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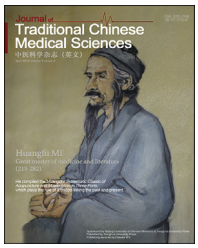

Network analysis of primary active compounds in Danqi analogous formulas for treating cardiovascular disease

Shichao Zheng, Yanling Zhang*, Yanjiang Qiao*

Research Center of Traditional Chinese Medicine Information Engineering, School of Chinese Pharmacy, Beijing University of Chinese Medicine, Beijing 100102, China

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Abstract *Objective:* Used extensively to treat cardiovascular disease, Danqi analogous formulas (DQAF) include prescriptions for Danqi (DQ), Fufang Danshen (FFDS) and Qishen Yiqi (QSYQ). Differences in prescription compatibility result in varying emphases of DQAF in clinical application.

Methods and results: Based on network analysis in this study, common and distinct mechanisms of DQAF actions on cardiovascular disease were analyzed at a systemic level. Components–targets–pathways models were developed by Cytoscape (http://www.cytoscape.org/); whereby, target information for active compounds was obtained based on the PharmMapper database (http://59.78.96.61/pharmmapper/), which was further used to search pathways using the Kyoto Encyclopedia of Genes and Genomes database (http://www.genome.jp/kegg/). Based on target and network analyses, we discovered RBP4 is a potential common target of DQAF, while mitogen-activated protein kinase 1 (MAPK1) and glutathione S-transferase P were potential targets of FFDS and QSYQ, respectively. Furthermore, the potential of DQAF to treat cardiovascular disease occurs through effects on the endocrine, immune, and digestive systems, in addition to lipid, sugar and amino acid metabolic pathways. Whereas FFDS exhibits effects on Toll-like receptor, transforming growth factor beta and MAPK signaling pathways; QSYQ exerts effects on cyclic adenosine monophosphate signaling, as well as metabolism of glutathione and arachidonic acid.

* Corresponding authors. Fax: +86 10 84738661.
E-mail addresses: collean_zhang@163.com (Y. Zhang), yjqiao@263.net (Y. Qiao).
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Conclusion: This study not only reflects the formulas-effect modality of multiple compounds, targets and pathways, but also provides clues to better understand physiological mechanisms of DQAF.

Introduction

Traditional Chinese Medicine (TCM) has been recognized as a typical representative of complementary and alternative medicine.[1] Using prescriptions called "formulas", clinical applications of TCM advocate combinatory therapeutic strategies. In contrast to modern pharmacology, which often focuses upon a single chemical entity, TCM formulas can affect multiple therapeutic targets to produce a synergistic effect resulting from multiple ingredients.[2,3] In TCM theory, analogous formulas (AF) refer to series of similar prescriptions based on common herb pairs. Elucidating mechanisms of AF is significant to clarify principles for rational use, with significant emphasis on applicable indications and prevention of misuse. Network analysis has provided methodologies and opportunities to reveal mechanisms of action for TCM formulas based on complex biological systems present in the human body.[4]

Danqi analogous formulas (DQAF), a series of prescriptions primarily derived from the herb pair *Salvia miltiorrhiza* and *Panax notoginseng*, include prescriptions for Danqi (DQ), Fufang Danshen (FFDS) and Qishen Yiqi (QSYQ). The basic formula of DQAF is DQ, which consists of *S. miltiorrhiza* and *P. notoginseng*. Different emphases of DQAF in clinical applications arise from their varied composition, as shown in Table 1. DQAF are commonly prescribed to treat cardiovascular disease, the leading health problem worldwide.[5] However, many previous studies have only investigated the effects of a single DQAF composition. For example, FFDS was found to protect myocardial ischemia and reperfusion injury though the Akt-eNOS signaling pathway[6]; whereas, QSYQ was found to inhibit platelet aggregation.[7] Most of these studies have elucidated one or several pharmacological effects of a specific formula, providing the foundation for further study of common and distinct mechanisms of DQAF.

In this study, a network analysis approach was employed to analyze active mechanisms of DQAF. Based on PharmMapper (http://59.78.96.61/pharmmapper/) and Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) databases, targets and pathways information for active compounds was obtained. Subsequently, components–targets–pathways network models of DQAF were constructed by Cytoscape (http://www.cytoscape.org/).[8] Potential targets and pathways that were common and distinct to DQAF were then evaluated using network model analysis. Our study aimed to provide new clues to better understand mechanisms of DQAF actions, in a concerted effort to instruct rational application of antibiotics and reflect the formulas-effect modality of multiple compounds, targets and pathways.

Table 1 DQAF composition.

Formula	Composition
DQ	*Salvia miltiorrhiza*, *Panax notoginseng*
FFDS	*Salvia miltiorrhiza*, *Panax notoginseng*, *borneolum*
QSYQ	*Salvia miltiorrhiza*, *Panax notoginseng*, *Dalbergia odorifera*, *Astragalus membranaceus*

Note: DQ stands for Danqi formula, FFDS stands for Fufang Danshen formula, QSYQ stands for Qishen Yiqi formula.

Methods and experimental section

Target-mining of DQAF's main active components

As the compositions of TCM formulas are complex, it is impossible to study all of the components and reliably separate results. Based on literature retrieval and according to principles defining the main ingredient for efficacy as having a high content and entering the blood, the main active components of DQAF were selected to clarify common and distinctive mechanisms.

Information for targets of the main active components of DQAF was extracted from PharmMapper,[9] an updated platform for potential target identification integrating pharmacophore with statistical methods. PharmMapper automatically identifies the best mapping poses of query molecules against all pharmacophore models in its PharmTargetDB, a pharmacophore database annotated from all the target information in BindingDB, TargetBank, DrugBank and potential drug target databases, including over 7000 receptor-based pharmacophore models.

Analysis of the compounds–targets–pathways network of DQAF

The pathway annotation of targets was performed based on the KEGG database, developed to facilitate understanding of high-level functions and utilities of biological systems from molecular-level information.[10]

Information regarding compounds, targets and pathways was collected to construct a compounds–targets–pathways network model using Cytoscape, a standard tool for visualization and integrated analysis of biological networks. In graphical networks, nodes represent compounds, protein targets and pathways; whereas, edges encode compound–target or target–pathway interactions. Based on such network analysis, common and distinct pathways of DQAF and the formulas-effect modality of multiple compounds, targets and pathways may be evaluated.

Table 2 Common and distinct active components of each DQAF.

Formulas	Common active components	Distinguished active components
DQ	Tanshinone IIA, cryptotanshinone, salianolic acid A, salvianolic acid B, tanshinol, protocatechuic aldehyde, dencichine, ginsenoside Rb1, ginsenoside Rg1, notoginsenoside R1[11–19]	None
FFDS		Borneol, isoborneol[20,21]
QSYQ		Butein, formononetin, isoliquiritigenin, nerolidol, calycosin, astragaloside and astragaloside[22–27]

Note: DQ stands for Danqi formula, FFDS stands for Fufang Danshen formula, QSYQ stands for Qishen Yiqi formula.

Table 3 Potential targets of the main active components of DQAF.

UniProt	Targets	Frequency	UniProt	Targets	Frequency	UniProt	Targets	Frequency
Danqi prescription								
P02753	RBP4	4	P10276	RARA	1	Q06520	SULT2A1	1
P01112	HRAS	4	P08581	MET[a]	1	P50225	SULT1A1	1
P09211	GSTP1[a]	4	P36873	PPP1CC[a]	1	P04035	HMGCR[a]	1
Q16539	MAPK14	3	P08254	MMP3	1	P49841	GSK3B	1
Q6P3U7	RXRA[a]	3	A4QPA9	MAP2K1[a]	1	Q03518	TAP1	1
P18031	PTPN1[a]	3	P00918	CA2	1	P54278	PMS2	1
P28845	HSD11B1[a]	3	Q92731	ESR2	1	O15540	FABP7	1
P35398	RORA[a]	2	Q86UC5	RARB	1	P14555	PLA2G2A	1
P49888	SULT1E1	2	P00734	F2	1	P42574	CASP3	1
P15121	AKR1B1	2	Q2VPJ6	HSP90AA1	1	P10275	AR[a]	1
P35968	KDR[a]	2	P05230	FGF1	1	P06730	EIF4E[a]	1
O00204	SULT2B1	2	P13716	ALAD	1	P29218	IMPA1	1
P53355	DAPK1	2	Q06187	BTK[a]	1	Q3KQS6	MME[a]	1
P12931	SRC	2	P11413	G6PD	1	P11309	PIM1	1
P27338	MAOB[a]	2	Q6FGU2	DTYMK	1	Q86WY9	INSR	1
Fufang Danshen prescription								
P28482	MAPK1	3	P10275	AR[a]	2	P28845	HSD11B1[a]	2
P35398	RORA[a]	2	A4QPA9	MAP2K1[a]	2	P27487	DPP4	1
Qishen Yiqi prescription								
P09211	GSTP1[a]	9	Q04609	FOLH1	2	P06730	EIF4E[a]	1
P18031	PTPN1[a]	4	Q6IRT1	ADH5	1	P27338	MAOB[a]	1
Q3KQS6	MME[a]	4	Q06187	BTK[a]	1	P14061	HSD17B1	1
Q6P3U7	RXRA[a]	3	P10827	THRA	1	P11712	CYP2C9	1
P36873	PPP1CC[a]	3	P0CG30	GSTT2B	1	P34896	SHMT1	1
P10275	AR[a]	3	P06744	GPI	1	P08581	MET[a]	1
O60760	HPGDS	3	P23368	ME2	1	P04035	HMGCR[a]	1
P08246	ELANE	2	P02679	FGG	1	P35968	KDR[a]	1

[a] The targets of analogous formulas are the same as the targets of DQ.

Table 4 Information of potential targets from main active components of DQAFs.

Related pathways	Number of pathways	Related pathways	Number of pathways
Human diseases	49	Nervous system	7
Signaling pathway	19	Amino acid metabolism	7
Endocrine system	15	Digestive system	5
Cellular processes	11	Genetic information processing	5
Immune system	10	Xenobiotics biodegradation	2
Carbohydrate metabolism	8	Sensory system	1
Lipid metabolism	7	Circulatory system	2

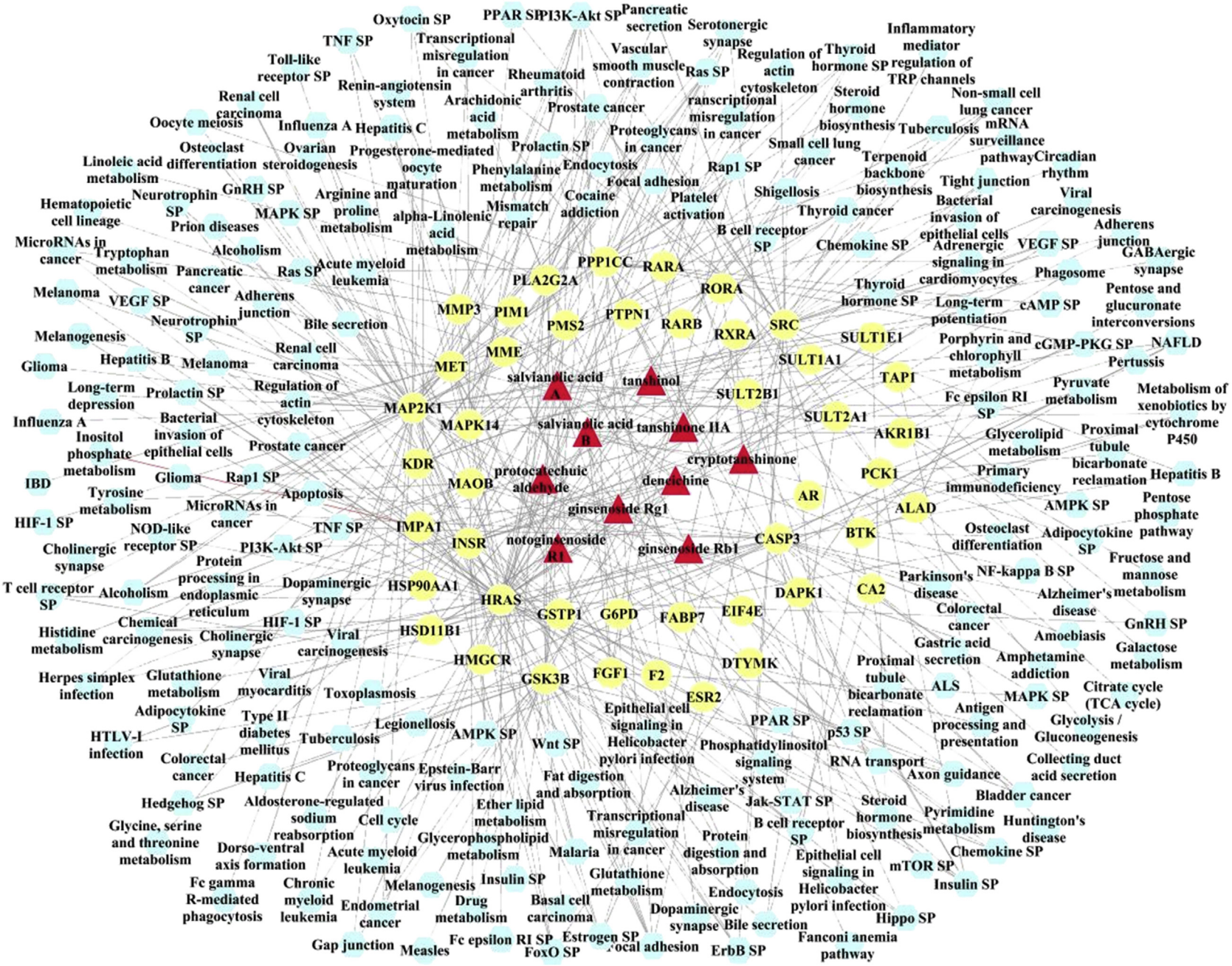

Figure 1 Components—targets—pathways network model of major active compounds of DQ. Note: Triangles represent active ingredients. Circles represent target proteins. Hexagons represent pathways. SP: signaling pathway.

Results and discussion

Analysis of targets for active components

Through literature retrieval, primary common and distinct active components of each DQAF were obtained, as shown in Table 2.

Potential targets of the main active components of each DQAF are shown in Table 3. In addition, 10 DQ compounds interacted with 45 targets, two distinct FFDS compounds interacted with six targets, and 7 distinct QSYQ compounds interacted with 24 targets.

The results of our target prediction suggested that primary targets of DQ are retinol binding protein 4 (RBP4), GTPase HRas (HRAS), glutathione S-transferase P (GSTP1), whose frequencies are high. These targets can simultaneously interact with multiple components to elicit a synergistic effect and enhance efficacy; thus, the higher frequency of the target, the more important it is to some extent. Among such targets is RBP4, an adipokine secreted primarily by liver and adipose tissue.[28] Li et al, demonstrated RBP4 is a novel risk factor for cardiovascular disease in patients with hyperinsulinemia and coronary artery disease.[29] Meanwhile, a study by Lambadiari et al, related serum RBP4 levels to the presence and severity of coronary artery disease.[30] This indicates RBP4 may be a potential target of DQ and other DQAF, as DQ is the basis for all DQAF.

Primary potential targets of other DQAF had different emphases. Mitogen-activated protein kinase 1 (MAPK1), also called ERK2,[31] was the primary target of FFDS; whereas, the primary target of QSYQ was glutathione S-transferase P (GSTP1). MAPK1 is a reported mediator of

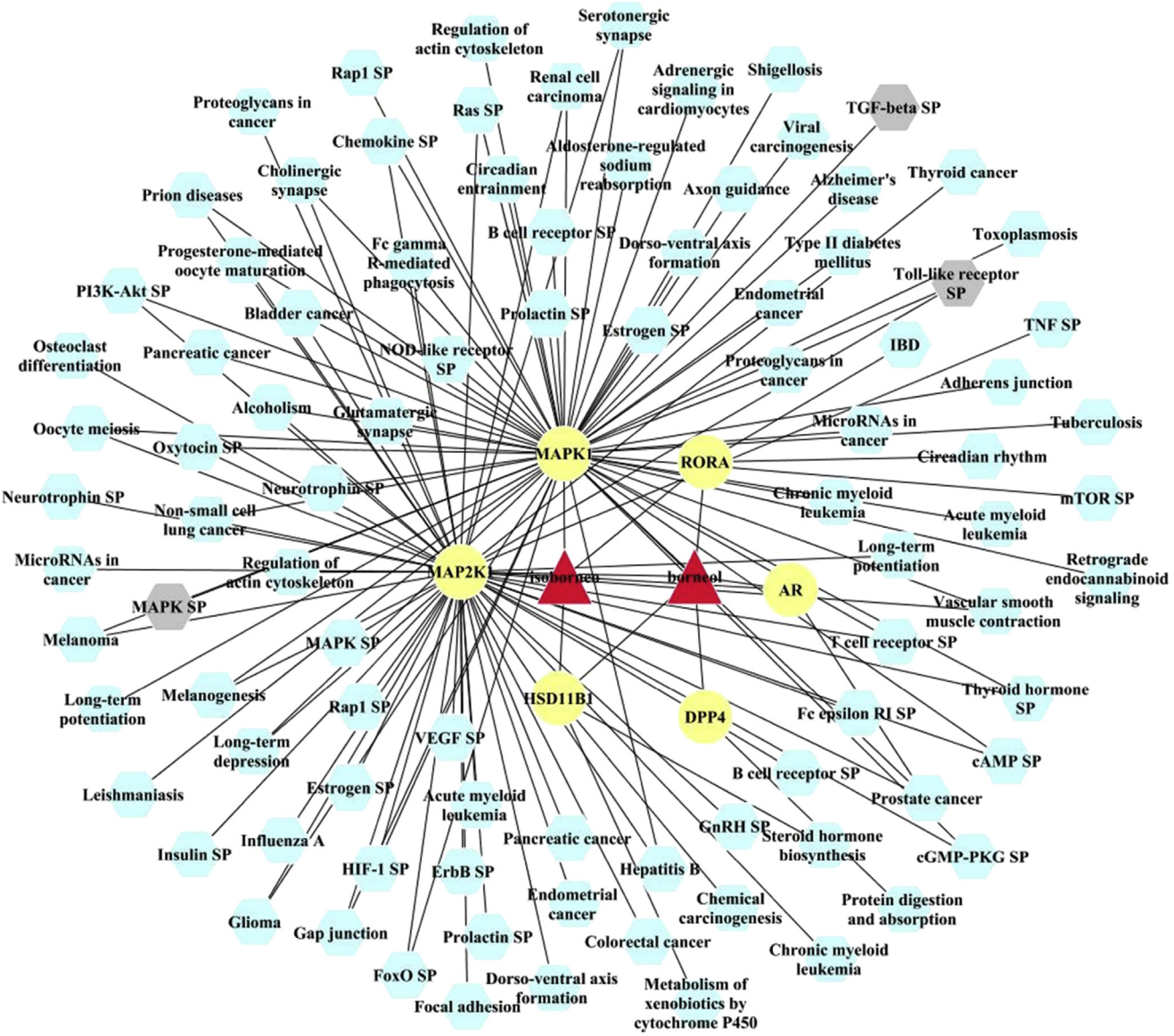

Figure 2 Components—targets—pathways network model of major active compounds of FFDS. Note: Triangles represent active ingredients. Circles represent target proteins. Hexagons represent pathways, with gray hexagons representing pathways for which FFDS has a greater effect. SP: signaling pathway.

estrogen action, which plays an important role in protecting pre-menopausal women from cardiovascular disease.[32] In addition, thrombin exhibits important effects on endothelial dysfunction in cardiovascular disease by activating MAPK1.[33] Therefore, MAPK1 plays a significant role in cardiovascular disease and the effect of FFDS may be related. GSTP1 is a member of the glutathione S-transferase (GST) family, which participates in metabolic detoxification of various environmental carcinogens. Specific genetic polymorphisms within GSTs have been reported to influence individual susceptibility to various pathologies, including cardiovascular disease.[34] As such, benefits of QSYQ for the treatment of cardiovascular disease may relate to mediation of GSTP1 polymorphisms.

Analysis of the compounds—targets—pathways network of DQAF

Based on KEGG pathway mapping, annotation of DQ targets included 148 related pathways, as shown in Table 4. The related compounds—targets—pathways network model constructed by Cytoscape is shown in Fig. 1.

DQ and other DQAF treat cardiovascular disease by mediating different pathways involved in various systems including the endocrine, immune, and digestive systems, as well as lipid, sugar and amino acid metabolic pathways. This network showed a clear three-level structure of the action modes of primary DQAF compounds, in which multiple compounds regulated multiple pathways by

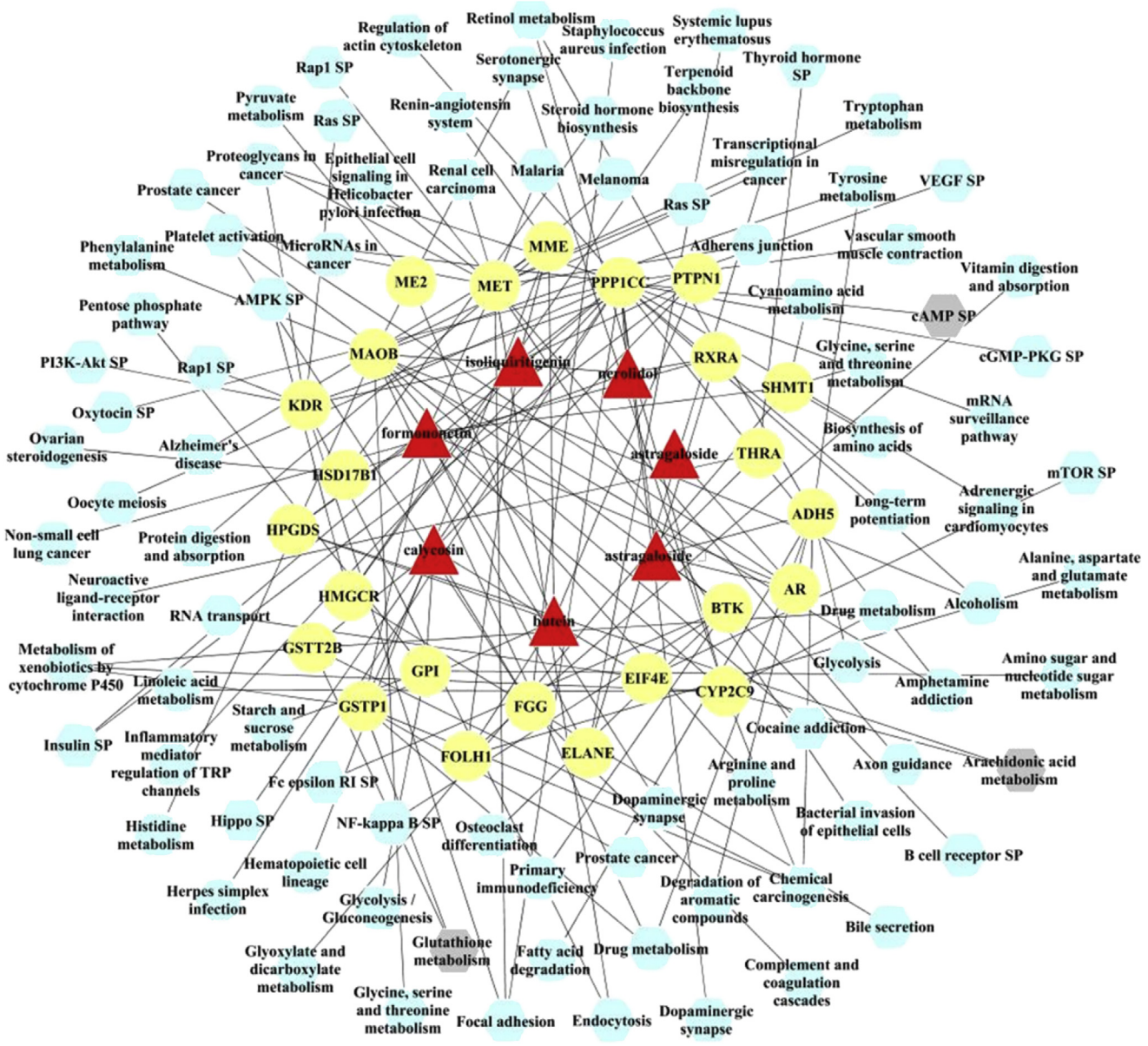

Figure 3 Components—targets—pathways network model of major active compounds of QSYQ. Note: Triangles represent active ingredients. Circles represent target proteins. Hexagons represent pathways, with gray hexagons representing pathways for which QSYQ has greater effects. SP: signaling pathway.

modulating groups of targets (and their associated pathways), which indicated their potential effects for treating cardiovascular disease.

As shown in Fig. 2, FFDS had a greater effect on Toll-like receptor, TGF-β and MAPK signaling pathways, which were not included in the network of QSYQ. Toll-like receptors, including TLR1, TLR2, TLR3, and TLR4, are a class of proteins that play a significant role in the innate immune system. In particular, TLR4 has been shown to mediate maladaptive left ventricular remodeling and damage of cardiac function after myocardial infarction.[35] Toll-like receptor signaling is also reported to be a key modulator of cardiac cell survival and ischemic injury.[36] TGF-β signaling has also been associated with cardiovascular disease, whereby complex regulation of TGF-β translates to a critical role in cardiovascular physiology, as TGF-β1 signaling appears to be deregulated in associated disorders such as atherosclerotic vascular disease.[37] Moreover, the MAPK signaling pathway has been reported to play a significant role in treating cardiovascular disease.[38,39] This indicates FFDS may exert its primary effects on cardiovascular disease through mediation of Toll-like receptor, TGF-β and MAPK signaling pathways.

QSYQ had greater effects on cyclic adenosine monophosphate (cAMP) signaling, and metabolism of glutathione and arachidonic acid (Fig. 3); glutathione and arachidonic acid metabolism were not included in FFDS. cAMP signaling pathways have been shown to exert inhibitory effects on pro-inflammatory cytokines, such as IL-6, which are strongly associated with coronary heart disease.[40] A previous report suggested antiplatelet-aggregation effects of QSYQ were related to improvement of cAMP metabolism.[41] Hence, this feature proved the accuracy of our network model to some extent. Glutathione and arachidonic acid metabolism have also been demonstrated to play critical roles in cardiovascular disease.[42,43] Therefore, it is suggested the primary effects of QSYQ for treating cardiovascular disease are associated with the cAMP signaling pathway and metabolism of glutathione and/or arachidonic acid.

Conclusions

In summary, RBP4 is potentially a common target of DQAF; whereas, MAPK1 is a potential target of FFDS and GSTP1 is a potential target of QSYQ. As such, DQAF treat cardiovascular disease through effects on endocrine, immune, and digestive systems, as well as lipid, sugar and amino acid metabolic pathways. While FFDS exhibited effects on Toll-like receptors, TGF-β and MAPK signaling pathways, QSYQ exerted effects on cAMP signaling and metabolism of glutathione and arachidonic acid. DQAF not only had common targets and pathways, but also displayed a specific emphasis to treat cardiovascular diseases by modulating groups of targets and a number of associated pathways. This reflected the formulas-effect modality of multiples compounds, targets and pathways. Although further "wet" experiments are needed to validate these conclusions, our results may provide clues to a faster and better understanding of DQAF mechanisms of action than what currently exists. Taking into consideration the complexity of TCM analogous formulas, our model also provides guidance for further exploration.

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