

Network pharmacology-based strategy to investigate pharmacological mechanisms of Ginkgo Biloba Extract for Aging

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Research

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Abstract

Background Aging represents the main risk factor for a number of debilitating diseases and contributes to increase in mortality. Previous studies have shown that ginkgo biloba extract (EGb) can prevent and treat aging-related diseases, but its pharmacological effects need to be further clarified. In this study, we proposed a network pharmacology-based method to identify the therapeutic pathways of EGb for aging.

Methods The active components of EGb and targets of sample chemicals were obtained from TCMSP database. Aging-related genes were obtained by retrieving the Human Ageing Genomic Resources database and the JenAge Ageing Factor Database. Then, a network containing the interactions between the putative targets of EGb and known therapeutic targets of aging was built, which was used to investigate pharmacological mechanisms of EGb for Aging.

Results 24 active components, 154 targets of active components of EGb and 308 targets of aging were obtained. Network construction and pathway enrichment were carried out after data integration. The research found that flavonoids (quercetin, luteolin, kaempferol) and beta-sitosterol might be the main active component of EGb. The top eight candidate targets, including PTGS2, PPARG, DPP4, GSK3B, CCNA2, AR, MAPK14, ESR1, were chosen as EGb's main therapeutic targets. The results of pathway enrichment participated in various pathways associated with inhibiting oxidative stress, inhibiting inflammation, ameliorating insulin resistance and regulating cellular biological processes, etc. Molecular docking results showed that PPARG had better binding capacity with beta-sitosterol and PTGS2 had better binding capacity with kaempferol and quercetin.

Conclusions The main components of EGb might act on multiple targets, such as PTGS2, PPARG, DPP4, GSK3B, etc., to regulate multiple pathways and played an anti-aging role by inhibiting oxidative stress, inhibiting inflammation, ameliorating insulin resistance.

1. Background

Aging is inevitable, but pathological aging poses a serious threat to human health, reduces the quality of life of the elderly, and promotes the occurrence of related diseases [1, 2]. Aging is an extremely important factor that induces many kinds of cardiovascular and cerebrovascular diseases such as atherosclerosis, myocardial infarction, stroke and heart failure [3, 4]. Among them, vascular aging plays a central role in morbidity and mortality of people [5]. After years of research, researchers have put forward many hypotheses about aging mechanism, such as oxidative stress, genetic determination theory, free radical theory, neuroendocrine theory, mitochondrial damage theory, etc. [4, 6, 7]. Most importantly, increasing oxidative stress, a major characteristic of aging, has been implicated in various age-related diseases [8]. The biological mechanisms of aging are still being explored. As fertility declines and life expectancy increases, the proportion of the world's population aging is increasing, and the presence of age-related diseases increases the socio-economic burden. Therefore, it has become one of the most important issues to study the biological mechanism of aging, explore effective drugs that can delay or reduce the

occurrence and development of age-related diseases and clarify the mechanism. It is believed that many of the medicinal herbs have anti-aging properties, but the mechanisms remain unclear.

Ginkgo biloba extract (EGb), one of famous medicinal plants, can treat many aging related diseases, such as cardiovascular and neurodegenerative aging-related diseases [9]. Due to its medicinal value, more and more attention has been paid to the basic research and clinical application of EGb. The study finds that the chemical composition of EGb is complex, including flavonoids, lactones, polyphenols, and alkyl phenolic acids, organic acids, steroids, trace elements, etc[10]. EGb preparation is a relatively successful case of plant medicine developed by modern science and technology, and occupies an important position in the field of anti-aging and clinical medication for cardiovascular and cerebrovascular diseases [9, 11]. Previous studies have shown that EGb has certain efficacy in the prevention and treatment of age-related diseases [9, 12–14]. However, its pharmacologic profiles of EGb for aging remain to be elucidated. Therefore, in this study, network pharmacology method was used to preliminarily explore molecular mechanism of EGb for aging.

2. Materials And Methods

2.1 Identification and screen of candidate compounds

The active constituents of EGb were obtained from TCMSP database (<http://lsp.nwsuaf.edu.cn/tcmsp.php>). Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) is an integrated systems pharmacology platform of Chinese herbal medicines that covers chemicals, targets and drug targets, as well as pharmacokinetic properties for compounds involving oral bioavailability, drug-likeness, blood-brain-barrier, intestinal epithelial permeability, aqueous solubility and etc [15]. In this study, oral bioavailability (OB) and drug-likeness (DL) were utilized to screen out the chemical constituents meeting $OB \geq 30\%$ and $DL \geq 0.18$ simultaneously [16]. In addition, the active ingredients with low oral bioavailability and remarkable efficacy reported in the literature were included.

2.2 Potential Targets of Active Components.

The validated targets proteins of the active components were obtained from TCMSP database. The conversion of the target protein name of the active ingredient to the standard target gene name was realized through the UniProt knowledge base (UniProtKB, <http://www.uniprot.org/>). The UniProtKB is the central hub for the collection of functional information on proteins, with accurate, consistent and rich annotation. The target protein names were inputted into UniProtKB, with the organism restricted to “Homo sapiens”, eventually gaining the official symbol.

2.3 Search of aging related targets and genes

Aging-related genes were obtained by retrieving the Human Ageing Genomic Resources database (HAGR, <http://genomics.senescence.info/>) and the JenAge Ageing Factor Database (AgeFactDB, <http://agefactdb.jenage.de>). HAGR is a collection of databases and tools for studying human aging

through modern approaches such as functional genomics, network analysis, systems biology, and evolutionary analysis[17]. The purpose of the JenAge aging factor database is to collect and integrate aging phenotype and longevity data and AgeFactDB also includes genes that are homologous to known ageing-related genes [18].The target matching analysis of aging related genes and EGb was conducted to select the target of EGb acting on aging.

2.4 Construction of networks and analyses

The Cytoscape software is adopted to visually display and analyze the network structure. It is an open source software platform for visualizing molecular interaction networks and biological pathways and integrating these networks with annotations, gene expression profiles and other state data[19]. There, two networks were constructed in this study as follows: (1) A component-component target network was established by joining active components of herbs and corresponding. (2) On the basis of the component-component target network, the core network was screened according to the degree value.

2.5 Gene Ontology and Pathway Enrichment Analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID) provided a comprehensive set of functional annotation tools to understand biological meaning behind large list of genes. DAVID tools were able to identify enriched biological themes, particularly GO terms which involved biological process(BP), cell component (CC), and molecular functions (MF) and visualize genes on KEGG pathway maps [20]. The target of EGb acting on aging was inputted into DAVID database with the organism limited to "Homo sapiens". The dividing value of recognized GO terms were set to FDR < 0.05 and KEGG pathways were set to P value < 0.05[21, 22].

2.6 Molecular docking

In the analysis of component-target action network, it is generally believed that the node with greater degree is the pivot node in the whole network. Therefore, the components of the core network were molecularly docked with the target. The 3D structure of the core components was obtained through the Pubchem (<https://pubchem.ncbi.nlm.nih.gov/>) and targets proteins were retrieved from the Protein Data Bank (<http://www.rcsb.org/pdb>). The main pharmacodynamic components of ginkgo biloba were docked with the core target gene by Autodock. The PyMOL was used to display the interaction diagram between receptor proteins and ligand small molecules.

3. Results

3.1 Identification of the active compounds in EGb

A total of 24 active ingredients of EGb were included according to OB and DL values in the TCMSP database and reported in the literature. The results were shown in Additional file 1: Chemical information of main compounds in EGb. The OB of ginkgolide A was < 30%, but it was a common compound in EGb

and shown to have effects on prevention and treatment of Alzheimer's disease (AD), a disease associated with aging [23, 24]. Hence, ginkgolide A was also considered to be a candidate compound.

3.2 Identification of targets in EGb

By searching the above database, 154 targets of active ingredients of EGb (Additional file 2: Targets of active ingredients) and 308 targets of aging (Additional file 3: Targets of aging) were obtained. 28 targets of active components of EGb for anti-aging were obtained by integrating the intersection of targets of active ingredients and aging target (Additional file 4: Potential targets of EGb for anti-aging).

3.3 Component-Component Target Network Analysis

The 24 active ingredients of EGb and 28 targets of active components of EGb for anti-aging were retrieved. We constructed networks to illustrate the relationship among EGb components, component targets with therapeutic effects against aging. The network consists of 52 nodes, 164 edges (see Fig. 1A). On the basis of the component-target network, the core network was screened according to the degree. The screening condition was that degree was greater than 10. There are four compounds, including quercetin (MOL000098), luteolin (MOL000006), kaempferol (MOL000422) and beta-sitosterol (MOL000358) and eight targets, including PTGS2, PPARG, GSK3B, DPP4, CCNA2, AR, MAPK14, ESR1, with a degree greater than 10 (see Fig. 1B). The results indicated that these four components might play a vital role in the anti-aging network of EGb through above targets gene.

(A) The network of EGb components, component targets with therapeutic effects against aging; (B) Filter the core network with a degree greater than 10 based on component-component target Network. The diamond shape represents the components, and the ellipse represents the target gene.

3.4 GO and KEGG Enrichment analysis

To illustrate the mechanism of EGb on aging, we performed GO analysis and KEGG pathway enrichment analysis for 27 putative therapeutic targets. Totally, we identified enrichment results in the related items 195 of biological process, 42 of molecular functions, and 25 of cell components. Then, 21 significant enriched GO terms with FDR < 0.05 were determined, including 13 biological processes, 2 cell components and 6 molecular functions (see Fig. 2). For biological process, these putative therapeutic targets were mainly enriched in positive regulation of positive regulation of transcription from RNA polymerase II promoter (GO:0045944), positive regulation of nitric oxide biosynthetic process (GO:0045429), positive regulation of transcription, DNA-templated (GO:0045893), positive regulation of superoxide anion generation (GO:0032930), negative regulation of apoptotic process (GO:0043066), peptidyl-serine phosphorylation (GO:0018105), positive regulation of smooth muscle cell proliferation (GO:0048661), Ras protein signal transduction (GO:0007265), response to drug (GO:0042493), positive regulation of ERK1 and ERK2 cascade (GO:0070374), cellular response to estradiol stimulus (GO:0071392), cellular response to lipopolysaccharide (GO:0071222), peptidyl-threonine phosphorylation (GO:0018107). For the cell component, the targets were enriched in nucleoplasm (GO:0005654) and nucleus (GO:0005634). For the molecular functions, the targets were enriched enzyme binding (GO:0019899), identical protein

binding (GO:0042802), transcription factor binding (GO:0008134), protein binding (GO:0005515), chromatin binding (GO:0003682) and core promoter sequence-specific DNA binding (GO:0001046). Therefore, the results indicated that EGb mainly exerted anti-aging therapeutic effects by regulating these biological processes, molecular functions, and cell components.

The X-axis shows the the counts of target genes, and the Y-axis shows significantly enriched GO categories of the target genes (FDR < 0.01).

We performed KEGG pathway enrichment analysis to expound the pathways for 27 putative therapeutic targets of EGb for anti-aging. The top 20 pathways were determined (see Fig. 3), mainly including Pathways in cancer (hsa05200), Neurotrophin signaling pathway (hsa04722), Proteoglycans in cancer (hsa05205), GnRH signaling pathway (hsa04912), PI3K-Akt signaling pathway (hsa04151), TNF signaling pathway (hsa04668), Insulin resistance (hsa04931), Sphingolipid signaling pathway (hsa04071), Focal adhesion (hsa04510), Non-alcoholic fatty liver disease (hsa04932), MAPK signaling pathway (hsa04010), ErbB signaling pathway (hsa04012), HIF-1 signaling pathway (hsa04066), T cell receptor signaling pathway (hsa04660), Toll-like receptor signaling pathway (hsa04620), Type II diabetes mellitus (hsa04930), NOD-like receptor signaling pathway (hsa04621), FoxO signaling pathway (hsa04068), VEGF signaling pathway (hsa04370), Wnt signaling pathway (hsa04310). The results indicate that these pathways might play an important role in anti-aging of EGb. It provided research ideas for exploring the targets of EGb for anti-aging.

3.5 Molecular docking

Eight proteins were docked with four components by AutoDock, and 20 conformations were obtained. The binding energy scoring and docking parameters are shown in Table 1. Low binding score is prone to interaction. Therefore, the three groups with the lowest score were selected for composition analysis. The conformation of receptor protein PPAR γ and beta-sitosterol ligand small molecules, receptor protein PTGS2 and kaempferol ligand small molecules, and receptor protein PTGS2 and quercetin ligand small molecules were constructed.

Table 1
Molecular docking of eight proteins and four components

Protein	Grid_size	Docking score(kcal/mol)			
		quercetin	luteolin	kaempferol	beta-sitosterol
GSK3B	40 × 40 × 40	-8.1	-8.5	-8.5	-9.0
DPP4	60 × 58 × 48	-8.6	-8.2	-8.2	-8.4
PPARG	40 × 40 × 40	-8.3	-8.3	-8.3	-9.7
CCNA2	40 × 40 × 40	-7.6	-7.7	-7.7	-7.4
AR	40 × 40 × 40	-8.8	-8.7	-8.7	-8.9
PTGS2	40 × 40 × 40	-9.6	-9.4	-9.5	-8.5
ESR1	42 × 44 × 44	-9.2	-9.0	-9.0	-7.9
MAPK14	40 × 40 × 40	-8.4	-8.6	-8.5	-8.3

The binding pattern between receptor protein PPARG and beta-sitosterol ligand small molecules was shown in Fig. 4A. Amino acid residues Glu343 and beta-sitosterol ligand small molecules formed hydrogen bond interactions, and amino acid residues Tyr473, Phe264, His266, Lys265, Ile262, Gly284, Ser342, Arg288, Glu291, Tyr477, Phe287 and beta-sitosterol ligand small molecules formed hydrophobic interactions.

The binding pattern between receptor protein PTGS2 and kaempferol ligand small molecules was shown in Fig. 4B. Amino acid residues Thr206, His207, His386 and kaempferol ligand small molecules formed hydrogen bond interactions, and amino acid residues Ala202, Ala199, Gln203, Leu391, Leu390, Asn382, Tyr385, Phe210 and kaempferol ligand small molecules formed hydrophobic interactions.

The binding pattern between receptor protein PTGS2 and quercetin ligand small molecules was shown in the Fig. 4C. Amino acid residues Glu465, His39, Arg44, Asp125 and quercetin ligand small molecules formed hydrogen bond interactions, amino acid residues Cys41, Gly45, Val46, Arg469, Tyr130, Leu152, Pro153, Gln461 and quercetin ligand small molecules formed hydrophobic interactions.

(A) The binding pattern between receptor protein PPARG and beta-sitosterol ligand small molecules;(B) The binding pattern between receptor protein PTGS2 and kaempferol ligand small molecules;(C) The binding pattern between receptor protein PTGS2 and quercetin ligand small molecules.

4. Discussion

Various compounds of EGb have anti-aging effect. Quercetin, luteolin, kaempferol and beta-sitosterol might play vital role in the anti-aging network of EGb based on the prediction method of network pharmacology. Quercetin, a polyphenol widely present in nature, has received the most attention in anti-

aging. Studies have shown that quercetin exert neuroprotective actions in the aging nervous system via stimulating cellular defenses against oxidative stress, activating sirtuins1 (SIRT1), inducing autophagy[25]. Quercetin played an anti-aging role via enhancement of cell proliferation and restoration of heterochromatin architecture [26]. In addition, several evidence-based studies suggest quercetin has significant research prospect in the prevention and treatment of vascular aging related diseases [27, 28]. Luteolin is found to possess the potential for antioxidative activity. Luteolin, as an anti-inflammatory and neuroprotective agent, can inhibit the generation of reactive oxygen species (ROS) via modulation of the AMPK-SIRT1 pathway to reduce age-related disorders [29]. The study also found that luteolin may improve cognitive performance in older mice by inhibiting microglia and neuroinflammation[30]. Luteolin may have the potentially useful in the prevention skin aging by inhibiting uva-induced production of collagen mmp-1[31]. A prospective study shows that vegetables and foods rich in kaempferol, lutein, folate, and etc may slow cognitive decline with aging[32]. Kaempferol is an antioxidant and anti-inflammatory by regulating NF-kappaB signaling cascade and inhibiting NADPH oxidase activation, hence kaempferol is considered as a potential anti-aging agent [33]. Beta-sitosterol may increased the proliferation and stimulated the differentiation of embryonic neural stem cells (eNSCs) to prevent neurodevelopmental syndromes, cognitive decline during aging [34]. Beta-sitosterol can extend lifespan of adult flies, possibly by activating AMP-activated protein kinase (AMPK) [35].

These eight targets, including DPP4, GSK3B, CCNA2, AR, ESR1, PTGS2, PPARG, MAPK14, might play a crucial role in the anti-aging network of EGb. DPP4 and GLP-1 had been found to play important roles in oxidative stress, lipid metabolism, insulin resistance and inflammation. Recently, the balance between DPP4 and GLP-1 might be a therapeutic target for the management of vascular aging and atherosclerosis in animals [36]. Studies have shown that GSK3B, which acted as egative regulator in the hormonal control of glucose homeostasis, Wnt signaling and regulation of transcription factors and microtubules, was closely related to Healthy Aging Index [37]. Silencing of CCNA2, which controled both the G1/S and the G2/M transition phases of the cell cycle, emarkably triggered the cellular aging, while CCNA2 overexpression delayed cellular aging [38]. The expression of PTGS with a particular role in the inflammatory response was up-regulated in diseases related to brain aging [39]. PPARG, key regulator of adipocyte differentiation and glucose homeostasis, was associated with inflammation [40]. MAPK14 played important roles in cell proliferation, differentiation, migration, transformation and programmed cell death [41].

In the GO enrichment analysis, the targets were closely related to positive regulation of transcription from RNA polymerase II promoter, nitric oxide biosynthetic process, smooth muscle cell proliferation, negative regulation of apoptotic process. Cell components involve nucleoplasm nucleus. Molecular functions involve the binding of enzymes, proteins and transcription factors.

KEGG pathway enrichment analyses demonstrated EGb to be involved with regulation of multiple pathways. Elevated oxidative stress and inflammation including HIF1 signaling pathway and TNF signaling pathway plays significant role in aging, especially vascular aging[42]. Neurotrophin signaling pathway plays an important role in higher order activities such as neural development and learning and

memory and several genes in the pathway are closely related to brain aging [43]. Insulin resistance is strongly associated with type II diabetes and Non-alcoholic fatty liver disease, the elderly often develop insulin resistance[44]. Oxidative stress, mitochondrial dysfunction, accumulation of intracellular lipid derivatives, and inflammation (via IL-6 and TNF α) contribute to decreased activation of signaling molecules including PI3K and AKT leading to insulin resistance[45–47]. The absence of sphingomyelin, a second messenger functions in a variety of cellular signaling pathways, leads to a shortened lifespan in animals and it suggests that sphingolipid signaling may play a role in neuronal function and animal stress response during aging[48]. The focal adhesion, mitogen-activated protein kinase (MAPK) signaling pathway and ErbB signaling pathway is module that is involved in cell proliferation, differentiation and migration [49–51]. Focal adhesion, a signaling molecule associated with cell survival, relies on the interaction between integrins and actin to connect cells to the extracellular matrix [50]. Studies have shown that extracellular matrix proteins–cell interactions gives rise to target organ damage, and inflammatory pathways leading to calcification or atherosclerosis [1, 52]. MAPK signaling pathway, including extracellular signal-regulated kinase 1 and 2 (ERK1/2), c-Jun N-terminal kinase (JNK) and p38, is closely related to the biological process of aging and the progression of the inflammatory process leads to dysregulation of MAPK that accelerates cell aging [53]. Inactivation of the ErbB signaling pathway leads to a loss of myocardial protective function during cardiac hypertrophy and to the onset of early failure [51]. T cell receptor (TCR) signaling pathway, Toll-like receptor (TLR) signaling pathway and NOD-like receptor (NLR) signaling pathway are responsible for generating innate immune responses and playing a critical role in inflammation. Toll-like receptors play a significant role in promoting aging adipose tissue inflammation, and the study shows that old TLR4-deficient mice have improved glucose tolerance compared to age-matched wild type mice[54]. A different set of NOD-like receptors induces caspase-1 activation and the activated of caspase-1 regulates maturation of the pro-inflammatory cytokines IL-1B, IL-18 and drives pyroptosis[55]. The forkhead box O (FOXO) family regulates the expression of genes in apoptosis, cell-cycle control, glucose metabolism, oxidative stress resistance, and longevity. Recent evidence indicates that the FOXO family plays a key role in the self-renewal of adult and embryonic stem cells, which could contribute to tissue regeneration [56]. Vascular endothelial growth factor (VEGF) can stimulate endothelial cell growth, promote angiogenesis and increase vascular permeability. The decreased angiogenesis associated with the aging of the body is related to the decreased expression of VEGF, and the proliferation ability of vascular endothelial cells in elderly animals is weakened. The addition of VEGF can help restore the proliferation ability of vascular endothelial cells leading delay vascular aging [57]. Wnt proteins are secreted morphogens that are required for basic developmental processes. The overactivation of Wnt/ β -catenin signaling pathway is closely related to stem cell aging[58].

5. Conclusion

In this study, the active components and mechanism of EGb for aging were analyzed based on network pharmacology. Four compounds, eight targets, and 20 significant pathways in EGb were identified by network analyse, which explained mechanism of EGb for aging by multiple components, targets and

pathways. Our study found that flavonoids (quercetin, luteolin, kaempferol) and beta-sitosterol, the main active components of EGb, might slow aging by inhibiting oxidative stress, inhibiting inflammation, ameliorating insulin resistance and regulating biological processes (including cell proliferation, differentiation and migration) and etc. The possible mechanism of EGb for aging is shown in **Figure 5**. Molecular docking results showed that PPARG had better binding capacity with beta-sitosterol and PTGS2 had better binding capacity with kaempferol and quercetin. However, additional experiments must be carried out to verify predicted these results for more evidence in the future.

Abbreviations

AD	Alzheimer's disease
BP	biological process
CC	cell component
DL	drug-likeness
EGb	Ginkgo biloba extract
EGFR	Epidermal growth factor receptor
FOXO	forkhead box O
HAGR	Human Ageing Genomic Resources database
IL6	Interleukin-6
JNK	c-Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
MF	molecular functions
OB	oral bioavailability
ROS	reactive oxygen species
SIRT1	sirtuins 1
TNF	Tumor necrosis factor
TP53	Cellular tumor antigen p53
VEGF	Vascular endothelial growth factor

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Declarations

Competing interests

The authors declare that there is no competing interest.

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Authors' Contributions

Weiliang Weng and Rui Gao contributed to the topic conception, manuscript revision, and decision to submit for publication and are the co-corresponding authors. Yanfei Liu and Yue Liu performed the network pharmacology analysis and writing of the manuscript together, are the co-first authors. Wantong Zhang and Mingyue Sun help to data analysis and revision of the manuscript. All authors discussed the results and wrote the manuscript.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Acknowledgments

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Figures

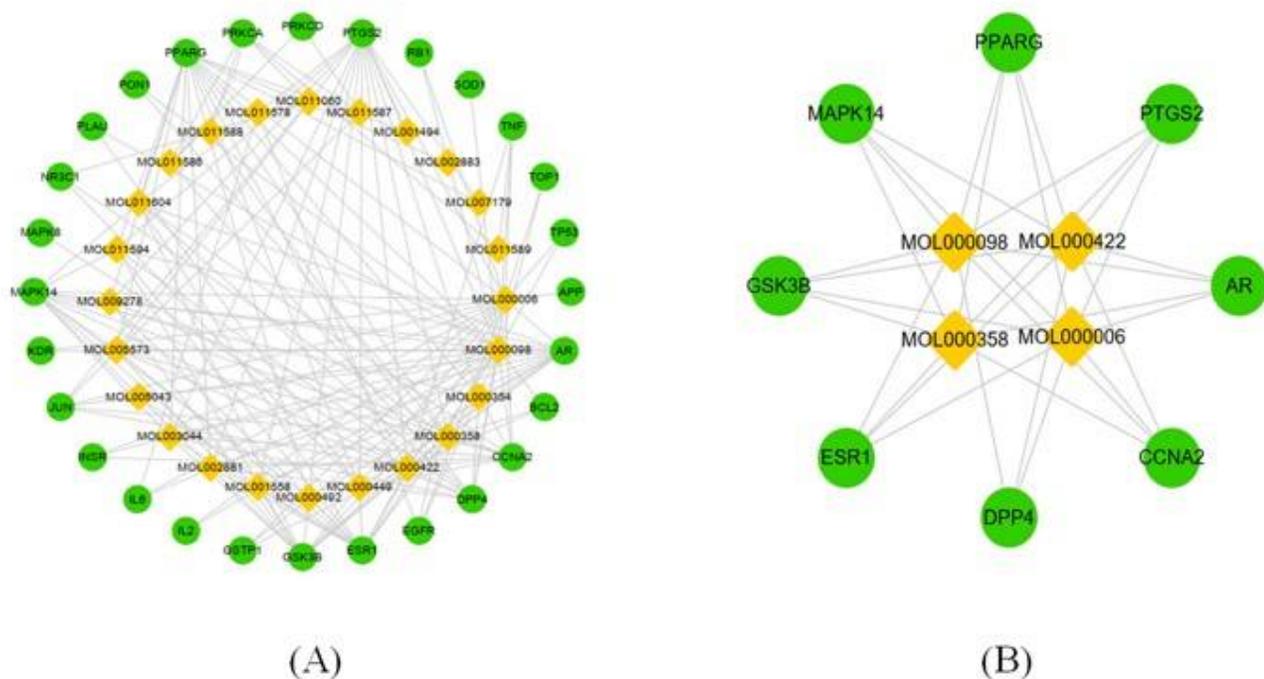


Figure 1

Component-Component Target Network. (A) The network of EGb components, component targets with therapeutic effects against aging (B) Filter the core network with a degree greater than 10 based on component-component target Network. The diamond shape represents the components, and the ellipse represents the target gene.

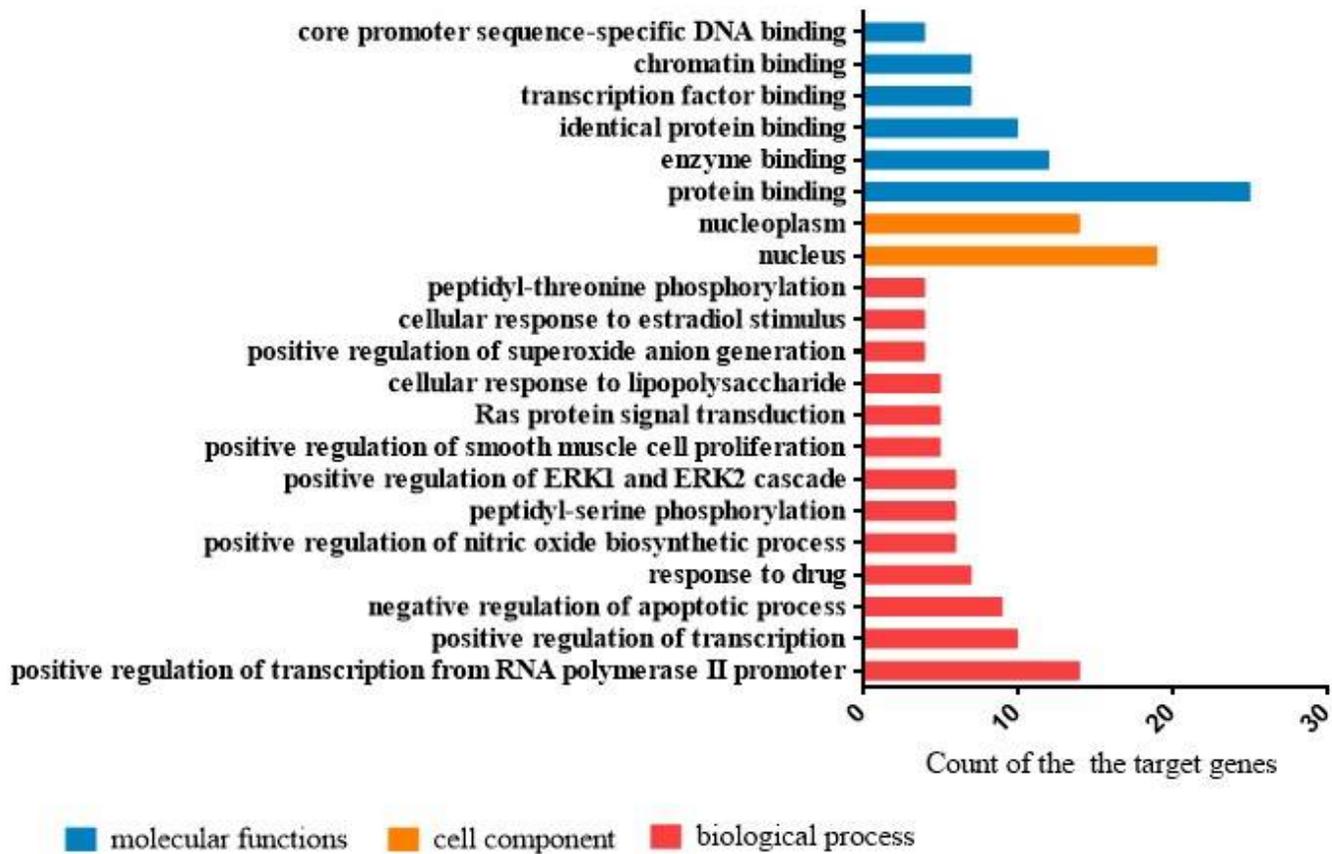


Figure 2

GO enrichment analysis of putative therapeutic targets. The X-axis shows the the counts of target genes, and the Y-axis shows significantly enriched GO categories of the target genes (FDR<0.01).

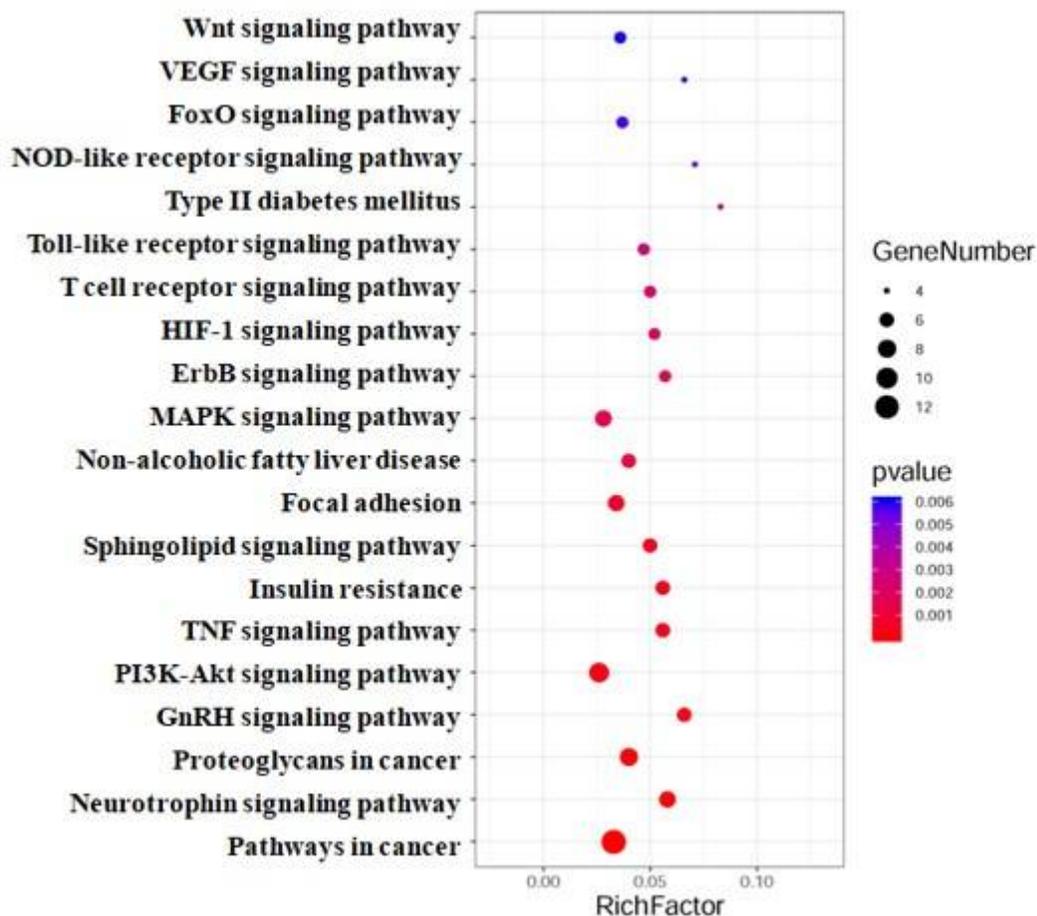


Figure 3

KEGG pathway enrichment analyses of putative therapeutic targets. The Y-axis represents significantly pathways of the target genes, and the X-axis shows the rich factor. The rich factor represents the ratio of the number of target genes belonging to a pathway to the number of all the annotated genes located in the pathway. A higher rich factor represents a higher level of enrichment. The color of the dot corresponds to different P values, and the size of the dot reflect the number of target genes expressed in the pathway.

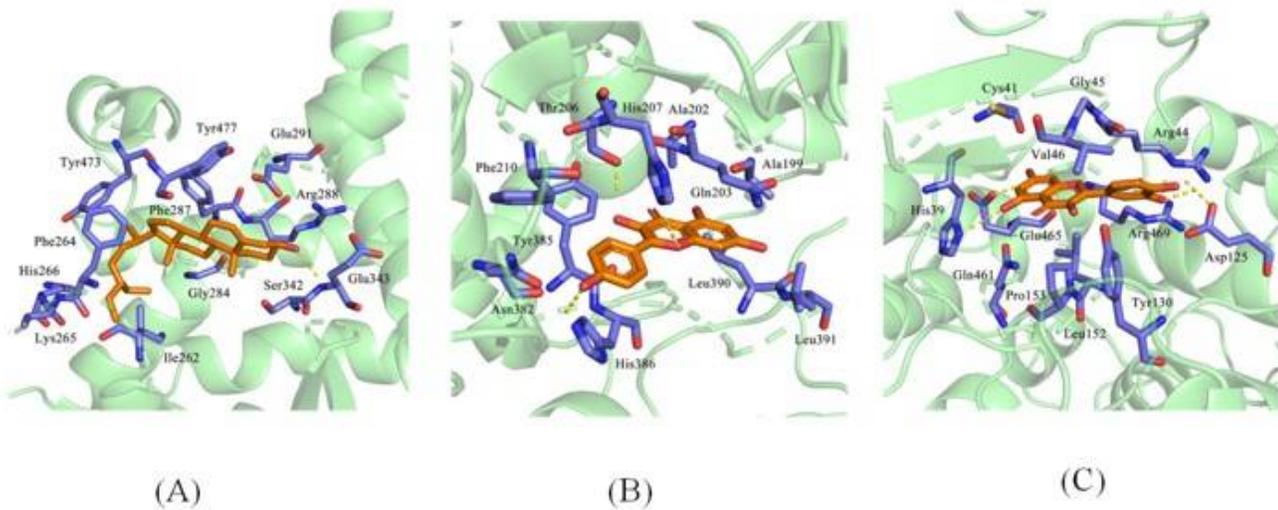


Figure 4

Docking diagram of components and protein molecules. (A) The binding pattern between receptor protein PPARG and beta-sitosterol ligand small molecules (B) The binding pattern between receptor protein PTGS2 and kaempferol ligand small molecules (C) The binding pattern between receptor protein PTGS2 and quercetin ligand small molecules.

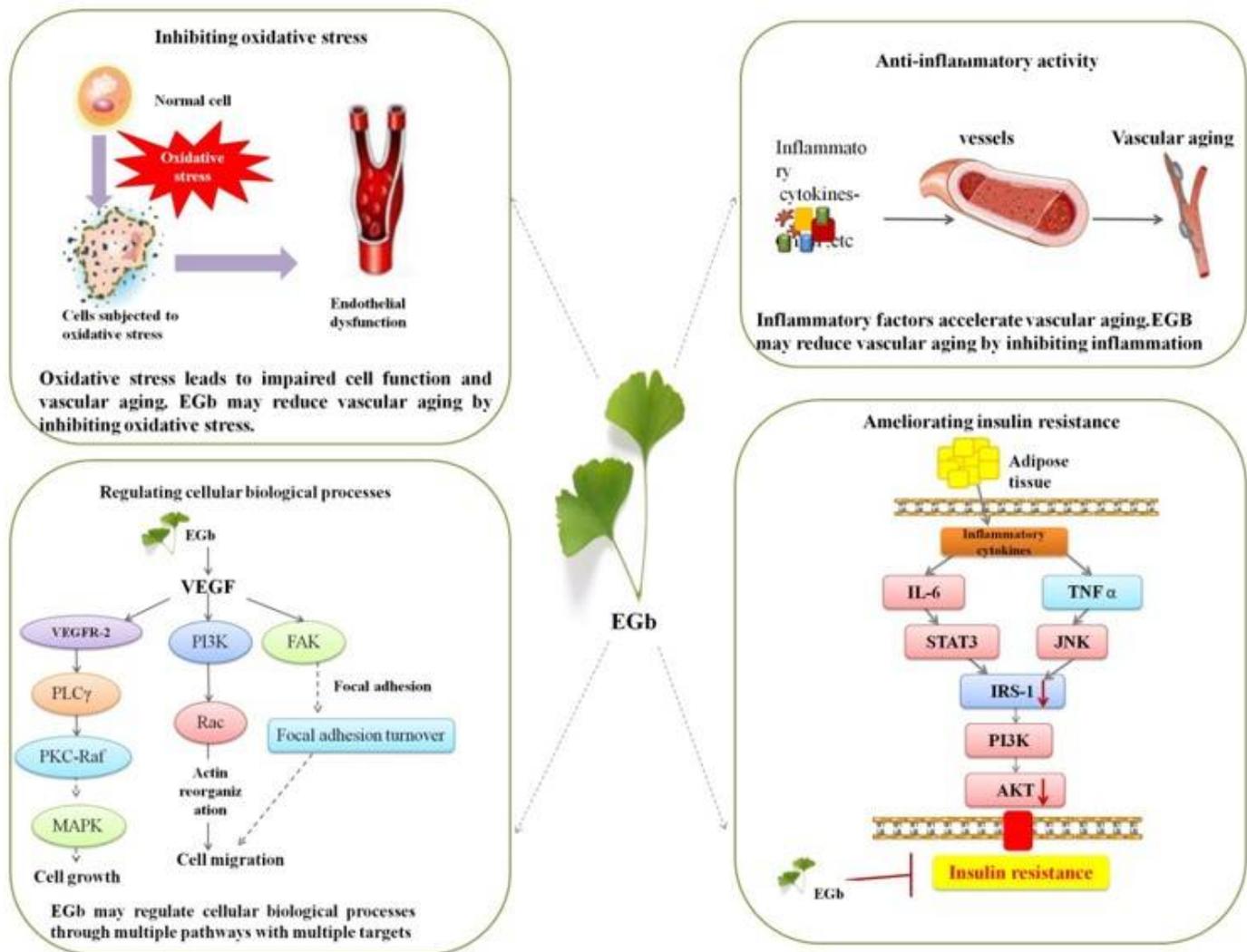


Figure 5

The possible mechanism of EGb for aging

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