New perspective: the multi-targets mechanism of hydroxychloroquine in the treatment of rheumatoid arthritis based on network pharmacology

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Abstract

Background

Network pharmacology is a new method of bioinformatics in exploring drug targets in recent 3 years. Hydroxychloroquine (HCQ) is a multi-targets drug that are clinically effective in rheumatoid arthritis (RA) but whose mechanism is not well understood.

Methods

The predicted targets of HCQ and the proteins related to RA were returned from databases. Followed by protein-protein interaction (PPI) network, the intersection of the two group of proteins was conducted. Furthermore, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment was used to analyse these proteins in a macro perspective. Finally, the candidate targets were verified by molecular docking.

Results

The results suggest that the efficacy of HCQ against RA is mainly associated with 4 targets of smoothened homolog (SMO), sphingosine kinase (SPHK) 1, SPHK2 and gatty-acid amide hydrolase (FAAH), with their related 3316 proteins' network which regulate ErbB, HIF-1, NF-κB, FoxO, Chemokine, MAPK, PI3K/Akt pathways and so forth. Biological process are mainly concentrated in the regulation of cell activation, myeloid leukocyte activation, regulated exocytosis and so forth. Molecular docking analysis shows that hydrogen bonding and π-π stacking are the main forms of chemical force.

Conclusions

Our research provides protein targets affected by HCQ in the treatment of RA. SMO, SPHK1, SPHK2 and FAAH involving 3316 proteins become the multi-targets mechanism of HCQ in the treatment of RA. As well, the research also provides a new idea for introducing network pharmacology into the evaluation of the multi-target drugs in internal medicine.

1. Background

Rheumatoid arthritis (RA) is defined as one of the chronic autoimmune diseases, characterized by destruction of joints and connective tissues with associated metabolic, vascular, bone and psychological comorbidities [1–2]. The dysregulated innate and adaptive immunity characterized by immune responses against autoantigens, disordered cytokine secretion, osteoclast and chondrocyte activation mediated by immune complex-complement pathway [2–3]. About 0.5-1.0% of adults affected by RA in developed countries while the ratio was around 0.4% in South East Asia and Eastern Mediterranean region. The

prevalence of RA is higher in female than that in male and the ration also rises with age. The quality of life is severely affected by persistent and progressive joint inflammation and damage which could result in disability finally [4].

Four categories of drugs are commonly used in the treatment of RA: □ glucocorticoid; □non-steroidal anti-inflammatory drugs (NSAIDs); □ disease-modifing anti-rheumatic drugs (DMARDs); □ biologics [5]. Different combinations of NSAIDs and glucocorticoids are mostly used to mitigate the pain and inflammatory effects. In addition, DMARDs such as hydroxychloroquine (HCQ), methotrexate, sulfasalazine and leflunomide are also widely used to protect joints by slowing down the inflammatory arthritis. In recent years, biologics that aim to relieve inflammation through depleting B lymphocytes or inhibiting inflammatory mediators such as interleukin 6 or tumor necrosis factor α pathways. It has been demonstrated that the early intervention of DMARDs and the availability of timely medications could greatly improve the prognosis in a large proportion of RA patients [6]. When combined with other DMARDs, HCQ could provide moderate clinical benefit to patients in terms of the control in RA activity [7]. Recently, HCQ has been revealed to benefit the metabolic profile and to a lesser extent cardiovascular events in RA patients [8].

However, the mechanism of HCQ in the treatment of RA is still unknown. With the rapid progress of Bioinformatics, Systematic Biology and Polypharmacology, network-based drug discovery and evaluation is considered a promising approach toward more cost-effective drug development. Network pharmacology introduces a paradigm shift from the current “one research-based target, one drug” strategy to a novel version of the “network multi-targets” strategy [9–12].

In our research, we evaluated potential targets of HCQ on RA patients by using the approach of network pharmacology. Firstly, we predicted potential molecular targets of HCQ. Then we investigated the intersection of these targets with RA-related genes. Protein-protein interaction (PPI) network was built to enlarge the amount of proteins which are closely related to the mutual genes. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment was conducted on the enlarged amount of proteins. Finally, we performed docking studies to verify the chemical force that allow HCQ binding to its predicted targets. Our results may be helpful to further find how HCQ can be effective against RA and and facilitate the development of novel drugs.

2. Methods And Materials

2.1.Predicted Target Proteins of HCQ

The chemical structure (SMILES) of HCQ was searched on PubChem website and made target prediction on different databases (SwissTargetPrediction, DrugBank and PharmMapper) based on it. The species was limited to “*Homo sapiens*”. A total of 100 human proteins that possibly targeted by HCQ were returned [13].

2.2.Collection of Related Genes associated with RA

Targets related to RA were returned from databases of OMIM and Genecards by searching with the key word of "*rheumatoid arthritis*" and "*Homo sapiens*". A total of 4329 human genes related to RA were returned [14].

2.3.Screening of Pivotal Target Proteins as well as GO and KEGG Analysis

The plug-in "Bisogenet" in Cytoscape software was used to conduct the PPI network of the mutual targets between HCQ and RA. The key targets were screened according to the parameters of "degree", "betweenness" and "closeness" calculated by Bisogenet. GO and KEGG enrichment analysis was conducted on the database of DAVID and Metascape to make macroscopic evaluation of target genes about their molecular function and systemic involvement [15].

2.4.Molecular Docking

AutoDock 4.2 software was used to analyze the chemical interactions between proteins and small molecules. The 3D crystal structures of potential targets of HCQ were found from the database of RCSB-PDB. Their structures were modified with AutoDock software including ligand and water removal, hydrogen addition, amino acid optimization and patching. ChemBioDraw 3D software was used to visualize the 3D chemical structures and minimize their energy. Results were saved in MOL.2 format. Molegro Virtual Docker predicted docking partners by comparing the predicted conformation with the observed crystal structure. A model was considered accurate if its root mean square deviation from the crystal structure was ≤ 2 Å; reliable if the deviation was ≤ 4 Å, and reliable or accurate if the deviation was less than 3 Å [16].

3. Results

3.1.The Predicted Targets of HCQ based on databases

The potential targets of HCQ were predicted by databases according to the 2-dimensional and 3-dimensional chemical structure (Fig. 1A). The top 100 targets (Fig. 1B) of them with high index of possibility were chose. Most of these targets are G protein-coupled receptors (29%), kinase (26%) and surface antigen (13%) (Fig. 1C).

3.2.Genes associated with RA and Topological Network Analysis

The intersected 64 of the total 4329 genes involved in RA with potential targets of HCQ were conducted with PPI network by cytoscape software through the plug-in of "Bisogenet". Then enlarged result of 3316 more proteins associated with the 64 proteins were returned. A total of 82340 edges (interactions) of the 3316 nodes (targets) could be seen in Fig. 2A. In order to show the most important nodes of these 3316 proteins, the index of degree≥56 (the median) was used as a criterion to screen them preliminarily (Fig. 2B). Further more, 913 nodes and 39807 edges were screened by the index of betweenness≥335 and

closeness≥0.515. The returned 147 related proteins and their 3767 interrelationships which may play important roles in the treatment of RA with HCQ could be seen in Fig. 2C. Finally, top 20 of them was screened (Table.1).

3.3. Gene Ontology and Pathways Enrichment of the 3316 Related Proteins

A total of 3316 human genes which participate in the mechanism of HCQ in curing RA, were conducted with GO and KEGG enrichment (Fig. 3). Macrobiological evaluation of these proteins was performed. According to GO enrichment: these proteins were mainly located in ficolin-1-rich granule lumen, ficolin-1-rich granule, vesicle lumen and so forth (Fig. 3A); as to molecular functions, these proteins mainly take part in ATP binding, adenyl nucleotide binding, RNA binding and so forth (Fig. 3B); the biological process of HCQ acted on the network mainly in inhibiting cell activation, myeloid leukocyte activation and regulating exocytosis (Fig. 3C); KEGG pathway analysis further showed that these proteins were mainly involved ErbB, HIF-1, NF-κB, FoxO, Chemokine, MAPK, PI3K/Akt pathway and so forth (Fig. 3D).

3.4.Molecular docking

A total of 20 candidate potential targets of HCQ (Table.1) were performed with molecular docking analysis which provided a visual explanation of the interaction between HCQ and its potential protein targets associated with RA (Table.2 and Fig. 4). The score below − 20 was considered to have better docking power. Smoothened homolog (SMO), sphingosine kinase (SPHK) 1, SPHK2 and gatty-acid amide hydrolase 1 (FAAH1) were the most possible target of HCQ in curing RA which had top 5 highest binding force and spatial fit with HCQ. We found that hydrogen bond, ionic bond and π-π stacking were the main forms of interaction. For instance, the hydroxyl, amino and carbonyl groups of HCQ formed hydrogen bonds with the proteins, while with the benzene ring and aromatic ring of HCQ engaged in π-π stacking (Fig. 4).

4. Discussion

HCQ is a multi-targets antimalarial drug, which is widely used in rheumatology. However, the exact pharmacological mechanism is still unclear. As one of the DMARDs, HCQ could relief RA activity and improve the prognosis of it. Antimalarial agents have numerous biological effects that are responsible for their immunomodulatory actions [5–8]. According to our results of network pharmacology, SMO, SPHK1, SPHK2 and FAAH1 play vital roles of HCQ in the treatment of RA.

Synovitis is the main characteristic of RA. Excessive proliferation of fibroblast-like synoviocytes (FLSs) and synovial angiogenesis are the most important contributors to the progression of RA synovitis and joint destruction. Sonic hedgehog (SHH) signaling pathway plays a pivotal role in FLSs proliferation in a SMO-dependent manner. Upregulation of SMO promotes proliferation of FLSs [17–18]. Targeting SHH signaling pathway may help control joint damage in patients with RA [19]. According to our analysis by

network pharmacology, the binding of HCQ with SMO is involved in the pathological process of synovitis through the SHH pathway (Fig. 5).

SPHK (including SPHK1 and SPHK2) is a key lipid kinase in sphingolipid metabolic pathway, which phosphorylate phingosine into sphingosine-1-phosphate (S1P) [20–21]. The importance of SPHK and S1P in inflammation and angiogenesis has been demonstrated in many hyperproliferative/inflammatory diseases such as RA [22]. The level of S1P exhibits significantly higher than those non-inflammatory osteoarthritis counterparts [23]. Furthermore, S1P receptor was found to be expressed in RA synovium, which means that inflammatory cytokines would further promote the progress of synovitis [21]. As mentioned above, excessive proliferation of FLSs was induced mainly through SHH pathway. In addition, inflammatory further accelerate the process through mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPK/ERK) signaling pathway. For instance, interleukin 6, tumor necrosis factor-α, angiopoietin 1, neuropilin 1 and vascular endothelial growth factor regulate the lesion of rheumatoid joint and the proliferation of FLSs through the MAPK/ERK pathway [24]. SphK blockade suppresses cytokines and MMP-9 release in RA peripheral blood mononuclear cells [23]. Targeting SPHK may help control joint damage in aspect of inflammation. According to our analysis by network pharmacology, the binding of HCQ with SPHK1 and SPHK2 plays important role in inhibiting the inflammatory process of synovitis (Fig. 5).

In recent years, the role of the endocannabinoid (EC) system in the pathogenesis of RA attracted more attention of researchers. EC system modulates function of immune cells and mesenchymal cells such as fibroblasts, which contribute to cartilage destruction in RA [25]. The action of EC system in immune system regulation, via primary cannabinoid receptor (CB) activation, followed by inhibition of production of pro-inflammatory cytokines, auto-antibodies and matrix metalloproteinase (MMPs), FLSs proliferation and T-cell mediated immune response [26]. Since FAAH is a major EC-degrading enzyme, the therapeutic possibility of FAAH inhibition is promising [27]. Thus, due to the result of network pharmacology, the binding of HCQ with FAAH acts as one of the multi-targets mechanism in the treatment of RA (Fig. 5).

5. Conclusions

Collectively, 4 key targets (SMO, SPHK1, SPHK1 and FAAH) (Fig. 5) involving 3316 proteins become the multi-targets mechanism of HCQ in the treatment of RA due to our research, through the pathway related to proliferation of fibroblast-like synoviocytes and inhibition of production of pro-inflammatory cytokines. According to GO analysis, they totally enrich in the functions of: regulation of cell activation, myeloid leukocyte activation, regulated exocytosis and so forth. ErbB, HIF-1, NF-κB, FoxO, Chemokine, MAPK, PI3K/Akt pathways and so forth are the main pathways that took part in the multi-targets machanism according to KEGG analysis. This paper introduced the new concept of network pharmacology into the clinical treatment of rheumatic diseases with multi-targets drugs, which is conducive to the exploration and evaluation of multi-targets drugs that are clinically effective in rheumatic treatment but whose particular mechanism is not well understood. In addition, it can also provide an explanation of drug usage and guidance for relevant scientific research.

6. List Of Abbreviations

RA: rheumatoid arthritis; NSAIDs: non-steroidal anti-inflammatory drugs; DMARDs: disease-modifing anti-rheumatic drugs; HCQ: hydroxychloroquine; PPI: protein-protein interaction; GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; SMO: smoothened homolog; SPHK: sphingosine kinase; FAAH: gatty-acid amide hydrolase; FLSs: fibroblast-like synoviocytes; SHH: sonic hedgehog; MMP: matrix metalloproteinase; EC: endocannabinoid; CB: cannabinoid receptor.

7. Declarations

Ethics approval and consent to participate: None declared.

Consent for publication: The article is approved by all the co-authors for publication.

Availability of data and materials: The data used or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no conflict of interests.

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Authors' contributions: The manuscript has been read and approved by all of the authors. Bo Xie, Xiuzu Song; performed the experiments: Bo Xie, Jinhui Xu, Haojie Lu, Haixin Luo, Yebei Hu, Yi Chen, Qingwei Geng; analyzed the data: Bo Xie, Xiuzu Song; wrote the paper: Bo Xie, Xiuzu Song.

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9. Tables

Table.1 The top 20 potential targets of HCQ associated with the treatment of RA according to PPI network

NO.	Gene Symbol	Name	Degree
1	NTRK1	high affinity nerve growth factor receptor	852
2	HSP90AA1	heat shock protein HSP 90-alpha	813
3	APP	amyloid-beta precursor protein	597
4	TP53	cellular tumor antigen p53	589
5	CUL3	cullin−3	570
6	EGFR	epidermal growth factor receptor	496
7	XPO1	Exportin−1	483
8	MDM2	E3 ubiquitin-protein ligase mdm2	457
9	SRC	proto-oncogene tyrosine-protein kinase src	448
10	GRB2	growth factor receptor-bound protein 2	447
11	CUL7	cullin−7	407
12	VCP	transitional endoplasmic reticulum ATPase	326
13	VCAM1	vascular cell adhesion protein 1	310
14	MYC	myc proto-oncogene protein	292
15	SPHK1	sphingosine kinase 1	206
16	SMO	smoothened homolog	167
17	SPHK2	sphingosine kinase 2	153
18	FAAH1	gatty-acid amide hydrolase 1	128
19	CSF1R1	macrophage colony-stimulating factor 1 receptor 1	117
20	SHH	sonic hedgehog protein	103

Table.2 The top 10 targets of RA according to docking score

NO.	Gene Symbol	The Name of Protein	Docking score
1	SMO	smoothened homolog	−31.139
2	SPHK1	sphingosine kinase 1	−28.258
3	SPHK2	sphingosine kinase 2	−27.900
4	FAAH1	gatty-acid amide hydrolase 1	−25.120
5	MYC	myc proto-oncogene protein	−19.590
6	HSP90AA1	heat shock protein HSP 90-alpha	−18.147
7	EGFR	epidermal growth factor receptor	−17.778
8	APP	amyloid-beta precursor protein	−15.159
9	TP53	cellular tumor antigen p53	−15.766
10	CUL3	cullin−3	−14.483

Figures

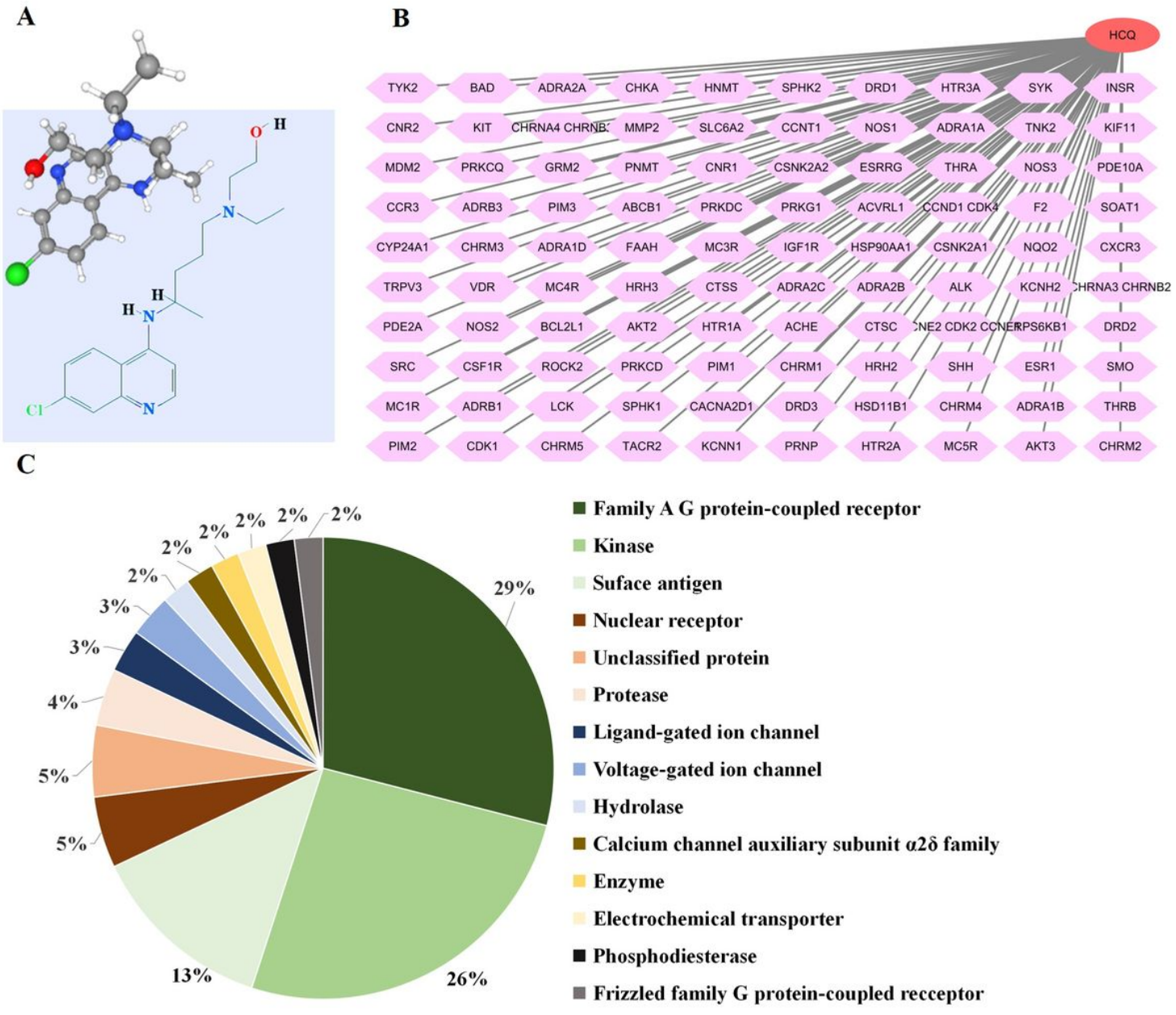

Figure 1

Top 100 potential targets of HCQ predicted by databases A: 3-dimensional and 2-dimensional structure of HCQ; B: top 100 predicted targets of HCQ; C: categories of the predicted targets.

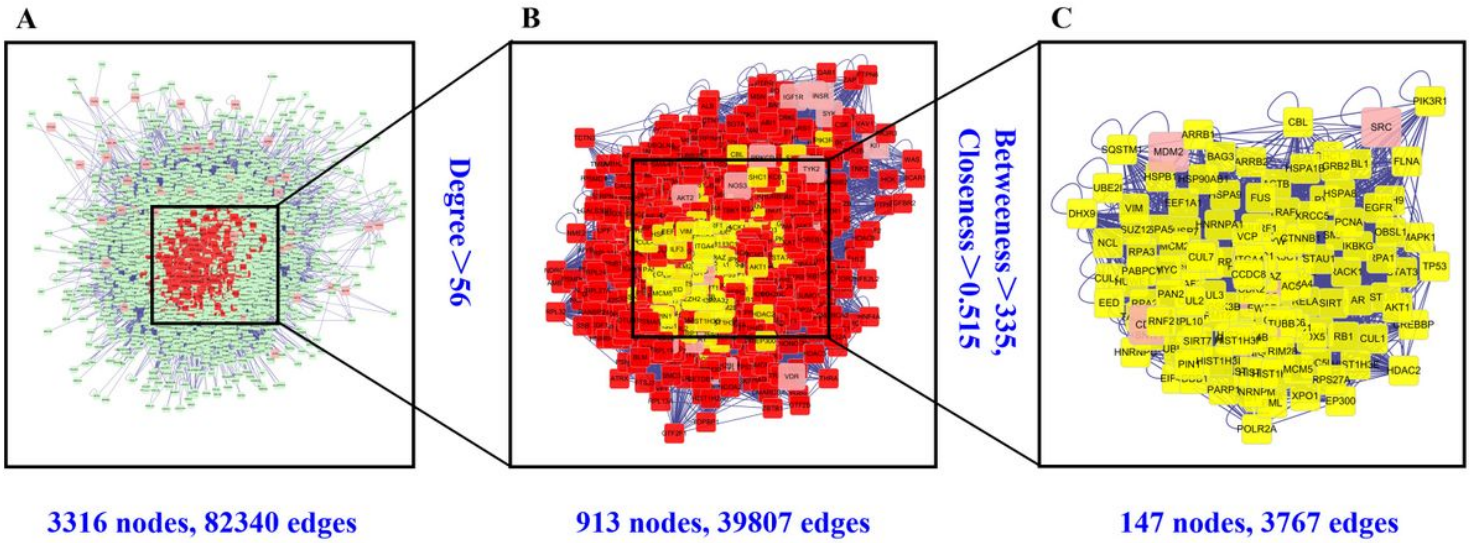

Figure 2

The PPI network conducted with 64 mutual genes of RA and HCQ's potential targets A: PPI network of the enlarged 3316 proteins; B: the 913 nodes and 39807 edges after the first screening; C: the 147 nodes and 3767 edges after the second screening.

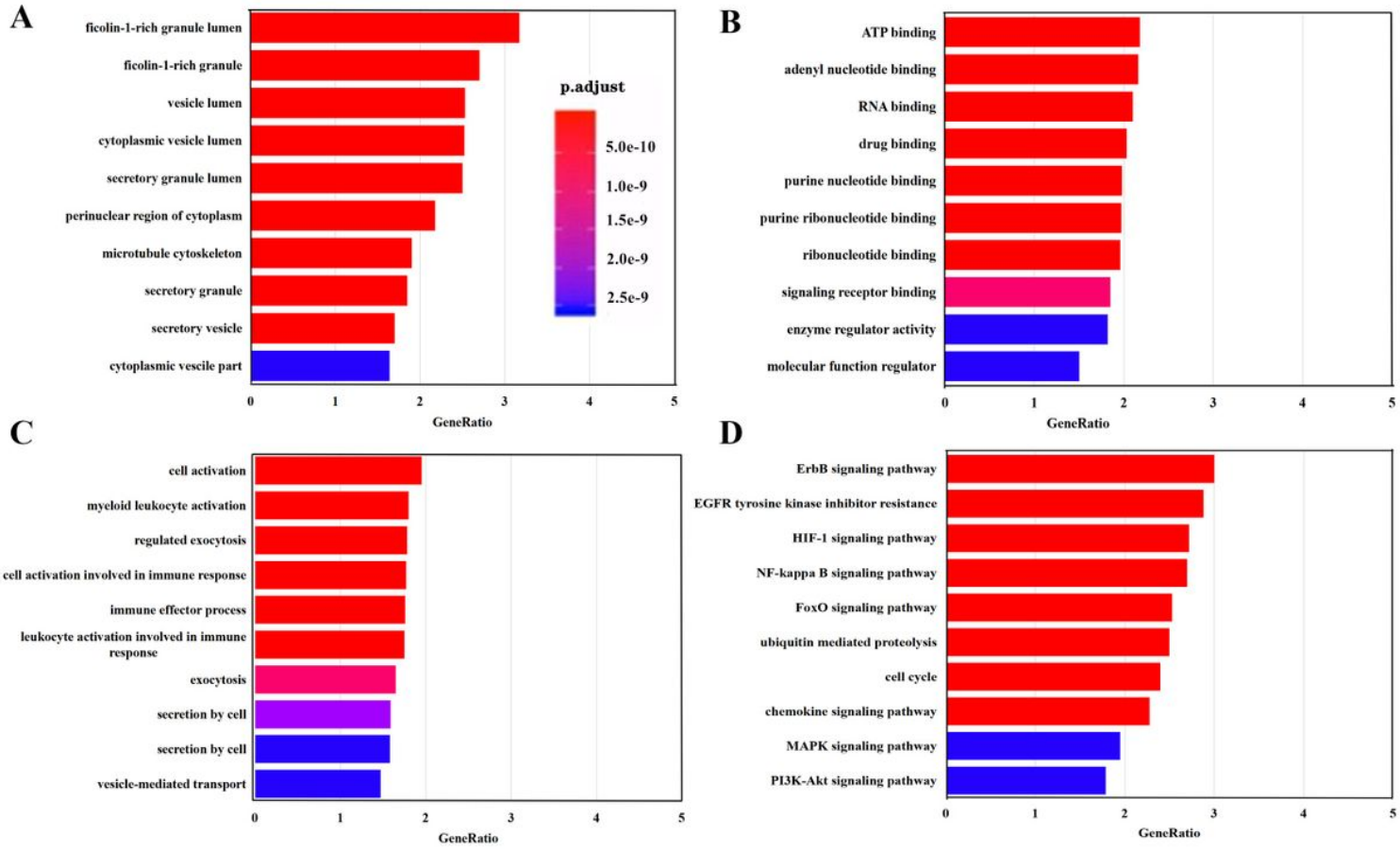

Figure 3

GO enrichment analysis of the 3316 genes A: cellular components; B: molecular function; C: biological process; D: KEGG pathways.

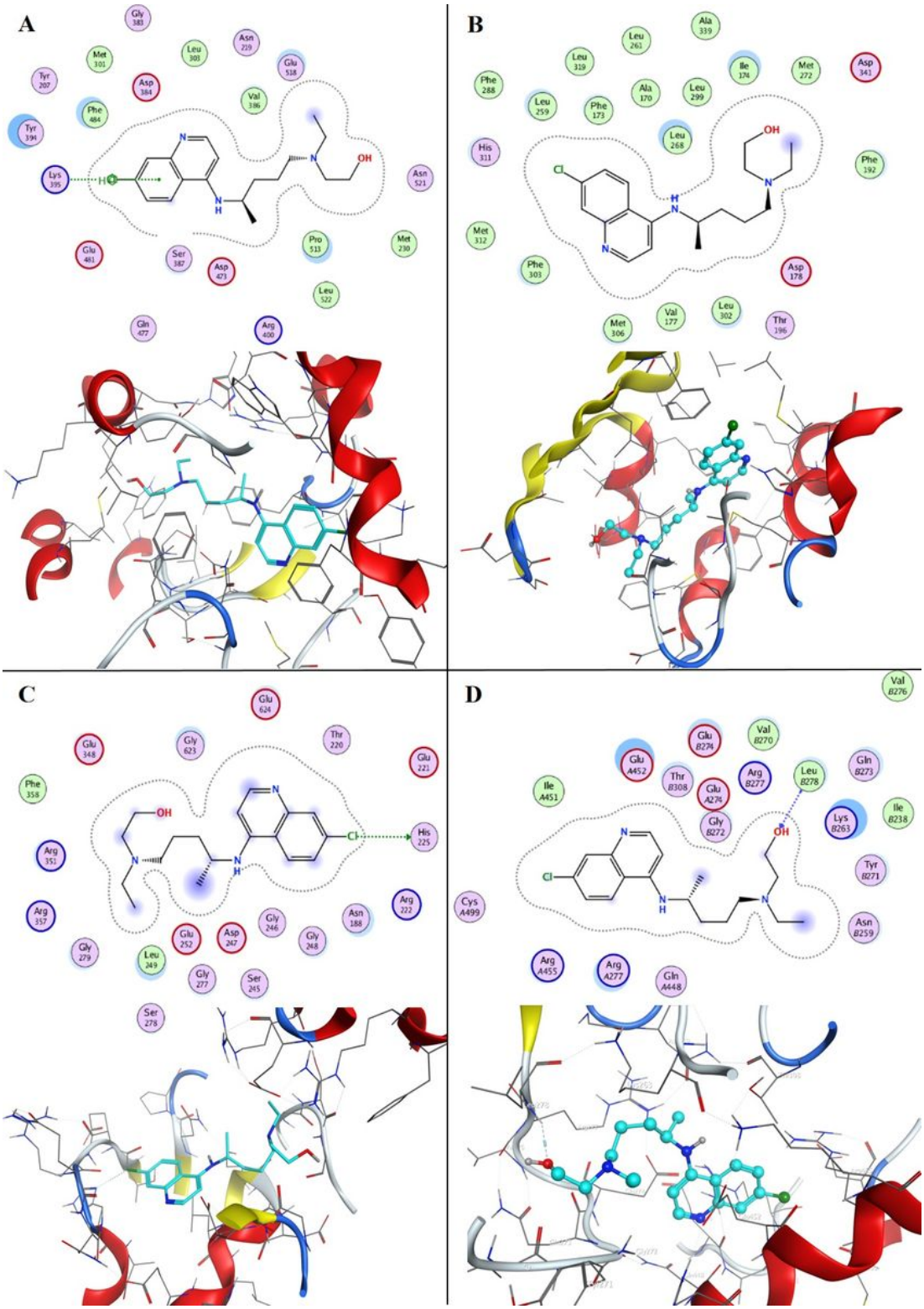

Figure 4

Molecular models of HCQ binding to its predicted protein targets. Proteins (A) SMO, (B) SPHK1, (C) SPHK2, (D) FAAH are shown interacting with a HCQ molecule, represented by a green stick model. hydrogen bonds and demarcate π-π interactions are shown in these models.

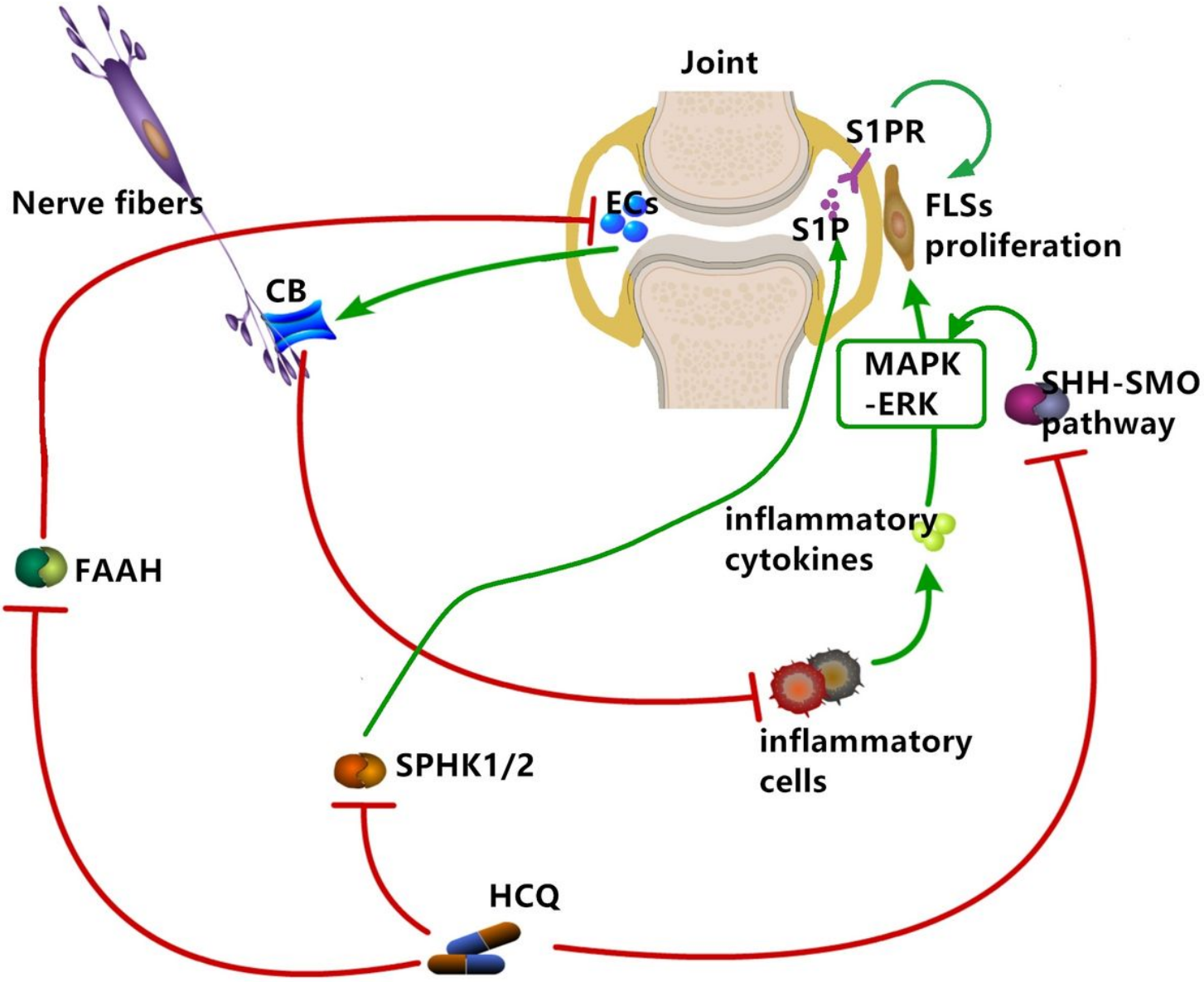

Figure 5

The multi-targets mechanism of HCQ in the treatment of RA HCQ: hydroxychloroquine; CB: cannabinoid receptor; FAAH:gatty-acid amide hydrolase ; SPHK: sphingosine kinase; SMO: smoothened homolog; SHH: sonic hedgehog; FLSs: fibroblast-like synoviocytes; S1P: sphingosine-1-phosphate; S1PR: sphingosine-1-phosphate receptor; ECs: endocannabinoids.