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Application of Network Pharmacology to Explore the Anti-Aging Molecular Mechanism of *Radix notoginseng*

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Abstract: *Objective:* To study the anti-aging effects of *Radix Notoginseng* and to explore its molecular network mechanism. *Methods:* Aging and *Radix notoginseng* gene targets were searched and downloaded from the Genecards website, then Venn intersection analysis was performed to find common genes for diseases and drugs to explore candidate targets for *Radix notoginseng* in the treatment of aging. Bioinformatics was then used to analyze the biological processes, cellular components, molecular functions, and KEGG signaling pathways of the shared target network. Protein molecular network construction was carried out to find the core molecular network genes of the drug *Radix notoginseng* for the treatment of aging. A final PubMed literature comparison was performed to assess the value of the potential role of core network genes. *Results:* The keywords "Aging" and "*Radix notoginseng*" were queried in Genecards and 25,000 aging-related targets were obtained, 17 for *Radix notoginseng*. GO and KEGG analysis of the intersecting genes obtained from the Venn intersection analysis then showed that the BP with the highest potential to be associated with disease and drugs is positive regulation of protein phosphorylation, CC is macromolecular complex and MF is identical protein binding. The KEGG with the higher correlation is lipid and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, and proteoglycans in cancer. A total of 10 hub genes were identified in the PPI network construction, including EGFR, MMP9, TNF, VEGFA, RHOA, CDKN1A, CASP3, CCND1, AKT1, and IL1B. Among these, it found that a large number of MMP9 and TNF genes were reported in the literature, with the remaining hub genes less frequently reported in the literature. *Conclusion:* This study uses bioinformatics and network pharmacology to explain the core network mechanisms of the drug *Radix notoginseng* in the treatment of aging using the latest databases. The results show that hub genes such as CDKN1A, EGFR, and AKT1 are involved in the core biological processes of aging. The results of the study provide an important reference for resolving the core molecular network mechanism of anti-aging properties and provide a validation basis for future experimental validation.

Keywords: Aging; *Radix notoginseng*; Network pharmacology; Core network; Network factors

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1. Introduction

Aging, a complex network characterized by multiple changes occurring at different biological levels, is a pathological process that leads to the deterioration of cellular, tissue, and organismal functions [1–2]. Aging needs to meet ICD-11 criteria to be considered a disease [3]. Mammalian aging is often attributed to molecular cross-linking, free radical-induced damage, telomere shortening, and methylation of DNA [4–7]. Aging is a major risk factor for most late-onset diseases, such as cancer, cardiovascular disease, diabetes, neurodegenerative diseases, and so on [8]. The accumulation of senescent cells in the nervous system increases the probability of Alzheimer's disease (AD) and Parkinson's disease (PD), and aging is the greatest risk factor for late-onset Alzheimer's disease (LOAD) [8–10]. In addition, aging can indirectly lead to obesity, diabetes, and insulin resistance by altering lipid metabolic pathways and inducing adverse metabolic conditions. Targeting anti-aging mechanisms may contribute to the progression of diabetes and the development of diabetic complications [11]. Senescent cells are often carcinogenic and are also closely associated with the development of premature cardiovascular failure [12–13]. Although very little is known about aging, it is unlikely that a panacea for it will be discovered. However, there may be new drugs available to intervene in the development of aging [14]. Vitorino et al. argue that aging is not an inevitable fate for all organisms and that it can be delayed [1]. As the world ages and the economic burden of aging-related diseases continues to increase for society and individuals, there is an urgent need for effective ways to prevent and treat aging.

The Chinese herb *Radix notoginseng* (RN), which contains the important bioactive components Radix notoginseng saponin (PNS) and ginsenoside (Rg1), is used as a medicinal and functional herb [15]. Chen et al. showed that Rb1 delays the replicative aging of endothelial progenitor cells, protecting human umbilical vein endothelial cells and human fibroblasts, and counteracts the onset of aging [16]. PNS also exhibits anti-inflammatory, antioxidant, and anti-aging pharmacological properties in some cells [16–19]. Xu et al. reported that RN has been used not only for the treatment of neurological and immune diseases, but also exerts significant pharmacological effects in cardiovascular protection and tumor suppression, and that RN also has important pharmacological effects in a variety of chronic diseases, such as inflammatory bowel disease, arthritis, diabetes, and others [15, 20–21]. In addition, PNS is used clinically to treat diabetes and obesity. *Radix notoginseng* has been shown to have anti-aging effects on the brain and is thought to possibly help prolong life [2]. Such evidence provides a good explanation for the possible use of *Radix notoginseng* as a treatment for aging. However, no studies so far have reported on the molecular network mechanisms by which RN improves the aging process.

Bioinformatics plays an important role in the progress of human aging research. Genes and pathways that control aging may be hidden in human genomic data [14]. Cyber pharmacology offers a new way of explaining diseases, starting from the analysis of the mechanism of action of herbal formulas on diseases and using the relationship between drugs and disease gene networks. Through big data analysis, the core biological mechanism of aging and the interaction of known drug RN targets can be analyzed, which will help to explore the anti-aging network mechanism of *Radix notoginseng* from a holistic and systemic perspective, and this new therapy has the potential to find anti-aging targets [22].

This study combines modern bioinformatics and network pharmacology to shift from a "one target, one drug" model to a "network target, herbal therapy" model [23]. The *Radix notoginseng* aging intersection genes were analyzed and GO and KEGG signaling pathway analysis was used to construct PPI network maps and find hub genes to predict the interaction of aging targets with *Radix notoginseng* drug components. This use of the link between structural formulas of disease molecules and drugs provides direction and reference for the development of drugs for anti-aging and the treatment of aging diseases [8].

2. Methods

2.1. *Radix notoginseng* and aging-related database search

The human aging-related genes were searched on the Genecards database in Baidu (https://www.genecards.org) using the keyword "Aging" and the results were exported to an Excel sheet. Similarly, Genes related to *Radix notoginseng* were searched using the keyword "*Radix notoginseng*", and the obtained gene data was exported to the local Excel result named RN.

2.2. Analysis of the data Venn intersection related to *Radix notoginseng* and aging

The Draw Venn Diagram website in Baidu (https://bioinformatics.psb.ugent.be/webtools/Venn/) was searched and the genes related to aging and Radix notoginseng were pasted into the lists labeled "Aging" and "RN" respectively, which were then submitted for cross-analysis of aging and *Radix notoginseng* genes. The intersection Venn diagram was saved in svg. format.

2.3. Functional enrichment and KEGG signaling pathway analysis of anti-aging targeting *Radix notoginseng*

The David database in Baidu (https://david.ncifcrf.gov) was searched as follows. The functional annotation in the shortcut to DAVID tools was clicked, mapping the intersecting genes of aging and *Radix notoginseng*. Afterward, OFFICIAL_GENE_SYMBOL was clicked and *Homo sapiens* was selected, then the gene list was clicked and submitted. In gene ontology, the chart was downloaded and pasted into an Excel sheet. The results of the GO analysis were obtained for cellular composition (CC), molecular function (MF), and biological processes (BP). The Kyoto Encyclopedia of Genes and Genomes (KEGG) was obtained in pathways and the 10 most significant signaling pathways were selected in ascending order of P-value and visualized using the Microbiology website (https://www.bioinformatics.com.cn) for visual analysis. To generate horizontal bar graphs with color gradients, the data from the GO and KEGG analysis were imported into Micrographics for plotting. Then the width, height, and X-axis maximum of the bar chart were modified with the Times New Roman font style, and Adobe Illustrator 2020 was used to beautify the exported bar chart layout.

2.4. Construction of PPI network map of the common target of aging and *Radix notoginseng* and prediction of hub gene

The string database in Baidu (https://string-db.org/) was searched by selecting multiple proteins, uploading the 17 intersecting genes of aging and *Radix notoginseng*, defining the species as *Homo sapiens*, and clicking on search and continue. This protein-protein-interaction network analysis (PPI) used to predict Hub genes accordingly were exported as svg. and tsv. format files.

The tsv. format file exported in String was then imported into Cytoscape 3.7.2 software (http://www.Cytoscape.org/) and topologized using the cytoHubba plugin to analyze the protein interactions network to obtain degree values. The top 10 most critical genes were filtered in descending order according to degree values, which are EGFR, MMP9, TNF, VEGFA, RHOA, CDKN1A, CASP3, CCND1, AKT1, and IL1B.

2.5. Hub gene search in Pubmed for comparison

The 10 core molecules identified by the PPI were entered into the Pubmed database (https://pubmed.ncbi.nlm.nih.gov/) together with the keyword "Aging", and the literature was queried for comparison to evaluate the investigative activity and novelty of the hub genes.

3. Results

3.1. Query of targets related to aging and *Radix notoginseng*

The Genecards website was searched for human aging-related genes using the keyword "Aging" and 25,000 results were generated as shown in **Table 1**. Similarly, all the genes of *Radix notoginseng* were searched by the keyword "*Radix Notoginseng*" and 17 results were derived as shown in **Table 2**.

Table 1. Selected Aging-related genes

Aging-related genes						
APOE	TRAK2	PTEN	LEP	BRAF	BDNF	BRCA2
PDGFRB	SOD2	IGF1R	TLR4	KRAS	ESR1	VEGFA
TP53	COMT	CST3	TDRD5	C3	LMNA	ARMS2
IGF1	TERT	CFI	MLH1	APP	ACE	ALB
CFH	SERPINE1	ERCC2	MMP9	CDKN2A	HTRA1	MAPT
INS	CFB	NOS3	ERCC6	HMCN1	IL1B	CRP
IL6	MSH2	HIF1A	ADIPOQ	AKT1	FBLN5	MTHFR
ABCA4	SOD1	ATM	MT-TL1	IGF2	PPARG	EGFR
BRCA1	NFKB1	AGER	TGFB1	AR	ERBB2	APOB
TNF	CFHR3	CXCL8	SNCA	VDR	WRN	IL10

Table 2. *Radix notoginseng*-related genes

***Radix Notoginseng*-related genes**			
BAX	TNF	SELP	CDKN1A
BCL2	IL1B	MAPK1	NFE2L2
KDR	EGFR	AKT1	VCAM1
VEGFA	CCND1	MMP9	RHOA
CASP3			

3.2. Venn intersection analysis

To obtain the intersection genes of aging and *Radix notoginseng*, the genes related to aging and Radix notoginseng were crossed and analyzed through the Draw Venn Diagram website, and 17 aging-*Radix notoginseng* intersection genes were obtained, which were BAX, BCL2, KDR, VEGFA, CASP3, CDKN1A, NFE2L2, VCAM1, RHOA, SELP, MAPK1 AKT1, MMP9, TNF, IL1B, EGFR, and CCND1. The Venn diagram plotted is shown in **Figure 1**.

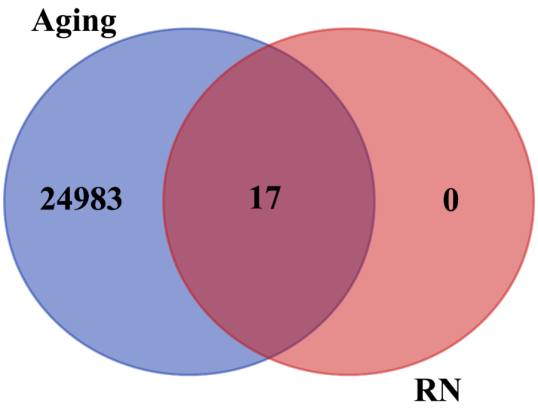

Figure 1. Venn diagram of aging-*Radix notoginseng* crossover genes, with aging-associated genes on the left, *Radix notoginseng*-associated genes on the right, and aging-*Radix notoginseng* intersection genes in the middle

3.3. GO functional enrichment and KEGG signaling pathway analysis of aging-*Radix notoginseng* intersection genes

To further investigate the localization distribution, molecular function, and biological processes of aging-*Radix notoginseng* crossover genes in cells, the GO functional enrichment and KEGG signaling pathway analysis data were downloaded separately using the David database. There are 239 signaling pathways in BP, and the top 10 pathways were screened in ascending order of P value, namely, positive regulation of protein phosphorylation, positive regulation of cell migration, negative regulation of apoptotic process, positive regulation of peptidyl-serine phosphorylation, response to UV-A, cellular response to DNA damage stimulus, cellular response to vascular, endothelial growth factor stimulus, cellular response to reactive oxygen species, negative regulation of extrinsic apoptotic signaling pathway in absence of ligand and lipopolysaccharide-mediated signaling pathway. Similarly, CC has a total of 29 results, with the top 10 results for macromolecular complex, membrane raft, extracellular space, external side of plasma membrane, nucleus, pore complex, cytosol, cell surface, cell junction, and nuclear membrane, in that order. There are a total of 22 signaling pathways in MF, the first 10 being identical protein binding, integrin binding, protein kinase binding, protein binding, BH3 domain binding, BH3 domain binding, nitric-oxide synthase regulator activity, cyclin-dependent protein serine/threonine kinase inhibitor activity, cytokine activity, protein binding, and kinase activity, in that order. KEGG has a total of 120 signaling pathways, the top 10 being lipid and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, Proteoglycans in cancer, fluid shear stress and atherosclerosis, colorectal cancer, human cytomegalovirus infection, pathways in cancer, endocrine resistance, pancreatic cancer, and EGFR tyrosine kinase inhibitor resistance. The first 10 pathways from the GO and KEGG analysis were then plotted as horizontal bars using the Microbiotics website, and the results are shown in **Figure 2**.

The results show that a variety of CC, BP, and MF are involved, including cellular components such as macromolecular complex, membrane raft, extracellular space, cellular response to DNA damage stimulus, cellular response to reactive oxygen species, response to UV-A, protein kinase binding and other signaling pathways are relevant to the topic of this paper.

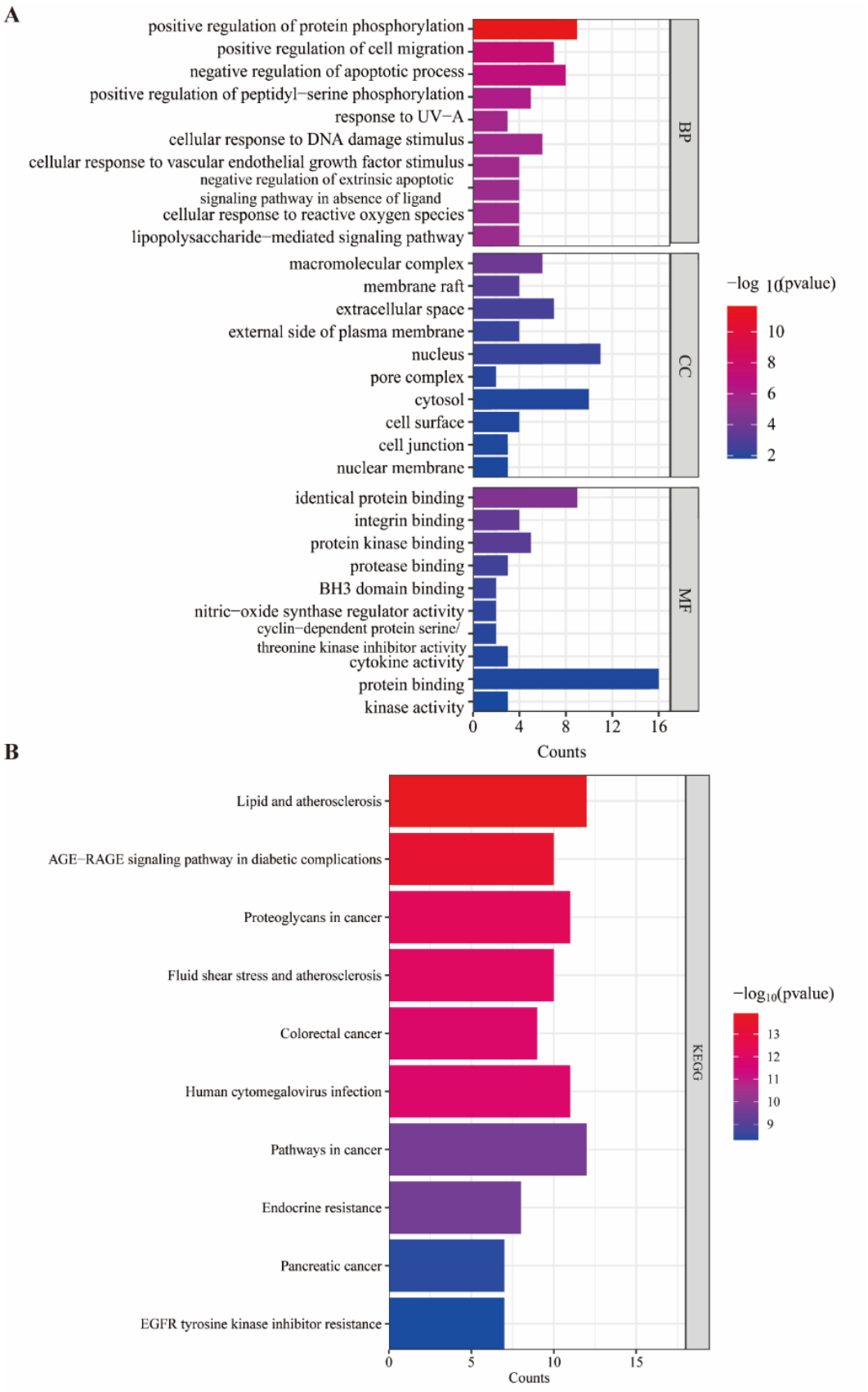

Figure 2. GO functional enrichment and KEGG pathway analysis

Note: A. GO functional enrichment histogram (BP, CC, MF) B. KEGG signaling pathway histogram. The horizontal coordinate is the number of enriched genes (counts), and the vertical coordinate is GO terms/KEGG terms. The gradient of the bars indicates the number of enriched genes, the redder the color indicates the smaller the p-value, the more statistically significant it is.

3.4. Prediction of PPI network and hub gene of the common targets of aging and *Radix notoginseng*

The 17 cross-targets of aging and *Radix notoginseng* were imported into the String website and the protein interaction analysis and protein interaction network (PPI) construction for the aging-*Radix notoginseng* crossover genes were performed as shown in **Figure 3**. The number of edges is 104, the average node degree is 12.2, the average local clustering coefficient is 0.863, the expected number of edges is 35, and the PPI enrichment p-value is 35, $< 1.0e^{-16}$. A total of 104 interactive network edges were obtained, indicating that aging is highly correlated with *Radix notoginseng*. Three panels with high linkage density are present in the figure, and AKT1 is found to be the densest, surrounded by a core network of CDKN1A, CCND1, MAPK1, RHOA, and CASP3, suggesting the possible existence of core proteins in this part. In the PPI network diagram, a strong correlation between aging and *Radix notoginseng* was found in 17 intersecting genes. The top 10 pairs of genes with the strongest linkage strength were taken in descending order according to the combined score values as shown in **Table 3**, in order of CCND1: CDKN1A; KDR: VEGFA; BAX: BCL2; AKT1: RHOA; CASP3: CDKN1A; AKT1: CDKN1A; MMP9: VEGFA; TNF: VCAM1; IL1B: TNF; and EGFR: RHOA. The top 10 hub genes were filtered by degree value in descending order as shown in **Figure 3**, which are EGFR, MMP9, TNF, VEGFA, RHOA, CDKN1A, CASP3, CCND1, AKT1, and IL1B. The hub genes in the KEGG pathway were mapped and 10 hub genes were found distributed in different pathways. Nine of the hub genes were related to proteoglycans in cancer and human cytomegalovirus infection signaling pathways. Among these, lipid and atherosclerosis, AGE-RAGE signaling pathways in diabetic complications, proteoglycans in cancer, and endocrine resistance pathways are relevant to the topic of this paper. AKT1 and EGFR are the most enriched genes, suggesting that *Radix notoginseng* may act on the biological process of aging through a related pathway with AKT1 and EGFR.

Table 3. The connection strength ranked among the top ten pairs of targets.

Node 1	Node 2	Node 1 string ID	Node 2 string ID	Co-expression	Experimentally determined interaction	Database annotated	Automated text mining	Combined score
CCND1	CDKN1A	9606.ENSP00000227507	9606.ENSP00000384849	0.085	0.983	0.9	0.99	0.999
KDR	VEGFA	9606.ENSP00000263923	9606.ENSP00000478570	0.062	0.984	0.9	0.992	0.999
BAX	BCL2	9606.ENSP00000293288	9606.ENSP00000381185	0	0.981	0.9	0.758	0.998
AKT1	RHOA	9606.ENSP00000451828	9606.ENSP00000400175	0.062	0.14	0.9	0.945	0.995
CASP3	CDKN1A	9606.ENSP00000311032	9606.ENSP00000384849	0	0.77	0.8	0.883	0.994
AKT1	CDKN1A	9606.ENSP00000451828	9606.ENSP00000384849	0	0.789	0.9	0.71	0.993
MMP9	VEGFA	9606.ENSP00000361405	9606.ENSP00000478570	0	0	0.9	0.879	0.987
TNF	VCAM1	9606.ENSP00000398698	9606.ENSP00000294728	0	0	0.9	0.884	0.987
IL1B	TNF	9606.ENSP00000263341	9606.ENSP00000398698	0.462	0	0.5	0.942	0.983
EGFR	RHOA	9606.ENSP00000275493	9606.ENSP00000400175	0	0.115	0.9	0.667	0.968

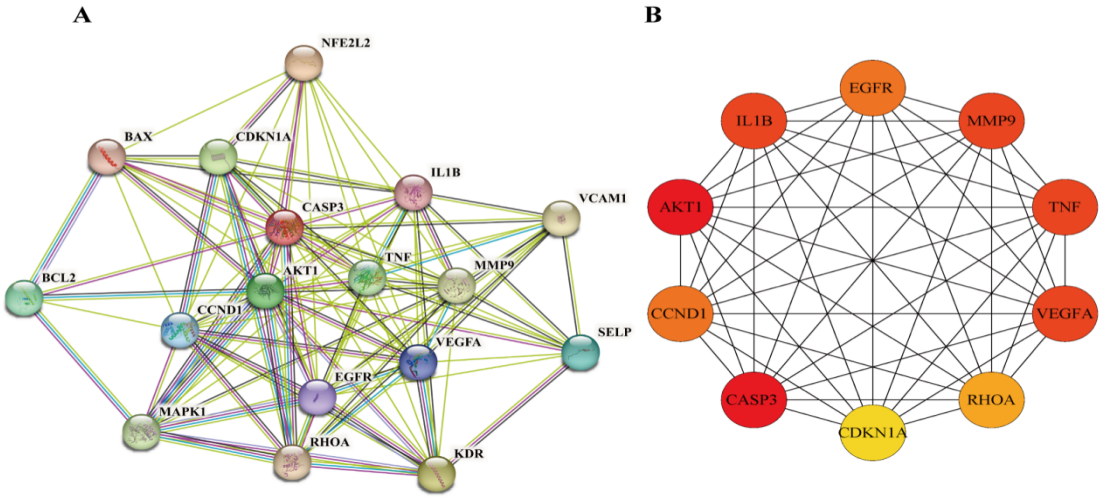

Figure 3. PPI protein interactions (A) and hub gene maps (B)

3.5. Evaluation of the innovation value of hub genes

The 10 hub genes were entered into the Pubmed database simultaneously with the keyword "Aging" to search the literature. There were 1751, 862, 4459, 395, 249, 913, 2005, 135, 311, 296 results found for EGFR, MMP9, TNF, VEGFA, RHOA, CDKN1A, CASP3, CCND1, AKT1, IL1B respectively. Among them, EGFR, MMP9, TNF, CDKN1A, and CASP3 have been extensively reported in the literature, indicating that these genes are hot spots for current research and have greater clinical application prospects. VEGFA, RHOA, CCND1, AKT1, and IL1B have been less reported in the literature, indicating that these genes are highly novel and of greater research value.

3.6. Core genetic relationships between PPI, GO enrichment analysis, and the KEGG pathway

To investigate the relationship between the PPI, GO, and KEGG pathways and the core genes, the positions of the 10 hub genes in the GO and KEGG pathways were compared and the hub gene-enriched pathways were collected as shown in **Table 4**. Results show that all hub genes were involved in the BP and KEGG signaling pathways, with the protein binding pathway in BP enriched for 10 hub genes, suggesting that this pathway may be highly relevant to the theme. AKT1 was enriched in seven BP and ten KEGG pathways, which is the most enriched pathway gene, suggesting that AKT1 may play an important molecular function in biological processes. EGFR enriches 13 pathways of CC and MF, suggesting that EGFR may be expressed in numerous cellular components, thereby regulating biological processes. However, the absence of hub gene enrichment in the BH3 domain binding pathway and pore complex suggests that it may not be related to biological processes. The macromolecular complex is the main result of CC, suggesting its possible thematic relevance. The MMP9 gene appears only in the extracellular space, suggesting that MMP9 may be under- or non-expressed in biological processes. The hub genes are all recurrent in the protein binding pathway, suggesting that the protein binding pathway may be highly thematically relevant. In addition, proteoglycans in cancer and human cytomegalovirus infection pathways were enriched with the most hub genes, and the EGFR tyrosine kinase inhibitor resistance pathway was enriched with the least hub genes. Furthermore, AKT1, EGFR, VEGFA, and CCND1 genes were simultaneously localized in the first 10 KEGG pathways, implying that the above co-expressed genes may interact with each other through different signaling pathways during biological

processes, thus providing a potential molecular explanation for the anti-aging mechanism of *Radix notoginseng*.

Table 4. Core gene relationships between PPI, GO enrichment analysis, and KEGG pathway

	Description	Gene ID							
BP	Positive regulation of protein phosphorylation	EGFR	MMP9	TNF	VEGFA	CDKN1A	CCND1	AKT1	IL1B
BP	Positive regulation of cell migration	EGFR	MMP9	VEGFA	RHOA	IL1B			
BP	Negative regulation of the apoptotic process	EGFR	MMP9	VEGFA	CDKN1A	CASP3	AKT1		
BP	Positive regulation of peptidyl-serine phosphorylation	EGFR	TNF	VEGFA	AKT1				
BP	Response to UV-A	EGFR	CCND1	AKT1					
BP	Cellular response to DNA damage stimulus	CDKN1A	CASP3	CCND1	AKT1				
BP	Cellular response to vascular endothelial growth factor stimulus	VEGFA	AKT1						
BP	Cellular response to reactive oxygen species	EGFR	MMP9	AKT1					
BP	Negative regulation of extrinsic apoptotic signaling pathway in the absence of ligand	TNF	AKT1	IL1B					
BP	Lipopolysaccharide-mediated signaling pathway	TNF	AKT1	IL1B					
CC	Macromolecular complex	EGFR	TNF	CDKN1A	AKT1				
CC	Membrane raft	EGFR	TNF	CASP3					
CC	Extracellular space	EGFR	MMP9	TNF	VEGFA	IL1B			
CC	External side of plasma membrane	TNF							
CC	Nucleus	EGFR	RHOA	CDKN1A	CASP3	CCND1	AKT1		
CC	Pore complex								
CC	Cytosol	RHOA	CDKN1A	CASP3	CCND1	AKT1	IL1B		
CC	Cell surface	EGFR	TNF	VEGFA					
CC	Cell junction	EGFR	RHOA						
CC	Nuclear membrane	EGFR	CCND1						
MF	Identical protein binding	EGFR	MMP9	TNF	VEGFA	AKT1			
MF	Integrin binding	EGFR	IL1B						
MF	protein Kinase binding	EGFR	RHOA	CDKN1A	CCND1	AKT1			
MF	Protease binding	TNF	CASP3						
MF	BH3 domain binding								
MF	Nitric-oxide synthase regulator activity	AKT1	EGFR						
MF	Cyclin-dependent protein serine/threonine kinase inhibitor activity	CDKN1A	CASP3						
MF	Cytokine activity	IL1B	TNF	VEGFA					

Table 4 (Continued)

Description		Gene ID									
MF	Protein binding	EGFR	MMP9	TNF	VEGFA	RHOA	CDKN1A	CASP3	CCND1	AKT1	IL1B
MF	Kinase activity	CDKN1A	AKT1	EGFR							
KEGG	Lipid and atherosclerosis	MMP9	TNF	RHOA	CASP3	AKT1	IL1B				
KEGG	AGE-RAGE signaling pathway in diabetic complications	TNF	VEGFA	CASP3	CCND1	AKT1	IL1B				
KEGG	Proteoglycans in cancer	EGFR	MMP9	TNF	VEGFA	RHOA	CDKN1A	CASP3	CCND1	AKT1	
KEGG	Fluid shear stress and atherosclerosis	MMP9	TNF	VEGFA	RHOA	AKT1	IL1B				
KEGG	Colorectal cancer	EGFR	RHOA	CDKN1A	CASP3	CCND1	AKT1				
KEGG	Human cytomegalovirus infection	EGFR	TNF	VEGFA	RHOA	CDKN1A	CASP3	CCND1	AKT1	IL1B	
KEGG	Pathways in cancer	EGFR	MMP9	VEGFA	RHOA	CDKN1A	CASP3	CCND1	AKT1		
KEGG	Endocrine resistance	EGFR	MMP9	CDKN1A	CCND1	AKT1					
KEGG	Pancreatic cancer	EGFR	VEGFA	CDKN1A	CCND1	AKT1					
KEGG	EGFR tyrosine kinase inhibitor resistance	EGFR	VEGFA	AKT1							

4. Discussion

The genes associated with aging and *Radix notoginseng* in Genecards were screened and the Venn intersection was mapped to show crossover genes, yielding 17 intersecting genes. The GO enrichment and KEGG signaling pathways show that aging development is associated with macromolecular complex, cellular response to DNA damage stimulus, cellular response to reactive oxygen species, response to UV-A, and protein kinase binding signaling pathways, respectively. Then, PPI network analysis reported the possible molecular mechanisms of CDKN1A, EGFR, and AKT1 involved in the treatment of aging by *Radix notoginseng*. The study provides an important reference for resolving the core molecular network mechanism of the drug *Radix notoginseng* in the treatment of aging. Unfortunately, due to the limitations of the timeliness and comprehensiveness of genetic data, the predicted results are biased from the actual situation. Additional experimental methods are needed to further validate the predicted results subsequently, but new clues are provided to explain the mechanism by which *Radix notoginseng* improves aging.

4.1. Gene target database

To obtain the latest genes, a total of 17 related genes for *Radix notoginseng* and 25,000 related genes for aging in the GeneCards database were queried as of March 20, 2023. Unfortunately, using "*Radix Notoginseng*" as a keyword search did not take into account the possible differences in the way the names of Chinese medicines are translated. Furthermore, due to the limitations of the timeliness and comprehensiveness of Genecards data, only the introduction of the most recent valid data in the Genecards is guaranteed and therefore some bias may exist. Admittedly, there are limitations to the data and these should be taken into account when interpreting the results, but at the same time, they open up greater possibilities for subsequent discoveries.

4.2. Intersecting genes

In this paper, the Draw Venn Diagram website was used to perform a cross-tabulation analysis of genes related

to aging and *Radix notoginseng* and obtained a total of 17 aging-*Radix notoginseng* cross-tabulation genes. It is found that all the targets of *Radix notoginseng* were contained in aging-related targets, suggesting that *Radix notoginseng* may be closely related to the development of aging, while this evidence also provides new insights into the promise of *Radix notoginseng* to delay aging.

4.3. GO analysis

To analyze the biological functions of the genes, the David database was used to download BP, CC, and MF data. For GO functional enrichment results, this can be interpreted as targeting the set of genes obtained experimentally, thus finding the enrichment of hub genes in BP, CC, MF, and so on. Specifically, cellular response to DNA damage stimulus, cellular response to reactive oxygen species, and response to UV-A processes in BP are highly relevant to the topic of this paper. According to Wang et al., UV-A irradiation can damage cells, induce cellular aging, and eventually lead to loss of function [24].

In addition, changes in the levels of reactive oxygen species (ROS), one of the most potent biological effectors in cellular metabolism, are a key mechanism contributing to the onset of aging and disease [25]. Excessive changes in intracellular ROS content, known as oxidative damage can lead to telomere shortening. In vitro experiments by Lin et al. found that fibroblasts cultured under enhanced oxidative stress, such as mild hyperoxia (40% normoxia), prematurely shortened telomeres, and correspondingly shortened lifespan [26–27]. DNA damage triggers a signaling cascade effect whereby once DNA damage is identified in the nuclear genome, cells will avoid replicating the damaged gene at all costs, driving apoptosis or irreversible cell cycle arrest in aging to occur. Moreover, the result of DNA damage, whether endogenous or exogenous, is accelerated aging. Mutations in DNA unwinding enzymes are also associated with a variety of diseases that accelerate aging [28]. All of this evidence supports the idea that DNA damage may be the cause and manner in which aging occurs. Furthermore, oxidative damage leads to telomere shortening, causing telomere dysfunction, which indirectly leads to a DNA damage response and ultimately to the loss of cell proliferation and aging [29]. In summary, the cellular response to reactive oxygen species can be intervened to regulate the DNA damage mechanism, providing new ideas and methods for effective anti-aging processes.

At the cellular component (CC) level, the data show that intersecting genes are located in macromolecular complexes, such as mitochondria. Mitochondria are the most important site of endogenous ROS production in the human body [30]. According to Jauhari et al., neuronal mitochondrial DNA (mtDNA) damage is caused by ROS-induced oxidative stress during neurodegeneration [31]. This corroborates both the cellular response to reactive oxygen species in BP and the cellular response pathway to DNA damage stimuli. A great deal of research is currently focused on finding ways to eliminate or counteract mtDNA mutations to extend human lifespan [32].

The most important MF is protein kinase binding. Zhang et al. identified the interferon gene (STING)-PKR endoplasmic reticulum kinase (PERK)-eIF2α pathway. The binding of STING to cyclic GMP-AMP synthase (cGAMP) directly activates the kinase PERK and activates PERK phosphorylates eIF2α, which in turn regulates cellular aging [33]. Data from Lin et al. show that receptor-interacting protein kinase 1 (RIPK1) also delays cellular aging by kinase-dependently regulating cell aging [26]. In summary, it is believed that the conclusions can be subsequently validated by experimentally modulating the above pathways, offering the potential for new therapies against aging.

4.4. KEGG analysis

KEGG signaling pathway analysis of intersecting genes was performed using the String database, where it was

found that lipid and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, Proteoglycans in cancer, and endocrine resistance pathway may be closely related to the development of aging. The above pathways suggest an increased probability of developing atherosclerosis, diabetes, cancer, and endocrine diseases during the aging process. Perdomo et al. reported that increased production of apolipoprotein D (apoD) in *Drosophila* leads to increased lifespan [34]. Also, they observed significantly elevated apoD in aging brains, suggesting that apoD is an important molecule that affects aging. The receptor for advanced glycosylation end products (RAGE) mediates multiple signaling and plays an important role in diabetic complications and aging-related diseases, making the AGE-RAGE signaling pathway a promising therapeutic target for anti-aging processes and treatment of aging-related diseases [35]. The association of aging with the increased incidence of several cancers, including gastrointestinal malignancies, was reported in a study by Nautiyal et al. [36]. In addition, aging, whether in a healthy or pathological state, leads to corresponding changes in the endocrine system. Chahal et al. found that peripheral levels of estrogen, growth hormone, and testosterone decreased in the elderly, while levels of luteinizing hormone, follicle-stimulating hormone, and sex hormone binding globulin increased [37]. These changes suggest that endocrine defects are also associated with a higher prevalence of aging-related diseases. However, current research has not found a cure for reversing the aging process. The herb *Radix notoginseng* may offer a new reference as a potential youth hormone in the fight against aging.

4.5. PPI analysis

The relationship between hub genes and PPI, GO, and KEGG pathways was analyzed and it was found that AKT1 was distributed in 9 BPs and 10 signaling pathways, and EGFR, VEGFA, and CCND1 were distributed in 7 signaling pathways. These hub genes were distributed in different pathways, suggesting that they may be highly associated with biological processes.

Numerous studies have shown that CDKN1A (p21) is closely associated with the development of aging. Stein et al. found that the CDK inhibitor p21 can accumulate in senescent cells and bind to the cell cycle protein E-Cdk2 complex to inactivate it, leading to the onset of cell cycle arrest or aging [38]. Prolonged activation of CDKN1A (p21) induces mitochondrial dysfunction and reactive oxygen species production, as reported by Passos et al. [39]. On the one hand, this corroborates the previous discussion of the possible contribution of cellular response to reactive oxygen species in BP and mitochondrial dysfunction in CC to the development of aging. On the other hand, it also suggests that CDKN1A (p21) is closely associated with the development of cellular aging. The EGFR receptor family plays an important regulatory role in senescent cells as Majumdar et al. reported that aging is associated with increased activation of downstream PI3K/Akt signaling regulated by EGFR signaling [40]. Meanwhile, Schmelz et al. observed a 30-35% increase in tyrosine phosphorylated EGFR levels in the colon of 30-35 month-old rats compared to 4-6 month-old rats in their experiments, suggesting that aging may be associated with an increase in EGFR expression and activation [41]. These results suggest a potential role for EGFR in the development of the aging process.

After APOE and FOXO3, AKT1 is thought to be the third longevity gene [42]. The study by Bao et al. found that the gene encoding the nematode insulin-like tyrosine kinase receptor (daf-2) inhibited Akt1 activity through inactivation, thereby increasing the lifespan of *Cryptobacterium hidradenum* [43]. This suggests to us that Akt1 is closely linked to the aging process. In addition, Zhao et al. also reported that AKT1 overexpression promotes cellular aging [44]. Several studies have shown that many intron single nucleotide polymorphisms (SNP) in the insulin/IGF-1 signaling pathway are significantly associated with human lifespan, including AKT1 [44–45]. However, Nygaard et al. showed in 2996 long-lived individuals who are non-elderly and centenarians, and 1840 young controls from Denmark and Germany that AKT1 was unlikely to be associated with human

longevity [42]. Li et al. similarly found no association between the AKT1 gene and human lifespan in a Chinese Han population [46]. Therefore, further studies are needed to confirm whether AKT1 is associated with human lifespan.

5. Conclusion

In summary, this paper explains the network mechanism of *Radix notoginseng* for the treatment of aging by screening targets through disease gene network-drug mechanism of action and evaluating the innovation and translational value in Pubmed. The results show that hub genes such as CDKN1A, EGFR, and AKT1 are involved in the core biological processes and signaling of aging. The factors that cause aging are numerous and complex, and this study provides important evidence for understanding the molecular events of aging in *Radix notoginseng* treatment, providing clues and a basis for future experimental validation.

Disclosure statement

The authors declare no conflict of interest.

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