Biochemical Targets and Molecular Mechanism of Ginsenoside Compound K for Treating Osteoporosis Based on Network Pharmacology

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Research Article

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Abstract

Ginsenosides have been proven to be potential beneficial in treatment of osteoporosis. To investigate the potential of ginsenosides in osteoporosis, ginsenoside compound K (GCK) was selected to explore the potential therapy targets and mechanism based on network pharmacology (NP). 206 and 6590 targets were obtained for GCK and osteoporosis, respectively, in which 138 targets were identified as co-targets of GCK and osteoporosis based on intersection analysis. Five central gene clusters and hub genes (STAT3, PIK3R1, VEGFA, JAK2 and MAP2K1) were identified through protein-protein interaction network analysis. Gene Ontology (GO) enrichment implied that phosphatidylinositol-related biological process, molecular modification and function may play an important role for GCK in treatment and prevention of osteoporosis. Functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis suggested that 16 targets were enriched in the osteoclast differentiation. Also, except for being identified as hub targets, MAPK and phosphatidylinositol-related proteins were enriched in the downstream signaling of c-Fms in the osteoclast differentiation pathway. Molecular docking further confirmed that GCK could interact with active cavity on the surface of c-Fms (osteoclast differentiation-related membrane receptor), and their complex could be stabilized by three H-bonds with residues including Glu 664 (3.19 Å), Glu 664 (2.62 Å) and Cys 666 (2.78 Å). Summarily, GCK could interfere the occurrence and progress of osteoporosis through c-Fms-mediated MAPK and phosphatidylinositol-related signaling regulating osteoclast differentiation.

1. Introduction

Osteoporosis is a metabolic and systemic bone disorder disease characterized by loss of bone mass and micro-architectural deterioration of bone tissue, susceptibly resulting in bone fragility and fracture [1, 2]. Osteoporosis causes over 8.9 million fractures worldwide each year, the most of which are located in hip, spine, distal forearm, and proximal humerus[3]. With the progressive aging of the global population, the incidence of osteoporotic fractures with high mortality and morbidity is increasing dramatically[4]. It is reported that the morbidity of hip fractures will increase by the 3.5-time between 1990 and 2050 all over the world[5]. Unfortunately, there are not enough effective drugs for the treatment of osteoporosis. In current clinical management of osteoporosis, relieving bone fractures is the major aim based on drugs, in which the most common drugs are bisphosphonates, such as Alendronate, Risedronate, Zoledronic acid, Ibandronate, and so on[6, 7]. However, there are several limitations for bisphosphonate drugs, including acute renal failure, gastrointestinal intolerability, musculoskeletal pain, and in rare cases, an increased risk of fracture upon their long-term use, particularly of atypical femoral fractures and osteonecrosis of the jaw[7, 8]. Therefore, it has been one of the more and more urgent problems for discovering new effective drugs with less side effects for the treatment of osteoporosis.

Natural products are important and reliable resources for the prevention and treatment of osteoporosis because they have fewer side effects and are more suitable for long-term application compared with chemosynthetic medicines[9]. Ginseng, the root of Panax ginseng C.A. Meyer, has been used as a tonic remedy for more than 2000 years in Asia[10]. Ginsenosides are the main pharmacologically active compounds in ginseng, pharmacological effects of which include resistance to tumors, inhibition of neurodegeneration in patients with Alzheimer's disease, promotion of brain development and memory improvement, exhibition of anti-inflammatory and antioxidant effects, prevention of diabetes, resistance to fatigue, protection of the heart and anti-osteoporosis, etc[11]. Ginsenoside Rb1, Rb2, Rg1, Rg3 and Rh2 can prevent osteoporosis based on *in vitro* or/and *in vivo* experiment[10, 12–14]. However, the molecular mechanism is not totally understood for ginsenosides to prevent and treat osteoporosis. Ginsenoside compound K (GCK) (20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol) does not naturally exist in ginseng, but it is a major metabolite of natural ginsenoside Rb1, Rb2 and Rc in the intestine under the effects of intestine bacteria[15]. GCK is considered as a rare ginsenoside and has received more attention because of its more soluble, bioavailability and bioactivities than its parent ginsenosides[15, 16]. Therefore, GCK may also have more beneficial effects on prevention and treatment of osteoporosis than its parent ginsenosides. However, investigation about role of GCK on the osteoporosis is quite poorer than its parent ginsenosides.

Network pharmacology (NP) has been recently proposed as a promising approach integrating database mining, bioinformatics analysis, topological analysis and molecular simulation, which is widely applied to discover potential medicinal ingredients from herb medicine and to predict their possible pharmacology mechanism at the molecular level[17]. Given the above considerations, we have investigated the role of GCK in osteoporosis based on NP and further explored the possible molecular mechanism in this study.

2. Materials And Methods

2.1 Targets Of GCK

The related targets of GCK were predicted and obtained from PharmMapper (http://www.lilab-ecust.cn/pharmmapper/), Similarity ensemble approach (SEA) (http://sea.bkslab.org/) and SwissTarget Prediction (http://www.swisstargetprediction.ch/) databases under the condition of Homo sapiens. Gene names and organisms were standized by manual retrieval based on Uniprot database (https://www.uniprot.org/) to avoid over-annotation of similar proteins like paralogs and putative products of pseudogenes.

2.2 Targets Of Osteoporosis

The osteoporosis-related target proteins were obtained through online search "osteoporosis", "fragile bones" and "bone fragility" under the condition of Homo sapiens in the following databases: GeneCards (https://www.genecards.org/), Online Mendelian Inheritance in Man (OMIM) (http://www.omim.org/), Therapeutic Target Database (TTD) (http://db.idrblab.net/ttd/), The Human Phenotype Ontology (HPO) (http://www.human-phenotype-ontology.org/), DisGeNET, (http://www.disgenet.org/), DigSee (http://210.107.182.61/geneSearch/) and home-for-researchers (https://www.home-for-researchers.com/static/index.html#/project_assistant). The duplicate and redundant proteins or genes are deleted.

2.3 Putative Targets Of GCK-treated Osteoporosis

The overlapping targets of both GCK and osteoporosis were considered potential targets of GCK-treated osteoporosis, and obtained through taking the intersection and Venn diagram analysis.

2.4 Protein-Protein Interaction（PPI）Network Construction And Analysis

Intrinsic relationships between these putative targets of ginsenoside CK-treated osteoporosis were analyzed further based on STING database (http://www.string-db.org/). The conditions of PPI network construction were limited to “Homo sapiens” with the highest confidence score >0.9. Furthermore, six topological properties (Betweenness, BottleNeck, Degree, Closeness, MCC and EcCentricity) of the PPI network were calculated to screen the hub genes based on topological importance by using Cytoscape software (ver. 3.8.0) and Cytoscape plugin cytoHubba. Additionally, Molecular Complex Detection (MCODE) (a plug-in of Cytoscape) was applied to investigate the node composition for gaining the links between significantly enriched network clusters and hub genes with a high degree of connectivity. In MCODE analysis, Degree cutoff value, Node Score Cutoff value and K-Core value were set 2, 0.2 and 2, respectively.

2.5 Enrichment Analysis

Gene Ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed to reveal the potential biological mechanism for GCK in the treatment of osteoporosis. GO and KEGG enrichment were conducted through the online analysis tool of The Database for Annotation, Visualization and Integrated Discovery (DAVID, ver. 6.8) (https://david.ncifcrf.gov/) with p <0.05. GO terms included three categories: biological process (BP), molecular function (MF), and cellular component (CC).

2.6 Molecular Docking Of Compound-Target Interaction

Molecular docking was employed to validate the potential mechanism of key protein in osteoporosis-related pathway by using Auto Dock software in this study. The 3D structure of the key target protein was obtained from the RCSB (Research Collaboratory for Structural Bioinformatics) PDB (Protein Data Bank) database (http://www.rcsb.org/). The 2D structure of GCK was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and its conformation of minimum energy was obtained by ChemOffice software. Protein and ligand were prepared in the AutoDock Tools prior to performing the docking process. The crystal structure of the target protein is pretreated, including removal of water molecules, (organic and heteroatom) and adding hydrogenation (charge and atom type). Auto Dock was utilized to semi-flexibly couple the GCK to the target protein.

3. Results

3.1 Molecular Targets Of GCK And Osteoporosis

A total of 206 and 6590 molecular targets were obtained for GCK and osteoporosis in this study, respectively. The overlapping targets of GCK and osteoporosis were considered as the potential targets of GCK-treated osteoporosis. Based on intersection analysis, a total of 138 molecular targets were identified as co-targets of GCK and osteoporosis, which were shown through a Venn diagram (Fig.1).

3.2 PPI Network Analysis Of Co-targets Of GCK And Osteoporosis

For investigating internal connection and important targets in these co-targets of GCK and osteoporosis, PPI network analysis was applied further here. After importing GCK-osteoporosis co-targets into STRING, we get GCK-osteoporosis target PPI network with the highest confidence ($p\geq$ 0.900), which contains 95 nodes and 365 edges (Fig.2). In PPI network, the degrees of node PIK3R1, PIK3CA, STAT3, SRC, GRB2, PLCG1 and VEGFA were greater than 20, which was 35, 34, 30, 23, 22, 21 and 20, respectively. Targets information in PPI network queried from STRIING database was analyzed further by MCODE and cytoHubba tool of Cytoscape software for identifying the key targets. MCODE analysis returned five central gene clusters (Fig.3 and Table 1). In these clusters, just the top 1 module was with score > 6 in this PPI network (Fig.3a and Table 1), which contained SYK, STAT3, PLCG1, PIK3CD, PIK3CB, MAP2K1, JAK1, IL2, HSP90AA1, HCK, GRB2. Additionally, STAT3, PIK3R1, VEGFA, JAK2 and MAP2K1 were identified as hub genes in PPI network with cytoHubba tool based on six methods (Fig. 4 and Table 2), which involved in the three of five central gene clusters identified by MCODE analysis (Fig. 3a, 3b and 3c). This suggests that the related molecules have an important role in GCK-treated osteoporosis, especially for STAT3, PIK3R1, VEGFA, JAK2 and MAP2K1.

3.3 GO And KEGG Analysis Of Co-targets Of GCK And Osteoporosis

Go and KEGG analysis were further used to investigate the BP, CC, MF and signaling pathway of co-targets of GCK and osteoporosis. The results indicated that 210, 55 and 66 terms were enriched for BP, CC and MF, respectively. The top 20 BP, CC and MF terms (which were ranked based on p value) were shown in Fig.6a, 6b and 6c, respectively. These enriched BPs mainly included cell growth- and death-related processes (such as negative regulation of apoptotic process, positive regulation of MAP kinase activity, positive regulation of cell proliferation, regulation of phosphatidylinositol 3-kinase signaling and so on), protein synthetic and modification processes (such as phosphatidylinositol-3-phosphate biosynthetic process, phosphatidylinositol phosphorylation, peptidyl-tyrosine (auto)phosphorylation, protein (auto)phosphorylation, protein processing, and so on), and some stress response processes (such as drug, hypoxia, inflammatory and innate immune response). Based on CC enrichment, these co-targets were found in plasma membrane, nuclear membrane, organelle, cytoplasm, extracellular matrix, extracellular secretion and exosomes, in which targets from membrane were the most, and the second is from cytoplasm and extracellular components. In MF enrichment analysis, these co-targets mostly involved in kinase activity,

protein/receptor/enzyme/small molecular-binding function. It was worth noting that phosphatidylinositol-related biosynthetic process, protein modification, complex assembly, mediated-signaling and kinase activities were among the top in BP, CC and MF enrichment analysis. This suggested that phosphatidylinositol-related bioprocess and signaling pathway may be a potential target for GCK to treat osteoporosis.

KEGG analysis showed that co-targets of GCK and osteoporosis mainly involved in 88 pathways (the top 20 pathways were shown in Fig.6), most of which were cancer-related pathways (such as pathways in cancer, proteoglycans in cancer, prostate cancer, pancreatic cancer, non-small cell lung cancer and so on). However, there was only one pathway (osteoclast differentiation pathway, shown in Fig.7) which was significantly related to osteoporosis. There were 16 genes (c-Fms, MAP2K1, SYK, PIK3CD, PIK3CB, PIK3R1, MAPK14, PIK3CG, MAPK12, IKBKB, MAPK8, PIK3CA, CAMK4, GRB2, PPARG, JAK1) enriched in osteoclast differentiation pathway with a *p* value of 4.81×10^{-9}. In these 16 genes, just c-Fms was located in the cell membrane, and the others were located in the cytoplasm and nucleus. This indicated that c-Fms may play a more key role than other targets through regulating downstream signal transduction in the osteoclast differentiation pathway for GCK-treated osteoporosis.

3.4 Molecular Docking Of GCK- c-Fms Interaction

Based on the results mentioned above, c-Fms-mediated signaling may be the most significant through interfering osteoclast differentiation for GCK-treated osteoporosis. To validate possible biological interaction between GCK and c-Fms, molecular docking was used here. The results showed that GCK could well insert into and bind to the active cavity on the surface of c-Fms with -7.21 Kcal/moL of binding energy (Fig.8 a and 8 b). The complex GCK-c-Fms was stabilized by three strong hydrogen bonds with residues including Glu 664 (3.19 Å), Glu 664 (2.62 Å) and Cys 666 (2.78 Å) (Fig.8c), respectively. It confirmed that GCK could interfere osteoclast differentiation through interacting with c-Fms, by which it exerts the efficacy in the treatment of osteoporosis.

4. Discussion

Osteoporosis is a considerable clinical and public health burden because of its association with age-related fractures[2]. With the progressive aging of the global population, the incidence of osteoporotic fractures with high mortality and morbidity is increasing dramatically[4]. Especially for China, the China' population over 65-age will be 399 million by 2049, accounting for more than 28.9% of the total population[18], which means that population suffered from osteoporosis will sharply increase in China and exacerbate clinical and public health burden in the next 20 years. So, it is significant to develop the drugs or functional food for the prevention and treatment of osteoporosis. Ginsenosides, as the main pharmacologically active compounds in ginseng, show a promising potential for the prevention and treatment of age-related diseases including osteoporosis. Although the effects of ginsenosides on osteoporosis have been investigated based on *in vitro* or/and *in vivo* experiment for Rb1, Rb2, Rg1, Rg3, Rh2 and so on, it is a pity for that the efficacy of GCK (which is more soluble, bioavailable and bioactive than its parent ginsenosides) on osteoporosis has been unknown, not to mention the molecular mechanism. In this study, we found there were 138 molecular targets at least that could be responded to osteoporosis and GCK simultaneously. Although these targets have not been obviously reported for other studies about ginsenoside-treated osteoporosis, some of them were verified indirectly based on the related-targets in the same pathway. For example, MAP2K1,MAPK12, MAPK14 and MAPK8 were related to MAPKs signaling which was trigged by Rb1[19] and Rh2[13], and mTOR was related to mTOR signaling which could be activated by Rg3[12]. So, this not only implies that GCK has the potential to prevent and treat osteoporosis, but also indicates that there is still large unknowability and necessity for exploring the molecular mechanism of ginsenoside-treated osteoporosis.

In this study, five osteoclast differentiation-related pathways (PI3K-AKT signaling pathway, NF-κB signaling pathway, MAPK signaling pathway, Calcium signaling pathway and Jak-STAT signaling pathway) were significantly enriched in KEGG analysis, which is closely related to osteoporosis. It was reported that ginsenosides have a poor permeability of cells with the apparent permeability coefficient of $<1 \times 10^{-6}$ cm/s[20, 21]. So, the membrane receptor-mediated signaling may be more efficient and important than the signaling proteins in the cytoplasm, and it was also confirmed indirectly by the CC enrichment in GO analysis. In these targets enriched in osteoclast differentiation-related pathways, c-Fms is the only membrane receptor protein. Furthermore, we found that GCK could insert into and bind to the active cavity on the surface of c-Fms, which works like the small-molecule inhibitors of c-Fms[22, 23]. The receptor-tyrosine kinase c-Fms (also known as CSF-1-R) is encoded by FMS or CSF-1-R proto-oncogene, which is the cell surface receptor for the (macrophage) colony-stimulating factor-1 (CSF-1 or M-CSF)[22]. c-Fms is expressed in macrophages, microglia, and osteoclasts is one type of receptor for M-CSF, and plays an important role in initiating inflammatory, cancer, and bone disorders when it binds with its ligand CSF[24]. Previous studies reported that c-Fms inhibition could prevent against osteoporosis by inhibiting osteoclast formation[24, 25]. So GCK binding with the active cavity on the surface of c-Fms may inhibit the activity of c-Fms and negatively regulate the downstream signaling pathways of c-Fms further so that osteoclast differentiation and formation were inhibited.

In the five osteoclast differentiation-related pathways mentioned above, PI3K-AKT and MAPK signaling pathways were just right in the downstream of c-Fms signaling. In addition, we found that phosphatidylinositol-related bioprocesses, proteins and signaling may play an important role in the treatment of osteoporosis with GCK based on GO and KEGG enrichment analysis. It was especially for PI3K-related signaling because subunit proteins of PI3K were included in the topped cluster of PPI network and hub genes which were verified by MCODE and Hub genes analysis. Although it has not been reported for PI3K-related signaling in other studies about ginsenoside-treated osteoporosis, PI3K/AKT pathway was considered to participate in anti-osteoporosis through promoting proliferation of osteoblast precursors and osteoblastic differentiation of BMSCs (Bone marrow stromal cells), as well as autophagy and differentiation of osteoclast [26–29]. Meanwhile, in KEGG analysis, PI3K/AKT pathway was enriched in the c-Fms-mediated osteoclast differentiation. So GCK may inhibit osteoporosis though inhibition of PI3K-mediated osteoclast differentiation. Although it is firstly reported about this molecular mechanism whether based on *in vitro* and *in vivo* tests or computing and simulation with NP approach, it still needs to be validated further with

more evidence of scientific experiments. To note that, cancer-related pathways were enriched at the top of the list in KEGG analysis for co-targets of GCK and osteoporosis. In the previous studies, it has been verified that PI3K pathway plays an important role in bone metastasis of lung cancer and bladder cancer[30, 31]. Therefore, it suggested that GCK may be involved in bone metastasis-induced osteoporosis through PI3K-related signaling in cancers.

In addition to phosphatidylinositol-related bioprocesses, proteins and signaling, GRB2-ERK was the other signaling pathway of c-Fms-mediated osteoclast differentiation. ERK is a member of the MAPK family, which transduces extracellular stimuli to alter gene expression and has been shown to play a role in diverse cellular events ranging from proliferation, differentiation to apoptosis[32]. It was reported that M-CSF could activate ERK via phosphorylation of c-Fms, which then recruits GRB2/SOS and stimulates the Ras/Raf/MEK(MAPK/ERK kinase)/ERK pathway[33]. Additionally, insulin may exert its anabolic effect on osteoblast through IR-GRB2-ERK mediated pathway[34]. Therefore, GCK may exert a similar or opposite effect on osteoclast differentiation through the regulation of c-Fms-GRB2-ERK signaling axis. Except for ERK, JNK and p38 of MAPK family were also enriched in osteoclast differentiation pathway, and MAPK-related protein MAP2K was verified that play a major role for GCK-treated osteoporosis in MCODE and Hub genes analysis. These results suggested that GCK could activate MAPK pathway which also involved in c-Fms-mediated downstream signaling in osteoclast differentiation. Although it has been reported that ginsenosides Rb1 and Rg3 could inhibit osteoclastogenesis and osteoclast differentiation by modulating MAPK pathway[19, 35], it was rarely reported that GCK could inhibit osteoclastogenesis and osteoclast differentiation by modulating Fms-mediated MAPK pathway. So, it is necessary to be validated further for GCK inhibiting osteoclast differentiation through the c-Fms-MAPK axis with more evidence.

5. Conclusions

GCK has been verified to be potential beneficial in treatment of osteoporosis in this study based on NP. Moreover, GCK could influence the occurrence and progress of osteoporosis through interacting with 138 potential targets at least, in which phosphatidylinositol-related biosynthetic process, protein modification, complex assembly, mediated-signaling and kinase activities were enriched among the top in BP, CC and MF analysis based on GO enrichment. Additionally, 16 targets were enriched in the osteoclast differentiation pathway based on KEGG analysis, in which c-Fms-mediated signaling may be the potential key targets and mechanisms for GCK in treatment and prevention of osteoporosis. Furthermore, it was proven that GCK could well insert into and bind to the active cavity on the surface of c-Fms based on molecular docking. Also, PIK3 and MAPK-related proteins were not only identified as the hub targets but also were enriched in the downstream signaling of c-Fms. Therefore, we proposed that c-Fms-mediated MAPK and phosphatidylinositol–related signaling may be the potential important mechanisms for GCK in treatment and prevention of osteoporosis through interfering osteoclast differentiation (An overview of the possible molecular mechanism is shown in Fig. 9). These results are just from the rational and systematic computing and simulation on a chip based on NP approach, so it still needs to be deeply explored and validated with more *in vitro* and *in vivo* experiments, which will be focused on the next work in our lab.

Abbreviations

GCK, ginsenoside compound K; NP, network pharmacology; SEA, similarity ensemble approach; OMIM, online mendelian inheritance in Man; TTD, therapeutic target database; HPO, the human phenotype ontology; PPI, protein-protein interaction; MCODE, molecular complex detection; GO, gene ontology; KEGG, kyoto encyclopedia of genes and genomes; DAVID, The Database for Annotation, Visualization and Integrated Discovery; BP, biological process; MF, molecular function; CC, cellular component; RCSB, research collaboratory for structural bioinformatics; PDB, protein data bank; CSF-1 or M-CSF, colony-stimulating factor-1; BMSCs, bone marrow stromal cells.

Declarations

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Author contributions

Sen Zhang: Conceptualization, Investigation, Formal analysis, Writing – original draft, Project administration. Shihong Shen: Conceptualization, Investigation, Formal analysis, Writing – review and editing. Pei Ma: Investigation, Writing – review and editing. Daidi Fan: Conceptualization, Supervision.

Data availability

All data collected and analyzed during the current study are available from the first author and corresponding author on reasonable request.

Conflicts of interest:

The authors declare that they have no competing interests.

Ethical approval

This article does not include human or animal experiments.

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Tables

Table 1 Five central gene clusters of ginsenoside CK-osteoporosis PPI network identified based on MCODE analysis

Cluster	Nodes	Edges	Score	Genes
1	11	36	7.200	SYK; STAT3; PLCG1; PIK3CD; PIK3CB; MAP2K1; JAK1; IL2; HSP90AA1; HCK; GRB2
2	15	40	5.714	S1PR1; PSENEN; PSEN2; PSEN1; PIK3R1; PIK3CA; NCSTN; KDR; JAK2; FGF2; FGF1; FES; EPHB4; APH1B; APH1A;
3	5	10	5.000	RXRG; RXRB; RARB; HDAC3; HDAC1
4	11	20	4.000	VEGFB; VEGFA; THBS1; SRC; SERPING1; PTPN1; MAPK8; KIT; IGF1R; CFD; AR
5	3	3	3.000	MMP9; MMP3; MMP1

Table 2 Top 20 hub genes of ginsenoside CK-osteoporosis PPI network identified with Betweenness, BottleNeck, Degree, Closeness, MCC and EcCentricity method based on cytoHubba tool

Methods	Betweenness		BottleNeck		Degree		Closeness		MCC		EcCentricity	
	Gene	Score	Gene	Score	Gene	Score	Gene	Score	Gene	Score	Gene	Score
1	STAT3	1858.83	PIK3R1	32.00	PIK3R1	35.00	PIK3R1	60.00	PIK3R1	33711.00	PIK3CA	0.32
2	PIK3R1	1514.88	STAT3	17.00	PIK3CA	34.00	PIK3CA	59.33	PIK3CA	33710.00	PIK3R1	0.32
3	PIK3CA	1166.88	MMP9	12.00	STAT3	30.00	STAT3	57.08	GRB2	23136.00	MMP9	0.24
4	EPHB4	1159.36	GRB2	9.00	SRC	23.00	SRC	52.00	PIK3CB	22560.00	HCK	0.24
5	VEGFA	568.08	EPHB4	8.00	GRB2	22.00	GRB2	51.75	PLCG1	21770.00	SYK	0.24
6	MTOR	477.35	VEGFA	7.00	PLCG1	21.00	VEGFA	50.17	JAK1	20934.00	PTPN6	0.24
7	PLCG1	449.22	MAPK14	6.00	VEGFA	20.00	JAK2	49.67	SYK	17220.00	IGF1R	0.24
8	CCND1	400.60	MTOR	4.00	JAK2	19.00	PLCG1	49.08	HCK	16812.00	PIK3C2B	0.24
9	MAPK14	384.62	KIT	4.00	JAK1	19.00	JAK1	49.08	SRC	11096.00	MAP2K1	0.24
10	JAK2	377.93	S1PR1	4.00	IL2	17.00	IL2	48.42	JAK2	10906.00	STAT3	0.24
11	MMP2	328.71	PTPN6	3.00	PIK3CB	17.00	HSP90AA1	46.42	IL2	8767.00	MTOR	0.24
12	KIT	317.35	MAP2K1	3.00	SYK	15.00	PIK3CB	46.00	STAT3	7819.00	JAK2	0.24
13	IL2	299.91	JAK2	3.00	KDR	15.00	KDR	46.00	PIK3CD	7440.00	MMP1	0.24
14	SRC	296.80	PPARG	3.00	PTPN6	14.00	PTPN6	45.42	MAP2K1	5929.00	PIK3CD	0.24
15	MMP9	262.75	RARB	3.00	MAPK14	14.00	HCK	45.33	KDR	2580.00	MMP3	0.24
16	HDAC3	260.66	CHEK1	3.00	HCK	13.00	KIT	45.00	VEGFA	2392.00	VEGFA	0.24
17	S1PR1	237.33	MMP2	3.00	HSP90AA1	13.00	SYK	44.92	S1PR1	1538.00	KIT	0.24
18	GRB2	225.02	F10	3.00	MAP2K1	12.00	MAPK14	44.87	FGF2	1344.00	PTPN1	0.24
19	PTPN6	212.20	KDR	3.00	S1PR1	12.00	MAP2K1	44.75	HSP90AA1	1130.00	ANXA1	0.24
20	MAP2K1	205.76	HDAC3	3.00	MMP9	11.00	FGF2	44.75	EPHB4	724.00	GNRHR	0.24

Figures

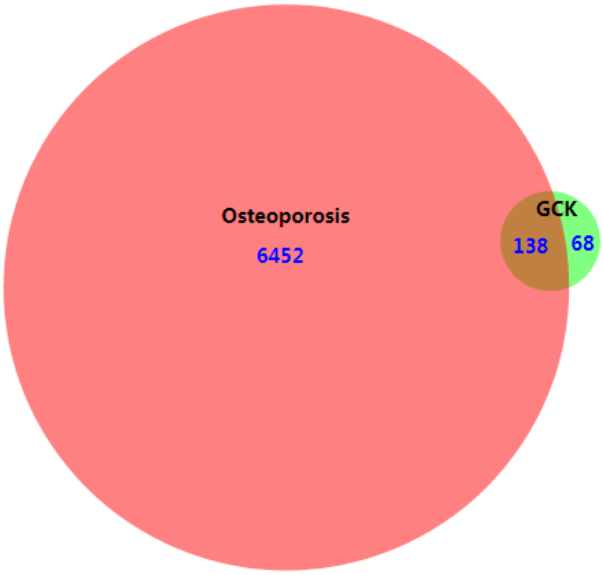

Figure 1

Co-targets of GCK-osteoporosis based on Venn diagram analysis.

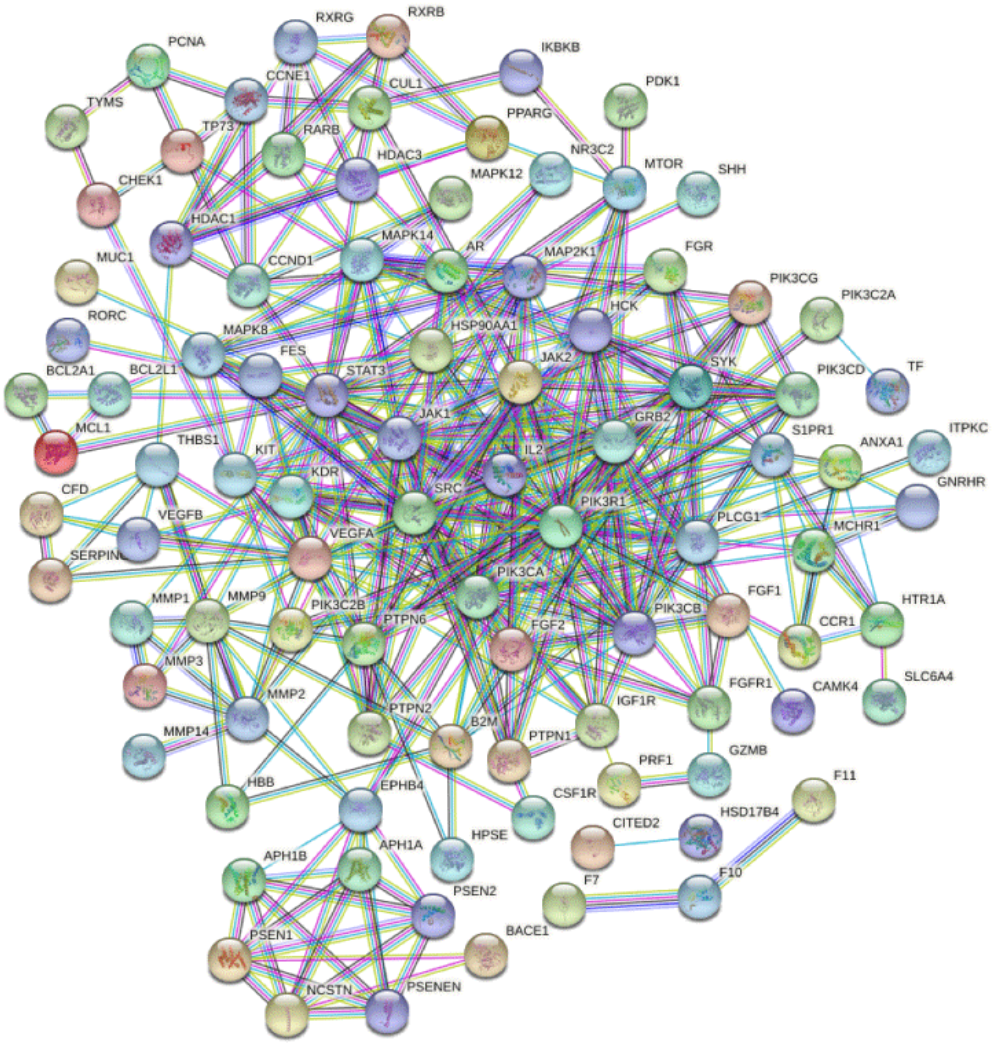

Figure 2

PPI network of co-targets of GCK-osteoporosis.

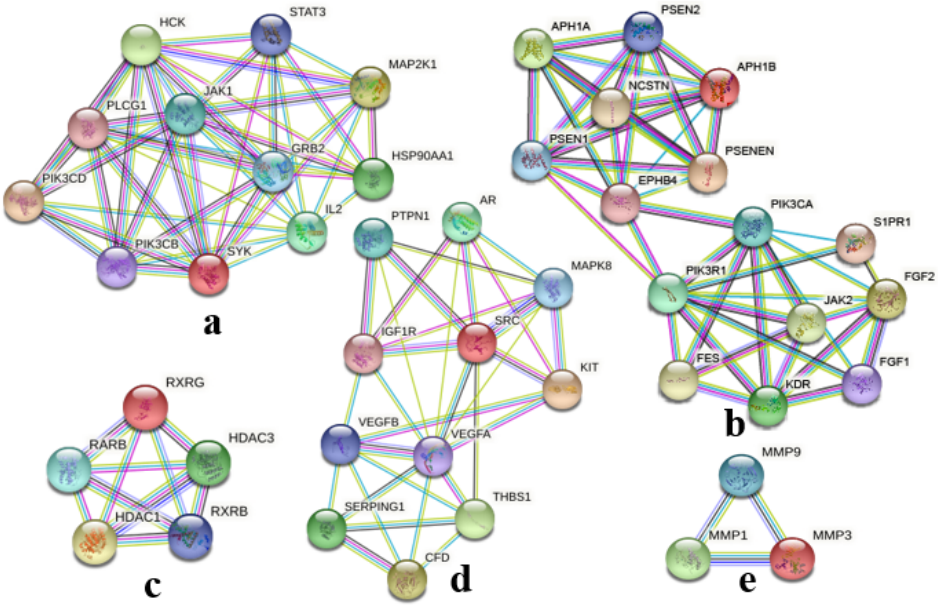

Figure 3

Central gene clusters identified in GCK-osteoporosis PPI network based on MCODE analysis.

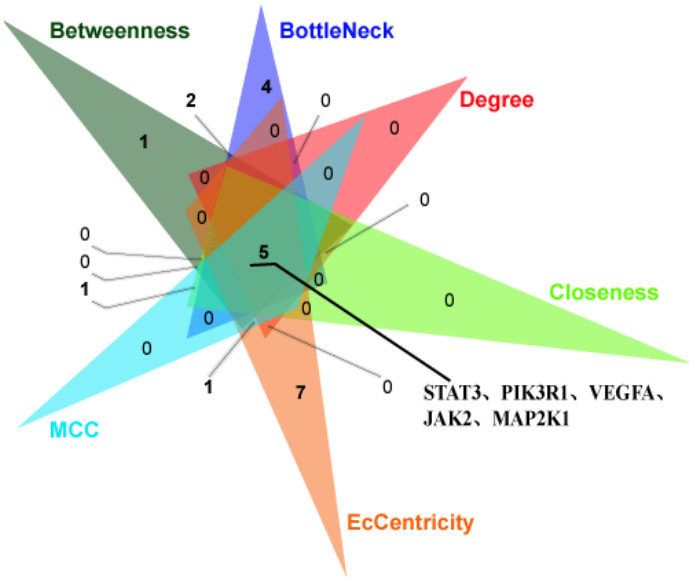

Figure 4

Hub genes identified in GCK-osteoporosis PPI network based on Betweenness, BottleNeck, Degree, Closeness, MCC and EcCentricity method.

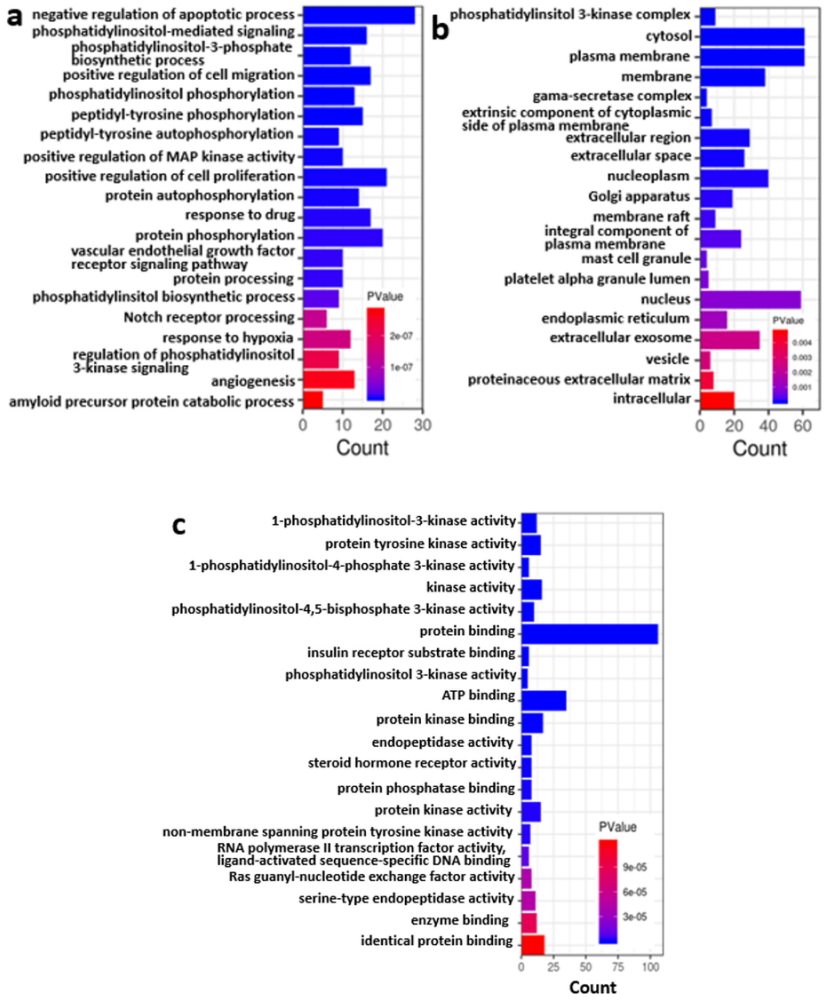

Figure 5

GO term enrichment for co-targets of GCK-osteoporosis (a. biological process; b. cellular component; c. molecular function).

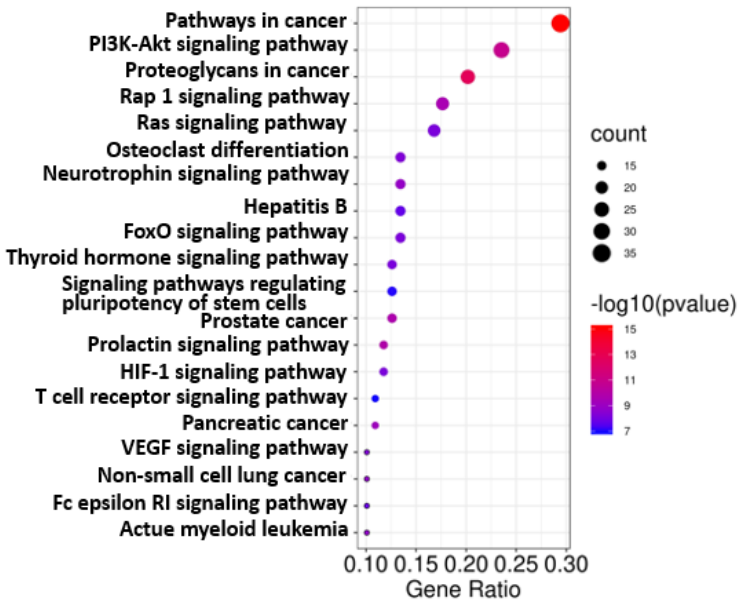

Figure 6

Top 20 pathways of KEGG enrichment analysis of co-targets of GCK-osteoporosis.

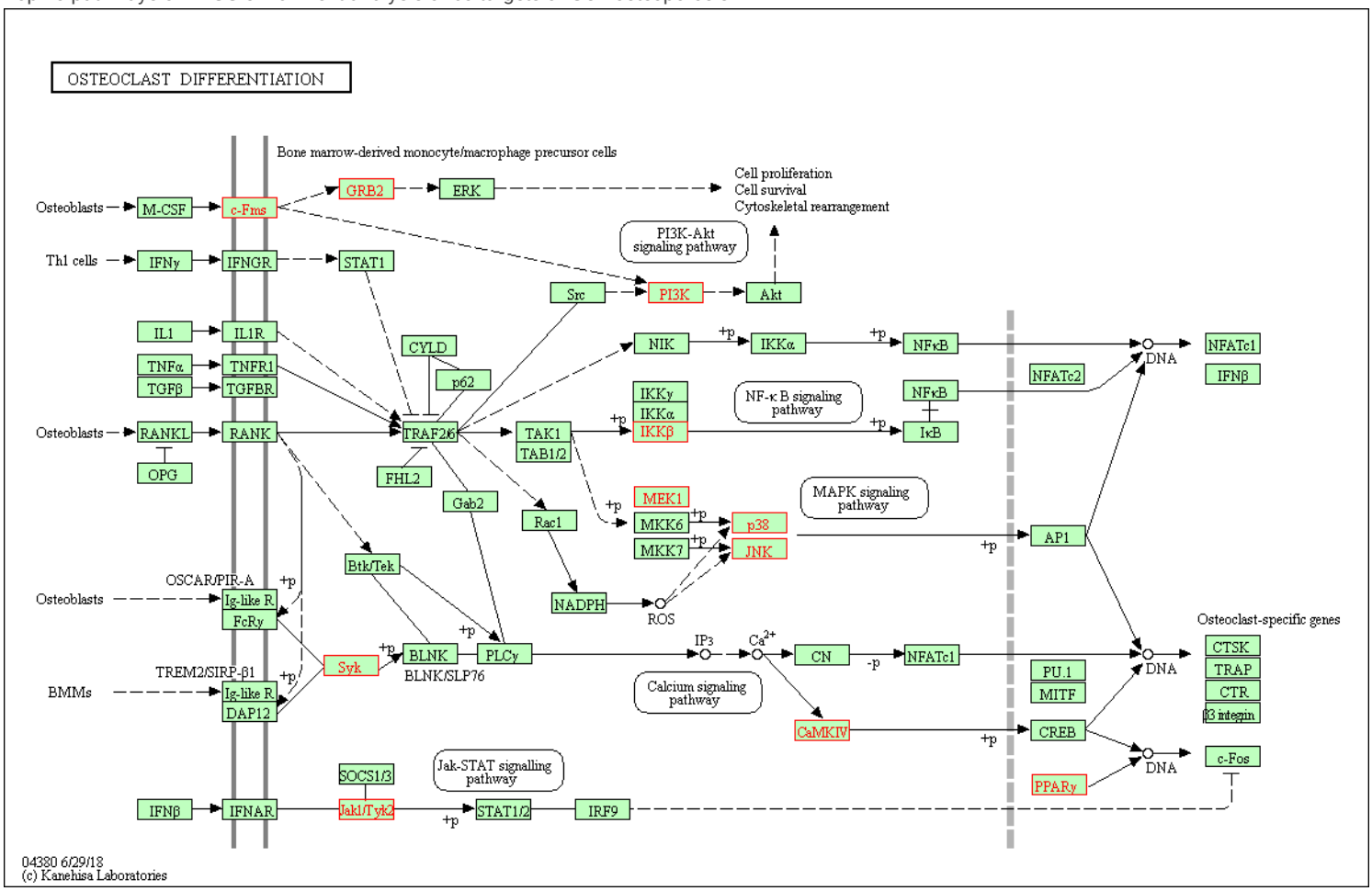

Figure 7

Osteoclast differentiation pathway enriched by co-targets of GCK-osteoporosis (the related targets were marked in red).

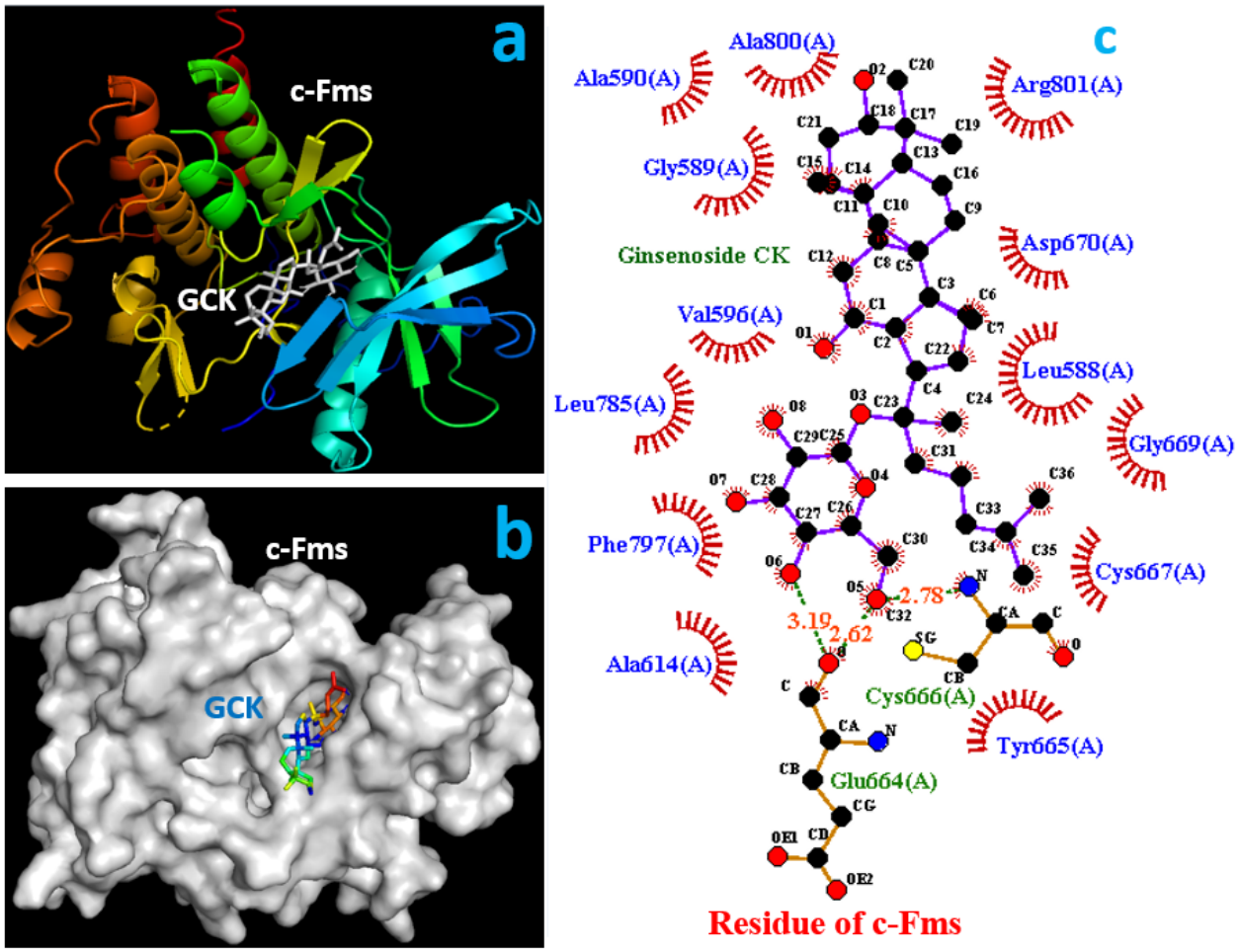

Figure 8

Molecular docking of c-Fms and GCK (a: secondary structure; b: senior structure; c: hydrogen bond and hydrophobic interaction between GCK and amino acid residues of c-Fms).

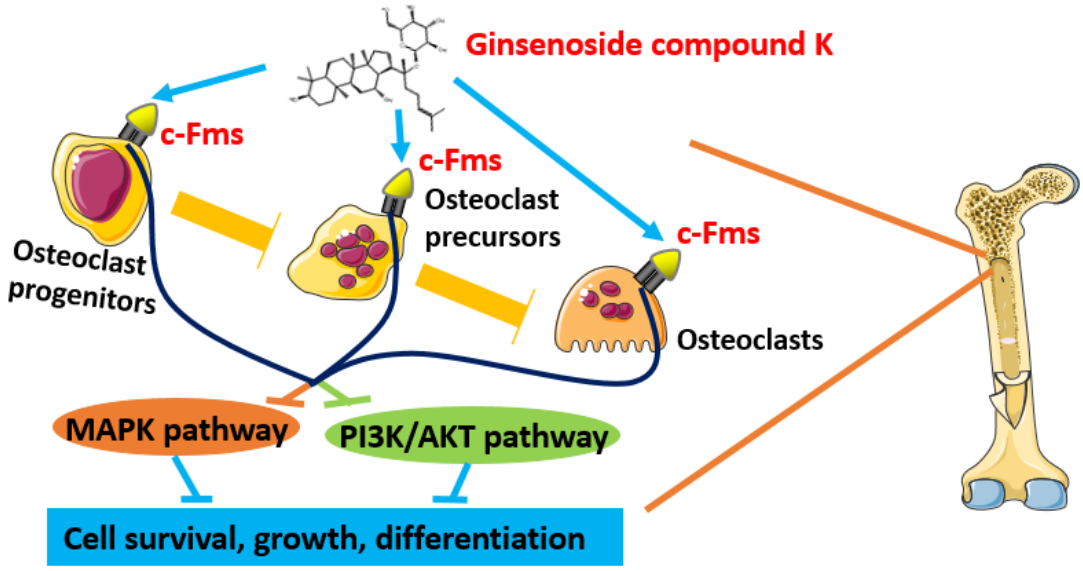

Figure 9

The putative schematic representation of the molecular mechanism of GCK in treating osteoporosis based on network pharmacology and in silico molecular docking.