Cannabis and Cannabinoid Research
Volume 6, Number 4, 2021

DOI: 10.1089/can.2020.0025

SPECIAL SECTION: CANNABINOIDS AND THE IMMUNE SYSTEM

A Network Pharmacology Approach to Identify Potential Molecular Targets for Cannabidiol's Anti-Inflammatory Activity

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Abstract

Introduction: Published preclinical and clinical studies support the anti-inflammatory activity of CBD, but the molecular targets (e.g., genes and proteins) that are involved in its mechanisms of action remain unclear. Herein, a network-based pharmacology analysis was performed to aid in the identification of potential molecular targets for CBD's anti-inflammatory activity.

Materials and Methods: Target genes and proteins were obtained from several online databases, including Swiss target prediction, Online Mendelian Inheritance in Man, and the DrugBank database. A compound-target-disease network was constructed with Cytoscape tool, and a network of protein–protein interactions was established with the Search Tool for the Retrieval of Interacting Genes/Proteins database. Lead proteins identified from the compound-target-disease network were further studied for their interactions with CBD by computational docking. In addition, biological pathways involved in CBD's anti-inflammatory activity were identified with the Gene Ontology enrichment and the Kyoto Encyclopedia of Genes and Genomes analysis.

Results: A panel of proteins, including cellular tumor antigen p53, NF-kappa-B essential modulator, tumor necrosis factor (TNF) receptor, transcription factor p65, NF-kappa-B p105, NF-kappa-B inhibitor alpha, inhibitor of nuclear factor kappa-B kinase subunit alpha, and epidermal growth factor receptor, were identified as lead targets involved in CBD's anti-inflammatory activity. This finding was further supported by molecular docking, which showed interactions between the lead proteins and CBD. In addition, several signaling pathways, including TNF, toll-like receptor, mitogen-activated protein kinases, nuclear factor kappa-light-chain-enhancer of activated B cells, and nucleotide-binding oligomerization domain-like receptors, were identified as key regulators in the mediation of CBD's anti-inflammatory activity.

Conclusion: A network-based pharmacology analysis identified potential molecular targets and signaling pathways for CBD's anti-inflammatory activity. Findings from this study add to the growing body of data supporting the utilization of CBD as a promising anti-inflammatory natural product.

Keywords: cannabidiol (CBD); anti-inflammation; network pharmacology; NFκB; target identification; drug-diseases network

Introduction

Inflammation is a vital immune response to combat harmful stimuli, irritants, cell damage, and tissue injury.[1] These stimuli trigger a series of physiological consequences, including increased blood flow, dilation of capillary blood vessels, and impaired vascular permeability, which can further lead to numerous cell and tissue dysfunctions. Inflammation-mediated pathological progressions are regulated by complex immune systems involving different immune cells and various signaling

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pathways.[2] Among these various regulatory mechanisms, one of the most studied signaling pathway is the signal transduction of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB).[3] NFκB is a complex of proteins with five family members, including NFκB1 (subunit p50), NFκB2 (subunit p100), RelA (subunit p65), RelB, and c-Rel. These proteins are usually formed in dimers (e.g., p50-p65 dimer) to enable a wide range of biological functions and mediate the inflammation processes, such as coordinating the expression of multiple inflammatory genes. NFκB is considered as a central mediator for inflammation responses.

NFκB can be collectively regulated by its upstream signal transduction cascade, including inhibitors of nuclear factor kappa-B kinase subunit alpha, beta, and gamma (also known as IKK-$\alpha/\beta/\gamma$), which are associated with the propagation of cellular response to inflammation.[3] Activation of the NFκB pathway also leads to a cascade of downstream signaling, including the expressions of several proinflammatory cytokines, including interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α).

The NFκB pathway in the inflammation process is an example showing that the regulation of inflammation is orchestrated by complex mechanisms involving multiple genes, proteins, and signaling pathways. Therefore, identification of molecular targets for the development of anti-inflammatory agents as therapeutics for inflammatory-mediated diseases remains a challenge.

A promising preventive and/or therapeutic strategy against inflammatory-mediated disorders is immunomodulation by natural products from medicinal plants.[4] Cannabis (*Cannabis sativa*) has been widely used in empirical and traditional remedies for various inflammation-related ailments.[5,6] Notably, the anti-inflammatory activity and related molecular targets of a major phytocannabinoid in Cannabis, namely CBD, are supported by several reported studies. For instance, CBD showed anti-neuroinflammatory effects in murine microglial cells through the downregulation of the NFκB pathway and upregulation of transcription factors, including signal transducer and activator of transcription 3 (STAT3).[7] CBD's anti-inflammatory effects in microglial cells are also supported by other studies, which showed that CBD's anti-inflammatory activity was associated with its modulation of downstream transcriptional events, including the regulation of gene expression and transcription.[8,9]

In addition, functional subsets of genes and gene networks, such as molecular and cellular functions, of CBD's anti-inflammatory effects, were identified by bioinformatic analysis tools, including Ingenuity Pathway Analysis, Database for Annotation, Visualization, and Integrated Discovery (DAVID) bioinformatics resources, and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.[8,9] These findings suggest that CBD's anti-inflammatory activity is mediated by a complex combination of multiple molecular targets, rather than certain individual proteins.

In addition to experimental "omic" methods, computational modeling of biological pathways and molecular interactions has been developed as a "network" approach for a broad view of the interactions between ligands and their multiple targets.[10,11] The network-based pharmacology approach has been used to predict molecular targets, including genes and proteins, protein–protein interactions (PPIs), and signaling pathways for drug candidates.[10] A computational pharmacology network analysis has been reported to aid in the identification of CBD's pharmacological targets.[12] This network analysis utilized a combination of chemogenomics-knowledgebase network analysis and integrated *in silico* modeling for the identification of three human neuro-related rhodopsin-like G-protein-coupled receptors as CBD's molecular targets.

However, to date, a network analysis approach has not been utilized for the prediction of potential molecular targets that are related to CBD's anti-inflammatory activity. Herein, the aims of the current study are to (1) identify putative genes and proteins that contribute to CBD's anti-inflammatory activity; (2) explore the interactions between CBD and inflammation-related proteins; and (3) analyze signaling pathways that are involved in CBD's anti-inflammatory activity.

Materials and Methods

Target prediction

Potential targets for CBD's anti-inflammatory effects were obtained from several online databases, including the Swiss Target Prediction database (www.swisstargetprediction.ch), the Online Mendelian Inheritance in Man (OMIM, www.omim.org), and the DrugBank database (https://www.drugbank.ca/). Human target connexins were obtained from the interactive protein database (DIP; http://dip.doe-mbi.ucla.edu). All the tested targets were transformed into the UniProt database in the protein ID format using the Retrieve/ID mapping tool (www.uniprot.org).

Construction of network and topology analysis

To elucidate the relationship between CBD and its anti-inflammatory targets, CBD and target genes and

enriched pathways were imported into Cytoscape 3.6.1 software (www.cytoscape.org) to build a "component-target-disease" network. Then the topological parameters of the network analysis were calculated to identify key nodes. Among the topologic parameters, closeness centrality (a measure of how close a molecule is to other nodes)[13] and betweenness centrality (the number of nonredundant shortest paths traveling through a node in the network)[14] were used as crucial factors to describe the most influential nodes in the network. Thus, the nodes of degree and betweenness centrality that are two times higher than the median value of the total nodes, and the nodes of closeness centrality are higher than the median value of the total nodes were selected as the hubs for the anti-inflammatory activity.

Molecular docking

The three-dimensional structural coordinates of CBD were obtained from the human metabolome database (www.HMDB.ca), and the PDB files were generated with BIOVIA Discovery Studio Visualizer 4.5. The structural coordinates of target proteins were retrieved in PDB format from the RCSB protein data bank (www.rcsb.org). Chimera 1.11.2 was applied to delete the solvent and ligands from the target proteins. AutoDockTools 1.5.6 was used to perform molecular docking with AutoDock 4.2 algorithm. BIOVIA Discovery Studio Visualizer 4.5 was applied to analyze the interactions between CBD and target proteins.

Binding energies of CBD and its target proteins were obtained from parameters, including intermolecular energy (1), internal energy (2), torsional free energy (3), and unbound system's energy (4). The total binding energy was calculated as energy of binding = [(1) + (2) + (3) − (4)].

PPI network and cluster analysis

The hub targets from the aforementioned screening process were further imported into the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (http://string-db.org/) to explore the interactions between the known and predicted proteins. The topological parameters of mean and maximum degrees of freedom in PPI network were analyzed with Cytoscape 3.6.1 software, and then the network was analyzed with the MCODE plug-in for cluster analysis.

Enrichment analysis for target proteins

The plug-in ClueGO in Cytoscape 3.6.1 software was used to perform the Gene Ontology (GO), including cell component, molecular function, and biological process enrichment and the KEGG pathway annotation. The localization of the biological and molecular functions of the proteins was identified based on high confidence with a p value (<0.01) calculated by the two-side hypergeometric test method.

Results and Discussion

Protein targets and topological parameters

Potential targets for CBD's anti-inflammatory activity were obtained from several databases. CBD's protein targets were identified through the Swiss Target Prediction database and inflammation-related genes and protein targets were obtained through the OMIM and DrugBank databases. An interactive network of CBD's anti-inflammatory activity was constructed through network pharmacological analysis (Fig. 1). The red triangle represents CBD and the circles are proteins for pharmacological targets of CBD (in yellow), inflammation (in violet), and PPI (in cyan-blue). The yellow rectangles represent protein targets that are directly involved with CBD and inflammation pathways. Through the analysis of this "compound-target-disease" interactive network, 100 protein targets are affected by CBD and 570 protein targets are affected by inflammation. Among these protein targets, 20 proteins are common targets shared by CBD and inflammation-related pathways (as shown in yellow triangles; Fig. 1). Analysis of the topological parameters of all nodes in the network showed that the median of degree, betweenness centrality, and closeness centrality values are 1, 0, and 0.19641626, respectively. The thresholds of topological parameters were setup as degree value (>2), betweenness centrality (>0), and closeness centrality values (>0.19641626) and a total of 44 potential targets for CBD's anti-inflammatory activity were obtained (Supplementary Table S1). Targets with degree value greater than 10 are listed in Table 1. The protein target with the highest degree was cellular tumor antigen p53 (p53; 58 degree), followed by NF-kappa-B essential modulator (as known as IKK-γ), TNF receptor-associated factor 6 (TRAF6), transcription factor subunit p65 (NFκB p65), nuclear factor NF-kappa-B p105 subunit (NFκB p105), NF-kappa-B inhibitor alpha (IκBα), IKKα, and epidermal growth factor receptor with comparable degrees (21, 20, 19, 19, 18, 18, and 17, respectively).

Notably, several proteins among the identified CBD's anti-inflammatory targets, namely, IκBα, IKKα, IKKβ, and KKγ are the key regulators of the upstream NFκB

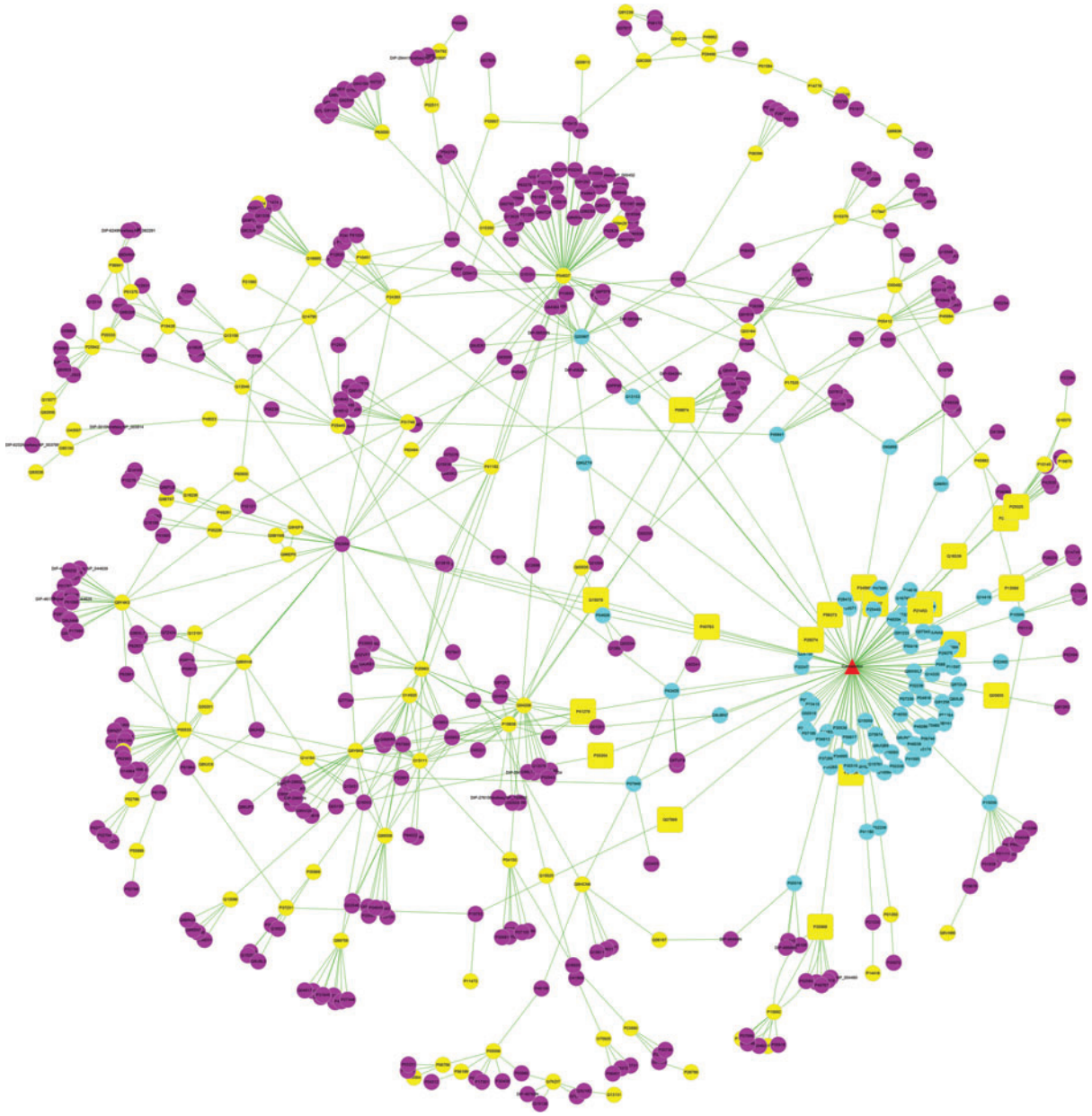

FIG. 1. CBD-target-inflammation network created by software Cytoscape (www.cytoscape.org). All target proteins are represented by their UniProt IDs. Red triangle: CBD; yellow circles: CBD's pharmacological target proteins; violet circle: proteins related to inflammation; cyan-blue circle: protein–protein interaction; yellow rectangles: proteins targets that are directly involved with CBD and inflammation pathways. Color images are available online.

signal cascade.[3] NFκB is in the inactivated state when IκBα masks the nuclear localization signals of NFκB and sequesters NFκB in the cytoplasm. Phosphorylation of IκBα proteins by IκB kinase, including IKKα, IKKβ, and KKγ, leads to the activation of NFκB via allowing it to enter into the cell nucleus from the cytoplasm. Therefore, given that IκB and IκB kinase are the top predicted protein targets, it is possible that CBD's anti-inflammatory effects are primarily associated with the NFκB signal cascade. This is in agreement with

Table 1. Topological Parameters of Potential Targets for CBD's Anti-Inflammatory Activity in the "Compound-Target-Disease" Network

UniProt ID	Protein name	Betweenness centrality	Closeness centrality	Degree
P04637	Cellular tumor antigen p53	0.3046	0.3012	58
Q9Y6K9	NF-kappa-B essential modulator	0.0648	0.2502	21
Q9Y4K3	TNF receptor-associated factor 6	0.0643	0.2377	20
Q04206	Transcription factor p65	0.0323	0.2513	19
P19838	Nuclear factor NF-kappa-B p105 subunit	0.0472	0.2468	19
P25963	NF-kappa-B inhibitor alpha	0.0426	0.2471	18
O15111	Inhibitor of nuclear factor kappa-B kinase subunit alpha	0.0196	0.2340	18
P00533	Epidermal growth factor receptor	0.0591	0.2427	17
P31749	RAC-alpha serine/threonine-protein kinase	0.0457	0.2653	13
O14920	Inhibitor of nuclear factor kappa-B kinase subunit beta	0.0241	0.2456	13
P05412	Transcription factor AP-1	0.0404	0.2321	13
Q99558	Mitogen-activated protein kinase 14	0.0246	0.2300	13
P09874	Poly [ADP-ribose] polymerase 1	0.1020	0.2864	12
Q16665	Hypoxia-inducible factor 1-alpha	0.0332	0.2254	12
P24385	G1/S-specific cyclin-D1	0.0347	0.2441	10
Q99759	Mitogen-activated protein kinase 3	0.0279	0.1993	10

in vitro experimental data from several previously reported studies. In these studies, CBD showed anti-inflammatory effects by the inhibition of NFκB pathways in BV-2 microglial cells,[7] PC12 neuronal cells,[15] and RAW264.7 murine macrophage cells.[16] To further validate the prediction of CBD's target proteins, computational docking was performed to evaluate the interactions between CBD and lead target proteins (top 16 proteins in Table 1).

Computational docking analysis

Computational docking revealed the interactions between CBD and the lead target proteins (top 16 from the protein targets analysis in Table 1). CBD can fit into the binding pocket of the target proteins and interact with protein amino acid residues by forming interactions, including van der Waals force, conventional hydrogen bonding, carbon hydrogen bonding, pi-donor hydrogen bonding, and pi-sigma, pi-alkyl, pi-anion, amide-pi stacked, and pi-sulfur forces (Fig. 2). In addition, the binding affinities between CBD and lead target proteins were ranked by their predicted free energy of binding and inhibition constant (Table 2). Proteins IKKβ and mitogen-activated protein kinase (MAPK) 14 had the lowest binding energy (-7.99 and -7.35 kcal/mol, respectively) and inhibition constant (1.4 and 4.1 μM, respectively). Comparably, p53, which had the highest degree in the "compound-target-disease" network, also had a third ranked low-binding energy and inhibition constant (-6.08 kcal/mol and 35.0 μM, respectively).

The utilization of computational docking as a complementary approach to validate the prediction of target proteins for CBD's neurophysiological effects has been reported.[12] In addition, molecular docking has also been used to evaluate the interactions between lupenone, a natural anti-inflammatory agent, and its predicted target proteins.[17] In the current study, data from the computational docking study supported the prediction of CBD's anti-inflammatory target proteins. Four of the top 6 proteins, including p53, TRAF6, p65, and IκBα, with lower binding affinity, matched within the top 6 lead target proteins predicted in Table 1. In addition, findings from computational docking are supported by reported experimental studies. For instance, CBD had the second lowest free binding energy and inhibition constant with protein MAPK 14 (also known as p38-α), which is a protein known as a mediator for cellular responses to external proinflammatory signals.[18] Animal experimental studies have reported that CBD exerted anti-inflammatory effects by the regulation of p38-α pathway in mouse models of type I diabetic cardiomyopathy[19] and alcohol-induced steatosis.[16] Nevertheless, further biological investigations are warranted to validate other predicted target proteins for CBD's anti-inflammatory activity.

PPI network analysis and cluster analysis of hub targets

The identified potential targets were imported into the STRING database. A PPI network of protein targets for CBD's inflammation activity was obtained with 44 nodes (target protein; shown as circles) and 288 edges (PPI; shown as lines; Fig. 3). In addition, the clustering of the target interaction network was further analyzed by the Cytoscape software MCODE plug-in to

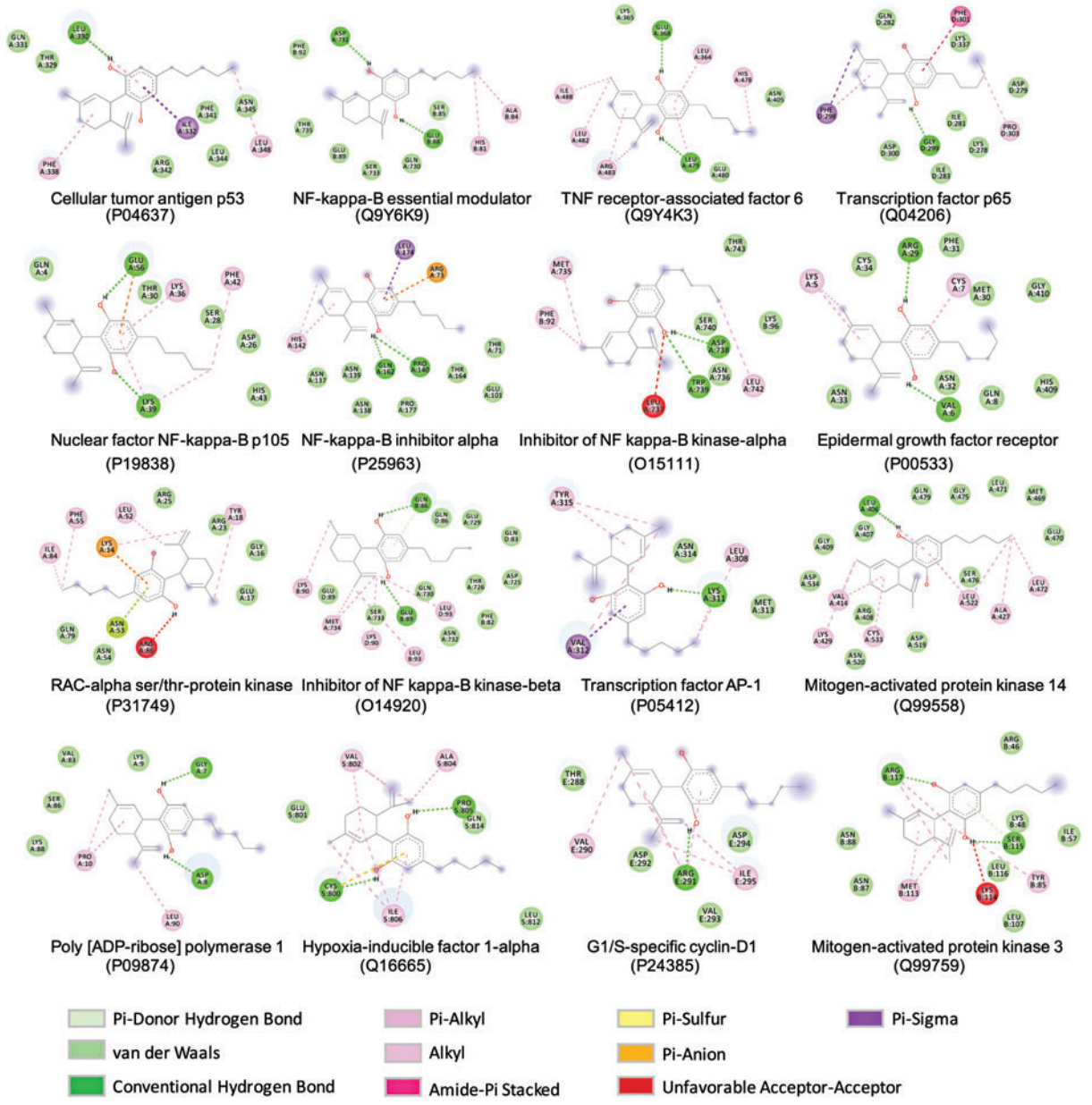

FIG. 2. Computational docking for interactions between CBD and potential target proteins for CBD's anti-inflammatory activity. Color images are available online.

Table 2. Interactions Between CBD and Target Proteins

UniProt-PBD ID	Protein name	Free energy of binding (kcal/mol)	Inhibition constant (Ki; μM)
O14920-3BRT	Inhibitor of nuclear factor kappa-B kinase subunit β	−7.99	1.4
Q99558-4IDT	Mitogen-activated protein kinase 14	−7.35	4.1
P04637-IAIE	Cellular tumor antigen p53	−6.08	35.0
P25963-1IKN	NF-kappa-B inhibitor alpha	−5.82	53.7
Q9Y4K3-1IB6	TNF receptor-associated factor 6	−5.74	62.3
Q04206-1NFI	Transcription factor p65	−5.66	71.5
P00533-1MOX	Epidermal growth factor receptor	−5.64	73.9
Q9Y6K9-3BRV	NF-kappa-B essential modulator	−5.36	117.8
P31749-1UNQ	RAC-alpha serine/threonine-protein kinase	−5.34	121.0
Q99579-2O2V	Mitogen-activated protein kinase 3	−5.34	122.6
P09874-2COK	Poly [ADP-ribose] polymerase 1	−4.88	264.4
Q16665-1H2K	Hypoxia-inducible factor 1-alpha	−4.41	581.3
O15111-3BRT	Inhibitor of nuclear factor kappa-B kinase subunit α	−4.32	686.9
P19838-IMDI	Nuclear factor kappa-B p105 subunit	−4.23	799.9
P24385- 5VZU	G1/S-specific cyclin-D1	−3.44	3010
P05412-1JUN	Transcription factor AP-1	−3.41	3180

Free energy of binding and inhibition constant predicted by computational docking.

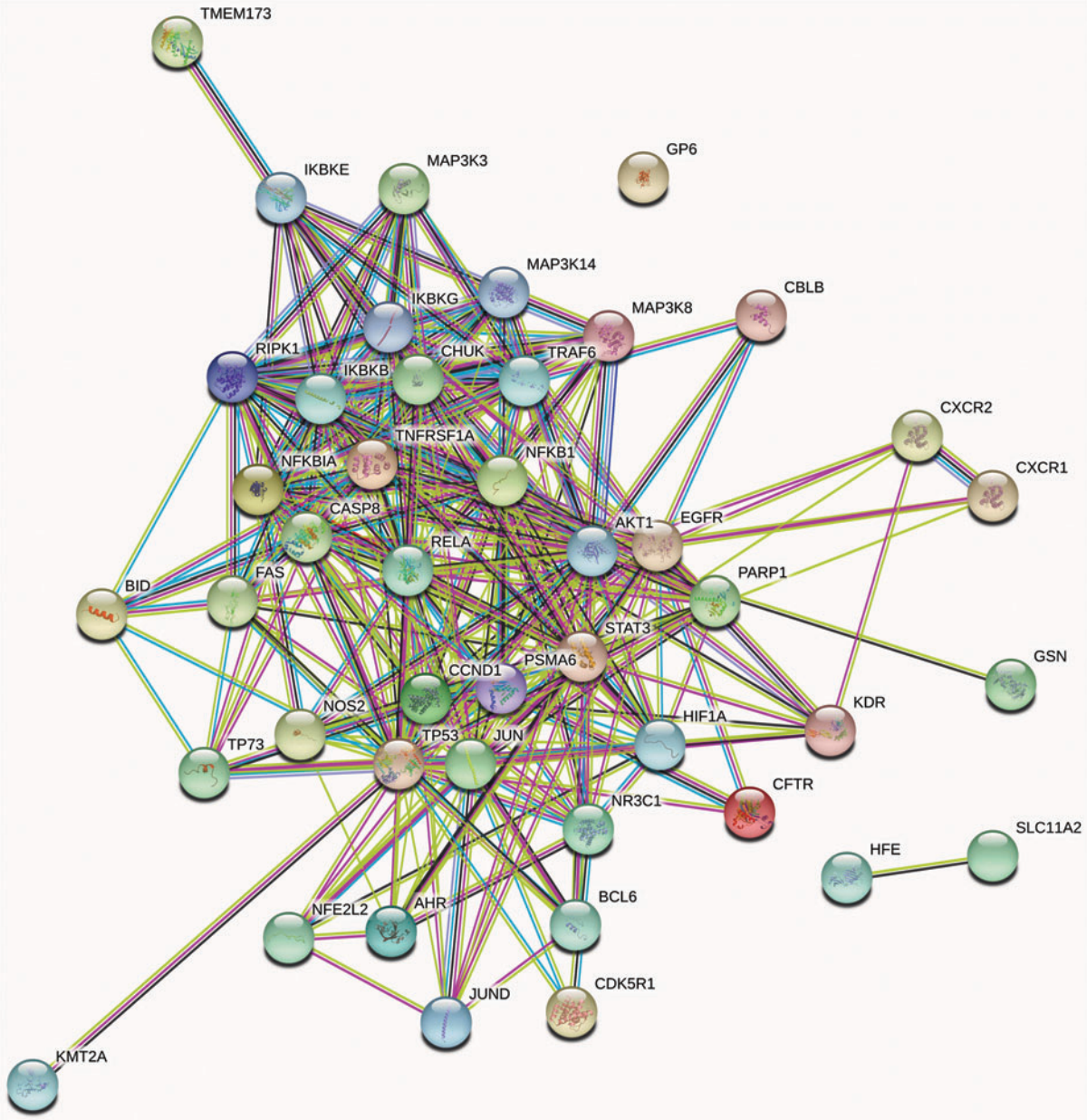

FIG. 3. Interaction network (created by software Cytoscape) of potential target proteins for CBD's anti-inflammatory activity. Color images are available online.

obtain two subnetworks (Fig. 4A, B), which represent possible independent pathways that contributed to the overall anti-inflammatory effects of CBD. In this PPI network, target proteins, including STAT3, RAC-alpha serine/threonine-protein kinase (also known as AKT1), tumor necrosis factor receptor superfamily member 1A (TNFRSF1A), and cysteine-aspartic acid protease-8 (CASP8), had greater number of degrees (as shown in a darker color) suggesting that they may play a pivotal role in the anti-inflammatory effects of CBD. This is in agreement with reported experimental studies. For instance, CBD was reported to exert anti-inflammatory effects by the downregulation of the proinflammatory STAT1 pathway and the activation of anti-inflammatory STAT3 pathway in murine microglial BV-2 cells.[7] In addition, CBD's anti-inflammatory

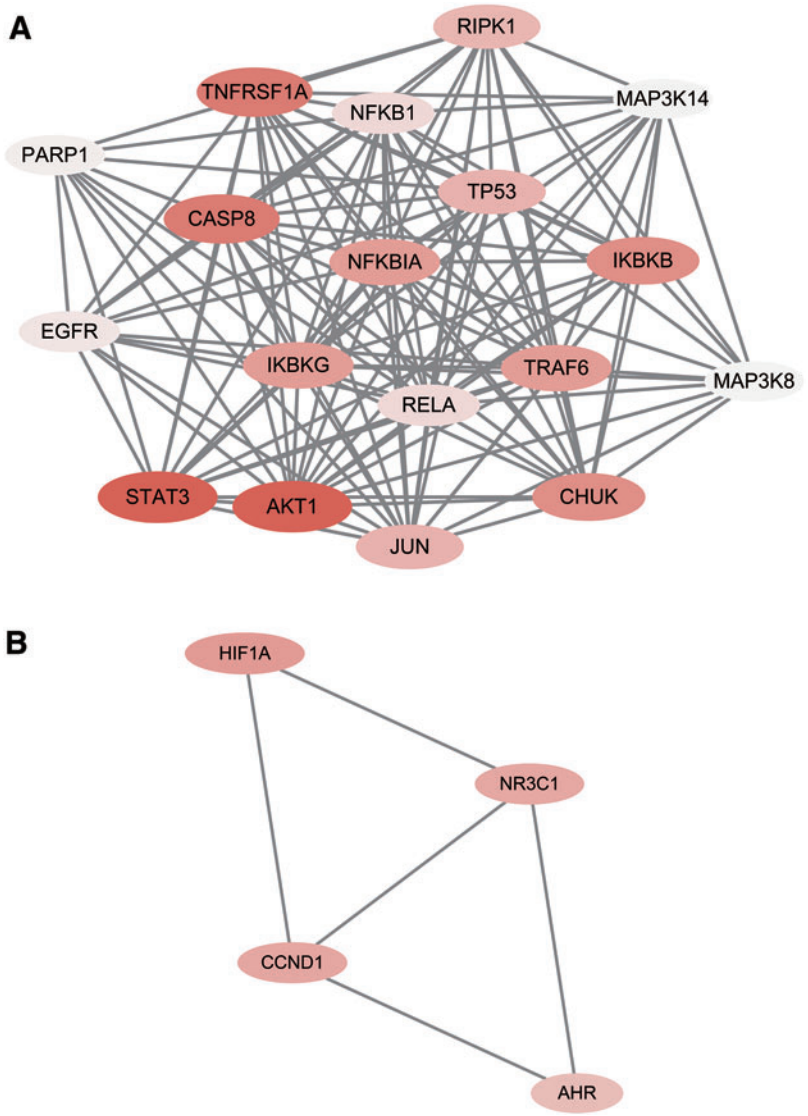

FIG. 4. Interaction network (created by software Cytoscape) of two protein core subnetworks **(A, B)** of target proteins for CBD's anti-inflammatory activity. Darker color indicates greater number of degrees. Color images are available online.

effects were reported to be associated with the upregulation of AKT phosphorylation in a mouse model of experimental autoimmune encephalomyelitis.[20] However, further studies are needed to elucidate the roles of other pathways in the predicted PPI network.

Enrichment analysis for GO biological processes and KEGG signaling pathway analysis

A panel of 44 potential targets was analyzed by the ClueGO plug-in for systematical analysis of the enrichment of biological functions and biological processes. There were 155 biological processes that were enriched (p value ≤ 0.01) and they were related to cellular response to the interleukin-1 (IL-1) and IL1-mediated signaling pathways (Table 3). For the molecular functions, 11 biological processes (p value ≤ 0.01) were related to histone deacetylase binding and enhancer binding (Table 3). For the cell components, five biological processes (p value ≤ 0.01) were strongly related to IκB kinase complex and CD40 receptor complex

Table 3. The Functional Analysis of Identified Compound-Related Targets by Gene Ontology Analysis and Kyoto Encyclopedia of Genes and Genomes Signaling Pathway Analysis

Ontology source	GOID	GOTerm	Term *p* value corrected with Bonferroni step down
GO_BiologicalProcess	GO:0070498	Interleukin-1-mediated signaling pathway	2.56E-13
GO_BiologicalProcess	GO:0071347	Cellular response to interleukin-1	7.48E-12
GO_BiologicalProcess	GO:0043123	Positive regulation of I-kappaB kinase/NF-kappaB signaling	1.83E-11
GO_BiologicalProcess	GO:0070555	Response to interleukin-1	4.43E-11
GO_BiologicalProcess	GO:0009612	Response to mechanical stimulus	1.57E-09
GO_BiologicalProcess	GO:0033209	Tumor necrosis factor-mediated signaling pathway	6.06E-09
GO_BiologicalProcess	GO:0038061	NIK/NF-kappaB signaling	6.28E-09
GO_BiologicalProcess	GO:1902895	Positive regulation of pri-miRNA transcription by RNA polymerase II	3.38E-08
GO_BiologicalProcess	GO:0035666	TRIF-dependent toll-like receptor signaling pathway	6.22E-08
GO_BiologicalProcess	GO:0038095	Fc-epsilon receptor signaling pathway	8.02E-08
GO_MolecularFunction	GO:0042826	Histone deacetylase binding	2.81E-06
GO_MolecularFunction	GO:0035326	Enhancer binding	1E-05
GO_MolecularFunction	GO:0000980	RNA polymerase II distal enhancer sequence-specific DNA binding	3.95E-05
GO_MolecularFunction	GO:0051879	Hsp90 protein binding	0.000357
GO_MolecularFunction	GO:0097110	Scaffold protein binding	0.000576
GO_MolecularFunction	GO:0032813	Tumor necrosis factor receptor superfamily binding	0.00121
GO_MolecularFunction	GO:0033613	Activating transcription factor binding	0.001913
GO_MolecularFunction	GO:0005123	Death receptor binding	0.001915
GO_MolecularFunction	GO:0051059	NF-kappaB binding	0.002589
GO_MolecularFunction	GO:0001102	RNA polymerase II activating transcription factor binding	0.002872
GO_CellularComponent	GO:0035631	CD40 receptor complex	0.000252
GO_CellularComponent	GO:0008385	IkappaB kinase complex	0.000297
GO_CellularComponent	GO:1902554	Serine/threonine protein kinase complex	0.000316
GO_CellularComponent	GO:0031264	Death-inducing signaling complex	0.000402
GO_CellularComponent	GO:0045178	Basal part of cell	0.001531

GO, Gene Ontology.

(Table 3). In addition, KEGG analysis revealed that a total of 71 pathways related to CBD's anti-inflammatory activity were found to be enriched with the protein targets (p value ≤ 0.01; shown in Fig. 5). The KEGG analysis of CBD's anti-inflammatory activity revealed several related signaling pathways, including TNF, toll-like receptor (TLR), retinoic acid-inducible gene-I-like receptors (RLRs), MAPK, C-type lectin receptors (CLRs), IL-17, NFκB, T cell receptor (TCR), cytosolic DNA-sensing, adipocytokine, nucleotide-binding oligomerization domain-like receptors (NLRs), neurotrophin, B cell receptor, chemokine, and advance glycation end products (AGEs)-receptor for AGEs (RAGE). Notably, some of these signaling pathways are known to be associated with a specific anti-inflammation mechanism, namely the inflammasome pathway, which is responsible for the maturation and secretion of proinflammatory cytokines IL-1β.[21] Our group has previously reported that the inhibitory effect of CBD on NLRP3 inflammasome activation is associated with its modulation of a purinergic receptor, namely, P2X7 receptor, which regulates several signaling pathways to release proinflammatory cytokines.[22] Published studies have also reported that the activation of signaling pathways, including TLR,[23] TCR,[24] NLRs,[25] and RAGE,[26] are closely related to the regulation of inflammasome activation. Therefore, multiple signaling pathways may be involved in CBD's anti-inflammasome activity, which contribute to CBD's overall anti-inflammatory activity. In addition, the drug-target association network also suggested that CBD had significant correlations with several inflammatory-mediated diseases, including hepatitis B and C, herpesviruses infection, various types of cancers (e.g., lung cancer, pancreatic cancer, prostate cancer, bladder cancer, myeloid leukemia, colorectal cancer, and melanoma), nonalcoholic fatty liver diseases, inflammatory bowel disease (IBD), and amyotrophic lateral sclerosis (ALS) (Fig. 5). These findings are supported by several preclinical investigations of CBD as interventions for the aforementioned inflammatory diseases, including hepatitis,[27] viral infections,[28] cancers,[29,30] liver diseases,[31] IBD,[32,33] and ALS.[34] Further experimental studies using "-omic" approaches (e.g., genomics, proteomics, and metabolomics) with *in vitro* and *in vivo* models are warranted to verify the biological effects of predicted protein targets.[35] Several challenges still remain before CBD's therapeutic applications for inflammatory-mediated diseases can be fully explored, due to (1) only limited data from clinical trials are available;[36–39]

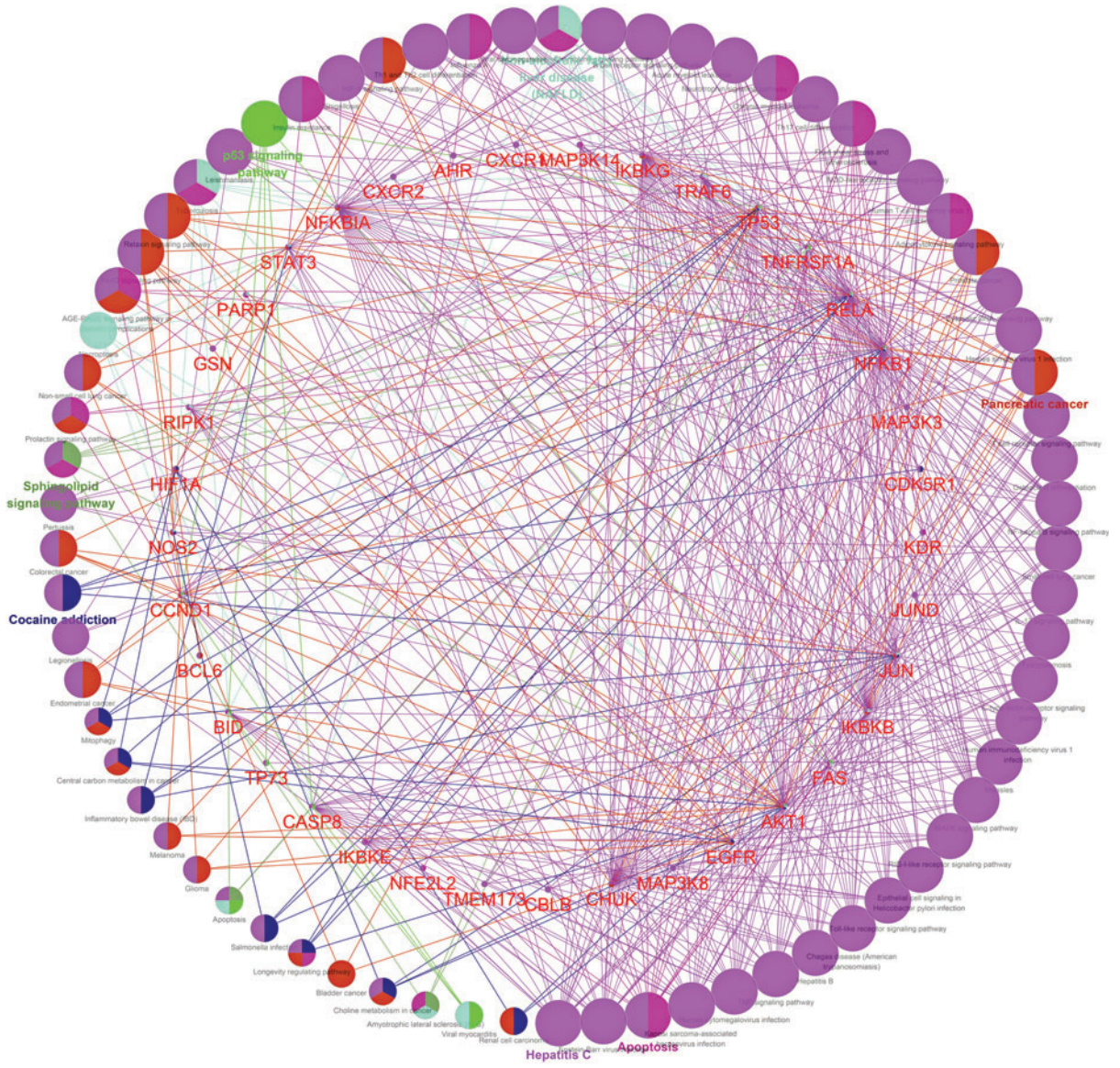

FIG. 5. Network diagram (created by software Cytoscape) of putative signaling pathways that are involved in CBD's anti-inflammatory activity and related diseases. Colors of nodes reflect the enrichment of biological function and classification of diseases associated with inflammation. Color images are available online.

(2) CBD may exert anti-inflammatory activity via multiple pharmacological targets (e.g., the endocannabinoid system)[40,41]; and (3) further mechanistic studies of CBD's pharmacological effects are warranted. It should be noted that the current study solely focused on the analysis of CBD's network for anti-inflammatory effects. However, there are numerous other phytochemicals, including over a hundred phytocannabinoids, such as THC, present in cannabis extracts. Similar to CBD, these phytochemicals (phytocannabinoids and nonphytocannabinoids) may also be involved in the modulation of the pharmacological targets identified herein and contribute to the overall anti-inflammatory effects of cannabis extracts. Therefore, it is possible that CBD and other phytochemicals, including THC in cannabis extracts, exert pharmacological effects in a complementary,

additive, and/or synergistic manner, but further studies are warranted to confirm this.

In summary, a network-based pharmacological analysis was utilized to predict the potential molecular targets for CBD's anti-inflammatory activity, which revealed the NFκB cascade as one of its primary anti-inflammatory mechanism of action. In addition, target proteins, including p53, IκBα, IKKs, and MAP kinases, as well as signaling pathways, including STAT3, AKT1, TNF, TLR, RLRs, and MAPK, were linked to CBD's anti-inflammatory activity. These molecular targets may contribute to CBD's overall anti-inflammatory activity and its potential therapeutic applications for several inflammatory-mediated diseases. Although further biological experiments are warranted to validate these molecular targets, our findings add to the growing body of data supporting the utilization of CBD as a promising anti-inflammatory natural product.

Author Disclosure Statement

N.P.S. serves on the Advisory Board of Alluvion Brands, LLC (Warwick, RI, USA) as a consultant for the biological evaluations of phytocannabinoids. Alluvion Brands did not influence the design of this study nor had any financial contributions to this work. The other authors declare no conflicts of interest.

Funding Information

No funding was received for this study.

Supplementary Material

Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3
Supplementary Figure S4
Supplementary Figure S5
Supplementary Table S1

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Cite this article as: Ma H, Xu F, Liu C, Seeram NP (2021) A network pharmacology approach to identify potential molecular targets for cannabidiol's anti-inflammatory activity, *Cannabis and Cannabinoid Research* 6:4, 288–299, DOI: 10.1089/can.2020.0025.

Abbreviations Used

AGEs = advance glycation end products
ALS = amyotrophic lateral sclerosis
CASP8 = cysteine-aspartic acid protease-8
CBD = cannabidiol
CLRs = C-type lectin receptors
DAVID = Database for Annotation, Visualization, and Integrated Discovery
GO = Gene Ontology
IBD = inflammatory bowel disease
IKK-$\alpha/\beta/\gamma$ = inhibitors of nuclear factor kappa-B kinase subunit alpha/beta/gamma
IL-1 = interleukin-1
IκBα = NF-kappa-B inhibitor alpha
KEGG = Kyoto Encyclopedia of Genes and Genomes
MAPK = mitogen-activated protein kinase
NFκB = nuclear factor kappa-light-chain-enhancer of activated B cells
NFκB p105 = nuclear factor NF-kappa-B p105 subunit
NFκB p65 = transcription factor p65
NLRs = nucleotide-binding oligomerization domain-like receptors
OMIM = Online Mendelian Inheritance in Man
PPI = protein–protein interaction
RAGE = receptor for advance glycation end products
RLRs = retinoic acid-inducible gene-I-like receptors
STAT3 = signal transducer and activator of transcription 3
STRING = Search Tool for the Retrieval of Interacting Genes/Proteins
TCR = T cell receptor
THC = tetrahydrocannabinol
TLR = toll-like receptor
TNF = tumor necrosis factor
TNFRSF1A = tumor necrosis factor receptor superfamily member 1A
TNFα = tumor necrosis factor alpha
TRAF6 = TNF receptor-associated factor 6