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Bioinformatics analyses on molecular pathways and pharmacological properties of glabridin

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Abstract

Glabridin, a bioactive compound that originally isolated from the roots of licorice (*Glycyrrhiza glabra* L., Fam. Fabaceae), has a wide range of pharmacological properties for instance anti-inflammatory, anti-cancer, anti-microbial, anti-viral, anti-osteoporosis, anti-diabetic, anti-atherogenic, neuroprotective, estrogenic, and skin-whitening. Even though, biological activities and pharmacological properties of glabridin have already been determined, molecular signaling pathways, gene targets, and pharmacological properties based on bioinformatics analyses have not been fully elucidated. Thus, in the presented research, network-based bioinformatics approaches were applied to demonstrate targets of glabridin in human genomes and proteomes. The glabridin was input into the ChEBI database, and the targets of its were predicted using DIGEP-Pred, and then, top interacting genes were identified by GeneCards database. Afterward, STRING and KEGG enrichment database were used to construct a protein-protein interaction (PPI) network and molecular targeting pathway network, respectively. A total of 14 genes coding proteins such as UGT1A1, MAPK1, CYP2B6, MMP9, CHKA, CYP3A4, EGFR, PON1, SLC6A4, SRC, EPHX2, TYR, PTK2, and PPIG effected by glabridin were determined by gene set enrichment analysis. Furthermore, multiple pathways including endocrine resistance, bladder cancer, ErbB signaling pathway, VEGF signaling pathway, chemical carcinogenesis, proteoglycans in cancer, relaxin signaling pathway, and estrogen signaling pathway were also identified to be regulated by glabridin. This research showed that glabridin exhibits highly active pharmacological activity as an anti-infective agent, chemopreventive agent, membrane permeability inhibitor, melanin inhibitor, and apoptosis agonist. Taken together, this study is network-based scientific research that will be very useful in elucidating the biological, molecular and pharmacological properties of glabridin for clinical applications in detail.

Keywords: Bioinformatics, Glabridin, KEGG pathway, Network pharmacology, Protein-protein interactions

Introduction

A type of isoflavonoid, glabridin is an isoflavone and has many pharmacological activities including improving metabolic abnormalities to improve obesity, diabetes, and cardiovascular disease, protecting nervous system function, as an estrogen substitute, preventing Staphylococcus, Candida and other microorganism-caused infection (Vaillancour et al., 2021), anti-cancer anti-inflammatory, anti-osteoporosis (Li et al., 2021), antiviral (Gezici and Sekeroglu, 2020; Sekeroglu and Gezici, 2020), anti-atherogenic, regulation of energy metabolism, estrogenic and skin-whitening (Simmler et al., 2013). The main source of glabridin is licorice root (*Glycyrrhiza glabra* L., Fam. Fabaceae), and this magic plant has been used has widely used traditional Chinese medicine and has also many other bioactive components, such as glycyrrhizic acid, glycyrrhetinic acid, liquiritin, isoliquiritigenin, licochalcones apart from glabridin (Hosseinzadeh and Nassiri-Asl, 2015). As a major flavonoid extracted from licorice root, glabridin is found a small quantity about 0.2% in the licorice root. Its chemical structure is a prenylated isoflavone and the systematic name is 4-[(3R)-8,8-dimethyl3,4-dihydro-2H-pyrano[2,3-f]chromen-3-yl]benzene-1,3-diol (Li et al., 2021).

Glabridin was first described in 1976 and many scientific research was released about it up to now. After exploring glabridin, it has been used in many formulations and now it is a valuable natural product in food, dietary supplements (DSs) and cosmetic industries. Recent scientific literature claim that

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biological activity of glabridin primarily comes from its activity for down-regulating intracellular reactive oxygen species (ROS), binding to antioxidant effectors, and acting on estrogen receptors, potentially as a plant-based Selective Estrogen Receptor Modulator (phytoSERM) (Simmler et al., 2013).

However, a numerous studies have been performed for uncover biological properties of glabridin, network-based molecular and pharmacological activities of glabridin have not been proposed yet. Therefore, we aimed to clarify the potential interactions of glabridin by gene-set enrichment and network pharmacology analyses to provide a novel approach to reveal the therapeutic mechanisms of glabridin that will ease its future clinical applications in the treatment of diseases.

Materials and Methods

Chemical Compositions and Predicted Targets

Chemical Entities of Biological Interest (ChEBI) database, a part of ELIXIR Core Data Resources, was used for dictionary of molecular entities and chemical properties of glabridin (Hastings et al., 2016). The targets of glabridin were identified using DIGEP-Pred (Prediction of drug-induced changes of gene expression profile) based on structural formula of glabridin (Lagunin et al., 2013).

Gene Set Enrichment Analysis

GeneCards, The Human Gene Database, was used to determine probable interacting genes of glabridin. Based on this database, top interacting genes were analyzed using unique GeneCards identifiers (GC ids) and GeneCards Inferred Functionality Scores (GIFtS), provided by the GeneLoc Algorithm (Harel et al., 2009; Fishilevich et al., 2016).

Protein-Protein Interaction (PPI) Analysis

STRING database was used to annotate the role of probable interacting genes and proteins associated with glabridin. PPI network mapping was conducted on glabridin and protein targets using the Retrieval of Interacting Genes database with the species limited to "homo sapiens" and a confidence score > 0.4 (Wu et al., 2009; Athanasios et al., 2017).

KEGG Enrichment Analysis

KEGG (Kyoto Encyclopedia of Genes and Genomes) is an integrated database of genes and genomes used for mapping pathways at molecular level. KEGG enrichment analysis was performed for construction the network regulated by glabridin (Aoki-Kinoshita and Kanehisa, 2007; Kanehisa et al., 2017).

Results

Results of Chemical Compositions and Predicted Targets

Glabridin ($C_{20}H_{20}O_4$) belonging to the class of isoflavonoids, the most valuable natural compound, is found in the roots of licorice (*Glycyrrhiza glabra* L.). The systematic name of glabridin with an average molecular weight of 324.376 g/mol is 4-{8,8-dimethyl-2H,3H,4H-pyrano [2,3-f]chromen-3-yl}benzene-1,3-diol. Hispaglabridin A ($C_{25}H_{28}0_4$), 4'-O-Methylglabridin ($C_{21}H_{22}O_4$), 2'-O-Methylglabridin ($C_{21}H_{22}O_4$), (R)-Hispaglabridin A ($C_{25}H_{28}O_4$), (R)-Hispaglabridin B ($C_{25}H_{26}O_4$), 3'-Hydroxy-4'-methoxyglabridin ($C_{21}H_{22}O_5$), and 4'-O-Methylpreglabridin ($C_{21}H_{24}O_4$) are derivatives of glabridin, of which 4'-O-Methylglabridin, 2'-O-Methylglabridin, (R)-Hispaglabridin A, (R)-Hispaglabridin B belong to the class of isoflavonoids, while Hispaglabridin A and 4'-O-Methylpreglabridin are from the class of hydroxyisoflavans and flavonoids, respectively. The chemical structure of glabridin and its derivatives were given in the in the Figure. 1.

The targets of glabridin were investigated according to prediction of drug-induced changes of gene expression profile for proteins at the pharmacological activity (Pa) > 0.5. The findings were presented in the Table 1. Pa (probability to be active) means the chance that glabridin is belonging to the subclass of active compounds, while Pi (probability to be inactive) means the chance that glabridin is belonging to the subclass of inactive compounds. Based on the data presented in the table, glabridin exhibits quite active biological activities and pharmacological properties. Actually, capable of acting against infection as an antiinfective agent, expression inhibitor on Hypoxia-inducible factor 1-alpha, substrate of cytochrome P450, chemopreventive agent, membrane permeability inhibitor, melanin inhibitor, and apoptosis agonist were determined as the most important properties of glabridin (Pa > 0.7).

Table 1. Prediction of drug-induced changes of gene expression profile for glabridin

Pa	Pi	Activity
0,948	0,003	Antiinfective
0,911	0,005	HIF1A expression inhibitor
0,839	0,027	CYP2C12 substrate
0,786	0,004	Chemopreventive
0,775	0,014	Membrane permeability inhibitor
0,715	0,001	Melanin inhibitor
0,700	0,002	Skin whitener
0,676	0,017	Apoptosis agonist
0,658	0,002	RELA expression inhibitor

0,650	0,011	Histidine kinase inhibitor
0,648	0,017	Spasmolytic, urinary
0,640	0,003	NOS2 expression inhibitor
0,640	0,038	TP53 expression enhancer
0,618	0,071	Membrane integrity agonist
0,616	0,035	Antidyskinetic
0,611	0,014	Spasmolytic
0,609	0,012	AR expression inhibitor
0,597	0,062	Chlordecone reductase inhibitor
0,596	0,009	Lipid peroxidase inhibitor
0,590	0,101	Aspulvinone dimethylallyltransferase inhibitor
0,589	0,048	Antineoplastic
0,562	0,005	Antioxidant
0,533	0,029	Kinase inhibitor
0,519	0,009	CYP2E1 inhibitor
0,517	0,023	Cytostatic
0,507	0,148	Ubiquinol-cytochrome-c reductase inhibitor
0,503	0,104	Antiischemic, cerebral
0,502	0,107	Phosphatase inhibitor

Table 2. The list of top genes interacts with glabridin

	Symbol	Description	Category	GIFtS	GC id	Score
1	UGT1A1	UDP Glucuronosyltransferase Family 1 Member A1	Protein Coding	46	GC02P233760	1.81
2	MAPK1	Mitogen-Activated Protein Kinase 1	Protein Coding	50	GC22M021759	1.77
3	CYP2B6	Cytochrome P450 Family 2 Subfamily B Member 6	Protein Coding	45	GC19P040991	1.64
4	MMP9	Matrix Metallopeptidase 9	Protein Coding	51	GC20P046008	1.60
5	MIR148A	MicroRNA 148a	RNA Gene	19	GC07M025993	1.59
6	CHKA	Choline Kinase Alpha	Protein Coding	40	GC11M068052	1.35
7	CYP3A4	Cytochrome P450 Family 3 Subfamily A Member 4	Protein Coding	49	GC07M099759	1.32
8	EGFR	Epidermal Growth Factor Receptor	Protein Coding	51	GC07P055019	1.19
9	PON1	Paraoxonase 1	Protein Coding	44	GC07M095297	1.10
10	SLC6A4	Solute Carrier Family 6 Member 4	Protein Coding	46	GC17M030194	0.32
11	SRC	Proto-Oncogene, Non-Receptor Tyrosine Kinase	Protein Coding	50	GC20P037344	0.22
12	EPHX2	Epoxide Hydrolase 2	Protein Coding	46	GC08P027490	0.22
13	TYR	Tyrosinase	Protein Coding	45	GC11P089177	0.22
14	PTK2	Protein Tyrosine Kinase 2	Protein Coding	45	GC08M140657	0.22
15	PPIG	Peptidylprolyl Isomerase G	Protein Coding	40	GC02P169584	0.22

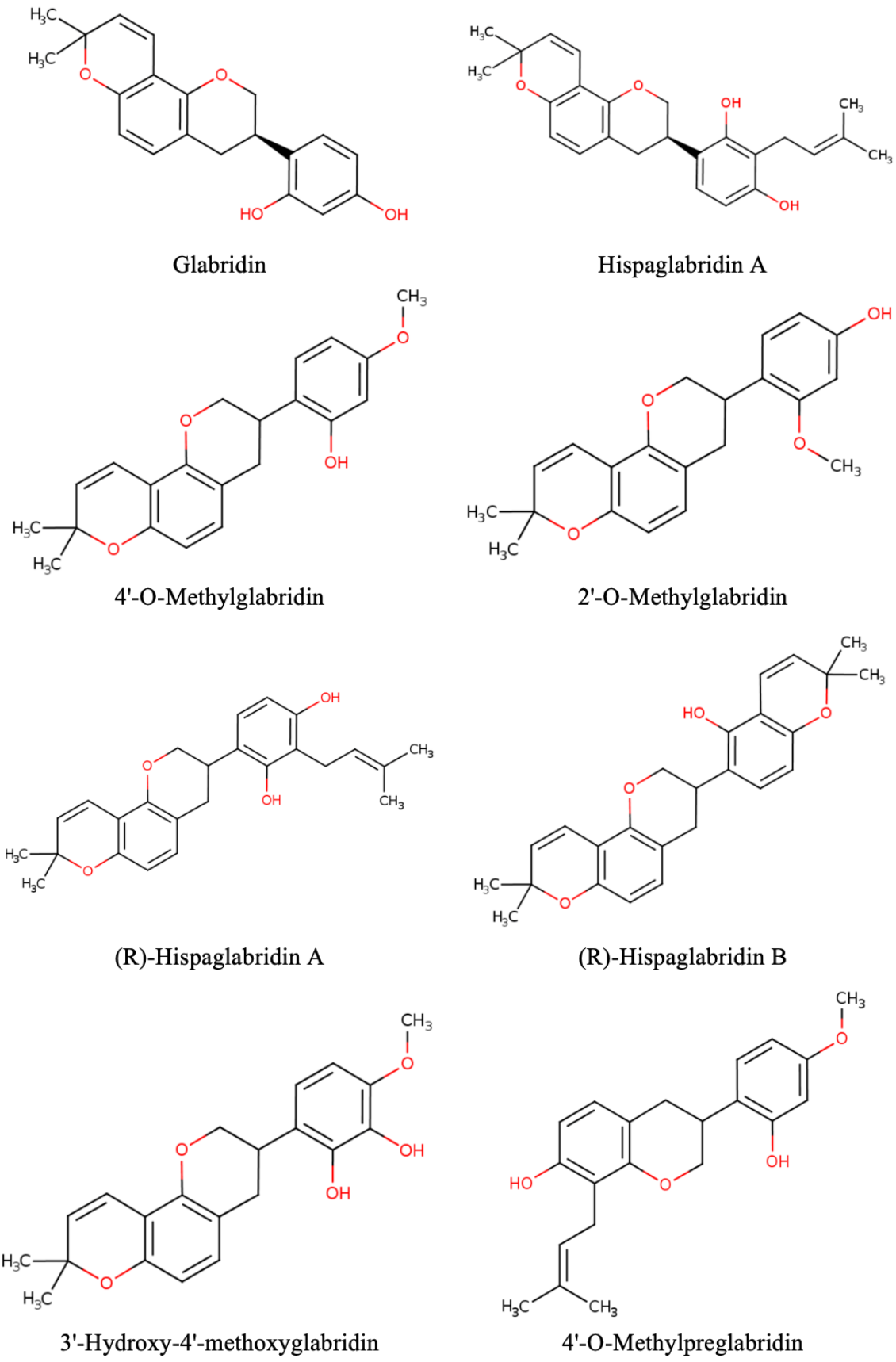

Figure 1. Chemical compositions of glabridin and glabridin derivatives

Results of Top Gene Enrichment Analysis

A total of fifteen genes regulated by glabridin were determined as the most interacting genes using gene enrichment analyses. All these genes, except MIR148A, are protein coding genes and MAPK1, MMP9, EGFR, SRC, UGT1A1, and CYP3A4 were found the most interacting genes, whereas PPIG, TYR, and PTK2 were identified as the least interacting genes with glabridin (Table 2).

Results of Protein – Protein Interaction (PPI) Network

The relationship of a total of 14 proteins between each other were constructed from STRING database with PPI enrichment p-value = 1.47e-06 (FDR < 0.05). PPPI network was presented in the Fig. 2. As can be seen in the Figure, SRC, MAPK1, PTK2, PPIG, and EGFR are the proteins that located in the center of the network (Figure. 2).

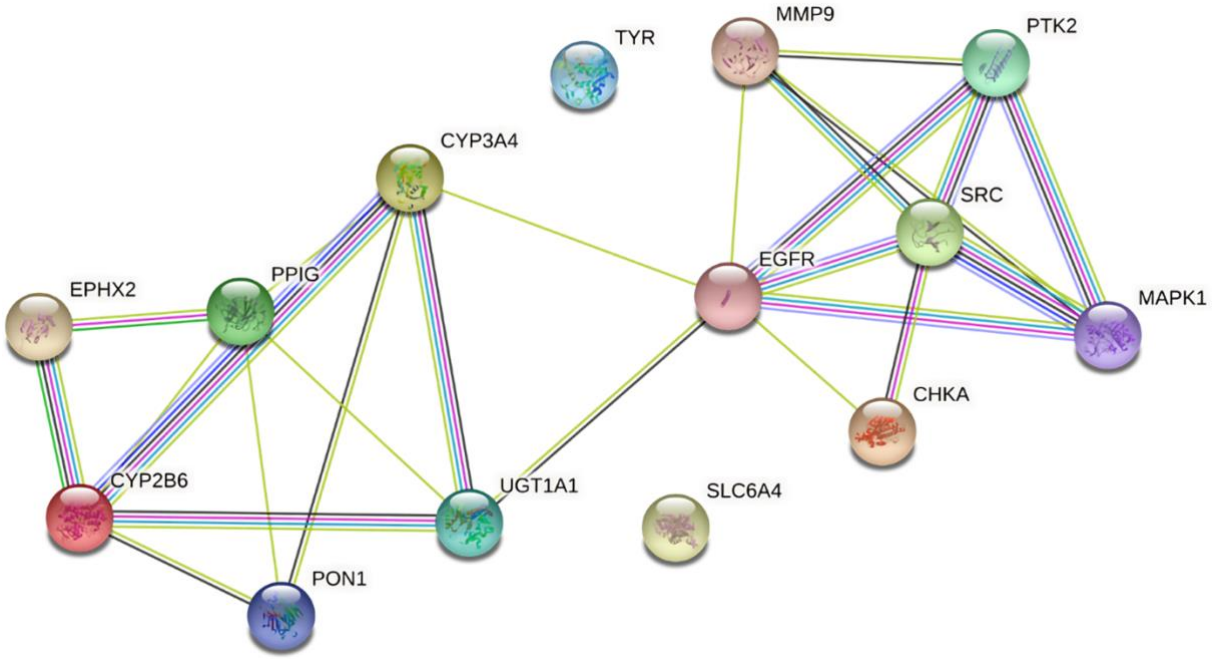

Figure 2. Protein-protein interaction proteins modulated by glabridin

Table 3. KEGG Enrichment analysis of proteins modulated by glabridin

#term ID	term description	observed gene count	background gene count	strength	false discovery rate	matching proteins in your network (labels)
hsa01522	Endocrine resistance	5	95	1.87	2.75e-06	MAPK1, EGFR, PTK2, MMP9, SRC
hsa05219	Bladder cancer	4	41	2.13	5.44e-06	MAPK1, EGFR, MMP9, SRC
hsa05205	Proteoglycans in cancer	5	196	1.55	3.05e-05	MAPK1, EGFR, PTK2, MMP9, SRC
hsa04012	ErbB signaling pathway	4	83	1.83	3.99e-05	MAPK1, EGFR, PTK2, SRC
hsa04915	Estrogen signaling pathway	4	133	1.62	0.00017	MAPK1, EGFR, MMP9, SRC
hsa04926	Relaxin signaling pathway	4	128	1.64	0.00017	MAPK1, EGFR, MMP9, SRC
hsa00830	Retinol metabolism	3	64	1.82	0.00063	CYP2B6, CYP3A4, UGT1A1

hsa00980	Metabolism of xenobiotics by cytochrome P450	3	69	1.78	0.00063	CYP2B6, CYP3A4, UGT1A1
hsa00982	Drug metabolism - cytochrome P450	3	64	1.82	0.00063	CYP2B6, CYP3A4, UGT1A1
hsa04370	VEGF signaling pathway	3	57	1.87	0.00063	MAPK1, PTK2, SRC
hsa04510	Focal adhesion	4	198	1.45	0.00063	MAPK1, EGFR, PTK2, SRC
hsa04520	Adherens junction	3	67	1.8	0.00063	MAPK1, EGFR, SRC
hsa04810	Regulation of actin cytoskeleton	4	209	1.43	0.00063	MAPK1, EGFR, PTK2, SRC
hsa05131	Shigellosis	4	218	1.41	0.00063	MAPK1, EGFR, PTK2, SRC
hsa05163	Human cytomegalovirus infection	4	218	1.41	0.00063	MAPK1, EGFR, PTK2, SRC
hsa01521	EGFR tyrosine kinase inhibitor resistance	3	78	1.73	0.00068	MAPK1, EGFR, SRC
hsa04540	Gap junction	3	87	1.68	0.00088	MAPK1, EGFR, SRC
hsa04912	GnRH signaling pathway	3	89	1.67	0.00089	MAPK1, EGFR, SRC
hsa05215	Prostate cancer	3	96	1.64	0.0010	MAPK1, EGFR, MMP9
hsa05231	Choline metabolism in cancer	3	96	1.64	0.0010	MAPK1, CHKA, EGFR
hsa05135	Yersinia infection	3	125	1.53	0.0020	MAPK1, PTK2, SRC
hsa05418	Fluid shear stress and atherosclerosis	3	130	1.51	0.0022	PTK2, MMP9, SRC
hsa04921	Oxytocin signaling pathway	3	149	1.45	0.0031	MAPK1, EGFR, SRC
hsa05161	Hepatitis B	3	159	1.42	0.0036	MAPK1, MMP9, SRC
hsa05206	MicroRNAs in cancer	3	160	1.42	0.0036	MAPK1, EGFR, MMP9
hsa04360	Axon guidance	3	177	1.37	0.0045	MAPK1, PTK2, SRC
hsa01100	Metabolic pathways	6	1447	0.76	0.0050	TYR, CHKA, CYP2B6, CYP3A4, UGT1A1, EPHX2
hsa04062	Chemokine signaling pathway	3	186	1.35	0.0050	MAPK1, PTK2, SRC
hsa04015	Rap1 signaling pathway	3	202	1.32	0.0059	MAPK1, EGFR, SRC

hsa05200	Pathways in cancer	4	517	1.03	0.0061	MAPK1, EGFR, PTK2, MMP9
hsa05213	Endometrial cancer	2	57	1.69	0.0116	MAPK1, EGFR
hsa00140	Steroid hormone biosynthesis	2	59	1.68	0.0120	CYP3A4, UGT1A1
hsa00590	Arachidonic acid metabolism	2	61	1.66	0.0124	CYP2B6, EPHX2
hsa04917	Prolactin signaling pathway	2	69	1.61	0.0145	MAPK1, SRC
hsa05100	Bacterial invasion of epithelial cells	2	70	1.6	0.0145	PTK2, SRC
hsa05120	Epithelial cell signaling in Helicobacter pylori infection	2	67	1.62	0.0145	EGFR, SRC
hsa05212	Pancreatic cancer	2	73	1.58	0.0145	MAPK1, EGFR
hsa05214	Glioma	2	72	1.59	0.0145	MAPK1, EGFR
hsa05218	Melanoma	2	72	1.59	0.0145	MAPK1, EGFR
hsa05223	Non-small cell lung cancer	2	68	1.61	0.0145	MAPK1, EGFR
hsa05230	Central carbon metabolism in cancer	2	69	1.61	0.0145	MAPK1, EGFR
hsa00983	Drug metabolism - other enzymes	2	75	1.57	0.0146	CYP3A4, UGT1A1
hsa05204	Chemical carcinogenesis	2	75	1.57	0.0146	CYP3A4, UGT1A1
hsa05165	Human papillomavirus infection	3	325	1.11	0.0153	MAPK1, EGFR, PTK2
hsa05210	Colorectal cancer	2	82	1.53	0.0161	MAPK1, EGFR
hsa04151	PI3K-Akt signaling pathway	3	350	1.08	0.0180	MAPK1, EGFR, PTK2
hsa04976	Bile secretion	2	89	1.5	0.0180	CYP3A4, UGT1A1
hsa05235	PD-L1 expression and PD-1 checkpoint pathway in cancer	2	88	1.5	0.0180	MAPK1, EGFR
hsa04657	IL-17 signaling pathway	2	92	1.48	0.0185	MAPK1, MMP9
hsa04916	Melanogenesis	2	95	1.47	0.0193	MAPK1, TYR
hsa04625	C-type lectin receptor signaling pathway	2	102	1.44	0.0217	MAPK1, SRC

hsa04928	Parathyroid hormone synthesis, secretion and action	2	103	1.43	0.0217	MAPK1, EGFR
hsa04066	HIF-1 signaling pathway	2	106	1.42	0.0225	MAPK1, EGFR
hsa04670	Leukocyte transendothelial migration	2	109	1.41	0.0229	PTK2, MMP9
hsa04726	Serotonergic synapse	2	108	1.41	0.0229	MAPK1, SLC6A4
hsa04668	TNF signaling pathway	2	112	1.4	0.0237	MAPK1, MMP9
hsa04919	Thyroid hormone signaling pathway	2	119	1.37	0.0257	MAPK1, SRC
hsa04935	Growth hormone synthesis, secretion and action	2	118	1.37	0.0257	MAPK1, PTK2
hsa04611	Platelet activation	2	122	1.36	0.0265	MAPK1, SRC
hsa04068	FoxO signaling pathway	2	127	1.34	0.0282	MAPK1, EGFR
hsa05224	Breast cancer	2	145	1.29	0.0353	MAPK1, EGFR
hsa05226	Gastric cancer	2	144	1.29	0.0353	MAPK1, EGFR
hsa04072	Phospholipase D signaling pathway	2	147	1.28	0.0355	MAPK1, EGFR
hsa04934	Cushing syndrome	2	153	1.26	0.0378	MAPK1, EGFR
hsa05160	Hepatitis C	2	156	1.25	0.0386	MAPK1, EGFR
hsa05225	Hepatocellular carcinoma	2	160	1.24	0.0399	MAPK1, EGFR
hsa05152	Tuberculosis	2	168	1.22	0.0431	MAPK1, SRC
hsa05202	Transcriptional misregulation in cancer	2	171	1.21	0.0440	PTK2, MMP9
hsa05203	Viral carcinogenesis	2	182	1.19	0.0488	MAPK1, SRC

Results of KEGG Enrichment Pathway

According to the KEGG enrichment pathway analyses, a total of 69 different pathways were defined as the probably modulated pathways by glabridin. The identified pathways were summarized in the Table 3, corresponding to 14 protein targets. As summarized in the Table 3, several target proteins are simultaneously involved in one pathway, while one target protein is also present in many pathways. A hierarchical clustering tree is schematized in Figure 3, summarizing the correlations between the major paths listed in the enrichment tab. Although pathways containing many common genes are clustered together; larger dots indicate more significant P values.

Accordingly, endocrine resistance, bladder cancer, proteoglycans in cancer, ErbB signaling pathway, estrogen signaling pathway, relaxin signaling pathway, retinol metabolism, metabolism of xenobiotics by cytochrome P450, drug metabolism - cytochrome P450, VEGF signaling pathway, focal adhesion, adherens junction, regulation of actin cytoskeleton, shigellosis, human cytomegalovirus infection, EGFR tyrosine kinase inhibitor resistance pathways and so forth were the most enrichment pathways-modulated by glabridin with the p-value cutoff (FDR<0.05). In addition, endocrine resistance,

bladder cancer, ErbB signaling pathway, VEGF signaling pathway, chemical carcinogenesis, proteoglycans in cancer, relaxin signaling pathway, and estrogen signaling pathway were determined as the top pathways associated with glabridin-regulated proteins with the lowest false discovery rate (FDR<0.05) (Figure. 3).

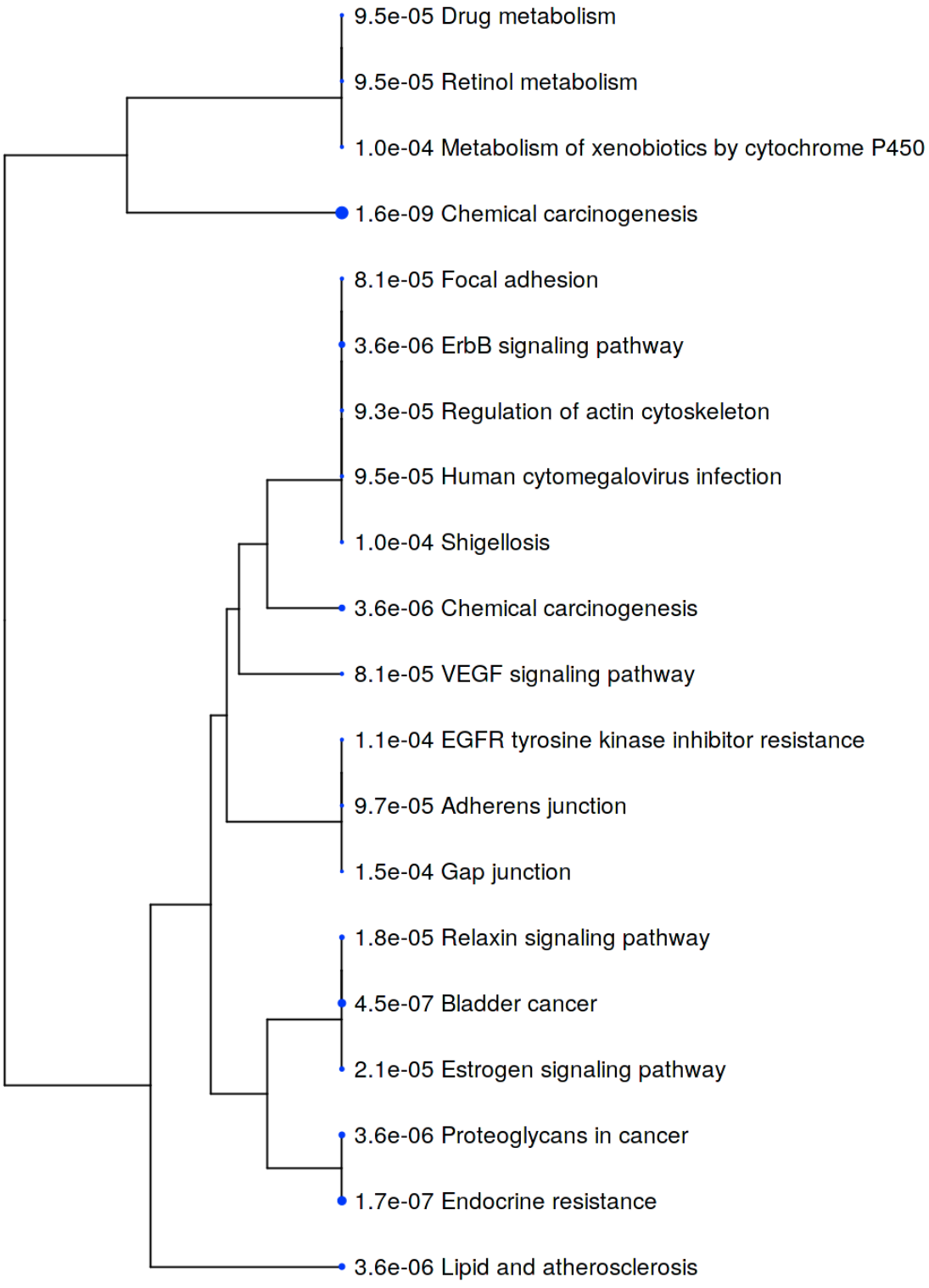

Figure 3. Chart of top related pathways construction with KEGG enrichment from STRING database

Discussion

Bioinformatics integrates systems-level network-based pharmacology and can provide insight into the molecular mechanisms of herbal formulas used for treatment of complex diseases. Currently, network-based analysis has been implemented to natural

compounds isolated form medicinal plants in order to search for multitargeted compounds that act in biological networks to explore multiple molecular mechanisms (Lee et al., 2018; Huang et al., 2020). Even though biological effects and pharmacological profiling of glabridin have been studied, network based genomic and proteomics prediction of glabridin and its target pathways has not been conducted yet (Simmler et al., 2013; Hosseinzadeh and Nassiri-Asl, 2015; Li et al., 2021). Thus, we investigated the potential interactions of glabridin by gene-set enrichment and network-based molecular pharmacology analyses to reveal the therapeutic mechanisms of glabridin in the current research. Glabridin regulates the activities of these genes in a way that causes an increase in the expression levels of some of the genes, when it causes a decrease in others. Glabridin both inactivates the active forms of the SRC (proto-oncogene, non-receptor tyrosine kinase) gene and enhances levels of phosphorylated SRC that functions as a proto-oncogene (Simmler et al., 2013; Su Wei Poh et al., 2015).

As for the PPI analyses of targeting proteins, SRC, MAPK1, PTK2, PPIG, and EGFR are identified as the core proteins that located in the center of the network. Among these proteins, SRC is a tyrosine kinase protein that involved in many biologicals signaling pathways such as gene transcription, immune response, cell adhesion, cell cycle progression, migration, and apoptosis. MAPK1, a serine/threonine kinase protein, is a major component of MAP kinase signal transduction pathway, and acts a significant role in the MAPK/ERK cascade, which mediates various biological functions including cell growth, adhesion, survival, and differentiation via the regulation of transcription, translation, cytoskeletal rearrangements. PTK2 is the other tyrosine kinase protein found in the center of network that it acts important roles in regulating cell migration, cell adhesion, metastasis, cell protrusions, cell cycle progression, cell proliferation and apoptosis, as well as reorganization of the actin cytoskeleton, and formation of focal adhesions. PPIG, peptidyl-prolyl cis-trans isomerase G, is a protein with catalytic activity that is involved in the folding, transport and assembly of proteins, besides in regulating pre-mRNA splicing. Lastly, EGFR, also called as proto-oncogene c-ErbB-1 and receptor tyrosine-protein kinase ErbB-1, is an essential protein participated in cell signaling pathways mainly associated with many types of cancer. Moreover, drugs that arrest epidermal growth factor receptor proteins are used in the treatment of some types of cancer (Hsu et al., 2011; Tsai et al., 2011; Huang et al., 2014; Lee et al., 2020; Li et al., 2021).

Additionally, target signaling pathways modulated by glabridin were determined in this research. It is well-known that multiple signaling pathways interact with each other in the metabolic processes normally occurred in living organisms. Based on the KEGG enrichment pathway analyses, most of the genes regulated by glabridin are closely associated with estrogen receptor modulator, oxidative stress, immune system, neurodegeneration, inflammation, and angiogenesis, as well as cancer. MAPK1, EGFR, PTK2, MMP9, and SRC genes are participated in endocrine resistance and estrogen signaling pathway, which are closely related to cancer development and progression. In agreement with the findings from this bioinformatics-based study, previous reports indicated that glabridin is used to treat of menopausal symptoms and thus has a

Herewith, glabridin prevent the cancer progression in many types of cancer through inhibition of migration, invasion, and angiogenesis. Glabridin also suppresses migration and invasion by transcriptionally inhibiting MMP9 (matrix metalloproteinase 9) via the modulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and activator protein 1 (AP-1) activity in human cancer cells. (Hsieh et al., 2016; Liu et al., 2019). Therefore, glabridin may function as a powerful immune stimulator and cancer preventive agent. Additionally, glabridin inhibits BRAF/MEK signaling pathway through arresting cell cycle and inhibiting proliferation in many types of cancers such as hepatocellular carcinoma, lung cancer, osteosarcoma. Meanwhile, it also reduces inflammation which caused by the inhibition of p38 mitogen activated protein kinase/extracellular regulated protein kinases (p38MAPK/ERK) signaling pathway that is a key pathway in the regulation of cellular processes including cell proliferation, survival, and differentiation. Besides, p38MAPK/ERK signaling pathway, glabridin induces cell death in the cancer cells by inducing apoptosis through the p38 MAPK and JNK1/2 pathways (Zhang and Li, 2016; Wang et al., 2016; Liu et al., 2019). Conversely, Glabridin induces the expression level of UDP-glucuronosyltransferase (UGT1A1) gene, which catalyzes the conjugation of bilirubin in the liver, supporting hepatoprotective properties of glabridin. Likewise, induction of the expression of UGT1A1 enzyme can improve the detoxification process and thereby releasing oxidative stress and contributing to reduce the burden of cancer development (Leung, 2001; Simmler et al., 2013). In contrast, the expression level of EGFR is suppressed by glabridin, resulting in decreased in cell proliferation, migration and angiogenesis as well as increased apoptotic process (Tsai et al., 2011; Zhu et al., 2019). It can be clearly stated that glabridin functions as a chemopreventive agents and apoptosis agonist. In addition to these activities, glabridin have been shown to induce the expressions of cytochrome P450 family 2 subfamily B member 6 (CYP2B6) and cytochrome P450 family 3 subfamily A member 4 (CYP3A4), which are involved in the metabolism of various endogenous substrates, including the metabolism of steroid, arachidonate, and retinol and also play a role in the oxidative metabolism of xenobiotics (Shahabi et al., 2014).

possible role in estrogen replacement therapy (ERT) (Tsai et al., 2011; Su Wei Poh et al., 2015). The genes of MAPK1, EGFR, MMP9, and SRC, whose expression levels are regulated by glabridin, are involved in relaxin signaling pathway signaling pathways. Relaxin, a polypeptide hormone with antifibrotic properties, inhibits fibrosis through numerous cellular targets and signaling pathways. Previous reports, supporting the findings from this study show that glabridin is an anti-inflammatory and anti-fibrotic agent (Ng et al., 2019). Furthermore, glabridin has also involved in ErbB signaling pathway and pathways of proteoglycans in cancer that revealed by previous studies. As revealed in previous studies, Insufficient ErbB signaling or expression of EGFR family are associated with the development of neurodegenerative diseases, whilst excessive ErbB signaling and increased EGFR expression are associated with the development of a wide variety cancer types (Zhang et al., 2016; Zhu et al., 2019; Karthikkeyan et al., 2020). In another research showed that glabridin regulates the MAPK1/3 and PI3K/AKT pathways (Karthikkeyan et al., 2020). Accordingly, glabridin has been shown to be a substantial pharmaceutical resource for drug targets, consistent with these findings from network-based molecular and pharmacological analyzes.

Conclusion

Glabridin is a natural isoflavonoid that found mainly in licorice roots and proven to possess remarkable biological and pharmacological activities in the human metabolism. In the current study, endocrine resistance, bladder cancer, ErbB signaling pathway, VEGF signaling pathway, chemical carcinogenesis, proteoglycans in cancer, relaxin signaling pathway, and estrogen signaling pathways involved in estrogen receptor modulator, oxidative stress, immune system, neurodegeneration, inflammation, and angiogenesis, as well as cancer were identified as the top pathways regulated by glabridin. Moreover, SRC, MAPK1, EGFR, PTK2, and MMP9, as well as CYP2B6, CYP3A4, and UGT1A1 were determined main core proteins involved in the top signaling pathways. Taken together, the targets of these core proteins may play a key role in the therapeutic potentials of glabridin. According to our network-based analysis, glabridin may exert various pharmacological effects through multiple targets, pathways, and biological processes, thus having potential use as a drug. Although it is rare to use glabridin alone as a medicine today, it is expected that glabridin will be most likely to be used in the future drug discovery. Further studies, especially clinical trials are necessary to confirm the metabolic efficacy of glabridin, as well as to reveal the possible side effects of glabridin.

Compliance with Ethical Standards

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