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Exploring the Effect of Ginsenosides on Thymocyte-Thymic Epithelial Intercellular Mitochondrial Transfer Based on Network Pharmacology

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Abstract

Background: The function of immune cells in thymus microenvironment is closely related to mitochondria. Ginsenoside, as the main active metabolite of ginseng, has immunomodulatory effects, but its effect on mitochondrial transfer between thymocytes and thymic epithelial cells is not clear. To predict the effect of ginsenosides on mitochondrial transfer between thymocytes and thymic epithelial cells based on network pharmacology. **Methods:** With the database and analysis platform of systematic pharmacology of traditional Chinese medicine, PharmMapper and Swiss Target Prediction were used for ingredient screening and corresponding target prediction. The targets of mitochondria, thymocytes and thymic epithelial cells were obtained by using the Genome Annotation Database Platform (GeneCards) database. The Metascape database was applied for enrichment analysis of gene ontology (GO) and Kyoto encyclopedia of Genes and genomes (KEGG) to predict the mechanism of action. **Results:** KEGG pathway enrichment analysis showed that it was related to mitochondria-related pathway, including phosphatidylinositide 3-kinases/protein kinase B (PI3K-Akt) signaling pathway, cyclic adenosine monophosphate (cAMP) signaling pathway, cyclic guanosine monophosphate/protein kinase G (cGMP-PKG) signaling pathway, and Ras-related protein 1 (Rap1) signaling pathway. GO function positively regulated phosphorus metabolism, protein serine kinase activity, and cell response to estrogen. **Conclusions:** Ginsenogenin may promote mitochondrial transfer between thymocyte and thymic epithelial cells and improve mitochondrial dysfunction. 20(s)-Protopanaxadiol (PPD) promotes mitochondrial generation through cAMP signaling pathway, while Propanaxantriol (PPT) regulates mitochondria through cGMP-PKG signaling pathway and Rap1 signaling pathway. The above analysis results provide new clues and hypotheses for understanding the immunomodulatory effects of ginsenosides, but their exact effects and molecular mechanisms need to be confirmed by further experimental studies.

1 Introduction

Mitochondria are organelles that exist in most cells and are enclosed by double membranes, which can produce energy in cells and are the main site for aerobic respiration, known as “power houses” [1]. Mitochondrial transfer refers to the process in which intact, functional mitochondria from donor cells (such as mesenchymal stem cells [2], fibroblasts, etc.) enter adjacent recipient cells through intercellular connections [3]. This process is of great significance to cells. For example, in the repair of tissue damage, mesenchymal stem cells can rescue damaged alveolar epithelial cells or cardiomyocytes through mitochondrial transfer [4], and in immune regulation, mitochondrial transfer can affect the differentiation and function of T cells [5]. In addition, this process also plays a key role in various physiological and pathological processes such as neuroprotection and acute kidney injury [3].

Network pharmacology is a new discipline based on the theory of systems biology, which analyzes the network of biological systems and selects specific signal nodes for multi-target drug molecule design [6]. Network pharmacology emphasizes the multi-pathway regulation of signaling pathways to improve the therapeutic effect of drugs, reduce toxic side effects, increase the success rate of clinical trials for new drugs and save on drug development costs.

Thymocytes, the precursor of T cells, are densely packed in the cortex and account for 85% to 90% of the total number of thymic cortical cells [7]. The viability of T cells, the main cells responsible for cellular immunity, has an important relationship with mitochondria. Thymic epithelial cells are important stromal cells in the thymus, with their protrusions interconnected to form a mesh-like structure, playing a crucial role in the differentiation, development, and selection of thymocytes.

Ginsenoside is a steroid compound, also known as triterpenoid saponin, which is considered an active ingredient in ginseng and has become a highly popular research target [8]. Ginsenosides all share similar basic structures and contain steroid nuclei composed of 30 carbon atoms arranged in four rings. They are divided into two groups based on the different glycosidic structures: dammarane type and oleanane type. The dammarane type includes two categories: 20(s)-Protopanaxadiol (PPD) [9] and Propanaxantriol (PPT).

Ginsenosides have been clinically confirmed to enhance human immunity and assist in the treatment of cancer [10]. The anti-tumor mechanism of ginsenosides mainly focuses on regulating tumor cell autophagy [11], inhibiting tumor cell proliferation [12], and promoting tumor cell apoptosis [13]. Recent studies have found that the anti-apoptotic mechanism of mesenchymal stem cells increases the number of mitochondrial transfers [14], which conversely promotes cancer cell proliferation, contradicting the purpose of cancer treatment. Therefore, fathoming the mechanism of mitochondrial transfer and the regulatory mode of ginseng on this process may be a new direction for further elucidating the role of ginsenosides in anti-tumor therapy. In recent years, studies have further shown that ginsenosides and their components can directly target mitochondria and regulate their function, metabolism and autophagy, which provides a theoretical basis for studying their regulation of mitochondrial transfer between cells [15,16]. Ginsenosides have a protective effect on thymocytes. Different from tumor cells, thymocytes and thymic epithelial cells are immune cells and supportive cells for immune cell differentiation and development. The effect of ginsenosides on mitochondrial transfer between thymocytes and thymic epithelial cells is not yet clear. By exploring the effect of ginsenosides on mitochondrial transfer between thymocytes and thymic epithelial cells, we can further understand the impact and mechanism of

ginsenosides on mitochondrial transfer from the perspective of immunoregulation, which undoubtedly contribute positively to elucidating the immunomodulatory role of ginseng. This paper aims to preliminarily explore the mechanism of ginsenosides on mitochondrial transfer between thymocytes and thymic epithelial cells using network pharmacology methods.

2 Materials and methods

2.1 Acquisition of ginsenoside target predictions

The Traditional Chinese Medicine Systems Pharmacology Analysis Platform [17] (https://tcmspw.com/tcmsp.php, TCMSP) was used to search for the chemical components in ginseng using the keyword "ginseng". By differentiating the chemical and structural formulas of PPD and PPT, the Molecule Names of PPD and PPT can be identified. The Organic Small Molecule Biological Activity Data [18] (http://pubchem.ncbi.nlm.nih.gov, PubChem) was applied to obtain the 3D structures and SMILES of the chemical components of PPD and PPT through their Molecule Names. Subsequently, with PharmMapper (http://lilab-ecust.cn/pharmmapper) Database [19], component target predictions were obtained through the 3D structures of the chemical components of PPD and PPT. The Swiss Target Prediction (http://www.swisstargetprediction.ch) Database [20] was adopted to obtain component target predictions through SMILES of PPD and PPT, thereby identifying the targets of PPD and PPT. The component target predictions obtained by PharmMapper and Swiss Target Prediction were screened to improve the reliability of the prediction results. Specifically, targets with a Z-score > 0 in the PharmMapper database were retained, and for the Swiss Target Prediction database, targets with a Probability > 0 are retained. Subsequently, the screening results of the two databases were integrated, and the intersection was taken as an effective target for PPD and PPT.

2.2 Acquisition of targets of mitochondria, thymocytes and thymic epithelial cells

GeneCards (https://www.genecards.org/) database [21] was utilized to obtain the related targets of mitochondria, thymocytes and thymic epithelial cells. In this study, 'mitochondrial target' is defined as a gene-encoded protein that is closely related to mitochondrial function, including but not limited to proteins located in mitochondria, and proteins that indirectly affect mitochondria by regulating mitochondrial biosynthesis, dynamics, metabolism and function (such as oxidative phosphorylation). Similarly, 'thymic cell target gene' and 'thymic epithelial cell target gene' are also defined as functionally related, referring to genes that are known or predicted to be expressed in the cell and participate in its physiological activity or pathological process. This broad definition helps to fully capture the potential targets of ginsenosides that may affect mitochondria and immune cells directly or indirectly. The mitochondrial targets were separately screened against the effective targets of PPD and PPT to identify common targets between mitochondria and PPD, as well as between mitochondria and PPT. The GeneCards database was also used to acquire targets for thymocytes and thymic epithelial cells. The targets of thymocytes and thymic epithelial cells, as well as effective targets of PPD and PPT were separately screen to obtain common targets of thymocytes and PPD/PPT, as well as common targets of thymic epithelial cells and PPD/PPT.

2.3 Target pathway enrichment analysis

To further understand the functions of the selected target protein genes and their roles in signaling pathways, the common targets were imported into the Metascape database [22]. By inputting a list of target gene names and limiting the species to humans with a threshold of $p < 0.01$, Gene Ontology (GO) biological process enrichment analysis and Kyoto Encyclopedia

of Genes and Genomes (KEGG) signaling pathway enrichment analysis were performed. The results obtained were subsequently analyzed.

3 Results

3.1 Results of targets obtainment

Through the PharmMapper database, 231 targets for PPD and 254 targets for PPT were identified. Using the Swiss Target Prediction database, there were 100 targets each for PPD and PPT. After data integration, 57 valid targets for PPD and 80 valid targets for PPT were obtained, as shown in Table 1, Table 2, and Figure 1.

A total of 2676 mitochondrial targets, 15 common targets for mitochondria and PPD, and 22 common targets for mitochondria and PPT were obtained through the GeneCards database, as described in Table 3 and Table 4.

A total of 1628 thymocyte targets and 336 thymic epithelial cell targets were obtained from the GeneCards database. There were 18 common targets between thymocytes and PPD, and 23 common targets between thymocytes and PPT (Table 5-6). Also, there were 2 common targets between thymic epithelial cells and PPD (c-Jun N-terminal kinase 1 and MAP kinase p38 alpha), and 7 common targets between thymic epithelial cells and PPT (Cyclooxygenase-2, MAP kinase p38 alpha, PI3-kinase p110-gamma subunit, Tyrosine-protein kinase JAK1, Tyrosine-protein kinase JAK2, c-Jun N-terminal kinase 1, and Stem cell growth factor receptor).

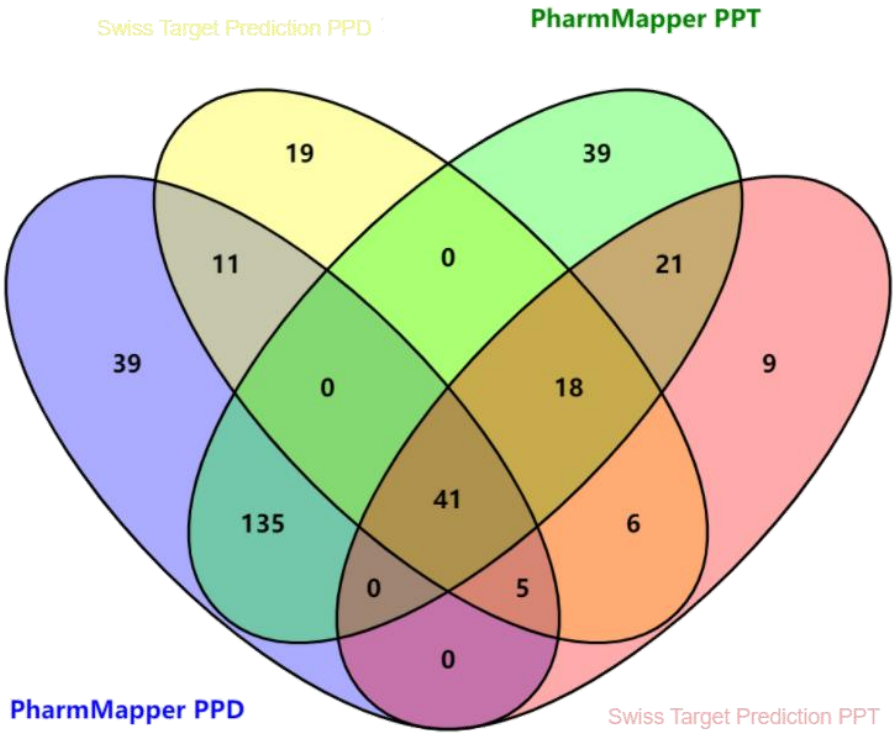

Figure 1 The Venn diagram of targets of PPD and PPT in the PharmMapper database and the Swiss Target Prediction database. Unit: number

Table 1 57 valid targets of PPD.

Targets	Common name
Protein-tyrosine phosphatase 1B	PTPN1
Norepinephrine transporter	SLC6A2

Androgen Receptor	AR
Cytochrome P450 2C19	CYP2C19
Estrogen receptor alpha	ESR1
Serotonin transporter	SLC6A4
Muscarinic acetylcholine receptor M2	CHRM2
Cytochrome P450 51	CYP51A1
HMG-CoA reductase	HMGCR
Cytochrome P450 19A1	CYP19A1
Nuclear receptor subfamily 1 group I member 3	NR1I3
LXR-alpha	NR1H3
Acetylcholinesterase	ACHE
11-beta-hydroxysteroid dehydrogenase 1	HSD11B1
Potassium-transporting ATPase alpha chain 2	ATP12A
Niemann-Pick C1-like protein 1	NPC1L1
Nuclear receptor ROR-gamma	RORC
Cytochrome P450 17A1	CYP17A1
Estrogen receptor beta	ESR2
Vitamin D receptor	VDR
Testis-specific androgen-binding protein	SHBG
Carboxylesterase 2	CES2
UDP-glucuronosyltransferase 2B7	UGT2B7
Cytochrome P450 24A1	CYP24A1
Butyrylcholinesterase	BCHE
Tyrosine-protein kinase BRK	PTK6
C-C chemokine receptor type 1	CCR1
Phosphodiesterase 10A	PDE10A
Aldo-keto-reductase family 1 member C3	AKR1C3
Interleukin-6 receptor subunit beta	IL6ST
Epoxide hydratase	EPHX2
Smoothened homolog	SMO
Adenosine A1 receptor	ADORA1
Adenosine A2a receptor	ADORA2A
Protein kinase C gamma	PRKCG
Protein kinase C delta	PRKCD
Protein kinase C beta	PRKCB
Protein kinase C epsilon	PRKCE
Pregnane X receptor	NR1I2
Cyclin-dependent kinase 6	CDK6
Cyclin-dependent kinase 4	CDK4

ALK tyrosine kinase receptor	ALK
Bile acid receptor FXR	NR1H4
Phosphodiesterase 2A	PDE2A
Phosphodiesterase 4B	PDE4B
Voltage-gated potassium channel subunit Kv1.3	KCNA3
11-beta-hydroxysteroid dehydrogenase 2	HSD11B2
P53-binding protein Mdm-2	MDM2
C-Jun N-terminal kinase 1	MAPK8
MAP kinase p38 alpha	MAPK14
Cytochrome P450 2C9	CYP2C9
Cytochrome P450 3A4	CYP3A4
Subtilisin/kexin type 7	PCSK7
G-protein coupled receptor 55	GPR55
N-arachidonyl glycine receptor	GPR18
Calcitonin gene-related peptide type 1 receptor	CALCRL
Serine/threonine-protein kinase Chk1	CHEK1

Table 2 80 valid targets of PPT.

Targets	Common name
Protein-tyrosine phosphatase 1B	PTPN1
Cytochrome P450 2C19	CYP2C19
Androgen Receptor	AR
Muscarinic acetylcholine receptor M2	CHRM2
Norepinephrine transporter	SLC6A2
Serotonin transporter	SLC6A4
Cytochrome P450 19A1	CYP19A1
Acetylcholinesterase	ACHE
11-beta-hydroxysteroid dehydrogenase 1	HSD11B1
Nuclear receptor subfamily 1 group I member 3	NR1I3
Estrogen receptor alpha	ESR1
Potassium-transporting ATPase alpha chain 2	ATP12A
Cytochrome P450 51	CYP51A1
Niemann-Pick C1-like protein 1	NPC1L1
HMG-CoA reductase	HMGCR
Nuclear receptor ROR-gamma	RORC
LXR-alpha	NR1H3
Sterol regulatory element-binding protein 2	SREBF2
Cytochrome P450 17A1	CYP17A1
Vitamin D receptor	VDR
C-C chemokine receptor type 1	CCR1

UDP-glucuronosyltransferase 2B7	UGT2B7
Cyclooxygenase-2	PTGS2
Phosphodiesterase 10A	PDE10A
G-protein coupled receptor 55	GPR55
N-arachidonyl glycine receptor	GPR18
Proteinase-activated receptor 1	F2R
Butyrylcholinesterase	BCHE
Serine/threonine-protein kinase mTOR	MTOR
ALK tyrosine kinase receptor	ALK
Phosphodiesterase 2A	PDE2A
Phosphodiesterase 4B	PDE4B
PI3-kinase p110-beta subunit	PIK3CB
Pregnane X receptor	NR1I2
Prostaglandin E synthase	PTGES
Gamma-secretase	PSEN2
	PSENEN
	NCSTN
	APH1A
	PSEN1
	APH1B
Corticotropin releasing factor receptor 1	CRHR1
MAP kinase p38 alpha	MAPK14
Testis-specific androgen-binding protein	SHBG
Sodium channel protein type IX alpha subunit	SCN9A
Smoothened homolog	SMO
Mu opioid receptor (by homology)	OPRM1
Delta opioid receptor (by homology)	OPRD1
PI3-kinase p110-delta subunit	PIK3CD
PI3-kinase p110-gamma subunit	PIK3CG
Cytochrome P450 2C9	CYP2C9
Cytochrome P450 3A4	CYP3A4
PI3-kinase p110-alpha subunit	PIK3CA
P2X purinoceptor 3	P2RX3
Cytochrome P450 24A1	CYP24A1
Serine/threonine-protein kinase Aurora-A	AURKA
Tubulin--tyrosine ligase	TTL
Vasopressin V1a receptor	AVPR1A
CDK8/Cyclin C	CCNC
	CDK8
Collagen type IV alpha-3-binding protein	COL4A3BP

Cell division protein kinase 8	CDK8
Leucine-rich repeat serine/threonine-protein kinase 2	LRRK2
Cyclin-dependent kinase 1/cyclin B	CCNB3
	CDK1
	CCNB1
	CCNB2
Tyrosine-protein kinase JAK1	JAK1
Tyrosine-protein kinase JAK2	JAK2
Carboxylesterase 2	CES2
C-Jun N-terminal kinase 1	MAPK8
Epoxide hydratase	EPHX2
Cyclooxygenase-1	PTGS1
Insulin receptor	INSR
Cytochrome P450 2D6	CYP2D6
Nicotinamide phosphoribosyltransferase	NAMPT
Phosphodiesterase 5A	PDE5A
Histone deacetylase 6	HDAC6
Neurokinin 2 receptor	TACR2
Cyclin-dependent kinase 9	CDK9
Histone deacetylase 1	HDAC1
Serine/threonine protein phosphatase PP1-gamma catalytic subunit	PPP1CC
Serine/threonine protein phosphatase 2A, catalytic subunit, alpha isoform	PPP2CA
Stem cell growth factor receptor	KIT
Subtilisin/kexin type 7	PCSK7
Vascular endothelial growth factor receptor 2	KDR
Melanin-concentrating hormone receptor 1	MCHR1
Calcitonin gene-related peptide type 1 receptor	CALCRL
Tyrosine-protein kinase BRK	PTK6

Table 3 15 common targets for mitochondria and PPD.

Targets	Common name
Androgen Receptor	AR
C-Jun N-terminal kinase 1	MAPK8
Cyclin-dependent kinase 4	CDK4
Cyclin-dependent kinase 6	CDK6
Cytochrome P450 24A1	CYP24A1
Estrogen receptor alpha	ESR1
Estrogen receptor beta	ESR2
HMG-CoA reductase	HMGCR

MAP kinase p38 alpha	MAPK14
P53-binding protein Mdm-2	MDM2
Phosphodiesterase 2A	PDE2A
Protein kinase C delta	PRKCD
Protein kinase C epsilon	PRKCE
Protein-tyrosine phosphatase 1B	PTPN1
Voltage-gated potassium channel subunit Kv1.3	KCNA3

Table 4 22 common targets for mitochondria and PPT.

Targets	Common name
Protein-tyrosine phosphatase 1B	PTPN1
Androgen Receptor	AR
Estrogen receptor alpha	ESR1
HMG-CoA reductase	HMGCR
Cyclooxygenase-2	PTGS2
Proteinase-activated receptor 1	F2R
Serine/threonine-protein kinase mTOR	MTOR
Phosphodiesterase 2A	PDE2A
MAP kinase p38 alpha	MAPK14
PI3-kinase p110-gamma subunit	PIK3CG
PI3-kinase p110-alpha subunit	PIK3CA
Cytochrome P450 24A1	CYP24A1
Serine/threonine-protein kinase Aurora-A	AURKA
Leucine-rich repeat serine/threonine-protein kinase 2	LRRK2
Tyrosine-protein kinase JAK1	JAK1
Tyrosine-protein kinase JAK2	JAK2
C-Jun N-terminal kinase 1	MAPK8
Insulin receptor	INSR
Histone deacetylase 6	HDAC6
Serine/threonine protein phosphatase 2A, catalytic subunit, alpha isoform	PPP2CA
Stem cell growth factor receptor	KIT
Vascular endothelial growth factor receptor 2	KDR

Table 5 18 common targets between thymocytes and PPD.

Targets	Common name
Androgen Receptor	AR
C-Jun N-terminal kinase 1	MAPK8
Cyclin-dependent kinase 6	CDK6
Estrogen receptor alpha	ESR1
Estrogen receptor beta	ESR2
MAP kinase p38 alpha	MAPK14
P53-binding protein Mdm-2	MDM2
Protein kinase C delta	PRKCD
Protein-tyrosine phosphatase 1B	PTPN1
11-beta-hydroxysteroid dehydrogenase 1	HSD11B1
Acetylcholinesterase	ACHE
Adenosine A1 receptor	ADORA1
Adenosine A2a receptor	ADORA2A
Calcitonin gene-related peptide type 1 receptor	CALCRL
Interleukin-6 receptor subunit beta	IL6ST
Nuclear receptor ROR-gamma	RORC
Protein kinase C gamma	PRKCG
Serine/threonine-protein kinase Chk1	CHEK1

Table 6 23 common targets between thymocytes and PPT.

Targets	Common name
Protein-tyrosine phosphatase 1B	PTPN1
Androgen Receptor	AR
Estrogen receptor alpha	ESR1
Cyclooxygenase-2	PTGS2
Serine/threonine-protein kinase mTOR	MTOR
MAP kinase p38 alpha	MAPK14
PI3-kinase p110-gamma subunit	PIK3CG
PI3-kinase p110-alpha subunit	PIK3CA
Tyrosine-protein kinase JAK1	JAK1
Tyrosine-protein kinase JAK2	JAK2
C-Jun N-terminal kinase 1	MAPK8
Insulin receptor	INSR
Serine/threonine protein phosphatase 2A, catalytic subunit, alpha isoform	PPP2CA
Stem cell growth factor receptor	KIT
Acetylcholinesterase	ACHE
11-beta-hydroxysteroid dehydrogenase 1	HSD11B1
Nuclear receptor ROR-gamma	RORC
PI3-kinase p110-delta subunit	PIK3CD

Cyclooxygenase-1	PTGS1
Cyclin-dependent kinase 9	CDK9
Histone deacetylase 1	HDAC1
Serine/threonine protein phosphatase PP1-gamma catalytic subunit	PPP1CC
Calcitonin gene-related peptide type 1 receptor	CALCRL

3.2 KEGG pathway enrichment analysis

The common targets shared by PPD/PPT with mitochondria, thymocytes, and thymic epithelial cells were imported into Metascape for KEGG pathway enrichment analysis. Items with a corrected p value < 0.01 were screened, and after removal of duplicates, a total of 22 signaling pathways were identified. However, due to the limited number of shared targets between PPD and thymic epithelial cells, effective KEGG pathway enrichment analysis could not be obtained. The results were depicted in Figure 2. In KEGG pathway enrichment analysis, all results contained cancer pathways.

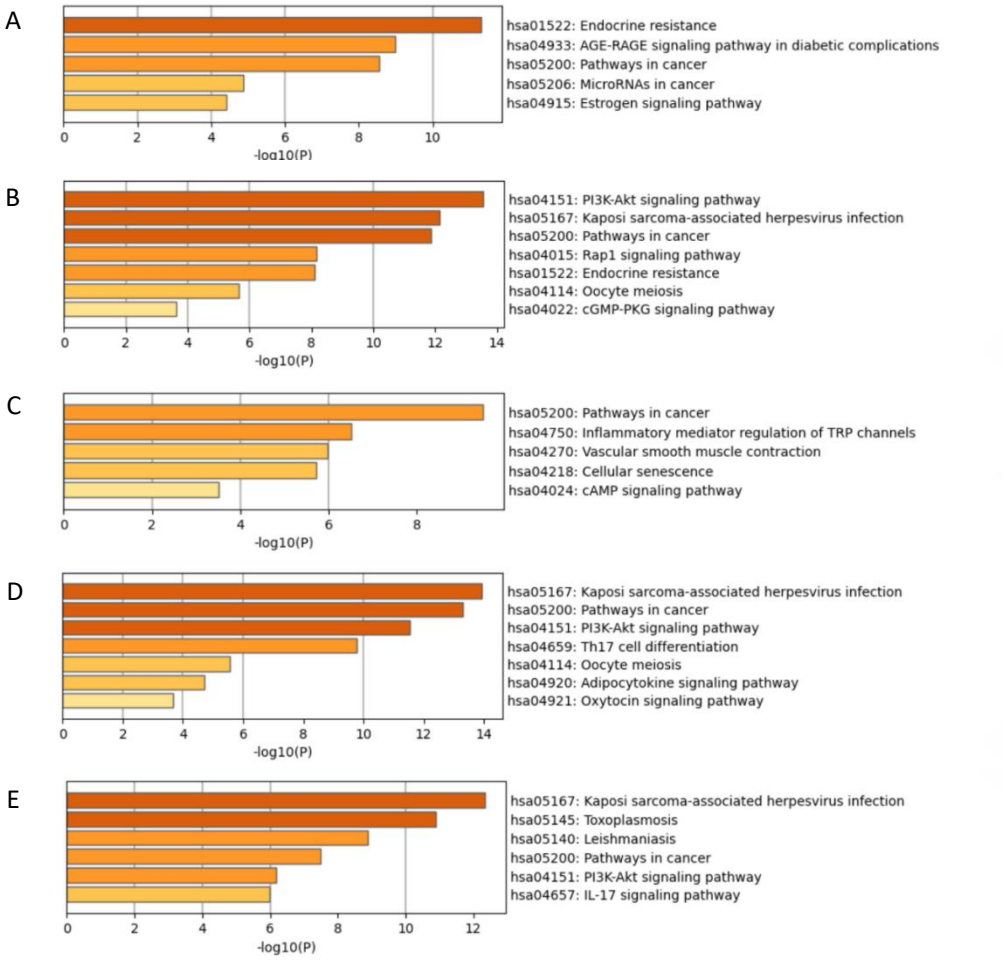

Figure 2 KEGG pathway enrichment analysis results. (A) 5 pathways from the KEGG enrichment analysis of PPD and mitochondrial targets; (B) 7 pathways from the KEGG enrichment analysis of PPT and mitochondrial targets; (C) 5 pathways from the KEGG enrichment analysis of PPD and thymocyte targets; (D) 7 pathways from the KEGG enrichment analysis of PPT and thymocyte targets; (E) 6 pathways from the KEGG enrichment analysis of PPT and thymic epithelial cell targets.

3.3 GO functional enrichment analysis

The common targets of PPD/PPT shared with mitochondria, thymocytes, and thymic epithelial cells were imported into Metascape for GO functional enrichment analysis. The items with a corrected *p* value < 0.01 were selected, and the duplicate items were removed. Finally, a total of 52 GO functions were screened out. However, due to the insufficient common targets of PPD and thymic epithelial cells, an effective GO functional enrichment analysis could not be conducted. The results were delineated in Figure 3.

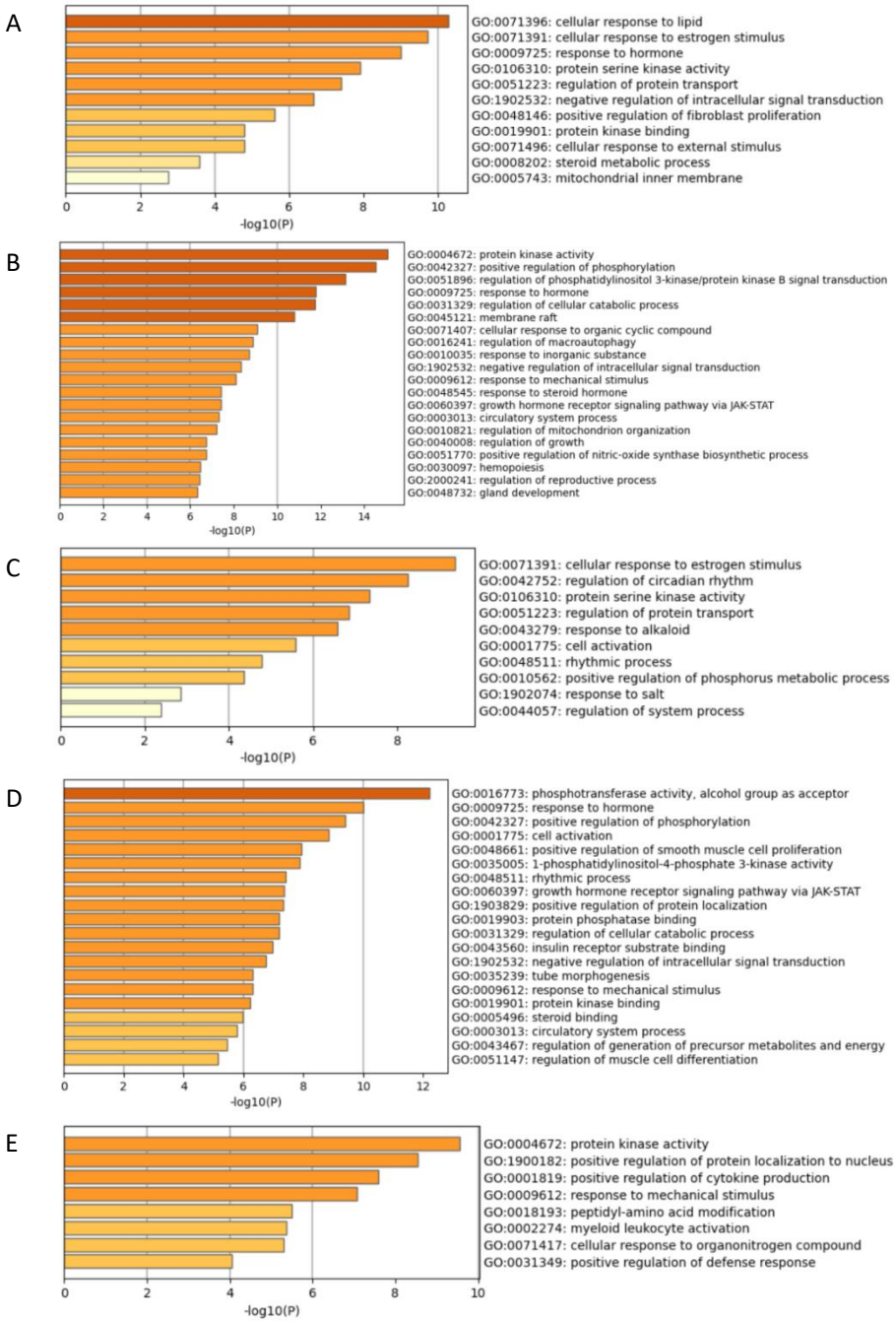

Figure 3 Functional enrichment analysis results. (A) 11 GO items from the GO enrichment analysis of PPD and mitochondrial targets; (B) 20 GO items from the GO functional analysis of PPT and mitochondrial targets; (C) 10 GO items from the GO functional analysis of PPD and thymocyte targets; (D) 20 GO items from the GO functional analysis of PPT and thymocyte targets; (E) 8 GO items from the GO functional analysis of PPT and thymic epithelial cell targets.

4 Discussion

In recent years, significant progress has been made in the early diagnosis and chemotherapy of cancer, but many cancers are still incurable. Fortunately, ginsenosides have comprehensive anti-cancer effects. In addition to regulating autophagy, suppressing proliferation, and promoting apoptosis of tumor cells, ginsenosides can also enhance the body's immune system. Thymocytes and thymic epithelial cells play an important role in the immune system, mediating the immune balance of the body and maintaining immune stability. Ginsenosides can increase the proportion of thymic cortex area, enhance thymic cell proliferation ability, and exert a reliable protective effect on thymocytes [23]. Therefore, it is speculated that ginsenosides can also affect mitochondrial transfer between thymocytes and thymic epithelial cells.

In this study, it was predicted by network pharmacology that PPD and PPT may affect the function of mitochondria and the transfer between cells in the thymic microenvironment through different signaling pathways and biological functions. Specifically, PPD may mainly promote mitochondrial biosynthesis, while PPT focuses on regulating mitochondrial dynamics and autophagy.

Generally, under the influence of microenvironment, damaged endothelial cell mitochondria can trigger the anti-apoptotic mechanism of mesenchymal stem cells, thereby alleviating cell damage. For cancer cells, mesenchymal stem cells mediate mitochondrial transfer to cancer cells through tunnel nanotubes (TNTs), extracellular vesicles (EVs), and cell fusion, restoring their oxidative phosphorylation and causing drug resistance and proliferation of cancer cells. PPD and PPT both can assist in cancer treatment in clinical practice. They interfere with tumor cell proliferation, mediate mitochondrial autophagy pathway [24], and promote cell apoptosis [25] via inhibiting the expression of cell cycle-related protein CDK4/CyclinD, reducing Haspin kinase activity, and disrupting mitosis.

The core prediction of this study is that PPD and PPT may regulate mitochondrial function through distinct signaling pathways. Besides the cancer pathway, PPD and PPT can directly affect mitochondria through different pathways. PPD can promote the activity of mitochondrial respiratory chain enzyme complex I through the cAMP signaling pathway [26], enhance the electron transfer process, which promotes intracellular phosphorylation level, and further enhance mitochondrial biogenesis and activity, manifested as an increase in mitochondrial quantity. PPT can mobilize white adipose tissue and increase fat breakdown and phosphorylation through the cGMP PKG signaling pathway [27], ultimately achieving the effect of increasing mitochondrial quantity.

In different pathways, PPD impacts AMPK signaling pathway [28]. It acts on β2 receptors, increases the transcription and activity of PGC-1α, and regulates NRF and TFAM transcription via activating the cAMP/PKA pathway and the pathway protein CREB. It also promotes mitochondrial biogenesis, activates AMPKα [29], boosts cellular glucose uptake and elevates the phosphorylation level of AMPKα, with the involvement of TFAM, thereby activating fatty acid oxidation pathways and further enhancing mitochondrial biogenesis and metabolic activity.

Compared to PPD, PPT influences mitochondria mainly through mitochondrial dynamics balance. On the one hand, PPT can activate the PI3K-Akt signaling pathway [30], upregulate the expression of the anti-apoptotic gene Bcl-2, and downregulate the expression of the pro-apoptotic gene Bax, thereby reducing mitochondrial autophagy. On the other hand, PPT activates its downstream protein Rap1 through the Rap1 signaling pathway [31], promoting signal transduction, inhibiting mitochondrial oxidative phosphorylation [32], thereby suppressing

mitochondrial fusion, enhancing mitochondrial fission, disrupting mitochondrial homeostasis and function, and activating the mitochondria-mediated apoptosis pathway.

The GO functions of PPD and PPT together include positive regulation of phosphorylation, protein-kinase binding, and response to hormones. The GO function contains cellular responses to estrogen stimulation, as PPD and PPT have estrogen-like effects [33], which can inhibit endothelial cell oxidative stress, activate inflammatory responses, mediate cell apoptosis, and reduce Akt and ERK1/2 protein phosphorylation levels through binding to estrogen receptors, thus achieving estrogen-like vascular protection. Their antagonistic effect on Ca^{2+} protects mitochondria in ischemic brain neurons from damage and loss by enhancing the fluidity of the mitochondrial membrane in the ischemic brain, reducing mitochondrial membrane phospholipid degradation, mitigating mitochondrial swelling, and inhibiting the decrease in activity of NADH complex I, cytochrome C oxidase, and succinate dehydrogenase [34]. Furthermore, it decreases mitochondrial MDA content, enhances mitochondrial SOD activity, and repress excessive Ca^{2+} uptake, thereby protecting mitochondria, although the efficacy is weaker than that of estrogen. The numerous GO functions of PPD and PPT provided conditions for direct or indirect mitochondrial transfer. For example, the function of PPD related to protein serine kinase activity was broad, encompassing regulating cell proliferation and differentiation, promoting cell survival, and mediating and correcting cell metabolism. The PPT can positively regulate phosphorus metabolism function, and accelerate mitochondrial oxidative phosphorylation to provide conditions for mitochondrial ATP synthesis.

It must be emphasized that all the conclusions of this study are derived from bioinformatics predictions. As a powerful hypothesis generation tool, network pharmacology has successfully outlined the potential blueprint of PPD and PPT for us. However, these computational predictions have inherent limitations. First of all, the target prediction database itself is still in continuous improvement; secondly, enrichment analysis suggested pathway associations, but could not confirm specific causal regulatory relationships (e.g., activation or inhibition). Therefore, the mechanism of action proposed in this paper, including PPD promoting mitochondrial formation through cAMP signaling pathway, and PPT regulating mitochondrial dynamics through cGMP-PKG and Rap1 signaling pathways, is a scientific hypothesis that needs to be verified by experiments. Future studies urgently need to use *in vitro* co-culture models, gene knockout, mitochondrial fluorescence labeling and other techniques to directly observe and confirm the direct effect of ginsenosides on mitochondrial transfer between thymocytes and thymic epithelial cells, and to empirically test the above predictive pathways.

Mitochondrial transfer is of great significance to the immune system, and generates different effects in different cells. Normal cells treat themselves through mitochondrial transfer. For example, mesenchymal stem cell mitochondrial transfer has therapeutic effects on various diseases such as cerebral ischemia, myocardial infarction, and acute renal failure [4]. Moreover, mesenchymal stem cells can dampen immune cell differentiation through mitochondrial transfer, and exert immunosuppressive effects through downregulating T-bet expression to suppress Th1 response [5]. Mitochondrial transfer has a broad prospect in the treatment of tissue damage repair [35] and mitochondrial diseases. As a novel treatment method, the mechanism of mitochondrial transfer needs further elucidation.

Based on network pharmacology, KEGG and GO enrichment analysis results revealed that PPD and PPT elevate mitochondrial quantity growth through various pathways, which may directly or indirectly promote

mitochondrial transfer and improve mitochondrial dysfunction. PPD can increase mitochondrial quantity by promoting cellular phosphorylation levels, such as promoting mitochondrial generation via the cAMP signaling pathway, enhancing mitochondrial biogenesis and metabolic activity. PPT also has regulatory effects on mitochondria possibly through the cGMP-PKG signaling pathway and Rap1 signaling pathway, facilitating mitochondrial autophagy. These findings indicated that PPD and PPT have different effects on mitochondria.

Among the known cancer pathways, mitochondrial transfer of mesenchymal stem cells may affect cancer treatment. Therefore, exploring key factors that inhibit mitochondrial transfer of mesenchymal stem cells (such as blocking the formation of TNTs) may provide a new target for cancer treatment. In contrast, PPT that regulates mitochondrial function is more likely to affect tube morphogenesis. The effects of PPD and PPT on cellular metabolism [36] may also be a new research direction, as cellular metabolic activity can indirectly reflect mitochondrial activity. This study also mentioned related pathways, such as inhibited PI3K Akt signaling pathway that can promote mitochondrial autophagy and cell apoptosis. Moreover, the endocrine resistance is attributed to the participation of mitochondria in oocyte development via multi-pathways, and the endocrine dysfunction can induce abnormal or disrupted oocyte development, further damage ovary function and trigger reproductive endocrine diseases [37]. PPD and PPT can improve mitochondrial dysfunction [38], implying their potential in treating endocrine diseases through the mechanism of mitochondrial transfer. In conclusion, to further investigate the efficacy of ginsenosides, it is necessary to deeply understand the mechanism of mitochondrial transfer.

A major limitation of this study is that its conclusions are entirely based on predictive analysis of network pharmacology and bioinformatics. Although these computational methods can efficiently reveal potential drug-target-pathway interactions and provide valuable directions and hypotheses for subsequent research, they still need to be verified by *in vitro* and *in vivo* experiments to provide direct biological evidence. Future research will focus on experimental demonstration of this prediction.

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Conflicts of Interest

The author of this article, Jianli Gao, is a member of the editorial office of this journal. All procedures during the editorial review process were conducted strictly in accordance with the journal's policies, and the author was not involved in handling any part of the process.

Author Contributions

Substantial contributions to conception and design: Z.Z. Data acquisition, data analysis and interpretation: X.W. Drafting the article or critically revising it for important intellectual content: J.G. Final approval of the version to be published: All authors. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved: All authors.

Ethics Approval and Consent to Participate

No ethical approval was required for this article.

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Availability of Data and Materials

The data presented in this study are available on

request from the corresponding author.

Supplementary

Not applicable.

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