inflammatory and immune responses.

Discussion

Collagen peptides sourced from yellowfin tuna have attracted considerable interest owing to their functional attributes and prospective health advantages, encompassing antioxidant, antihypertensive, lipid-reducing effects, immunomodulatory properties, and positive impacts on skin, bones, and joints [30]. These diverse properties underscore the potential of YFTCP as a multifunctional therapeutic agent. In this study, proteomic analysis identified 17 peptides and mapped their interactions with key proteins involved in cancer progression, immune regulation, and antioxidant responses.

The analysis reveals a complex network of pathways that interact to influence cancer progression, inflammation, and oxidative stress, highlighting the integrated role of these processes in disease development and progression. Enriched pathways such as human cytomegalovirus infection, proteoglycans in cancer, Kaposi sarcoma-associated herpesvirus infection, human papillomavirus (HPV) infection, NOD-like receptor (NLR) signaling, necroptosis, C-type lectin receptor (CLR) signaling, and TNF signaling play critical roles in modulating tumor growth, immune responses, inflammation, and oxidative stress.

Viral infections, including human cytomegalovirus and HPV, contribute to both inflammation and oncogenesis. HPV infection, for example, drives inflammation by disrupting cell cycle regulation and immune responses, particularly in cervical cancer, where this viral-induced inflammation facilitates carcinogenesis and immune evasion [31,32]. The activation of NLR signaling, which is crucial in recognizing pathogen- and damage-associated molecular patterns, amplifies inflammatory responses by triggering cytokine release, contributing to tumorigenesis and cancer-related inflammation [33,34].

Proteoglycans, known for their involvement in modulating tumor growth, angiogenesis, and metastasis, also play a role in immune responses and inflammation. Their contribution to cancer progression is intricately linked to their ability to modulate the tumor microenvironment, which can include influencing oxidative stress, further enhancing the inflammatory and cancer-promoting processes [35]. In parallel, endocrine resistance, particularly in hormone-dependent cancers like breast and prostate cancer, not only contributes to treatment resistance but also promotes chronic inflammation, exacerbating cancer progression. Pathways such as necroptosis and CLR signaling, while involved in mediating inflammatory responses, are also implicated in the amplification of inflammation and oxidative stress, intensifying tumor progression and resistance to therapies [36,37].

C-type lectin receptors (CLRs) play a significant role in modulating immune responses via dendritic cells and macrophages, with established functions in infections, autoimmune diseases, and cancer [38]. Though these pathways exhibit moderate enrichment, indicating a secondary role in inflammation, they underscore the interconnectedness between viral infections, immune receptors, and cell death pathways in driving inflammation and contributing to disease progression. These findings align with existing literature, which highlights the potential of these pathways as therapeutic targets in cancer and inflammatory disorders [39].

Furthermore, pathways such as apoptosis, TNF signaling, and those associated with diseases like Chagas disease, tuberculosis, and Alzheimer's disease show moderate enrichment in mechanisms related to oxidative stress and cellular defense. Apoptosis, triggered by oxidative stress, plays a protective role through regulated cell death, which aligns with studies suggesting that antioxidants can modulate this process to control cellular damage and inflammation [40,41]. TNF signaling, known to induce oxidative stress [42], also suggests a regulatory role for antioxidants in controlling inflammation and mitigating oxidative damage [43]. The enrichment of these pathways underscores their crucial role in managing oxidative stress, reinforcing the antioxidant activity of the YFTCP and their potential as therapeutic agents in addressing both cancer and inflammation.

Network pharmacology analysis revealed a complex network of interactions among proteins such as CCND1, SRC, AKT1, IL-1β, TNF, and mTOR, which are involved in various biological processes, particularly in cancer and immune responses. These interactions illustrate how growth signals, mediated by AKT1 and SRC, promote cell proliferation through CCND1, while simultaneously modulating inflammatory responses via IL-1β and TNF [44,45]. The mTOR pathway acts as a critical hub that integrates these signals, influencing both tumor growth and immune response dynamics [46]. This interconnectedness highlights the importance of these proteins in cancer biology and their potential as therapeutic targets for treating malignancies associated with inflammation and abnormal cell proliferation.

The Skyline query method identified six key proteins—AKT, SRC, CCND1, IL-1β, TNF, and mTOR—based on network metrics such as degree, betweenness centrality, and closeness centrality, highlighting their central roles in anticancer, anti-inflammatory, and antioxidant pathways. This balanced selection approach [26] ensured robust identification of critical targets within the network. Furthermore, molecular docking analysis provided the important insights into protein-peptide interactions, which may have implications for drug development, therapeutic targeting, and understanding receptor dynamics in cellular pathways [47].

Peptide A demonstrated strong and consistent binding affinities for CCND1, SRC, AKT1, IL-1β, TNF, and mTOR, highlighting its potential to inhibit cell proliferation and survival, modulate inflammation, and regulate metabolic and immune functions. Peptide A's high binding affinities for AKT1 could inhibit cell proliferation and survival, key processes in cancer biology. Its strong interaction with IL-1β and TNF, pivotal pro-inflammatory cytokines, indicates its potential to dampen inflammation in cancer and autoimmune disorders [48]. Additionally, Peptide A's binding to mTOR supports its role as a modulator of metabolic pathways involved in cancer and metabolic diseases. These findings position Peptide A as a promising candidate for developing therapies targeting multiple biological pathways simultaneously. Further studies are necessary to optimize its therapeutic efficacy and explore how environmental factors could influence receptor-ligand interactions, potentially improving drug specificity and reducing side effects.

Conclusion

In this study, the anticancer, anti-inflammatory, and antioxidant mechanisms of YFTCP were investigated using a network pharmacology-based approach. A total of 17 peptides were identified through proteomic analysis, and their interactions with key proteins were mapped to reveal pathways involved in cancer progression, immune regulation, and oxidative stress. Central proteins such as PPARG, TNF, IL-1β, and CASP1 were found to play significant roles in these processes. Enrichment analysis highlighted critical pathways, including the PI3K/AKT/MTOR and TNF signaling pathways, which are essential for cellular proliferation, apoptosis, inflammation, and antioxidant defense mechanisms. The findings suggest that the identified peptides exhibit multifunctional potential, particularly in their ability to target cancer, inflammation, and oxidative stress. Strong connections between these processes were identified, underscoring the therapeutic relevance of these peptides. Further studies, including preclinical and clinical validation, will be essential to advance these peptides as potential treatments for cancer, inflammatory conditions, and oxidative stress-related diseases.

Ethics approval

Not required.

Acknowledgments

None to declare.

Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

The authors declare that artificial intelligence (AI) tools, ChatGPT and Quillbot, were used during the preparation of the manuscript. These AI-based models assisted in refining the language, enhancing grammar, sentence structure, and readability. We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors

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