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Investigation of the key targets and pharmacological mechanisms of rhamnocitrin against oxaliplatin-induced neuropathic pain based on network pharmacology approach and experimental validation

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Abstract. Rhamnocitrin (RH) is a bioactive flavonoid of Astragali Radix, which exerts a wide variety of pharmacological effects. However, there are no reports focusing on the therapeutical effects and mechanisms of RH against neuropathic pain (NP). In this study, systematic pharmacology and *in vivo* experimental approaches were employed to identify the potential targets of RH for treating oxaliplatin-induced NP. Our findings indicated that the therapeutical effect of RH might be closely associated with key genes, including MAPK3, MAPK1, SRC, PTGS2, EGFR, MMP9, and MMP2, as well as potential signaling pathways such as PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, and Rap1 signaling pathway. The *in vivo* experimental findings demonstrated that RH could suppress oxidative stress, inflammatory response, and down-regulate MMP2 and MMP9 expressions to exert its therapeutic effects against NP. This study used network pharmacology and experimental validation to elucidate the potential targets and underlying mechanisms by which RH improves oxaliplatin-induced NP and offer new insight on drug development for NP.

Key words: Rhamnocitrin — Oxidative stress — Oxaliplatin — Neuropathic pain — Astragali radix

Introduction

Neuropathic pain (NP) is a pathological change in nervous system function (Kuner and Flor 2016; Peirs and Seal 2016). The previous report indicated that chemotherapy, autoimmune disease, cancer, infection, or diabetes could cause impairment of the nervous system, eventually resulting in NP (Tsuda 2019). Chemotherapy-induced peripheral neuropathy (CIPN) is a common dose-limiting side effect in cancer patients undergoing chemotherapy. About 30–40% of cancer patients undergoing chemotherapy suffer from CIPN, which significantly increased annual healthcare costs (Pike et al. 2012). The specific pathophysiology mechanism of CIPN has not been studied. Previous studies have revealed that diverse pathological mechanisms have been proposed in the development of CIPN, such as inflammatory responses, membrane remodeling, axon degeneration, altered calcium homeostasis, and oxidative stress (Jaggi and Singh 2012; Starobova and Vetter 2017). Currently, no drug has been proven effective in controlling CIPN. Oxaliplatin is a commonly used platinum-based chemotherapeutic agent for various types of cancer, such as pancreatic, gastric, and colorectal cancer (André et al. 2004; Carozzi et al. 2015). Unfortunately, despite the use of oxaliplatin being effective in the treatment of solid tumors, this chemotherapeutic drug has been demonstrated to evoke peripheral neurotoxicity, which results in the development of NP (Boyette-Davis et al. 2015). It has been revealed that the binding of oxaliplatin to DNA is the major inciting mechanism that causes chronic impair-

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ment in the dorsal root ganglia neuron, which could induce apoptosis and ultimately cause peripheral neuropathy (Gill and Windebank 1998; Staff et al. 2019). The chemotherapy-induced NP can seriously affect patients' quality of life. And there is currently no effective treatment to avoid or reduce oxaliplatin-induced NP.

Natural products and traditional Chinese medicine contain various types of pharmaceutical or bioactive components for the development of drugs. Astragali Radix (root of *Astragalus membranaceus*; Huangqi) is an important traditional Chinese medicine that has been widely used in China for centuries for the treatment of various diseases, such as uterine prolapse, uterine bleeding, chronic fatigue, fever, wounds, and weakness (Fu et al. 2014). Recent studies have revealed that the Astragali Radix extracts exert anti-neuropathic effects in the cellular and rat model of oxaliplatin-induced neuropathy (Di Cesare Mannelli et al. 2015, 2017). However, the pharmacodynamic material basis of Astragali Radix has not been systematically studied and its representative anti-neuropathic active components cannot be confirmed. A previous study showed that flavonoids are major bioactive ingredients in Astragali Radix (Yang et al. 2021). Kaempferol is a flavonoid compound found in Astragali Radix. And previous reports indicated that kaempferol and its glycosylated derivatives exert a multipotential neuroprotective effect in central nervous system diseases, such as neuropathic pain, epilepsy, ischemia stroke, and anxiety disorders (Kishore et al. 2018; Silva Dos Santos et al. 2020). Rhamnocitrin (RH) is also known as kaempferol-7-methyl ether, one of the bioactive flavonoids of Astragali Radix, which exerts different pharmacological activities, such as anti-proliferative (Tu et al. 2007), anti-inflammatory (Hu et al. 2017), anti-oxidant (Hong et al. 2009), and anticancer effects (Saleem et al. 2013). However, there are no reports focusing on the effects and mechanisms of RH against NP. Based on these studies, we speculated RH may have beneficial effects in the treatment of NP.

Network pharmacology is proposed as a new research approach to initially investigate the relationships of pathways, targets, diseases, and drugs, and elucidate their molecular mechanisms. In addition, this method has effectively bridged the gap between traditional Chinese medicine and modern medicine. Thus, the present study used the network pharmacology method to identify the main targeted genes and potential signaling pathways of RH against oxaliplatin-induced NP.

Materials and Methods

Identification of RH-associated targets

PubChem website (https://pubchem.ncbi.nlm.nih.gov/compound/5320946) was used to obtain the chemical structure of RH (Fig. 1A). The collection of genes of *Homo sapiens* related to RH in the following databases: Comparative Toxicogenomics Database (http://ctdbase.org/) and the SwissTargetPrediction database (http://www.swisstargetprediction.ch/).

Collection of oxaliplatin-associated targets

Targets collection of oxaliplatin was performed using the following databases: Comparative Toxicogenomics Database (http://ctdbase.org/) and Gene Cards database (https://www.genecards.org/). Access to these databases with the keywords "oxaliplatin" to collect oxaliplatin-associated targets, and duplicate genes were removed.

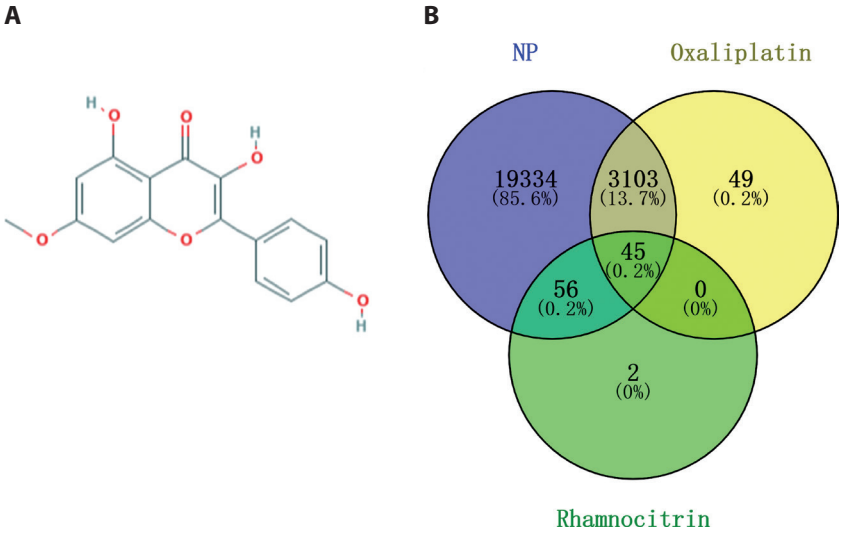

Figure 1. Potential targets of rhamnocitrin against oxaliplatin-induced neuropathic pain (NP). **A.** Chemical structure of rhamnocitrin. **B.** Venn diagram of rhamnocitrin-related genes, NP-related genes, and oxaliplatin-related genes.

Collection of NP-associated targets

We accessed the Comparative Toxicogenomics Database (http://ctdbase.org/), Gene Cards database (https://www.genecards.org/), and DisGeNet (http://www.disgenet.org/) with the keywords "neuralgia" or "neuropathic pain" to research NP-associated targets, and reproducible genes were deleted. Lastly, we used Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/index.html) tool to identify the common genes of RH, NP, and oxaliplatin.

Construction of protein-protein interaction (PPI) network

Firstly, these common genes were input into the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org/cgi/input.pl) to construct the PPI network, the species was defined as "Homo sapiens". Then, the TSV format file was downloaded and imported into Cytoscape3.8.2 to visualize the PPI network. Target genes were ranked based on the degree value, which was calculated by the CytoNCA plug-in. Then, the top 10 genes were selected according to a degree, closeness, and betweenness, and overlapped genes were identified as the key genes.

Functional enrichment analysis of RH-related genes in the treatment of oxaliplatin-induced NP

GO and KEGG enrichment analyses were performed to further explore the underlying mechanisms of RH as a therapeutic drug for NP. The 45 common genes were uploaded to metascape (http://metascape.org/gp/#/main/step1). Meanwhile, the species was defined as "Homo sapiens" and a p value < 0.05 was screened and considered significant. Lastly, a bioinformatics platform (http://www.bioinformatics.com.cn/) was used to visualize the results.

Animal experiment and drug interventions

All animal protocols were by the guidelines approved by the Institutional Animal Care and Use Committee of Ganzhou City People's Hospital. And experimental research on animals complies with the Directive 210/63/EU. Adult Sprague-Dawley rats (190–230 g body weight) were purchased from the Animal Experimental Center of Jiangxi province and maintained in a specific pathogen-free environment (a 12 h dark/light cycle in 50–60% humidity, 18–22°C temperature with food and water *ad libitum*). All rats were randomly divided into four experimental groups (n = 8) as follows: Control group (Con); oxaliplatin group (NP): rats were intraperitoneally (i.p.) injected with oxaliplatin (2.5 mg/kg) for four consecutive days to induce NP (Ni et al. 2021); NP + 5 mg/kg of RH group (NP+LRH): NP rats were i.p. injected with RH (5 mg/kg) for every 24 h for three consecutive weeks after the final injection with oxaliplatin; NP + 10 mg/kg of RH group (NP+HRH): NP rats were i.p. injected with RH (10 mg/kg) for every 24 h for three consecutive weeks after the final injection with oxaliplatin. The RH doses were based on the previous report and our preliminary experiment (Saleem et al. 2013). RH was dissolved in 5% DMSO and diluted in 0.9% saline. Pain behavioral assessment (mechanical allodynia and thermal hyperalgesia) was performed 1 h after the administration of RH, according to the previous studies (Ni et al. 2021; Xie et al. 2021).

Measurement of inflammatory cytokines and oxidative stress

After the last pain behavioral assessment, the animals were anesthetized by 50 mg/kg of pentobarbital sodium. Then, the dorsal root ganglion (DRG) from lumbar L4–L6 were collected and homogenated by ice-cold PBS (1:9, m:v). The protein concentration of homogenate was measured by bicinchoninic acid assay and the levels of SOD, CAT, MDA, TNF-α, and IL-6 were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, China) based on manufacturer's protocols.

Quantitative real-time PCR

Total RNA of DRG was isolated using TRIzol based on the manufacturer's protocols (Invitrogen, USA). Isolated RNA was reverse-transcribed into cDNA using Super-Script II Reverse Transcriptase (Thermo Fisher, USA), following the manufacturer's instructions. Then, the qPCR was carried out by the SYBR GREEN Master Mix (Thermo Fisher, USA) in an ABI ViiA 7 Dx instrument (Thermo Fisher, USA). The $2^{-\Delta\Delta Ct}$ method was performed to quantify the expression of the gene. The primer pairs (forward and reverse) were used as follows: 5'-GGGAGCGCAAGGATGGAGGCACGA-3' and 5'-CCAGCAGGCAGCACAGGACGCAGA-3' for MMP2; 5'-AGGCGCCGTGGTCCCCACTTACTT-3' and 5'- GCAGGGTTTGCCGTCTCCGTTGCC-3' for MMP9; 5'-CAACTTTGGCATTGTGGAAGG-3' and 5'-ACACATTGGGGGTAGGAACAC-3' for GAPHD.

Statistical analysis

The GraphPad 6 software was used to carry out the statistical analyses. Values were presented as mean ± standard deviation (SD) and analyzed *via* two-way ANOVA. Data from the behavioral test were analyzed *via* mixed factor designed ANOVA. A p-value below 0.05 represents statistically significant.

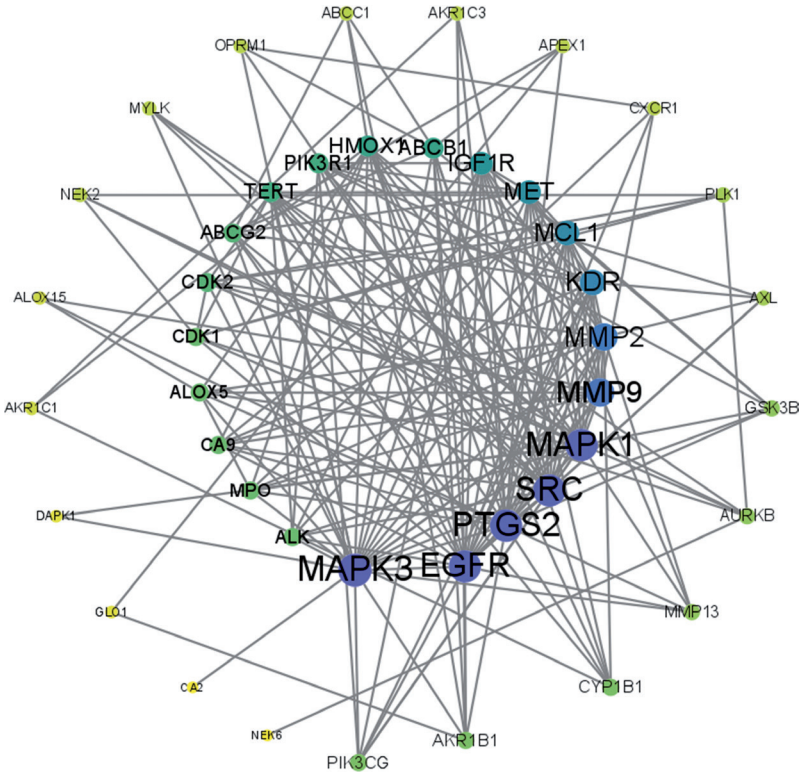

Figure 2. PPI (protein-protein interaction) network with common genes of rhamnocitrin and oxaliplatin-induced neuropathic pain (NP). The darker color and the larger nodes represent more importance in the network. Nodes are sorted from small to large based on the degree value.

Results

Screening of potential targets of RH against oxaliplatin-induced NP

In the present study, the Comparative Toxicogenomics Database and the SwissTargetPrediction database were utilized to collect 103 genes of RH. Comparative Toxicogenomics Database and Gene Cards database were used to obtain 3197 oxaliplatin-associated genes. Comparative Toxicogenomics Database, Gene Cards database, and DisGeNet were used to collect 22538 NP-associated genes. Lastly, a Venny tool was used to obtain the 45 genes overlapped between RH, oxaliplatin, and NP-related genes (Fig. 1B).

Table 1. Designations and topological parameter of top 7 key genes in the PPI network

Target genes	Closeness	Degree	Betweenness
MAPK3	0.6885	24	209.1300
MAPK1	0.6667	23	195.2991
SRC	0.6667	23	152.0414
PTGS2	0.6563	23	238.7450
EGFR	0.6667	23	196.8152
MMP9	0.6269	19	160.0713
MMP2	0.6176	18	70.9560

PPI network analysis

PPI network analysis was performed by the STRING database to explore the interactions between the 45 common genes. Then, the visualization of the network was carried out *via* inputting the PPI network file into Cytoscape 3.8.2 software. As shown in Figure 2, the network contained 45 nodes and 200 edges, indicating 45 interacting targets and 200 interactions, respectively. Nodes are sorted from small to large based on the degree value. The darker color and the larger nodes represent more importance in the network. Among these nodes, the top 10 genes, including MAPK3, EGFR, PTGS2, SRC, MAPK1, MMP9, MMP2, KDR, MCL1, and MET were the primary targets for RH against oxaliplatin-induced NP.

Screening of key genes

As shown in Figure 3A–C, the top 10 key genes of degree, betweenness, and closeness subnetworks were obtained. According to the results of a topological analysis, 7 overlapped genes were further screened as key targets, including MAPK3, MAPK1, SRC, PTGS2, EGFR, MMP9, MMP2 (Fig. 3D). The detailed node parameters were shown in Table 1.

These findings indicated that these 7 targets may be used as valuable targeted genes for RH in the prevention and treatment of oxaliplatin-induced NP.

GO and KEGG enrichment analysis

To further screen the relevant biological functions of RH in the treatment of oxaliplatin-induced NP, GO enrichment analysis identified 45 potential genes implicated in biological processes. As shown in Figure 4, there were the top 20 significantly enriched biological processes, mainly including cellular response to oxidative stress, response to oxidative stress, cellular response to chemical stress, response to reactive oxygen species, and cellular response to reactive oxygen species, were regulated by RH in the treatment of oxaliplatin-induced NP. The detailed results of GO enrichment analysis were shown in Table 2.

Besides, the KEGG enrichment analysis was performed to screen the potential signaling pathways of RH in the treatment of oxaliplatin-induced NP. As shown in Figure 5, there were the top 20 significantly enriched signaling pathways, mainly including proteoglycans in cancer, EGFR tyrosine kinase inhibitor resistance, focal adhesion, PI3K-Akt signaling pathway, Rap1 signaling pathway, and melanoma. The results indicated that RH could regulate these signaling pathways to exert its therapeutic effects in the treatment of oxaliplatin-induced NP. The detailed results of the KEGG enrichment analysis were shown in Table 3.

Compound-disease-pathway-target network analysis

Based on the results of KEGG pathway enrichment and genes identification, a network diagram of compound-disease-pathway-target was constructed using Cytoscape (v 3.8.2) to further investigate the relationship between disease, ingredient, signaling pathway, and common genes. As shown in Figure 6, there was a five-layer network containing 65 nodes (1 disease node, 1 compound node, 45 targeted gene nodes, and 18 signaling pathway nodes) and 233 edges. Besides, each gene corresponds to

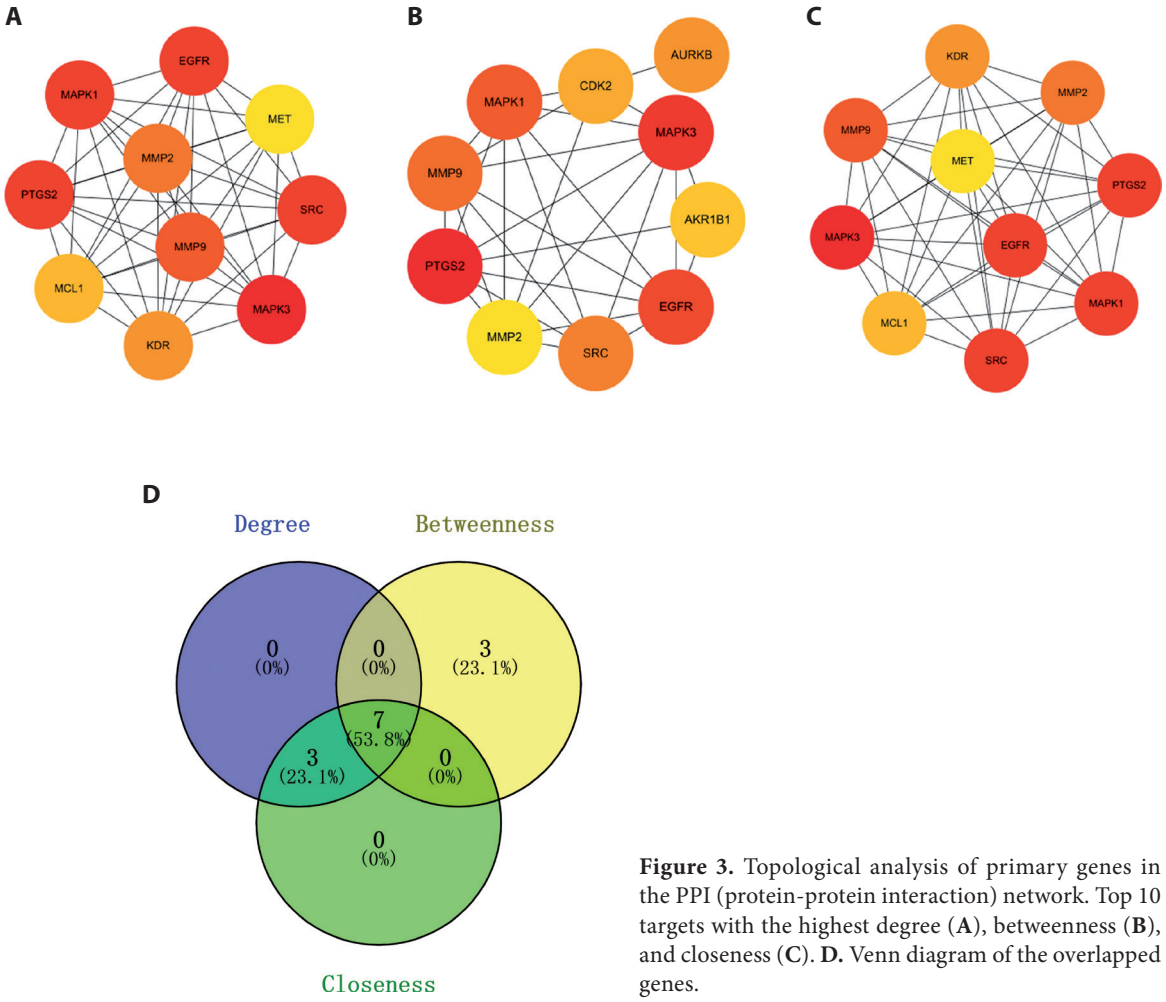

Figure 3. Topological analysis of primary genes in the PPI (protein-protein interaction) network. Top 10 targets with the highest degree (**A**), betweenness (**B**), and closeness (**C**). **D.** Venn diagram of the overlapped genes.

multiple signaling pathways, and each signaling pathway was related to multiple genes, indicating the multiple signaling pathways and multiple targeted genes of RH against oxaliplatin-induced NP. Multiple signaling pathways were interlinked by common genes, suggesting that each pathway plays a synergistic role in the treatment of oxaliplatin-induced NP.

RH alleviates oxaliplatin-induced NP symptoms

As shown in Figure 7, oxaliplatin injection caused a significant decline in the paw withdrawal threshold and paw withdrawal latency of the NP group as compared to the Con group on days 7–21 ($p < 0.01$). The NP symptoms were alleviated after HRH administration for three consecutive weeks ($p < 0.01$).

RH inhibits oxidative stress and neuroinflammation induced by oxaliplatin

As shown in Figure 8A–C, after injection of oxaliplatin, the activities of SOD [$F(1, 11) = 223.37$, $p < 0.0001$] and CAT [$F(1, 11) = 43.5$, $p = 0.0012$] declined, and the MDA [$F(1, 11) = 204.05$, $p < 0.0001$] levels significantly increased in

Table 2. Results of GO enrichment analysis

Term	GO function	Enrichment	*p* value	Related genes
GO:0034599	cellular response to oxidative stress	0.0399	2.082E−19	ALOX5, CDK2, EGFR, HMOX1, MCL1, MET, MMP2, MMP9, MPO, MAPK1, MAPK3, SRC, PTGS2, KDR, TERT
GO:0006979	response to oxidative stress	0.0291	2.391E−19	ALOX5, CDK2, EGFR, HMOX1, MCL1, MET, MMP2, MMP9, MPO, MAPK1, MAPK3, PTGS2, SRC
GO:0062197	cellular response to chemical stress	0.0344	1.252E−18	ALOX5, CDK2, EGFR, HMOX1, MCL1, MET, MMP2, MMP9, MPO, MAPK1, MAPK3, SRC
GO:0000302	response to reactive oxygen species	0.0444	1.493E−16	CDK2, EGFR, HMOX1, MET, MMP2, MMP9, MPO, MAPK1, MAPK3, SRC
GO:0034614	cellular response to reactive oxygen species	0.0566	7.384E−16	CDK2, EGFR, MET, MMP2, MMP9, MPO, MAPK1, MAPK3, SRC
GO:0010035	response to inorganic substance	0.0203	1.743E−14	CDK2, EGFR, HMOX1, KDR, MET, MMP9, MPO, MAPK1, MAPK3, SRC, TERT
GO:0030335	positive regulation of cell migration	0.0218	2.988E−16	EGFR, HMOX1, IGF1R, KDR, MET, MMP9, PIK3R1, MAPK1, MAPK3, PTGS2, SRC, TERT
GO:2000147	positive regulation of cell motility	0.0209	4.982E−16	EGFR, HMOX1, IGF1R, KDR, MET, MMP9, PIK3R1, MAPK1, MAPK3, PTGS2, SRC, TERT
GO:0051272	positive regulation of cellular component movement	0.0205	6.381E−16	EGFR, HMOX1, IGF1R, KDR, MET, MMP9, PIK3R1, MAPK1, MAPK3, PTGS2, SRC, TERT
GO:0040017	positive regulation of locomotion	0.0204	6.512E−16	EGFR, HMOX1, IGF1R, KDR, MET, MMP9, PIK3R1, MAPK1, MAPK3, PTGS2, SRC, TERT
GO:0014066	regulation of phosphatidylinositol 3-kinase signaling	0.0551	2.369E−12	EGFR, IGF1R, KDR, PIK3R1, MAPK1, MAPK3, SRC
GO:0014065	phosphatidylinositol 3-kinase signaling	0.0455	9.307E−12	EGFR, IGF1R, KDR, PIK3R1, MAPK1, MAPK3, SRC
GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway	0.0135	2.152E−11	EGFR, IGF1R, KDR, MET, MMP2, MMP9, PIK3R1, MAPK1, MAPK3, SRC
GO:0048015	phosphatidylinositol-mediated signaling	0.0365	4.412E−11	EGFR, IGF1R, KDR, PIK3R1, MAPK1, MAPK3, SRC
GO:0048017	inositol lipid-mediated signaling	0.0357	5.1E−11	EGFR, IGF1R, KDR, PIK3R1, MAPK1, MAPK3, SRC
GO:0080135	regulation of cellular response to stress	0.0128	4.64E−10	ALOX5, EGFR, IGF1R, MCL1, MET, PIK3R1, MAPK1, MAPK3, TERT
GO:0038083	peptidyl-tyrosine autophosphorylation	0.1905	1.098E−09	EGFR, IGF1R, KDR, MAPK3
GO:0032103	positive regulation of response to external stimulus	0.0138	3.824E−08	EGFR, KDR, MET, MAPK1, MAPK3, PTGS2, SRC
GO:0018108	peptidyl-tyrosine phosphorylation	0.0162	1.639E−07	EGFR, IGF1R, KDR, MET, MAPK3, SRC
GO:0018212	peptidyl-tyrosine modification	0.0161	1.718E−07	EGFR, IGF1R, KDR, MET, MAPK3, SRC

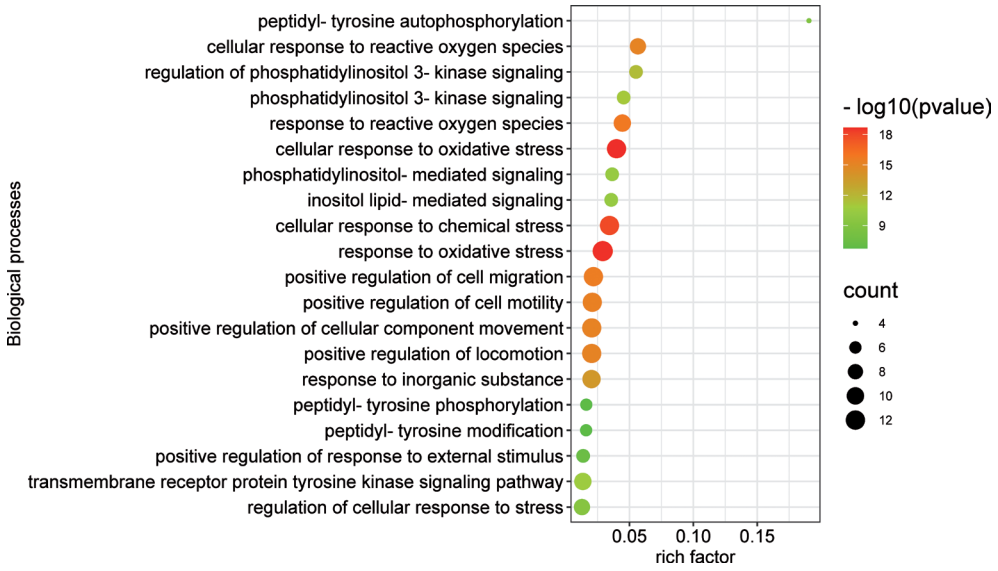

Figure 4. GO enrichment analysis of 45-common genes. The size of each air bubble represents enriched counts, the color of each air bubble represents *p*-value, and the abscissa means the enrichment factor.

the NP group compared to those in the Con group. HRH administration significantly improved the SOD [F (1, 11) = 101.23, $p = 0.0002$] and CAT [F (1, 11) = 131.0, $p < 0.0001$] activities, and reduced MDA [F (1, 11) = 81.57, $p = 0.0003$] levels in DRG.

As shown in Figure 8E–F, after injection of oxaliplatin, the levels of TNF-α [F (1, 11) = 296.52, $p < 0.0001$] and IL-6 [F (1, 11) = 327.60, $p < 0.0001$] increased in the NP group compared to those in the Con group. HRH administration significantly decreased the TNF-α [F (1, 11) = 137.74, $p < 0.0001$] and IL-6 [F (1, 11) = 63.5, $p = 0.0005$] levels in DRG.

Effects of RH on MMP2 and MMP9 expression

According to the results of PPI network analysis, MMP2 and MMP9 were identified as one of the top 7 genes involved in the therapeutic effect of RH against oxaliplatin-induced NP. Therefore, we then measured the mRNA expressions of MMP2 and MMP9 in DRG. As shown in Figure 9, after injection of oxaliplatin, the expressions of MMP2 [F (1, 11) = 173.08, $p < 0.0001$] and MMP9 [F (1, 11) = 143.69, $p < 0.0001$] up-regulated in the NP group compared to those in the Con group. HRH administration significantly down-regulated the MMP2 [F (1, 11)

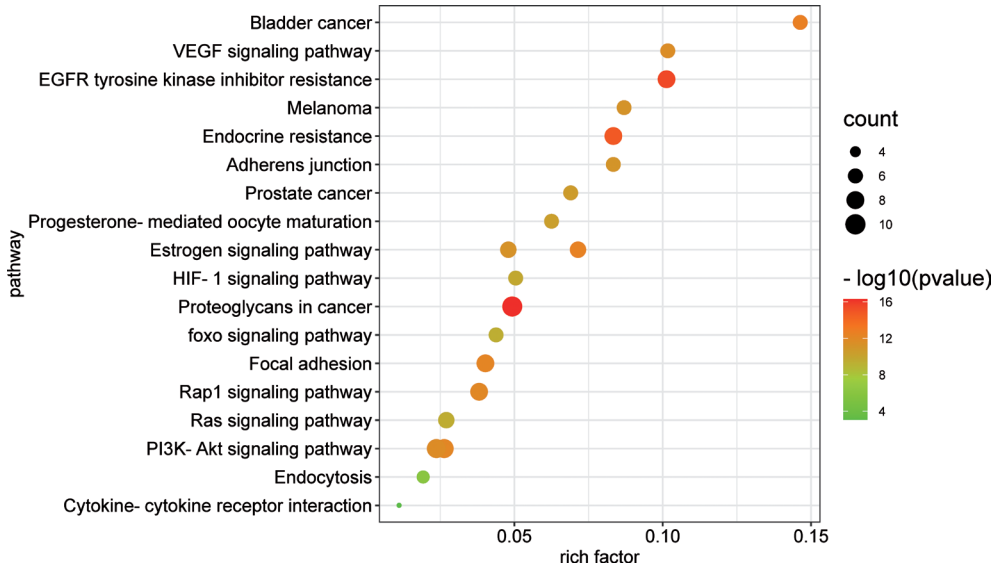

Figure 5. KEGG enrichment analysis of 45-common genes. The size of each air bubble represents enriched counts, the color of each air bubble represents *p*-value, and the abscissa means the enrichment factor.

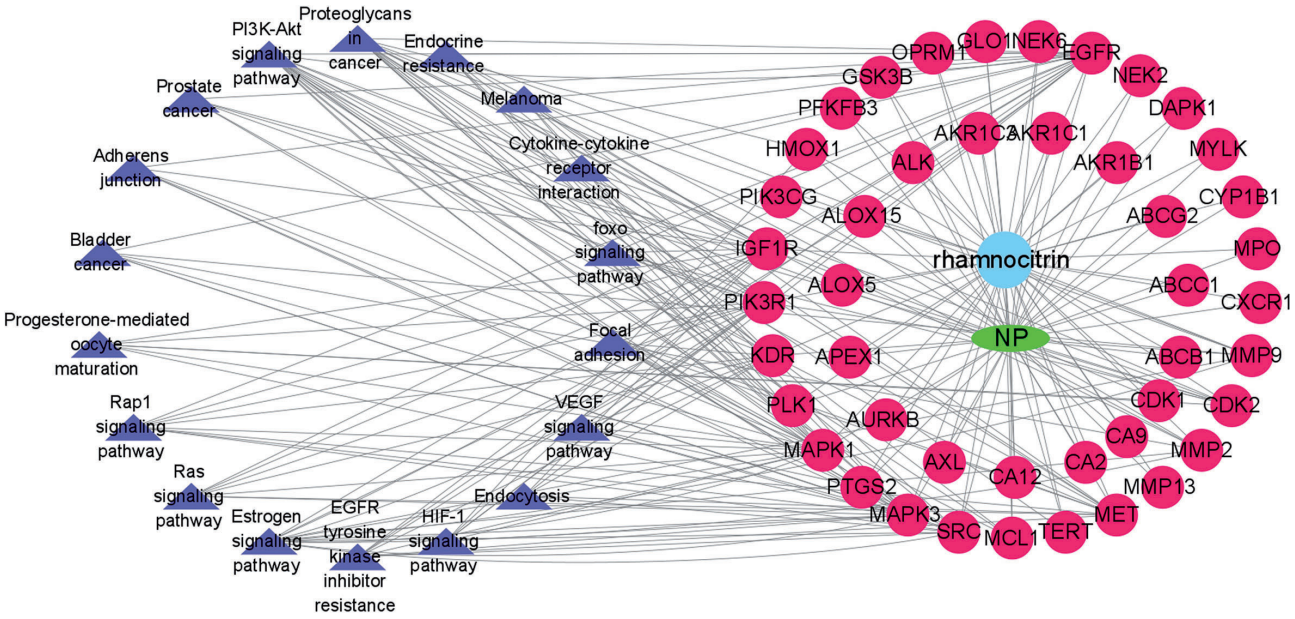

Figure 6. Compound-disease-pathway-target network analysis of rhamnocitrin in the prevention and treatment of oxaliplatin-induced neuropathic pain (NP). The red circles represent the genes; the remaining circular represents the RH; the green ellipse represents NP, and the blue inverted triangles represent KEGG pathways. (See online version for color figure.)

Table 3. Results of KEGG enrichment analysis

Term	Pathway	Enrichment	*p* value	Related genes
ko05205	proteoglycans in cancer	0.0493	5.26E−17	EGFR, IGF1R, KDR, MET, MMP2, MMP9, PIK3R1, MAPK1, MAPK3, SRC, HMOX1, PTGS2, TERT, CDK2, MCL1, ALOX5
hsa01521	EGFR tyrosine kinase inhibitor resistance	0.1013	3.26E−16	EGFR, IGF1R, KDR, MET, PIK3R1, MAPK1, MAPK3, SRC
ko04510	focal adhesion	0.0402	6.32E−13	EGFR, IGF1R, KDR, MET, PIK3R1, MAPK1, MAPK3, SRC
ko04151	PI3K-Akt signaling pathway	0.0263	7.72E−13	CDK2, EGFR, IGF1R, KDR, MCL1, MET, PIK3R1, MAPK1, MAPK3
ko04015	Rap1 signaling pathway	0.0381	9.75E−13	EGFR, IGF1R, KDR, MET, PIK3R1, MAPK1, MAPK3, SRC
hsa04151	PI3K-Akt signaling pathway	0.0237	1.99E−12	CDK2, EGFR, IGF1R, KDR, MCL1, MET, PIK3R1, MAPK1, MAPK3
hsa05218	melanoma	0.0870	6.53E−12	EGFR, IGF1R, MET, PIK3R1, MAPK1, MAPK3
ko04520	adherens junction	0.0833	8.5E−12	EGFR, IGF1R, MET, MAPK1, MAPK3, SRC
hsa04014	Ras signaling pathway	0.0270	3.59E−10	EGFR, IGF1R, KDR, MET, PIK3R1, MAPK1, MAPK3
hsa04144	endocytosis	0.0192	8.93E−07	EGFR, IGF1R, KDR, MET, SRC
ko04060	cytokine-cytokine receptor interaction	0.0111	0.00088	EGFR, KDR, MET
ko01522	endocrine resistance	0.0833	1.65E−15	EGFR, IGF1R, MMP2, MMP9, PIK3R1, MAPK1, MAPK3, SRC
ko05219	bladder cancer	0.1463	2.48E−13	EGFR, MMP2, MMP9, MAPK1, MAPK3, SRC
ko04915	estrogen signaling pathway	0.0714	3.71E−13	EGFR, MMP2, MMP9, PIK3R1, MAPK1, MAPK3, SRC
hsa04370	VEGF signaling pathway	0.1017	2.47E−12	KDR, PIK3R1, MAPK1, MAPK3, PTGS2, SRC
hsa04915	estrogen signaling pathway	0.0479	6.38E−12	EGFR, MMP2, MMP9, PIK3R1, MAPK1, MAPK3, SRC
hsa05215	prostate cancer	0.0690	2.73E−11	CDK2, EGFR, IGF1R, PIK3R1, MAPK1, MAPK3
ko04914	progesterone-mediated oocyte maturation	0.0625	4.99E−11	CDK1, CDK2, IGF1R, PIK3R1, MAPK1, MAPK3
hsa04066	HIF-1 signaling pathway	0.0504	1.85E−10	EGFR, HMOX1, IGF1R, PIK3R1, MAPK1, MAPK3
hsa04068	foxo signaling pathway	0.0438	4.35E−10	CDK2, EGFR, IGF1R, PIK3R1, MAPK1, MAPK3

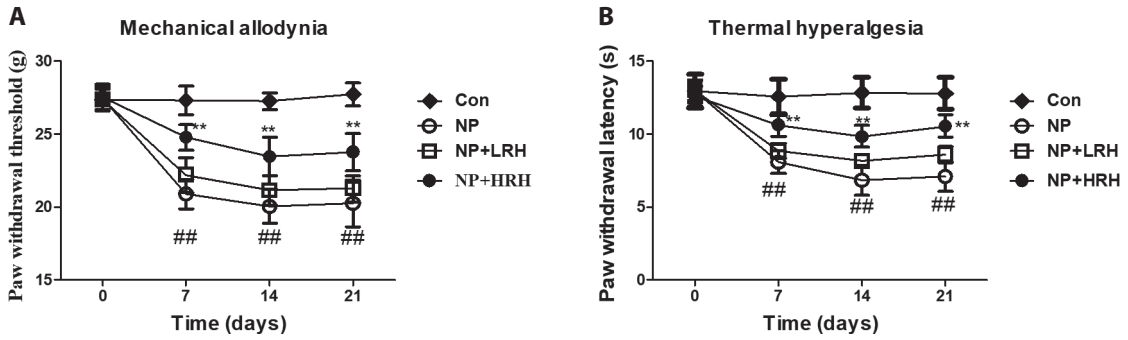

Figure 7. Effects of rhamnocitrin (RH) on behavioral alterations in oxaliplatin-induced neuropathic pain (NP). Prevention effects of oxaliplatin-induced mechanical allodynia (**A**) and thermal hyperalgesia (**B**) by injection of RH. Values were presented as mean ± SD (n = 8); $^{\#\#}p < 0.01$ *vs.* Con group; $^{**}p < 0.01$ *vs.* NP group (repeated measures ANOVA). Con, control group; NP, rats injected with oxaliplatin (2.5 mg/kg); NP+LRH, NP rats injected with RH (5 mg/kg); NP+HRH, NP rats injected with RH (10 mg/kg). For more informations, see Materials and Methods.

= 97.39, p = 0.0002] and MMP9 [F (1, 11) = 70.67, p < 0.0004] levels in DRG.

Discussion

Chemotherapy-induced NP is a commonly encountered disease after cancer chemotherapy, which is characterized by sensory peripheral neuropathy, including loss of sensation, allodynia, pain, autonomic neuropathy, and weakness (Hou et al. 2018). Although the exact etiology of NP remains poorly understood, multiple mechanisms are involved during the pathophysiology of chemotherapy-induced NP, such as oxidative stress, inflammation, potassium channels, sodium channels, and the activation of mitogen-activated protein kinase (MAPK) (Jaggi and Singh 2012). Besides, there was currently no effective treatment to control oxaliplatin-induced NP optimally.

Previous reports indicated that flavonoids could prevent the occurrence and development of chemotherapy-induced NP *via* suppressing apoptosis, oxidative stress, astrocyte and microglial activation, and inflammatory responses (Zhang

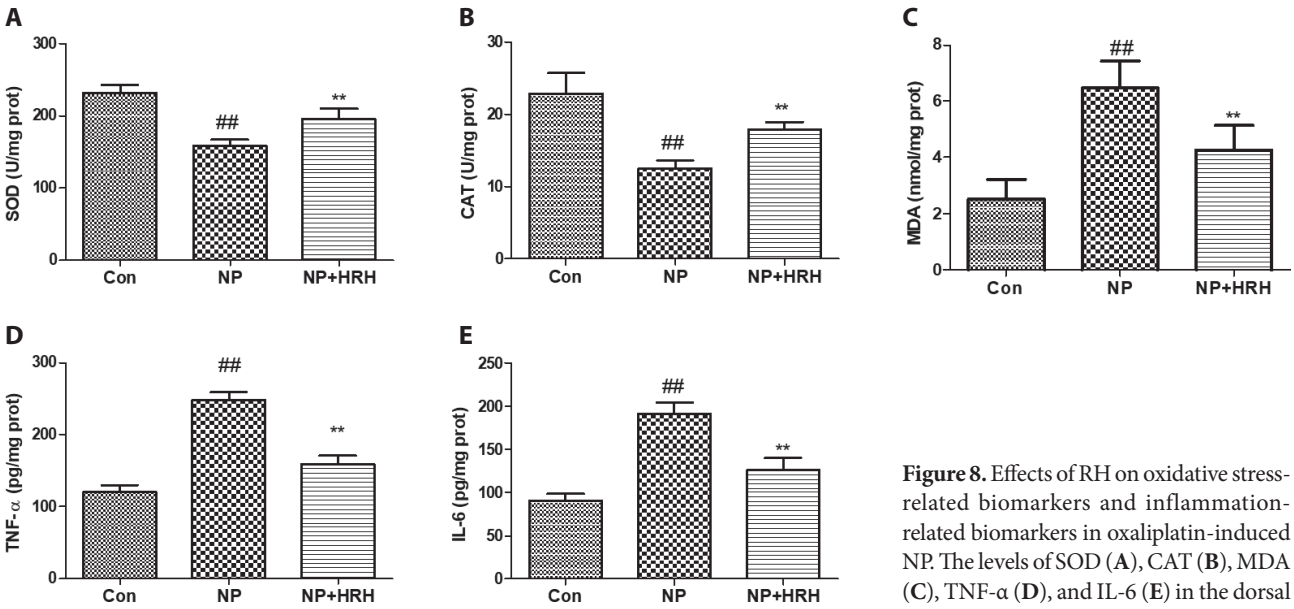

Figure 8. Effects of RH on oxidative stress-related biomarkers and inflammation-related biomarkers in oxaliplatin-induced NP. The levels of SOD (**A**), CAT (**B**), MDA (**C**), TNF-α (**D**), and IL-6 (**E**) in the dorsal root ganglion were measured by the corresponding kit. Values were presented as mean ± SD (n = 8); $^{\#\#}p < 0.01$ *vs.* Con group; $^{**}p < 0.01$ *vs.* NP group; significant difference measured *via* two-way ANOVA. For more abbreviations, see Figure 7.

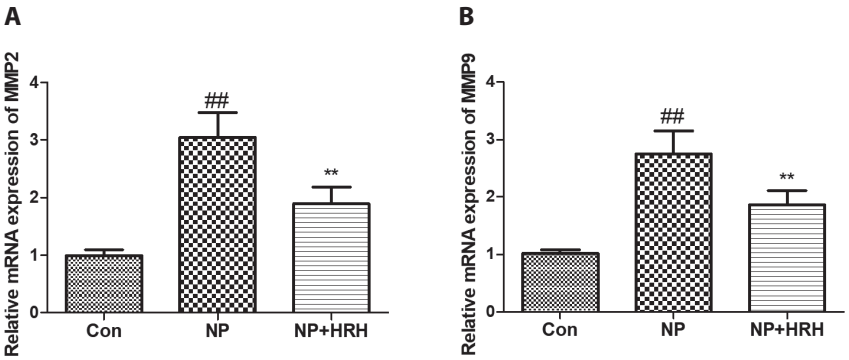

Figure 9. Effects of RH on MMP2 (**A**) and MMP9 (**B**) expressions in oxaliplatin-induced NP. Values were presented as mean ± SD ($n = 4$); $^{\#\#}p < 0.01$ *vs.* Con group; $^{**}p < 0.01$ *vs.* NP group; significant difference measured *via* two-way ANOVA. For more abbreviations, see Figure 7.

et al. 2019; Singh et al. 2020; Xie et al. 2020; Siddiqui et al. 2021). Therefore, flavonoids may be a novel analgesic drug in the prevention and treatment of chemotherapy-induced NP. RH is a monomethoxyflavone that is the 7-methyl ether derivative of kaempferol. To the best of our knowledge, there were no studies reported the therapeutic effects of RH against NP. However, kaempferol has been reported to show neuroprotective effects in the central nervous system and alleviate the progression of diabetic neuropathy (Kishore et al. 2018; Silva Dos Santos et al. 2020). Therefore, we speculated RH could also exert a beneficial effect in the treatment of oxaliplatin-induced NP.

Network pharmacology is a powerful new method to investigate the potential targets and underlying mechanism actions of traditional Chinese medicine. In the present study, we used the network pharmacology and bioinformatics approaches to identify the potential targets and signaling pathways of RH against oxaliplatin-induced NP.

Based on the results of a topological analysis, the top 7 key targets were screened as key targets, including MAPK3, MAPK1, SRC, PTGS2, EGFR, MMP9, and MMP2. MAPKs are a family of intracellular signaling molecules and play an important role in the regulation of inflammatory responses and neural plasticity. MAPK activation leads to the induction and maintenance of NP (Ji et al. 2009a). Therefore, specific MAPK inhibitors could target glial and neurons cells and maybe a novel therapy for pain management. The inflammation and NP were inhibited without deleterious consequences *via* targeting SRC (Liu et al. 2008). Dysregulation of Src-mediated enhancement of the NMDA receptor could cause pathological changes in the central nervous system (Salter and Pitcher 2012). Besides, a previous report showed that SRC contributed to orofacial ectopic pain following inferior alveolar nerve transection (Li et al. 2021). It has been reported that MMP9 could evoke NP *via* contributing to the cleavage of cytokines nerve damage (Kawasaki et al. 2008). MMP2 and MMP9 were the main molecules involved in the development of NP (Ji et al. 2009b). MMP9 and MMP2 inhibitors could alleviate mechanical allodynia in the dorsal root ganglion (Henry et al. 2015; Kwan et al. 2019). Consistent with these reports, our PPI result indicated that RH mainly targeted the multiple targets (MAPK3, MAPK1, SRC, PTGS2, EGFR, MMP9, and MMP2) in the treatment of oxaliplatin-induced NP. Moreover, our *in vivo* experiment further demonstrated that RH treatment significantly down-regulated the mRNA overexpression of MMP2 and MMP9 induced by oxaliplatin.

NP is a nervous system disease involving multiple signaling pathways. Based on the findings of GO and KEGG enrichment analyses, the following signaling pathways of RH involved in oxaliplatin-induced NP were screened for discussion: PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, and Rap1 signaling pathway. The activation of EGFR accelerates the progression in CCD-induced NP, and it is considered as a potential target for NP treatment (Wang et al. 2019). EGFR-inhibitors have been demonstrated to alleviate NP in clinical trials (Kersten et al. 2015). Previous studies revealed that kaempferol could inhibit the EGFR signaling pathway activation in human renal cell carcinoma (Song et al. 2014). Besides, kaempferol also may prevent the progression of pancreatic cancer *in vitro via* suppression of EGFR-related pathways (Lee and Kim 2016). These findings were in good agreement with the results of the KEGG analysis. In the present study, the KEGG result showed that RH mainly targeted the EGFR tyrosine kinase inhibitor resistance in oxaliplatin-induced NP treatment.

A PI3K-Akt signaling pathway is an important signaling pathway involved in the process of oxidative stress, apoptosis, and inflammation (Wen et al. 2018; Duan et al. 2019; Luo et al. 2019). Oxidative stress is also a vital pathogenic mechanism of chemotherapy-induced NP (Areti et al. 2014). Additionally, the NP status may be related to the inflammatory response that drives the development and persistence of NP (Ellis and Bennett 2013; Sommer et al. 2018). A recent study showed that both RH 3-O-β-isorhamninoside and kaempferol 3-O-β-isorhamninoside exerted the most potent suppression effects against superoxide anion (Bhouri et al.

2011). Besides, both RH and kaempferol could improve the ability of cell anti-oxidation defense *via* regulation of MAPK signaling pathway and HO-1 expression (Hong et al. 2009). Several physiological processes, including apoptotic cell death, cell differentiation, and growth, were regulated by the MAPK signaling pathway and oxidative stress was one of the major factors which caused pathogenesis *via* dysregulation of the MAPK signaling pathway (Rezatabar et al. 2019). Moreover, the MAPK signaling pathway activation was revealed to cause nociceptive responses in nerve damage state, and it may be proposed as a potential therapeutic target against NP (Ma and Quirion 2005). Eugenol, a natural phenolic compound, relieves NP accompanied oxidative stress and neuroinflammation *via* the down-regulation of MAPK signaling pathway in the damaged spinal cord (Ma et al. 2018). These reports offered the evidence to support our network pharmacology results that the main PI3K-Akt signaling pathway and targets (MAPK3 and MAPK1) affected by RH were mainly implicated in oxidative stress processes (cellular response to oxidative stress, response to oxidative stress, cellular response to chemical stress, response to reactive oxygen species, and cellular response to reactive oxygen species) in the treatment of oxaliplatin-induced NP. It has been demonstrated that overexpression of Rap1A induced inflammatory responses in chronic constriction injury rats and it may be a potential target in the treatment of NP (Fang et al. 2019; Gao et al. 2019). Consistent with these reports, our KEGG result indicated that RH mainly targeted the Rap1A signaling pathway in the treatment of oxaliplatin-induced NP. Overall, these findings indicated that RH exerted the therapeutic effect in oxaliplatin-induced NP *via* targeting the multiple signaling pathways to regulate apoptosis, oxidative stress, and neuroinflammation processes. Moreover, in our animal experimental model of NP, RH treatment improved the antioxidant enzymes activities (SOD and CAT), and inhibited the generation of pro-inflammatory cytokines (TNF-α and IL-6) in oxaliplatin-induced NP rats, further indicating that RH could inhibit oxaliplatin-induced oxidative stress and neuroinflammation. However, there are still several limitations in the present study. For example, more experiments will focus on the relationship between RH and safety on the animal experiment. Systematic animal toxicity experiments are necessary before this study could proceed to clinical trials.

Conclusions

In conclusion, the present report demonstrated the multi-targets and multi-pathways of RH in the therapeutical effects on oxaliplatin-induced NP. Based on network pharmacology and *in vivo* experimental results, we proposed that RH may be a potential drug that may be applied for drug development in the treatment of NP. However, further verification experiments were required to confirm the detailed mechanism actions of RH against NP.

Conflict of interest. The authors declare no conflict of interest, financial or otherwise.

References

André T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, de Gramont A (2004): Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N. Engl. J. Med. **350,** 2343-2351
https://doi.org/10.1056/NEJMoa032709

Areti A, Yerra V G, Naidu V, Kumar A (2014): Oxidative stress and nerve damage: role in chemotherapy induced peripheral neuropathy. Redox Biol. **2,** 289-295
https://doi.org/10.1016/j.redox.2014.01.006

Bhouri W, Sghaier MB, Kilani S, Bouhlel I, Dijoux-Franca MG, Ghedira K, Ghedira LC (2011): Evaluation of antioxidant and antigenotoxic activity of two flavonoids from Rhamnus alaternus L. (Rhamnaceae): kaempferol 3-O-β-isorhamninoside and rhamnocitrin 3-O-β-isorhamninoside. Food Chem. Toxicol. **49,** 1167-1173
https://doi.org/10.1016/j.fct.2011.02.011

Boyette-Davis JA, Walters ET, Dougherty PM (2015): Mechanisms involved in the development of chemotherapy-induced neuropathy. Pain Manag. **5,** 285-296
https://doi.org/10.2217/pmt.15.19

Carozzi VA, Canta A, Chiorazzi A (2015): Chemotherapy-induced peripheral neuropathy: What do we know about mechanisms? Neurosci. Lett. **596,** 90-107
https://doi.org/10.1016/j.neulet.2014.10.014

Di Cesare Mannelli L, Pacini A, Micheli L, Femia AP, Maresca M, Zanardelli M, Ghelardini C (2017): Astragali radix: could it be an adjuvant for oxaliplatin-induced neuropathy? Sci. Rep. **7,** 42021
https://doi.org/10.1038/srep42021

Cesare Mannelli L, Zanardelli M, Bartolucci G, Karioti A, Bilia AR, Vannacci A, Ghelardini C (2015): In vitro evidence for the use of Astragali Radix extracts as adjuvant against oxaliplatin-induced neurotoxicity. Planta Med. **81,** 1045-1055
https://doi.org/10.1055/s-0035-1546117

Duan M X, Zhou H, Wu QQ, Liu C, Xiao Y, Deng W, Tang QZ (2019): Andrographolide protects against HG-induced inflammation, apoptosis, migration, and impairment of angiogenesis via PI3K/AKT-eNOS signalling in HUVECs. Mediators Inflamm. **2019,** 6168340
https://doi.org/10.1155/2019/6168340

Ellis A, Bennett DL (2013): Neuroinflammation and the generation of neuropathic pain. Br. J. Anaesth. **111,** 26-37
https://doi.org/10.1093/bja/aet128

Fang B, Wei L, Dong K, Niu X, Sui X, Zhang H (2019): miR-202 modulates the progression of neuropathic pain through targeting RAP1A. J. Cell Biochem. **120,** 2973-2982
https://doi.org/10.1002/jcb.27025

Fu J, Wang Z, Huang L, Zheng S, Wang D, Chen S, Yang S (2014): Review of the botanical characteristics, phytochemistry, and pharmacology of Astragalus membranaceus (Huangqi). Phytother. Res. **28,** 1275-1283 https://doi.org/10.1002/ptr.5188

Gao L, Pu X, Huang Y, Huang J (2019): MicroRNA-340-5p relieved chronic constriction injury-induced neuropathic pain by targeting Rap1A in rat model. Genes Genomics **41,** 713-721 https://doi.org/10.1007/s13258-019-00802-0

Gill JS, Windebank AJ (1998): Cisplatin-induced apoptosis in rat dorsal root ganglion neurons is associated with attempted entry into the cell cycle. J. Clin. Invest. **101,** 2842-2850 https://doi.org/10.1172/JCI1130

Henry MA, Fairchild DD, Patil MJ, Hanania T, Hain HS, Davis SF, Sucholeiki I (2015): Effect of a novel, orally active matrix metalloproteinase-2 and -9 inhibitor in spinal and trigeminal rat models of neuropathic pain. J. Oral Facial Pain Headache **29,** 286-296 https://doi.org/10.11607/ofph.1350

Hong JT, Yen JH, Wang L, Lo YH, Chen ZT, Wu MJ (2009): Regulation of heme oxygenase-1 expression and MAPK pathways in response to kaempferol and rhamnocitrin in PC12 cells. Toxicol. Appl. Pharmacol. **237,** 59-68 https://doi.org/10.1016/j.taap.2009.02.014

Hou S, Huh B, Kim HK, Kim KH, Abdi S (2018): Treatment of chemotherapy-induced peripheral neuropathy: systematic review and recommendations. Pain Physician. **21,** 571-592 https://doi.org/10.36076/ppj.2018.6.571

Hu T, Liu QM, He XW, Huang F, Zhang MW, Jiang JG (2017): Identification of bioactives from Astragalus chinensis L.f. and their antioxidant, anti-inflammatory and anti-proliferative effects. J. Food Sci. Technol. **54,** 4315-4323 https://doi.org/10.1007/s13197-017-2902-3

Jaggi AS, Singh N (2012): Mechanisms in cancer-chemotherapeutic drugs-induced peripheral neuropathy. Toxicology **291,** 1-9 https://doi.org/10.1016/j.tox.2011.10.019

Ji RR, Gereau RW, Malcangio M, Strichartz GR (2009a): MAP kinase and pain. Brain Res. Rev. **60,** 135-148 https://doi.org/10.1016/j.brainresrev.2008.12.011

Ji RR, Xu ZZ, Wang X, Lo EH (2009b): Matrix metalloprotease regulation of neuropathic pain. Trends Pharmacol. Sci. **30,** 336-340 https://doi.org/10.1016/j.tips.2009.04.002

Kawasaki Y, Xu ZZ, Wang X, Park JY, Zhuang ZY, Tan PH, Ji RR (2008): Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. Nat. Med. **14,** 331-336 https://doi.org/10.1038/nm1723

Kersten C, Cameron MG, Laird B, Mjåland S (2015): Epidermal growth factor receptor-inhibition (EGFR-I) in the treatment of neuropathic pain. Br. J. Anaesth. **115,** 761-767 https://doi.org/10.1093/bja/aev326

Kishore L, Kaur N, Singh R (2018): Effect of Kaempferol isolated from seeds of Eruca sativa on changes of pain sensitivity in Streptozotocin-induced diabetic neuropathy. Inflammopharmacology **26,** 993-1003 https://doi.org/10.1007/s10787-017-0416-2

Kuner R, Flor H (2016): Structural plasticity and reorganisation in chronic pain. Nat. Rev. Neurosci. **18,** 20-30 https://doi.org/10.1038/nrn.2016.162

Kwan MY, Choo A, Hanania T, Ghavami A, Beltran J, Shea J, Sucholeiki I (2019): Biomarker analysis of orally dosed, dual active, matrix metalloproteinase (MMP)-2 and MMP-9 inhibitor, AQU-118, in the spinal nerve ligation (SNL) rat model of neuropathic pain. Int. J. Mol. Sci. **20,** 811 https://doi.org/10.3390/ijms20040811

Lee J, Kim JH (2016): Kaempferol inhibits pancreatic cancer cell growth and migration through the blockade of EGFR-related pathway in vitro. PLoS One **11,** e0155264 https://doi.org/10.1371/journal.pone.0155264

Li YL, Liu F, Zhang YY, Lin J, Huang CL, Fu M, Shen JF (2021): NMDAR1-Src-Pannexin1 signal pathway in the trigeminal ganglion contributed to orofacial ectopic pain following inferior alveolar nerve transection. Neuroscience **466,** 77-86 https://doi.org/10.1016/j.neuroscience.2021.04.032

Liu XJ, Gingrich JR, Vargas-Caballero M, Dong YN, Sengar A, Beggs S, Salter MW (2008): Treatment of inflammatory and neuropathic pain by uncoupling Src from the NMDA receptor complex. Nat. Med. **14,** 1325-1332 https://doi.org/10.1038/nm.1883

Luo X, Lin B, Gao Y, Lei X, Wang X, Li Y, Li T (2019): Genipin attenuates mitochondrial-dependent apoptosis, endoplasmic reticulum stress, and inflammation via the PI3K/AKT pathway in acute lung injury. Int. Immunopharmacol. **76,** 105842 https://doi.org/10.1016/j.intimp.2019.105842

Ma L, Mu Y, Zhang Z, Sun Q (2018): Eugenol promotes functional recovery and alleviates inflammation, oxidative stress, and neural apoptosis in a rat model of spinal cord injury. Restor. Neurol. Neurosci. **36,** 659-668 https://doi.org/10.3233/RNN-180826

Ma W, Quirion R (2005): The ERK/MAPK pathway, as a target for the treatment of neuropathic pain. Expert Opin. Ther. Targets **9,** 699-713 https://doi.org/10.1517/14728222.9.4.699

Ni W, Zheng X, Hu L, Kong C, Xu Q (2021): Preventing oxaliplatin-induced neuropathic pain: Using berberine to inhibit the activation of NF-κB and release of pro-inflammatory cytokines in dorsal root ganglions in rats. Exp. Ther. Med. **21,** 135 https://doi.org/10.3892/etm.2020.9567

Peirs C, Seal RP (2016): Neural circuits for pain: Recent advances and current views. Science **354,** 578-584 https://doi.org/10.1126/science.aaf8933

Pike CT, Birnbaum HG, Muehlenbein CE, Pohl GM, Natale RB (2012): Healthcare costs and workloss burden of patients with chemotherapy-associated peripheral neuropathy in breast, ovarian, head and neck, and nonsmall cell lung cancer. Chemother. Res. Pract. **2012,** 913848 https://doi.org/10.1155/2012/913848

Rezatabar S, Karimian A, Rameshknia V, Parsian H, Majidinia M, Kopi TA, Yousefi B (2019): RAS/MAPK signaling functions in oxidative stress, DNA damage response and cancer progression. J. Cell Physiol. **234,** 14951-14965 https://doi.org/10.1002/jcp.28334

Saleem S, Shaharyar MA, Khusroo MJ, Ahmad, P, Rahman, RU, Ahmad K, Imam F (2013): Anticancer potential of rhamnocitrin 4'-β-D-galactopyranoside against N-diethylnitrosamine-

induced hepatocellular carcinoma in rats. Mol. Cell Biochem. **384,** 147-153
https://doi.org/10.1007/s11010-013-1792-6

Salter MW, Pitcher GM (2012): Dysregulated Src upregulation of NMDA receptor activity: a common link in chronic pain and schizophrenia. FEBS J. **279,** 2-11
https://doi.org/10.1111/j.1742-4658.2011.08390.x

Siddiqui M, Abdellatif B, Zhai K, Liskova A, Kubatka P, Büsselberg D (2021): Flavonoids alleviate peripheral neuropathy induced by anticancer drugs. Cancers (Basel) **13,** 1576
https://doi.org/10.3390/cancers13071576

Silva Dos Santos J, Gonçalves Cirino JP, de Oliveira Carvalho P, Ortega MM (2020): The pharmacological action of kaempferol in central nervous system diseases: A Review. Front. Pharmacol. **11,** 565700
https://doi.org/10.3389/fphar.2020.565700

Singh P, Bansal S, Kuhad A, Kumar A, Chopra K (2020): Naringenin ameliorates diabetic neuropathic pain by modulation of oxidative-nitrosative stress, cytokines and MMP-9 levels. Food Funct. **11,** 4548-4560
https://doi.org/10.1039/C9FO00881K

Sommer C, Leinders M, Üçeyler N (2018): Inflammation in the pathophysiology of neuropathic pain. Pain **159,** 595-602
https://doi.org/10.1097/j.pain.0000000000001122

Song W, Dang Q, Xu D, Chen Y, Zhu G, Wu K, Li L (2014): Kaempferol induces cell cycle arrest and apoptosis in renal cell carcinoma through EGFR/p38 signaling. Oncol. Rep. **31,** 1350-1356
https://doi.org/10.3892/or.2014.2965

Staff NP, Cavaletti G, Islam B, Lustberg M, Psimaras D, Tamburin S (2019): Platinum-induced peripheral neurotoxicity: From pathogenesis to treatment. J. Peripher. Nerv. Syst. **24** (Suppl 2), S26-S39
https://doi.org/10.1111/jns.12335

Starobova H, Vetter I (2017): Pathophysiology of chemotherapy-induced peripheral neuropathy. Front. Mol. Neurosci. **10,** 174
https://doi.org/10.3389/fnmol.2017.00174

Tsuda M (2019): Microglia-mediated regulation of neuropathic pain: molecular and cellular mechanisms. Biol. Pharm. Bull. **42,** 1959-1968
https://doi.org/10.1248/bpb.b19-00715

Tu YC, Lian TW, Yen JH, Chen ZT, Wu MJ (2007): Antiatherogenic effects of kaempferol and rhamnocitrin. J. Agric. Food Chem. **55,** 9969-9976
https://doi.org/10.1021/jf0717788

Wang S, Liu S, Xu L, Zhu X, Liu W, Tian L, Huo FQ (2019): The upregulation of EGFR in the dorsal root ganglion contributes to chronic compression of dorsal root ganglions-induced neuropathic pain in rats. Mol. Pain **15,** 1744806919857297
https://doi.org/10.1177/1744806919857297

Wen Z, Hou W, Wu W, Zhao Y, Dong X, Bai X, Song L (2018): 6'-O-Galloylpaeoniflorin attenuates cerebral ischemia reperfusion-induced neuroinflammation and oxidative stress via PI3K/Akt/Nrf2 activation. Oxid. Med. Cell Longev. **2018,** 8678267
https://doi.org/10.1155/2018/8678267

Xie H, Chen Y, Du K, Wu W, Feng X (2020): Puerarin alleviates vincristine-induced neuropathic pain and neuroinflammation via inhibition of nuclear factor-κB and activation of the TGF-β/Smad pathway in rats. Int. Immunopharmacol. **89,** 107060
https://doi.org/10.1016/j.intimp.2020.107060

Xie H, Chen Y, Wu W, Feng X, Du K (2021): Gastrodia elata blume polysaccharides attenuate vincristine-evoked neuropathic pain through the inhibition of neuroinflammation. Mediators Inflamm. **2021,** 9965081
https://doi.org/10.1155/2021/9965081

Yang L, Han X, Xing F, Wu H, Shi H, Huang F, Wu X (2021): Total flavonoids of astragalus attenuates experimental autoimmune encephalomyelitis by suppressing the activation and inflammatory responses of microglia via JNK/AKT/NFκB signaling pathway. Phytomedicine **80,** 153385.
https://doi.org/10.1016/j.phymed.2020.153385

Zhang G, Liu N, Zhu C, Ma L, Yang J, Du J, Yu J (2019): Antinociceptive effect of isoorientin against neuropathic pain induced by the chronic constriction injury of the sciatic nerve in mice. Int. Immunopharmacol. **75,** 105753
https://doi.org/10.1016/j.intimp.2019.105753

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