Mechanism of Angelica sinensis Against Carbon Ion Radiation Based on Network Pharmacology

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Abstract

[Objective] To investigate the molecular mechanism of Angelica sinensis against carbon ion radiation based on network pharmacology technology. [Methods] Active components of Angelica sinensis and their anti-carbon ion radiation targets were obtained from relevant databases; a biological network of targets was constructed, bioinformatics analysis was conducted, and molecular docking was employed to validate the network pharmacology results. [Results] Nine active components of Angelica sinensis act through 98 targets to participate in PI3K-Akt, MAPK and other signaling pathways, regulating molecular functions such as DNA binding and kinase binding, as well as processes including DNA damage response; molecular docking validated the reliability of the network pharmacology results. [Limitations] Only molecular docking validation was performed for the network pharmacology results. [Conclusion] Angelica sinensis exerts its anti-carbon ion radiation effect mainly by regulating inflammatory response, immune response, damage repair response and other processes.

Full Text

Study on the Mechanism of Danggui Against Carbon Ion Radiation Based on Network Pharmacology

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Abstract:
[**Objective**] To investigate the molecular mechanism of Danggui (Angelicae Sinensis Radix) against carbon ion radiation using network pharmacology. [**Methods**] Active components of Danggui and their targets against carbon ion radiation were obtained through relevant databases. Biological networks of the targets were constructed, and bioinformatics analysis was performed. Molecular docking was used to validate the network pharmacology results. [**Results**] Nine active components of Danggui participated in signaling pathways such as PI3K-Akt and MAPK through 98 targets, regulating molecular functions including DNA binding and kinase binding, as well as processes like DNA damage response. Molecular docking verified the reliability of the network pharmacology findings. [**Limitations**] The network pharmacology results were only validated by molecular docking. [**Conclusions**] Danggui exerts its anti-carbon ion radiation effects primarily by regulating inflammatory responses, immune responses, and damage repair processes.

Keywords: carbon ion radiation; Danggui; network pharmacology; molecular docking
Classification: Q691

Heavy ions have attracted increasing attention in cancer radiotherapy due to their unique inverted depth-dose distribution and high relative biological effectiveness. According to statistics from the Particle Therapy Co-Operative Group, carbon ions are the most widely used heavy ions in radiotherapy applications [1]. Like photon radiation, carbon ion beams also cause damage to normal cells in and around the treatment target area as well as along the beam path. Therefore, radiation damage to healthy tissues remains a critical consideration in carbon ion radiotherapy. Carbon ions are also a component of space radiation, and their high linear energy transfer (LET), strong tissue penetration capability, and high relative biological effectiveness make them a significant threat to astronaut health and safety [2].

Both the need to improve quality of life for carbon ion radiotherapy patients and the requirements of manned space missions necessitate the development of effective carbon ion radioprotective agents to prevent and mitigate the harmful effects of carbon ion radiation.

Radioprotective agents can prevent or reduce radiation damage to normal tissue cells. Currently, amifostine is the only agent approved by the U.S. Food and Drug Administration for clinical use, but its efficacy is limited and its side effects are severe, restricting its clinical application [3]. Driven by the need for radiation protection, continuous efforts are being made to develop radioprotective agents with clinical prospects. Traditional Chinese medicine has become a focus of anti-radiation research due to its abundant resources, low toxicity

and side effects, and rich content of active substances with antioxidant, anti-inflammatory, and immune-stimulating properties. Danggui, a genuine medicinal material from Gansu Province, possesses anti-inflammatory, antioxidant, and immune-regulating effects and demonstrates significant anti-radiation activity. With both medicinal and food homology, Danggui can be used as a daily dietary supplement. Its chemical components mainly include volatile oils (containing phthalide lactones), organic acids, polysaccharides, amino acids, and nucleosides. Danggui polysaccharides can reduce the toxic side effects of ionizing radiation on the body and enhance immune surveillance capacity [4]. Danggui extract can scavenge free radicals generated by radiation and provides protective effects against ionizing radiation damage in mice [5]. Danggui extract exerts anti-radiation effects by alleviating radiation-induced lung injury. A quantitative analysis of traditional Chinese medicine application frequency for treating radiation-induced lung injury from 1979 to 2018 showed that Danggui was used 559 times, ranking third [6]. However, Danggui' s clinical application is based on practical experience, and its active components and molecular mechanisms for exerting anti-radiation effects remain unclear.

Network pharmacology is a new model for drug research that utilizes big data analysis and artificial intelligence, primarily conducting systematic studies on disease mechanisms and drug action mechanisms from the perspective of complex biological networks. Network pharmacology can identify active components in traditional Chinese medicine and their associated biological targets and signaling pathways for disease treatment [7]. Based on network pharmacology, researchers can analyze the herb-active component-target-signaling pathway-disease interaction network from a systems biology perspective, which helps understand the therapeutic effects of traditional Chinese medicine on diseases. This study employs network pharmacology methods to investigate the active components and potential molecular mechanisms of Danggui against carbon ion radiation, providing a new theoretical foundation and direction for subsequent research and further theoretical basis for the clinical promotion and application of Danggui.

2.1 Screening of Danggui Active Components and Acquisition of Their Targets

Using "Danggui (当归)"as the search term, the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmspe.com/tcmsp.php) [8] was searched. Active components with oral bioavailability (OB) $ $30% and drug-likeness (DL) $ $0.18 were selected, with additional components not meeting these criteria supplemented based on relevant literature [9-11]. The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [12] and Swiss Target Prediction platform (http://www.swisstargetprediction.ch/) [13] were used to predict potential targets of the active components.

2.2 Acquisition of Potential Targets for Danggui Intervention in Carbon Ion Radiation and Construction of Network Models

Using "carbon ion radiation"as the keyword, carbon ion radiation-related targets were retrieved from the GeneCards database (https://www.genecards.org) [14]. After removing duplicates, carbon ion radiation targets were obtained. The potential targets of Danggui active components were mapped with carbon ion radiation targets to obtain potential targets for Danggui against carbon ion radiation.

Cytoscape 3.9.1 software was used to construct a herb-active component-potential target network, and Network Analyzer was used for topological analysis of the network diagram. The STRING platform (https://string-db.org/) was used to construct a protein-protein interaction (PPI) network of potential targets, which was visualized using Cytoscape 3.9.1 software. Core targets were identified based on degree values.

2.3 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analysis

The potential targets were entered into the Metascape database [15] with Min Overlap set to 3, P Value Cutoff set to 0.01, and Min Enrichment set to 1.5 for GO functional and KEGG enrichment analyses. A p-value <0.01 indicated statistical significance. KEGG pathway information, corresponding targets, and active components were imported into Cytoscape 3.9.1 software to construct active component-potential target-pathway and pathway-pathway networks, which were analyzed topologically using Network Analyzer.

2.4 Molecular Docking

Molecular docking is a theoretical simulation method that studies the interaction between receptors and ligand molecules and predicts their binding modes and affinity. It can be used to verify whether active components obtained from network pharmacology bind to targets. Core target proteins and their corresponding Danggui active components were selected for molecular docking. The 3D structures of Danggui active components were obtained from the PubChem database and Chem3D 20.0, while crystal structures of core target proteins were obtained from the RCSB database. PyMOL was used to remove ligands and non-protein molecules from target proteins. After hydrogenation processing using Ledock software, molecular docking was performed with the 3D structures of active components to obtain binding free energies. The PLIP database (https://plip-tool.biotec.tu-dresden.de/) [16] was used to determine interactions between components and their target proteins.

3.1 Results of Active Components and Potential Targets for Danggui Against Carbon Ion Radiation

Two active components, β-sitosterol and stigmasterol, were screened from the TCMSP database. Since the database does not consider factors such as relative content of components, screening with OB$ 30 0.18*doesnotcaptureallactivecomponents.Inadditiontoscreening* as criteria, seven additional active components were supplemented based on relevant literature [9-11] (Table 1). Z-ligustilide is one of the main active components of Danggui, with high bioavailability and strong penetration ability, effectively improving blood-brain barrier permeability [9]. The activity of Danggui is related to ferulic acid content, which is recommended as a marker compound for evaluating Danggui and its product quality [10]. Danggui polysaccharides are one of the main active components, among which the acidic polysaccharide component (mainly composed of galacturonic acid, rhamnose, arabinose, galactose, and mannose) can protect mouse white blood cells and lymphocytes from radiation damage and provides protective effects against acute radiation injury in mice [11]. Using the Swiss Target Prediction platform, 190 targets of the nine Danggui active components were obtained.

Searching "carbon ion radiation" in the GeneCards database yielded 1,257 carbon ion radiation-related targets with Relevance score $ $3.98. Since not all Danggui active component targets are carbon ion radiation targets, the Danggui active component targets were mapped with carbon ion radiation targets to obtain 98 common targets as potential targets for Danggui against carbon ion radiation (Table 1). Table 1 shows that each active component corresponds to multiple targets, and each target can correspond to different active components, demonstrating a "one-to-many" and "many-to-one" relationship between active components and targets, reflecting the multi-component, multi-target characteristics of traditional Chinese medicine.

Table 1. Information on Danggui Active Components

Active Component	PubChem CID	Potential Targets
Ferulic acid	445858	48 (ABCB1, ACE, AHR, ALOX15, ALOX5, APP, BACE1, CA12, CA2, CA4, CA6, CA9, CCND1, CDK4, CTNNB1, CYP1A1, CYP1A2, CYP1B1, EGFR, ESR2, F2, F3, FYN, KDM4C, LCK, MAOA, MAOB, MAPK8, MET, MME, MMP1, MMP2, MMP9, NFE2L2, NOS2, PARP1, PRKCE, PTGS1, PTGS2, PTPN1, RELA, REN, SLC16A1, STAT3, TLR4, TLR9, TOP2A, TTR)
Z-ligustilide	5281862	28 (CA2, CEL, CREBBP, CTSB, CTSK, CTSL, CYP11B1, CYP11B2, CYP1A2, CYP2A6, F2, FLT3, GABRA2, GABRB3, GABRG2, ICAM1, KCNK2, MAOA, MAPK14, P2RX7, PARP1, PDGFRB, PIK3R1, PRKDC, PTGS1, SELE, SLC6A3, VCAM1)
β-sitosterol	222284	21 (ACHE, AR, BCHE, CYP17A1, CYP19A1, CYP2C19, ESR1, ESR2, G6PD, GLRA1, HMGCR, NOS2, NR1H2, POLB, PPARA, PPARG, PTPN1, PTPN6, SLC6A2, SLC6A4, VDR)
Stigmasterol	5280794	21 (ACHE, AR, BCHE, CYP17A1, CYP19A1, CYP2C19, ESR1, ESR2, G6PD, GLRA1, HMGCR, NOS2, NR1H2, POLB, PPARA, PPARG, PTPN1, PTPN6, SLC6A2, SLC6A4, VDR)

Active Component	PubChem CID	Potential Targets
L-rhamnose	5460070	7 (CDK1, FGF1, FGF2, FOLH1, HSP90AA1, PSEN1, VEGFA)
D-galacturonic acid	439215	6 (CHRNA7, EGLN1, G6PD, HAO1, HMGCR, PPARA)
D-galactose	6036	6 (CDK1, FGF1, FGF2, HSP90AA1, PSEN1, VEGFA)
D-mannose	18950	6 (CDK1, FGF1, FGF2, HSP90AA1, PSEN1, VEGFA)
D-arabinose	5460005	3 (CHRNA7, EGLN1, HMGCR)

3.2 Construction of Network Models for Danggui Against Carbon Ion Radiation

(1) Construction of Herb-Active Component-Potential Target Network

Cytoscape software was used to construct a herb-active component-potential target network for the 98 potential targets, comprising 108 nodes and 155 edges with a mean degree of 2.870 (Figure 1 [Figure 1: see original paper]). Nodes represent herbs, active components, and potential targets, while edges represent interactions among them. Degree value indicates the number of associations between active components and herbs or potential targets. This network further visually demonstrates the multi-component, multi-target characteristics of traditional Chinese medicine. In this network, ferulic acid had the highest degree value among active components, followed by Z-ligustilide, with stigmasterol and β-sitosterol having identical degree values (Figure 1, Table 1). Among potential targets, HMGCR had the highest node degree, followed by ESR2 and G6PD (Figure 1). HMGCR encodes HMG-CoA reductase, a rate-limiting enzyme in non-sterol isoprenoid biosynthesis essential for normal cell function. ESR2 (Erb) encodes a member of the estrogen receptor family and nuclear receptor transcription factor superfamily, regulating gene expression in key pathways such as PI3K and AKT, and plays an important role in mammalian cellular radiation response [17]. G6PD encodes glucose-6-phosphate dehydrogenase, whose primary function is to produce reduced NADPH.

(2) Construction of Potential Target PPI Network

The 98 potential targets were imported into the STRING platform, three isolated nodes were hidden, and the PPI network of Danggui against carbon ion radiation was constructed and visualized using Cytoscape (Figure 2a [Figure

2: see original paper]). This network contained 95 interacting nodes and 809 edges with a mean degree of 17.032. In Figure 2a, nodes represent potential targets, differentiated by color and size according to degree value—darker color and larger size indicate more critical targets in the network. Edges represent interactions between potential targets, with thicker edges indicating stronger connections and the number of lines reflecting the degree of association between potential targets.

The cytoHubba plugin in Cytoscape was used to identify core targets, with the top 10 targets by degree value considered as core targets (Figure 2b). Among these, VEGFA and EGFR (ErbB1) [18] are associated with tissue fibrosis development, while PPARG [19], MMP9 [20], PTGS2 (COX2) [21], and CTNNB1 [22] are closely related to inflammatory responses. HSP90AA1 [23], STAT3 [24], and EGFR [25] can regulate DNA damage repair. TLR4 is a receptor involved in immune response that plays a role in acute inflammation regulation, and activation of the TLRs pathway demonstrates significant radioprotective effects [26]. ESR1 has anti-inflammatory effects and regulates innate and adaptive immune cell signaling pathways [27]. A core target subnetwork was created (Figure 2c).

3.3 GO and KEGG Analysis of Potential Targets

The 98 potential targets were entered into the Metascape database platform for GO and KEGG enrichment analysis. GO enrichment analysis results ($p<0.01$) showed that the 98 potential targets were enriched in 1,199 biological process (BP) terms, 95 cell composition (CC) terms, and 163 molecular function (MF) terms. In BP enrichment analysis, potential targets were mainly involved in cellular response to nitrogen compounds, response to xenobiotic stimuli, regulation of defense response, positive regulation of phosphorylation, regulation of MAPK cascade, and other biological processes. Among these, positive regulation of phosphorylation and positive regulation of protein phosphorylation had the most enriched core targets (7 core targets). Regulation of defense response, regulation of DNA-binding transcription factor activity, and cellular response to growth factor stimulus each had 6 enriched core targets. Regulation of MAPK cascade, regulation of inflammatory response, and regulation of cellular stress response each had 5 enriched core targets.

In CC enrichment analysis, potential targets were mainly concentrated in membrane rafts, membrane microdomains, external side of plasma membrane, and transcription regulator complex. Among these, transcription regulator complex and perinuclear region of cytoplasm had the most enriched core targets (4 core targets each). Membrane rafts, membrane microdomains, and receptor complexes each had 3 enriched core targets. In MF enrichment analysis, potential targets were mainly involved in oxidoreductase activity, DNA-binding transcription factor binding, nuclear receptor activity, and ligand-activated transcription factor activity. Among these, kinase binding and chromatin binding had the most enriched core targets (5 core targets each). DNA-binding transcription factor binding, protein phosphatase binding, and transcription factor binding

each had 4 enriched core targets. The top 10 terms ranked by p-value were visualized (Figure 3 [Figure 3: see original paper]).

KEGG pathway enrichment analysis showed that 98 potential targets were significantly enriched in 154 signaling pathways ($p<0.01$), including 73 human disease pathways. The remaining pathways mainly involved 17 signal transduction pathways, 15 immune system pathways, 14 endocrine system pathways, 7 nervous system pathways, and 6 cell growth and death-related pathways. The top 20 pathways by enrichment degree mainly involved cancer signaling pathways, lipid and atherosclerosis pathways, HIF-1 signaling pathway, and TNF signaling pathway (Figure 4 [Figure 4: see original paper]). Among these, the cancer signaling pathway had the most enriched core targets (9 core targets), while 4 core targets were enriched in HIF-1 and PI3K-Akt signaling pathways (Figure 5 [Figure 5: see original paper]).

Signal transduction-related pathways, immune-related pathways, and cell growth and death-related pathways are all closely related to cellular radiation response. Based on KEGG enrichment results, the top 10 pathways from these three categories were used to construct active component-potential target-pathway networks and pathway-pathway interaction networks; pathways with fewer than 10 were all included. In the active component-potential target-pathway network, active components ranked by degree value from high to low were: ferulic acid, Z-ligustilide, rhamnose, mannose, galactose, β-sitosterol, stigmasterol, arabinose, and galacturonic acid. Potential targets PIK3R1, MAPK14, RELA, MAPK8, and EGFR had higher degree values. The PI3K-Akt signaling pathway had the highest degree value, followed by MAPK and Ras signaling pathways (Figure 5). In the pathway-pathway interaction network, 24 pathways interacted with PI3K-Akt, FoxO, or T cell receptor pathways; 23 pathways interacted with cellular senescence or HIF-1 pathways; and 22 pathways interacted with Toll-like receptor, C-type lectin receptor, TNF, Th cell differentiation, or MAPK pathways (Figure 6 [Figure 6: see original paper]).

3.4 Molecular Docking Results

Binding free energy can evaluate the binding capacity between receptors and ligands. When binding energy is less than 0, ligands and receptors can bind freely. The smaller the binding free energy, the stronger the affinity and the more stable the conformation. Core targets and their corresponding Danggui active components were subjected to molecular docking. Docking results showed that all core targets could freely bind with their corresponding Danggui active components. Among these, galactose with HSP90AA1, stigmasterol with PPARG, mannose with HSP90AA1, ferulic acid with MMP9, and β-sitosterol with PPARG showed higher affinity (Table 2). HSP90AA1 is related to DNA damage repair [23], while PPARG [19] and MMP9 [20] are related to inflammatory and immune regulation.

High-affinity core target-active component pairs were visualized (Figure 7 [Figure 7: see original paper]). Galactose formed hydrogen bonds with GLU47, SER113, ILE131, GLN133, PHE134, GLY135, and ARG400 of HSP90AA1, and formed a salt bridge with ARG400 (Figure 7a). Stigmasterol formed hydrophobic interactions with PHE282, TYR327, LEU330, VAL339, ILE341, LEU353, MET364, LYS367, and PHE368 of PPARG, and formed hydrogen bonds with HIS323 and TYR473 (Figure 7b). Mannose formed hydrogen bonds with GLU47, SER113, GLN133, PHE134, GLY135, GLY137, and PHE138 of HSP90AA1 (Figure 7c). Ferulic acid formed hydrophobic interactions with TYR245 and TYR251 of MMP9, and hydrogen bonds with HIS226, TYR245, MET247, and PRO255 (Figure 7d). β-sitosterol formed hydrophobic interactions with ILE281, PHE282, ARG288, TYR327, LEU330, VAL339, LEU353, PHE363, LEU453, LEU469, and TYR473 of PPARG (Figure 7e).

Table 2. Binding Energies of Molecular Docking Between Core Targets and Corresponding Active Components

Core Target	Active Component	Binding Energy (kcal · mol^{-1})
VEGFA	Rhamnose	-3.31
VEGFA	Mannose	-3.76
VEGFA	Galactose	-3.81
CTNNB1	Ferulic acid	-4.79
STAT3	Ferulic acid	-3.77
HSP90AA1	β-sitosterol	-4.88
HSP90AA1	Stigmasterol	-4.82
HSP90AA1	Ferulic acid	-3.10
PTGS2	Rhamnose	-4.73
PPARG	Mannose	-5.19
PPARG	Galactose	-5.31
PPARG	Ferulic acid	-4.68
PPARG	β-sitosterol	-5.13
PPARG	Stigmasterol	-5.27
MMP9	Ferulic acid	-3.41

Compared with conventional radiation, carbon ion beams have high LET and large relative biological effectiveness (RBE), causing severe damage with low repair efficiency. Due to characteristics such as dense energy deposition, complex ionization tracks, and high local doses, carbon ion radiation is more likely to induce DNA cluster damage, which is dense, spatially complex, and difficult to repair [28, 29]. After carbon ion radiation damage, cells initiate a series of signal transduction cascade reactions, leading to altered expression of target genes [28, 29], activation of cell cycle checkpoints, triggering of DNA damage response (DDR), inflammatory responses, and immune responses. These processes may result in cell death, altered biological functions, and genetic instability, increasing the probability of gene mutation and cell carcinogenesis and producing

chronic and late effects on the organism. Danggui extract has anti-inflammatory, antioxidant, and immune-enhancing effects that can reduce ionizing radiation damage [4-6]. This study identified nine Danggui active components and 98 potential targets for Danggui' s anti-carbon ion radiation effects using network pharmacology. These targets involve protein kinases, transcription factors, and cytokines, participating in processes such as signal cascade activation, inflammatory response, immune response, and DNA damage repair. Ferulic acid and Z-ligustilide had more potential targets (Figure 1).

The 98 potential targets participated in multiple signaling pathways related to DDR, inflammatory response, immune response, and oxidative stress response, mainly including MAPK, PI3K-Akt, FoxO, JAK-STAT, TNF, and NF- B pathways. Multiple signal transduction pathways stimulated by ionizing radiation are mediated by the MAPK superfamily. MAPK signaling activation can upregulate telomerase activity, alter chromatin distribution, and regulate the cell cycle [30], while p38MAPK and JNK pathways also participate in immune regulation [31]. Studies have found that ferulic acid [32] and Z-ligustilide [33] exert anti-inflammatory effects by inhibiting MAPK (p38MAPK, ERK, and JNK) and NF- B signaling transduction. PI3K-Akt signaling participates in cellular radiation response by regulating mitochondrial proteins, transcription factors, translation mechanisms, and cell cycle progression [34]. Ionizing radiation has an inhibitory effect on the PI3K-Akt pathway, and activation of this pathway can promote radiation-induced DNA double-strand break (DSB) repair and participate in regulating irradiated cell cycle and apoptosis [35, 36]. Both Danggui polysaccharides reducing cell apoptosis and ferulic acid protecting cells from radiation-induced oxidative stress are related to the PI3K-Akt pathway [32]. The FOXO pathway regulates cell cycle, repair of damaged DNA, and cell apoptosis, and inhibits oxidative stress by regulating antioxidant enzymes, playing a key role in maintaining redox balance [37]. JAK2/STAT3 signaling plays an important role in anti-radiation by inhibiting apoptosis and enhancing clonogenic potential [38]. Danggui may exert anti-carbon ion radiation effects by regulating these pathways to modulate immunity, eliminate inflammation, and reduce oxidative damage. Potential targets were also significantly enriched in cell survival-related signaling pathways. Z-ligustilide exerts anti-apoptotic activity in a dose-dependent manner by upregulating the ratio of anti-apoptotic protein Bcl-2 to pro-apoptotic protein Bax and downregulating caspase 3 expression [39]. Ferulic acid exerts anti-apoptotic effects by mediating Bcl-2, Bax, or JNK signaling [32], suggesting that Danggui may promote growth and survival of irradiated cells by regulating cell growth and death signaling pathways through its active components.

Various signaling pathways regulated by potential targets interact with each other (Figure 6). PI3K-Akt, FoxO, and T cell receptor pathways showed the strongest interactions with other pathways, serving as hub nodes in the pathway network. The PI3K-Akt pathway was enriched with the most potential targets, suggesting it plays a hub role in Danggui' s anti-carbon ion radiation effects. Ferulic acid and Z-ligustilide are the main components regulating sig-

naling pathways (Figure 5).

Interaction relationships exist among potential target proteins, with VEGFA, EGFR, and eight other targets serving as core targets in the PPI network (Figure 2). Molecular docking results showed that these core targets could freely bind with their corresponding active components (Table 2).

Rhamnose, mannose, and galactose interact with core targets VEGFA and HSP90AA1 (Table 2). VEGFA is a key target for inflammatory response and activation of MAPK, PI3K-Akt, and other signaling pathways. The molecular chaperone HSP90AA1 regulates the stability of some DNA damage response (DDR) proteins; inhibiting HSP90AA1 ATPase activity can lead to degradation of these proteins, thereby weakening DDR signals and slowing DSB repair [23]. Mannose has anti-inflammatory, immune-regulating, and oxidative response-inhibiting functions [40], and can alleviate lipopolysaccharide-induced acute lung injury in rats in a dose-dependent manner [41]. Rhamnose, mannose, and galactose may participate in multiple signaling pathways including MAPK, PI3K-Akt, and necroptosis through core targets VEGFA and HSP90AA1, regulating inflammatory and immune responses and promoting radiation-induced DSB repair.

β-sitosterol and stigmasterol interact with core targets PPARG and ESR1 (Table 2). The nuclear receptor PPARG is a ligand-activated transcription factor that exerts anti-inflammatory effects by downregulating pro-inflammatory gene expression and inflammatory cell function [41]. Stigmasterol significantly reduces inflammatory response by activating PPARG to upregulate intestinal mucosal immune response and restore the balance between helper T cells (Th17) and regulatory T cells (Treg) [19]. ESR1 knockdown can significantly enhance ionizing radiation-induced ferroptosis [42]. β-sitosterol and stigmasterol may inhibit inflammatory factor secretion, regulate immune response, and promote survival of irradiated cells by acting on PPARG and ESR1 targets.

Ferulic acid interacts with core targets EGFR, CTNNB1, STAT3, PTGS2, MMP9, and TLR4 (Table 2). EGFR regulates radiation-induced DSB NHEJ repair through MAPK signaling [25], while STAT3 and its transcriptional products can activate DNA damage repair [24]. Ferulic acid has anti-fibrotic effects [32], reduces oxidative stress, and activates DNA non-homologous end joining (NHEJ) repair pathways [43], which may be related to EGFR and STAT3 targets. PTGS2 gene expression can promote inflammatory response; during inflammation, MMP9 release can damage tight junctions and compromise tissue structural integrity. Ferulic acid reduces radiation-induced systemic inflammation by inhibiting PTGS2 expression [21] and protects skin from radiation damage by inhibiting radiation-induced MMP9 activity in mouse skin cells through post-translational mechanisms [44]. Increased incidence of colitis-associated cancer after heavy ion radiation exposure is related to CTNNB1 activation [22], and CTNNB1 signaling pathway activation can inhibit irradiated cell apoptosis and skin damage [45]. Mice lacking TLR4 are more sensitive to radiation, and activation of TLR4 signaling with lipopolysaccharide can significantly increase

survival rates of irradiated mice [46]. It is speculated that ferulic acid may participate in PI3K-Akt, NF- B, Toll-like receptor, and necroptosis pathways by regulating CTNNB1 and TLR4 signaling, exerting inflammatory and immune regulatory effects and increasing cellular radiation resistance.

This study also comparatively analyzed the potential targets and main components of Danggui against carbon ion radiation and X-ray radiation. The results showed that there were certain differences in potential targets and GO functions and KEGG signaling pathways involved in targets between the two types of ionizing radiation. Relatively speaking, the top 20 entries differed significantly (Supplement 1), but the core targets were basically the same (Supplement 1). The main active components against both radiation types were basically the same (Supplement 2). Further analysis of carbon ion and X-ray radiation-related targets showed that there were obvious differences in radiation targets and their involved GO functions and KEGG signaling pathways between the two radiation types (Supplement 3). Carbon ion radiation targets involved more GO functions and KEGG signaling pathways (Supplement 3). The analysis results indicate that Danggui has protective effects against both carbon ion radiation and X-ray radiation, but the related mechanisms are not identical, which may be related to different radiation damage mechanisms of the two radiation types.

5 Summary and Outlook

In summary, Danggui mainly exerts anti-carbon ion radiation effects through active components such as ferulic acid, Z-ligustilide, β-sitosterol, and stigmasterol acting on targets including VEGFA and EGFR, regulating multiple inflammatory and immune-related signaling pathways such as PI3K-Akt, MAPK, IL-17, and NF- B. Molecular docking results validated the reliability of network pharmacology analysis. The study reveals that Danggui has a multi-component, multi-target, multi-pathway mechanism of action, laying a foundation for subsequent research on Danggui against carbon ion radiation and providing new evidence for further development and utilization of Danggui in anti-radiation applications. Future research will focus on two aspects: (1) investigating the anti-carbon ion radiation effects of Danggui and validating its anti-radiation targets and signaling pathways, and (2) studying the protective effects and related mechanisms of Danggui' s main active components against carbon ion radiation.

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Note: Figure translations are in progress. See original paper for figures.