Original Article

A System-Level Investigation into the Mechanisms of Apigenin Against Inflammation

Natural Product Communications
Volume 14(9): 1–11

DOI: 10.1177/1934578X19878600
journals.sagepub.com/home/npx

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Abstract

Apigenin is a natural flavone that possesses excellent biological activities especially against aging and cancer. However, the underlying mode of its action is not yet revealed. The purpose of this study was to examine the pharmacological mechanisms of apigenin using the knowledge of network pharmacology, protein-protein interaction (PPI) databases and biological processes analysis through Cytoscape. Apigenin targets were retrieved through PASS Prediction and STITCH database and the interactive associations between these targets were studied using STITCH, followed by GO (gene ontology) and pathway enrichment analysis. As a result of target search, 125 protein targets were retrieved. Moreover, 216 GO terms related to various biological processes, 16 GO terms for various molecular processes, 5 GO terms for the cellular components, and 52 Kyoto Encyclopedia of Genes and Genomes pathway terms were achieved by analyzing gene functional annotation clusters and abundance values of these targets. Most of these terms are strongly associated with inflammation through various pathways, for example, FOXO, mammalian target of rapamycin, tumor necrosis factor, p53, AMP-activated protein kinase, p13K-AKT, and mitogen-activated protein kinase, which play an important role in inflammation, aging and cancer. Apigenin can be used to treat inflammation, aging, and cancer with an underlying mechanism of inflammation suppression. This study contributed excellent information for a better understanding of the modes of action of apigenin. However, further studies such as docking and MD simulation are required to understand the therapeutic and toxicological roles of these targets of apigenin.

Keywords

apigenin, biological effects, cytoscape, inflammation, mechanisms of action, molecular targets, STITCH

Received: July 19th, 2019; Accepted: August 27th, 2019.

Like other flavones, apigenin (4',5,7-trihydroxyflavone)[1] is abundantly found in several fruits, such as apples, oranges, and grapefruits,[2] and vegetables, including chamomile, celery, parsley, and onions,[3] which constitute the routine diet of human. Moreover, apigenin also exists in tea, celery, yarrow, tarragon, cilantro, foxglove, coneflower, licorice, flax, passion flower, horehound, spearmint, basil, oregano, red wine, and beer.[4] Apigenin (Figure 1) has been isolated from the plants *Matricaria recutita and Gingko biloba* as free apigenin[5] and in the form of various acylated derivatives, such as apigenin-7-*O*-glucoside.[6,7] Mediterranean food is also rich in apigenin, which is considered responsible for lower incidence of cardiovascular disorders, obesity, diabetes, and cancer.[8-10] Apigenin is synthesized through chemical modification of the flavanone naringenin by inserting a double bond to its ring C. However, apigenin and naringenin have significantly different bioactivities regardless of their structural similarity. In contrast to naringenin,[11] apigenin mediates apoptosis of various cell lines.[12-14] Apigenin possesses excellent activity against inflammation[15] via activation of the nuclear factor B (NF-B)-mediated pathway.[16] Moreover, there is reduced risk of ovarian risk owing to the consumption of apigenin, as evident from large population-based studies.[8] Besides, several dietary supplements also have apigenin in excess.[17] Therefore, it acts as an excellent natural compound from which to recognize the entire set of human target proteins as an initial

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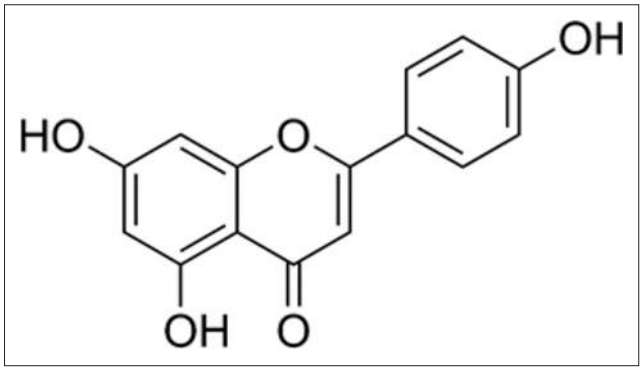

Figure 1. Chemical structure of apigenin.

step to recognize how dietary phytochemicals provide health advantages.

In recent times, there is an increasing trend of using network pharmacology to explore the action of herbals.[18,19] Network pharmacology is a systems biology-based approach. Its application in drug discovery has accelerated the process of scientific validation of drug action.[20,21] For instance, Gan et al used network pharmacology involving STITCH and Cytoscape plugin bingo to predict the molecular targets of curcumin that were involved in suppressing inflammation.[22] In addition, network pharmacology is also utilized to highlight the ameliorated drug efficacy by constructing the interactive channels between different signaling pathways.[23–25]

The living body performs various functions through a large number of signaling proteins, which work together as networks. These networks represent protein-protein interactions (PPIs), which work in association with a number of cellular signaling pathways[26,27] to accomplish the biological activities.[28–31] The gene ontology (GO) project[32] has played an important role in creating the ontologies for gene annotations. GO enrichment analysis is a statistical procedure that can be utilized to predict the association between annotations to GO and proteins. GO enrichment and network analysis together can be supportive to envisage the molecular mode of apigenin activities.

Apigenin has been proved to have anticancer activity, mainly through inhibition of oxidation and inhibition, triggering of cell-cycle arrest and apoptosis, attenuation of proliferation, and provoking of detoxification enzymes. Currently, anticancer activity data of apigenin describes only 1 or 2 pathways and are available in split form, showing the lack of an overall multi-target regulation of apigenin.

Owing to this breach in knowledge, this study was designed to examine the pharmacological mechanisms of apigenin using the knowledge of network pharmacology, PPI databases, and biological processes analysis through Cytoscape. This study will not only act as a reference for clinical trials of apigenin, but also assist to produce analog molecules.

Methodology

Target retrieval through PPI database and network development and analysis were collectively used to investigate molecular targets and biological effects of apigenin. In the first stage of this study, molecular targets of apigenin were searched for using 2 well-renowned databases, i.e. PASS Prediction and STITCH. Targets obtained from both databases were merged, excluding any duplication. Then, an apigenin-target interaction network was constructed and analyzed by using the STITCH database. Finally, GO enrichment analysis and biological processes analysis were carried out using Cytoscape-plugin ClueGO to examine the molecular basis of apigenin activities.

Search and Prediction of Apigenin Targets

PASS Prediction (http://www.pharmaexpert.ru/PASSOnline/) was utilized to retrieve the apigenin targets.[33] PASS Prediction is a database developed to find various types of biological actions of small organic molecules on the basis of their chemical structures, mainly before their chemical synthesis and biological analysis. This database requires "SMILES" or structural formula of a compound in MOLfile format or "SMILES" to identify molecular targets with a probability of Pa >0.7 (probability to be active). In addition, STITCH 5.0 database (http://stitch.embl.de/)[34] was utilized to retrieve known protein targets of apigenin. To explore protein targets of an active organic compound and their interactions, this online database searches several sources of information including experimental confirmations, genomic context, high-throughput experiments (conserved) coexpression, and text mining for a large number of organisms. The database presently is composed of 9 643 763 proteins from 2031 organisms. In addition, the interactions taken from STITCH are of 2 types including direct (physical) and indirect (functional) type. Probabilistic confidence score plays an important role in finding the interactions using the STITCH database, thus a probabilistic confidence score of greater than 0.7 was used to predict protein targets.

Network Construction, GO, and Pathway Enrichment Analysis

After retrieving targets through PASS Prediction and STITCH related to homo sapiens, a network comprising apigenin targets was constructed and analyzed. STITCH database exhibits an excellent feature of network construction and then its analysis. On the basis of biological terms, target genes were divided into a structured hierarchy by using GO biological processes for the identification and analysis of the characteristic features of potential targets. To investigate the modes of apigenin for aging and cancer, pathway enrichment analysis was introduced. GO terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway terms were acquired from STITCH. Moreover, KEGG

Table 1. List of Targets Retrieved Through PASS Prediction Analysis of Apigenin (Pa > 0.7).

No.	Pa	Activity	No.	Pa	Activity
0.973	0.001	Chlordecone reductase inhibitor	0.795	0.003	Leukotriene-B4 20-monooxygenase inhibitor
0.967	0.002	Membrane integrity agonist	0.794	0.002	NADPH oxidase inhibitor
0.963	0.003	HIF1A expression inhibitor	0.798	0.008	JAK2 expression inhibitor
0.946	0.002	Membrane permeability inhibitor	0.792	0.004	UGT1A1 substrate
0.946	0.004	CYP2C12 substrate	0.791	0.003	Histamine release inhibitor
0.942	0.002	2-Dehydropantoate 2-reductase inhibitor	0.791	0.004	CYP1A2 inhibitor
0.941	0.002	Kinase inhibitor	0.782	0.002	1-Alkylglycerophosphocholine *O*-acetyltransferase inhibitor
0.936	0.001	Aryl-alcohol dehydrogenase (NADP+) inhibitor	0.783	0.003	Pectate lyase inhibitor
0.937	0.003	Aldehyde oxidase inhibitor	0.797	0.019	Mucomembrane protector
0.931	0.001	P-benzoquinone reductase (NADPH) inhibitor	0.787	0.011	Dehydro-L-gulonate decarboxylase inhibitor
0.931	0.003	Anaphylatoxin receptor antagonist	0.780	0.004	MMP9 expression inhibitor
0.926	0.002	Antimutagenic	0.777	0.002	CYP1A1 inhibitor
0.924	0.002	Peroxidase inhibitor	0.773	0.003	UGT1A7 substrate
0.918	0.002	Histidine kinase inhibitor	0.778	0.008	CYP3A4 inducer
0.914	0.002	NADPH-ferrihemoprotein reductase inhibitor	0.765	0.004	CYP2A4 substrate
0.912	0.001	Quercetin 2,3-dioxygenase inhibitor	0.775	0.015	Antineoplastic
0.907	0.002	CYP1A inducer	0.763	0.003	UGT1A10 substrate
0.906	0.003	UGT1A6 substrate	0.757	0.002	CYP19A1 expression inhibitor
0.905	0.005	TP53 expression enhancer	0.758	0.003	Beta glucuronidase inhibitor
0.902	0.005	Ubiquinol-cytochrome-c reductase inhibitor	0.755	0.003	Xenobiotic-transporting ATPase inhibitor
0.899	0.003	HMOX1 expression enhancer	0.755	0.005	Pin1 inhibitor
0.895	0.001	SULT1A3 substrate	0.751	0.002	CF transmembrane conductance regulator agonist
0.895	0.002	Beta-carotene 15,15'-monooxygenase inhibitor	0.758	0.011	Glutathione thiolesterase inhibitor
0.896	0.004	CYP1A substrate	0.778	0.031	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0.891	0.003	Vasoprotector	0.749	0.004	Tetrahydroxynaphthalene reductase inhibitor
0.890	0.003	UGT1A9 substrate	0.749	0.004	Nitrite reductase [NAD(P)H] inhibitor
0.886	0.002	CYP1A1 inducer	0.745	0.007	CYP1A2 substrate
0.885	0.001	Glycerol dehydrogenase (NADP+) inhibitor	0.747	0.009	CYP3A inducer
0.884	0.001	2-Dehydropantolactone reductase (A-specific) inhibitor	0.739	0.002	Alcohol dehydrogenase [NAD(P)+] inhibitor
0.885	0.005	Antiseborrheic	0.750	0.013	Glucan endo-1,6-beta-glucosidase inhibitor
0.880	0.003	Alcohol dehydrogenase (NADP+) inhibitor	0.740	0.004	Antioxidant
0.888	0.011	Aspulvinone dimethylallyltransferase inhibitor	0.731	0.001	Creatine kinase inhibitor
0.879	0.003	27-Hydroxycholesterol 7alpha-monooxygenase inhibitor	0.732	0.002	NOS2 expression inhibitor
0.865	0.001	Cystathionine beta-synthase inhibitor	0.729	0.002	CYP19 inhibitor
0.866	0.003	4-Nitrophenol 2-monooxygenase inhibitor	0.730	0.005	Insulysin inhibitor
0.862	0.002	MAP kinase stimulant	0.726	0.002	Iodide peroxidase inhibitor
0.861	0.002	2-Enoate reductase inhibitor	0.723	0.003	UGT1A8 substrate
0.861	0.003	Cholestanetriol 26-monooxygenase inhibitor	0.729	0.010	NAD(P)+-arginine ADP-ribosyltransferase inhibitor

(Continued)

Table 1. Continued

No.	Pa	Activity	No.	Pa	Activity
0.861	0.004	CYP1A1 substrate	0.753	0.034	CYP2J substrate
0.854	0.005	Apoptosis agonist	0.720	0.002	UGT2B15 substrate
0.837	0.002	Antihemorrhagic	0.721	0.005	5 Hydroxytryptamine release inhibitor
0.827	0.002	AR expression inhibitor	0.720	0.004	UGT2B12 substrate
0.826	0.003	APOA1 expression enhancer	0.719	0.004	Free radical scavenger
0.825	0.004	Sulfotransferase substrate	0.709	0.002	CYP1B1 inhibitor
0.818	0.001	Testosterone 17-beta-dehydrogenase inhibitor	0.741	0.036	Gluconate 2-dehydrogenase (acceptor) inhibitor
0.819	0.003	Monophenol monooxygenase inhibitor	0.711	0.008	Ecdysone 20-monooxygenase inhibitor
0.820	0.005	Alkane 1-monooxygenase inhibitor	0.702	0.003	CYP1B substrate
0.818	0.004	CYP1A inhibitor	0.725	0.027	CYP2J2 substrate
0.813	0.003	UGT1A3 substrate	0.706	0.010	Thioredoxin inhibitor
0.813	0.004	CYP2B5 substrate	0.703	0.009	CYP2A6 substrate
0.813	0.004	UGT1A substrate	0.709	0.026	NADPH peroxidase inhibitor
0.803	0.007	UDP-glucuronosyltransferase substrate	0.706	0.032	Sugar-phosphatase inhibitor

Table 2. Predicted Targets of Apigenin Obtained Through STITCH Database (Confidence View).

Abbreviation	Predicted protein targets	Score	Degree	Abbreviation	Predicted protein targets	Score	
ESR1	Estrogen receptor 1	0.961	8	SPAM1	Sperm adhesion molecule 1	0.848	2
CDK1	Cyclin-dependent kinase 1	0.949	4	ESR2	Estrogen receptor 2	0.847	6
CASP3	Caspase 3, apoptosis-related cysteine peptidase	0.947	6	VEGFA	Vascular endothelial growth factor A	0.845	9
PARP1	Poly (ADP-ribose) polymerase 1	0.944	5	MMP9	Matrix metallopeptidase 9	0.842	5
UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1	0.938	3	CCND1	Cyclin D1	0.838	9
PTGS2	Prostaglandin-endoperoxide synthase 2	0.877	7	CYP19A1	Cytochrome P450, family 19, subfamily A, polypeptide 1	0.837	4
CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1	0.876	2	ELAVL1	ELAV -like 1	0.826	7
AKT1	v-akt murine thymoma viral oncogene homolog 1	0.876	12	HIF1A	Hypoxia inducible factor 1	0.824	7
TP53	Tumor protein p53	0.868	15	CAT	Catalase	0.822	3
MAOA	Monoamine oxidase A	0.848	1	TNFRSF10B	Tumor necrosis factor receptor	0.819	3

(http://www.kegg.jp/) database was utilized for more information about these pathways. Afterwards, the apigenin-target network was also analyzed by using ClueGO, a plug-in of Cytoscape to investigate the mode of apigenin and its pharmacodynamics significance. Cytoscape 3.4.0 has an excellent feature of network visualization and analysis and is supplied with numerous plug-ins.[35] Enrichment analysis by ClueGO was conducted by having set the level of significance at 0.05. Besides, a medium network type and 2-sided hypergeometric test with a Bonferroni correction was selected. In addition, the functional network was visualized via an organic layout algorithm.

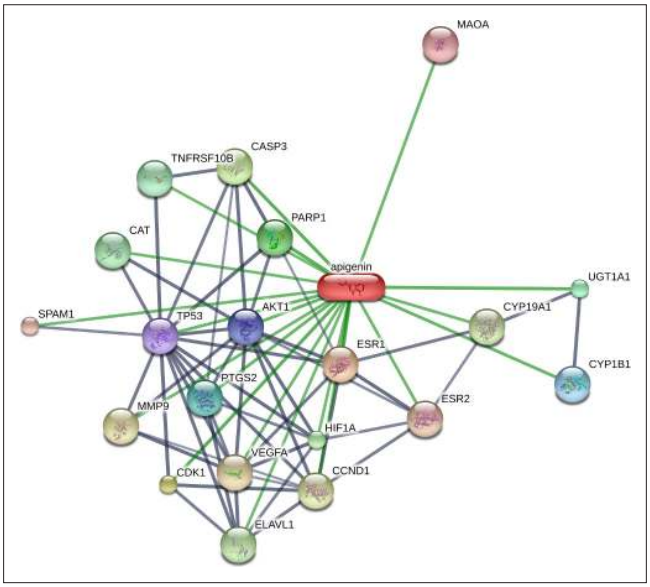

Figure 2. Confidence view of the protein network of apigenin. This view is composed of nodes and edges signifying the proteins and their interactions, respectively. Thick lines specify the stronger associations. Gray and green lines characterize the protein-protein and chemical-protein interactions.

Results

Retrieval and Identification of Protein Targets

Through PASS Prediction (accessed in April 2017), a total of 105 potential targets of apigenin in human with Pa >Pi (Table 1) were found. On the other hand, 20 potential human protein targets (Table 2) were retrieved via STITCH (accessed in April 2017) setting a confidence score at 0.7. Targets retrieved through both searches were merged, excluding any repeated target. Overall, 125 protein targets with numerous physiological and/or pathological activities were retrieved. These proteins are involved in pharmacokinetic and inflammatory processes.

Protein Interaction Network

The STITCH 5.0 database was utilized to construct the interaction network of target proteins (encoded by the respective genes) (Figure 2), which were indicated as nodes. These nodes were connected with each other through lines, known as edges leading to the formation of protein pairs. The obtained protein interaction network consisted of 20 proteins and 49 edges. PPI enrichment *P*-value for the obtained protein-protein network was 0.00642. This small value indicates that the observed number of edges is significant and that the nodes are not random. In addition, the number of edges, if the nodes are randomly selected, is denoted by a term known as the expected number of edges: the value in the presently discussed network is 33. Besides, the network stats indicated that the average node degree and clustering coefficient were 4.9 and 0.688, respectively. At the threshold score, the average of number of interactions of a protein in a network is known as the average node degree, while the extent of connectivity of nodes in a network is denoted as the clustering coefficient. A high value of the clustering coefficient indicates that the network is highly connected A node degree is a quantitative property of a node and reflects the number of interactions of a node. The nodes with

Table 3. Predicted Targets of Apigenin Obtained Through STITCH Database (Action View).

	Activation	Inhibition	Binding	Expression	**Score**
ESR1	•	•	•	•	0.961
CDK1		•			0.949
CASP3	•				0.947
PARP1	•				0.944
UGT1A1	•			•	0.938
PTGS2		•	•		0.877
CYP1B1		•	•		0.876
AKT1		•		•	0.876
TP53	•			•	0.868
MAOA		•	•		0.848
SPAM1		•			0.848
ESR2	•		•		0.847
VEGFA		•		•	0.845
MMP9		•			0.842
CCND1		•			0.838
CYP19A1		•	•	•	0.837
ELAVL1	•				0.826
HIF1A		•		•	0.824
CAT				•	0.822
TNFRSF10B	•				0.819

a number of interactions higher than the average node degree are called hubs. The degree of each node is given in Table 2. In the obtained network, there are 12 nodes with the average node degree higher than 4.9; thus, this network contains 12 hubs. For instance, the highest degree is noted for TP53 (15), followed by AKT1, vascular endothelial growth factor A, and cyclin D1 having 12, 9, and 9 degrees, respectively, and so on. Most of the protein nodes, especially hubs, in this network are involved in the process of inflammation, aging, and cancer development. Table 3 shows that apigenin activates ESR1, ESR2, CASP3, PARP1, UGT1A1, TP53, ELAVL1, and TNFRSF10B, while ESR1, CDK1, PTGS2, CYP1B1, AKT1, MAOA, SPAM1, VEGFA, MMP9, CCND1, CYP19A1, and HIF1A are inhibited by apigenin. Apigenin undergoes binding with ESR1, PTGS2, CYP1B1, MAOA, ESR2, and CYP19A1. In addition, apigenin plays a role in the expression of ESR1, UGT1A1, AKT1, TP53, VEGFA, CYP19A1, HIF1A, and TNFRSF10B. Their respective scores are mentioned in Table 3.

GO and Pathway Analysis

The STITCH 5.0 database was further used to analyze the biological functions of these potential targets through functional enrichment of the network. As a result of this analysis, 216 GO terms related to various biological processes were retrieved. It is worthy of mention that most of these targets are involved in the multiple biological responses such as positive regulation of reactive oxygen species metabolic process, regulation of cell proliferation, regulation of protein serine/threonine kinase activity, positive regulation of angiogenesis, response to hypoxia, intrinsic apoptotic signaling pathway, and positive regulation of cellular protein metabolic process. Moreover, the retrieval of 16 GO terms associated with various molecular processes was achieved in this analysis. Most of the molecular functions (GO) were linked with enzyme binding, histone acetyltransferase binding, estrogen receptor activity, and nitric-oxide synthase regulator activity. In addition, 5 GO terms related to various cellular components were also achieved. It mainly included the intracellular organelles such as mitochondria. In addition, the retrieval of 52 KEGG pathway terms with a false discovery rate (FDR) <0.05 (Table 4) was achieved in this analysis. Most KEGG pathway terms were related to xenobiotic metabolism, induction of hepatitis, aging, and development of various cancers such as those of the thyroid, prostate, bladder, colorectal, pancreatic, and renal. All these terms had the lowest FDR. These pathways are associated with FOXO, mammalian target of rapamycin, tumor necrosis factor (TNF), p53, AMP-activated protein kinase, p13K-AKT, and mitogen-activated protein kinase (MAPK), which play an important role in inflammation, aging, and cancer.

Additional enrichment analysis of this protein interaction network was conducted by using GO terms (GO terms) for annotation of the biological functions of apigenin-related targets, employing Cytoscape-based plugin ClueGO. Overall, the significant enrichment of 49 GO terms was obtained (Table 5). Figure 3 illustrates the grouping of these GO terms into 20 subclasses, which are largely involved in macrophage differentiation, intracellular estrogen receptor signaling pathway, response to estradiol, estrogen metabolic process, mitotic G1

Table 4. Functional Enrichments in Network (Kyoto Encyclopedia of Genes and Genomes Pathways).

Pathway ID	Pathway description	Count in gene set	False discovery rate	Pathway ID	Pathway description	Count in gene set	False discovery rate
05205	Proteoglycans in cancer	8	7.58e-09	05220	Chronic myeloid leukemia	3	0.000497
05206	MicroRNAs in cancer	7	1.08e-08	05204	Chemical carcinogenesis	3	0.000522
05200	Pathways in cancer	8	5.36e-08	05215	Prostate cancer	3	0.000863
05219	Bladder cancer	4	3.8e-06	04066	HIF-1 signaling pathway	3	0.00148
04919	Thyroid hormone signaling pathway	5	5.59e-06	04726	Serotonergic synapse	3	0.00169
05161	Hepatitis B	5	1.2e-05	04068	FoxO signaling pathway	3	0.00211
05210	Colorectal cancer	4	1.28e-05	04152	AMPK signaling pathway	3	0.00211
05212	Pancreatic cancer	4	1.48e-05	04151	PI3K-Akt signaling pathway	4	0.00284
04115	p53 signaling pathway	4	1.81e-05	05216	Thyroid cancer	2	0.00284
04917	Prolactin signaling pathway	4	1.95e-05	05203	Viral carcinogenesis	3	0.00642
04210	Apoptosis	4	3.59e-05	04510	Focal adhesion	3	0.00858
05222	Small-cell lung cancer	4	3.59e-05	05221	Acute myeloid leukemia	2	0.0106
04915	Estrogen signaling pathway	4	4.98e-05	05416	Viral myocarditis	2	0.0106
04668	NF signaling pathway	4	7.81e-05	00982	Drug metabolism—cytochrome P450	2	0.0135
00380	Tryptophan metabolism	3	0.000149	04010	MAPK signaling pathway	3	0.0141
05162	Measles	4	0.000149	05166	HTLV-I infection	3	0.0141
04913	Ovarian steroidogenesis	3	0.000268	00980	Metabolism of xenobiotics by cytochrome P450	2	0.0142
05014	Amyotrophic lateral sclerosis	3	0.000268	04064	NF-kappa B signaling pathway	2	0.0238
05213	Endometrial cancer	3	0.000268	01100	Metabolic pathways	5	0.0353
00140	Steroid hormone biosynthesis	3	0.000303	05145	Toxoplasmosis	2	0.0364
05223	Non-small-cell lung cancer	3	0.000306	04722	Neurotrophin signaling pathway	2	0.0375
04150	ammalian target of rapamycin signaling pathway	3	0.000364	04110	Cell cycle	2	0.0405
04370	Vascular endothelial growth factor signaling pathway	3	0.000364	04650	Natural killer cell-mediated cytotoxicity	2	0.0407
05214	Glioma	3	0.000386	04728	Dopaminergic synapse	2	0.0407
05211	Renal cell carcinoma	3	0.000428	05160	Hepatitis C	2	0.0425
05218	Melanoma	3	0.000497	04310	Wnt signaling pathway	2	0.0462

Table 5. GO Terms and Their Associated Genes Obtained Through ClueGO Analysis.

GO ID	GO term	Associated genes found	GO ID	GO term	Associated genes found
30225	Macrophage differentiation	MMP9, PARP1, VEGFA	08210	Estrogen metabolic process	CYP19A1, CYP1B1, UGT1A1
32355	Response to estradiol	CASP3, CAT, CCND1, ESR1, PTGS2, UGT1A1	31571	Mitotic G1 DNA damage checkpoint	CCND1, CDK1, TP53
30520	Intracellular estrogen receptor signaling pathway	ESR1, ESR2, PARP1	10634	Positive regulation of epithelial cell migration	AKT1, HIF1A, MMP9, PTGS2, SPAM1, VEGFA

GO, gene ontology.

DNA damage checkpoint and positive regulation of epithelial cell migration. These biological processes help us better comprehend the mechanisms of apigenin action.

Discussion

Apigenin possesses excellent antiaging and anticancer activities.[2] However, the precise modes of its action are still uncertain. Therefore, this system pharmacology study was designed and executed via a combination of drug target retrieval, network construction, and pathway analysis.

Our results elaborated a total of 125 potential targets of apigenin. GO analysis of protein targets and target network analysis revealed the excellent effect of apigenin against aging and cancer, largely by inhibiting the inflammatory response. At the same time, the pathway analysis in our work elaborates that apigenin could instantaneously regulate multitargets/pathways coupled with multiple therapeutic modules, for instance, the inhibition of inflammation.

Apigenin has already been documented to mediate various activities against cancer, such as inhibition of oxidation and inhibition, triggering of cell-cycle arrest and apoptosis, attenuation of proliferation, and provoking of detoxification enzymes. Apigenin has been proved to have an inhibitory effect on (i) mutagenicity against nitropyrene-induced genotoxicity in Chinese hamster ovary cells, [5] benzoapyrene, and 2-aminoanthracene-induced bacterial mutagenesis,[5] (ii) carcinoma of skin and colon,[3,7] (iii) ornithine decarboxylase,[36] and (iv) UV-induced cancer.[3] In addition, apigenin-mediated accumulation of intracellular glutathione enhances the endogenous defense against oxidative stress.[15] Apigenin exerts its effect against inflammation through inhibition of cyclooxygenase-2,[15] nitric oxide synthase-2 activity,[37] and TNF-induced nuclear factor (NF)-κB.[38] Moreover, literature study reveals multiple molecular signaling effects of apigenin,[39] for instance, inhibition of protein kinase C activity, MAPK,[40,41] protein-tyrosine kinases and peroxisome proliferation regulated kinase (ERK),[42] Na+/Ca2+-exchanger,[43] phosphorylated EGFR tyrosine kinase and nuclear substrate c-myc,[44] casein kinase 2,[45] and p34 (cdc2) kinase activity.[46,47] Antiapoptotic activity of apigenin is mediated through triggering of WAF1/p21,[48] altering the Bax/Bcl-2 ratio,[49] regulating protease production,[50] inhibiting TNF-induced intracellular adhesion molecule-1 upregulation,[51] inhibiting expression of HIF-1,[52] VEGF,[53] aromatase,[54] 17β-hydroxysteroid dehydrogenase,[55] and IGF-I.[56] Many other proteins also act as targets of apigenin, for example heat shock proteins,[57] telomerase,[58] fatty acid synthase,[59] matrix metalloproteinases,[60] aryl hydrocarbon receptor activity,[58] and HER2.[43] These proteins play an important role in developing cancer. However, reliable scientific confirmations are really required to associate these genes with the system effects of apigenin.

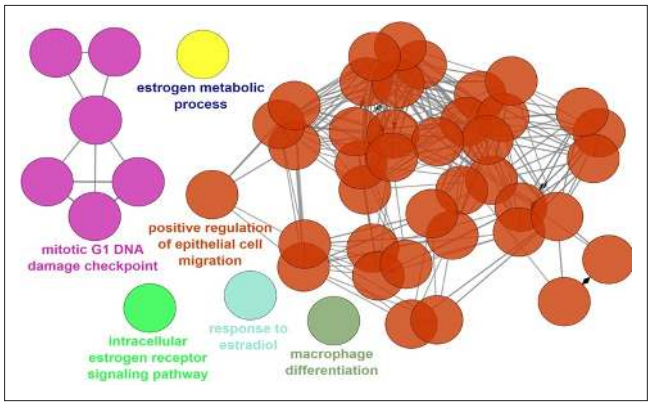

Figure 3. Functionally grouped networks, retrieved via ClueGO analysis to identify the potential targets of apigenin. Each class of targets consists of the most important terms only. The overlapped classes reflect their functional likeness.

It is also evident from the literature that apigenin could suppress inflammatory responses and treat the impairment caused by inflammation via inhibiting the expression of IL-6 and TNF-α and ameliorating the expression of IL-2, IL-10, and IFN-γ.[5] In this study, GO enrichment, network, and pathway analyses showed that apigenin considerably enriches target genes involved in suppressing the inflammation response and ameliorates the effects of aging and cancer. Figure 4 states the effect of apigenin on aging and cancer, where the anticancer feature of apigenin has been described mainly through the regulation of cellular response to oxidative stress, DNA damage, inhibition of inflammation, suppression of angiogenesis, reduction in cell proliferation, triggering of autophagy, and apoptosis. Out of all these, p53-mediated apoptosis and

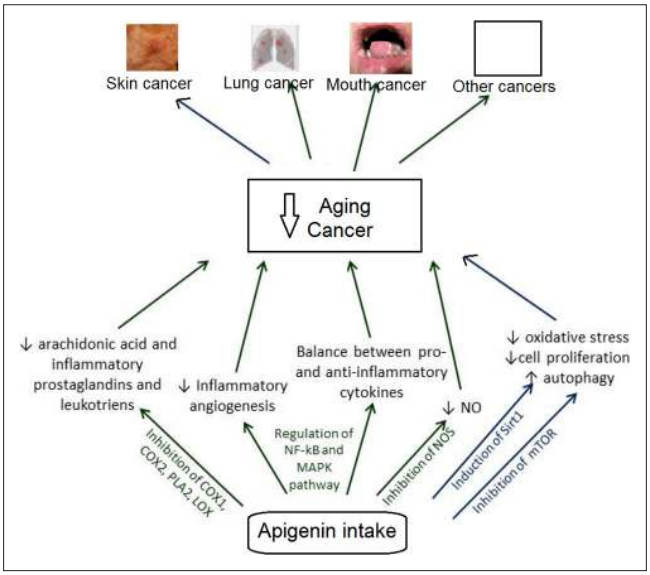

Figure 4. Effect of apigenin on aging and cancer. NF-kB, nuclear factor kB; MAPK, mitogen-activated protein kinase.

promotion of cell-cycle arrest is the well-described mode of apigenin.

Conclusions

This study reveals the dynamic targets and pathways of apigenin. These results support the conclusion that the modes of apigenin in aging and cancer largely include suppressing the inflammation response. This study not only contributes to an improved understanding of the modes of apigenin, but also suggests an approach to reveal novel drug candidates at a network pharmacology level. As a limitation of such in silico studies, this study could only retrieve apigenin targets that are available in published studies. Thus, additional experimentation in future studies is required to confirm the rationality of these findings as well as to investigate its clinical use in patients.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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