Comprehensive analysis of lumbar disc degeneration and autophagy-related candidate genes, pathways, and targeting Drugs

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Abstract

Background: Lumbar disc degeneration (LDD) is a major pathological process implicated in low back pain. At present, the research in the fields of spinal surgery has highlighted the complex mechanisms underlying LDD, with autophagy being considered as one of the important processes involved.

Objectives: This study was designed to identify the potential key genes and molecular pathways associated with LDD and autophagy using computational tools and publicly available data, and to explore drugs targeting the relevant genes associated with LDD and autophagy.

Materials and Methods: We used text mining to detect the LDD and autophagy-associated genes, and the intersection of the two gene sets was selected for gene ontology analysis using the DAVID program. We then constructed protein–protein interaction networks, followed by a functional enrichment analysis, from which we obtained three significant gene modules. Finally, the final list of genes was queried against the Drug Gene Interaction database to find drug candidates targeting relevant genes associated with LDD and autophagy.

Results: Our analysis identified 72 genes common to both the “LDD” and “Autophagy” text mining concepts. Gene enrichment analysis yielded three significant gene modules (22 genes), which represent four significant pathways and could be targeted by 32 Food and Drug Administration (FDA)-approved drug molecules, and identified the drug–gene interactions.

Conclusion: In summary, we presented a method to explore the potential key genes, molecular pathways and candidate drugs associated with LDD and autophagy. As a result, in this method, we identified a total of 22 potential genes, four significant pathways and 32 candidate drugs, which could provides a basis for new trials and the development of novel targeted therapies as potential treatments for LDD.

Introduction

Lumbar disc degeneration (LDD) is a major pathological process implicated in low back pain [1]. Studies have shown that lumbar disc herniation, Lumbar stenosis, degenerative lumbar instability, and other degenerative lumbar spinal diseases, such as hyperplasia of articular processes, wedging of vertebral bodies, and spinal hyperosteogeny, are often secondary to LDD [2, 3]. LDD is related to many factors, including spine biomechanics, biology, injury, inflammation, and nutrition [4, 5]. At present, the research in the fields of spinal surgery has highlighted the complex mechanisms underlying LDD [6], with autophagy being considered as one of the important processes involved.

Autophagy is an essential lysosome-dependent cellular catabolic pathway that degrades cytoplasmic proteins, protein aggregates, and organelles. Although under certain conditions pathologically increased autophagy has been implicated in cell death, it is considered cytoprotective under most circumstances [7].

Many gene expression profiling studies have focused on LDD and autophagy in the last decade, and hundreds of candidate genes have been identified [8, 9]. These genes have different functions and are involved in a variety of processes, including biological processes (BP), cellular components (CC), molecular function (MF), signal pathways and protein–protein interaction (PPI) networks. This study was designed to identify the potential key genes and molecular pathways associated with LDD and autophagy via bioinformatics methods, and to explore drugs targeting the relevant genes associated with LDD and autophagy. First, we made a preliminary list of related genes by mining the literature. Subsequently, we performed functional and signaling pathway analyses using the online bioinformatics resource DAVID. Next, we constructed PPI networks of the common genes and identified three significant gene modules. Finally, based on the drug–gene interaction analysis of the final genes, we identified candidate drugs. Using this approach, we identified some potentially important genes, significant pathways and candidate drugs, which could provides a basis for new trials and the development of novel targeted therapies as potential treatments for LDD.

Materials And Methods

Text mining

The web-based service GenCLip3 was used to perform text mining (http://ci.smu.edu.cn/genclip3/analysis.php). When a query is performed, GenCLip3 extracts all the gene names found in the available literature related to the search concepts [10]. We performed two queries: one for the concept termed Lumbar disc degeneration (LDD), and one for the concept termed autophagy. We then extracted all the unique gene hits from each result. The intersection of these two gene sets was used in the subsequent analyses.

Gene ontology (GO) enrichment and pathway analysis

Gene ontology [11] is a structured vocabulary of terms describing gene products according to their biological process (BP), molecular function (MF), and cellular component (CC). The Kyoto Encyclopaedia of Genes and Genomes (KEGG) [12] provides data resources of known biological metabolic pathways. We used DAVID [13], a web-accessible program that integrates functional genomic annotations with intuitive graphical summaries, to view the GO and KEGG enrichment of the common genes. A p value of <0.05 was considered statistically significant, and the criterion of having a p value of <0.05 was also appropriate for big data.

PPI networks and module analysis

The Search Tool for the Retrieval of Interacting Genes (STRING, Version 11.0) [14] database was used to retrieve the common genes, encoded proteins and PPI network information. This database contains over 24.6 million proteins and 2 billion interactions observed in 5,090 organisms. We uploaded the common genes to the STRING database and set the interaction score to >0.900 (highest confidence) as the significance threshold. Following this, PPI networks were constructed using the Cytoscape software [15].

The Molecular Complex Detection (MCODE) built in Cytoscape is an automated method that was used to analyse highly interconnected modules as molecular complexes or clusters. The analysis parameters were set to default. The functional enrichment analysis was executed for the common genes, from which three significant gene modules were identified with $p < 0.05$ set as the significance threshold.

Drug-gene interaction

We used the Drug Gene Interaction Database (DGIdb, http://www.dgidb.org) to explore drug–gene interactions in the final list of genes, which were used as the potential targets in a search for existing drugs [16]. Our criteria for drug selection required FDA-approval and the presence of defined drug–gene interactions. These candidate drugs targeting the genes/pathways relevant to LDD and autophagy may represent potential treatment strategies.

Results

Text mining

Based on the data mining strategy that is described in Fig. 1, 4208 genes identified were related to autophagy, 88 genes were related to LDD, and 72 genes were common to both lists (Fig. 2 and Table 1).

GO enrichment and pathway analysis

To further explore the potential targets of these common genes in LDD and autophagy, we performed GO and pathway analyses on these common genes with the criterion of having a p value of <0.05 (Fig. 3). Figure 3 shows the top six significant terms for each of the following: the BP, CC, MF, and KEGG pathways of the common genes, respectively.

We also show the annotation of the common genes. As shown in Table 2, in the BP group, the common genes were mainly enriched for genes involved in the regulation of cell proliferation, regulation of cell death, regulation of apoptotic process, and positive regulation of phosphorus metabolic process. In the CC group, the common genes were mainly enriched for genes associated with the extracellular space, extracellular region part, proteinaceous extracellular matrix, cell surface, and extracellular matrix. In the MF group, the common genes were primarily enriched for genes associated with cytokine activity, receptor binding, cytokine receptor binding, growth factor activity, and identical protein binding. In the KEGG pathway group, the enrichment was observed for genes in the Cytokine-cytokine receptor interaction, TNF signaling pathway, HIF-1 signaling pathway, Apoptosis, Hepatitis B, and Pathways in cancer.

PPI network and module analysis

All the common genes were uploaded to the STRING website and were analysed using the Cytoscape software. A total of 70 nodes with 185 edges with scores >0.900 (highest confidence) were selected to construct the PPI networks (Fig. 4). Three significant modules were selected using the MCODE plug-in.

Module 1 consisted of 7 nodes/genes and 21 edges (Fig. 5), which were mainly associated with egulation of chemokine production (BP), extracellular space (CC), cytokine receptor binding (MF), and the MAPK signaling pathway(KEGG) (Table 3). Module 2 consisted of 9 nodes/genes and 26 edges (Fig. 6), which were mainly associated with the extracellular matrix disassembly (BP), proteinaceous extracellular matrix (CC), metalloendopeptidase activity (MF), and Estrogen signaling pathway (KEGG) (Table 4). Module 3 consisted of 6 nodes/genes and 8 edges (Fig. 7), which were mainly associated with the negative regulation of apoptotic process (BP), transferase complex, transferring phosphorus-containing groups (CC), cytokine receptor binding (MF), and MAPK signaling pathway (KEGG) (Table 5).

Drug–gene interactions

Using the final list of 22 genes, which were identified as the potential targets by the three significant modules in the drug–gene interaction analysis, 32 autophagy-regulating drugs were selected as possible molecules that can be repurposed for LDD treatment (Table 6). Potential gene targets of the drugs in this list are *STAT3 (1 drug)、TNF、11 drugs、、AKT1 (3 drugs)、 TP53、2 drugs、, IL1B、3 drugs、、 PIK3CA、5 drugs、、 MMP1、3 drugs、、MMP、2 drugs、、MMP9、2 drugs、、MMP13、3 drugs、, IL1A、1 drug、、 IL6、1 drug、* . Common previously approved uses for these drugs include the treatment of psoriasis, cancer, inflammation, Osteoarthritis, angiocarpy disease, and Idiopathic Pulmonary Fibrosis.

Discussion

We classified and summarised the final list of 22 genes, their targeted drugs, and the signaling pathways involved.

、 Genes, gene-targeted drugs, and gene-mediated signaling pathways associated with the mechanism of LDD

(1) Genes, gene-targeted drugs, and gene-mediated signaling pathways associated with catabolism in discs.

Matrix metalloproteinases (MMPs) are enzymes responsible for the degradation of almost all extracellular matrix proteins (ECM). The over-expression of MMPs or tissue inhibitors of metalloproteinases (TIMPs) may disrupt the dynamic balance of the ECM.

MMP-1: Deng et al. demonstrated that MMP-1 expression is increased in LDD, with higher expression observed in more severe cases, whereas TIMP-1 expression was similarly expressed in both normal and degenerated discs [17]. In terms of candidate drugs targeting genes, doxycycline calcium, doxycycline hyclate, and doxycycline hydrate are specific inhibitors of MMP1 and MMP13.

MMP-2: Rastogi et al. suggested that MMP-2 may have a functionally significant role in the etiology of degenerative disc disease and could be a potential therapeutic target [18]. In terms of candidate drugs targeting genes, tiludronic acid and captopril are special inhibitors of MMP-2.

MMP-9: Li et al. showed that the levels of IL-1α and MMP-9 in degenerated lumbar disc tissues are higher than normal levels, and the increasing levels are positively correlated with the disease condition [19]. In terms of candidate drugs targeting genes, glucosamine is a special antagonist of MMP-9, and captopril is a special inhibitor of it.

MMP-3 and MMP-13: Ao et al. showed that 17β-estradiol protects nucleus pulposus cells from serum deprivation-induced apoptosis and regulates expression of MMP-3 and MMP-13 through promotion of autophagy [20]. Furthermore, Wang et al. showed that BRD4 inhibition regulates MAPK, NF-κB signals, and autophagy to suppress MMP-13 expression in diabetic intervertebral disc degeneration [21].

TIMP1: This gene belongs to the TIMP gene family. The proteins encoded by this gene family are natural inhibitors of the matrix metalloproteinases (MMPs), a group of peptidases involved in degradation of the extracellular matrix. In addition to its inhibitory role against most of the known MMPs, the encoded protein is able to promote cell proliferation in a wide range of cell types, and may also have an anti-apoptotic function. Transcription of this gene is highly inducible in response to many cytokines and hormones. Kwon et al. showed that IL-8, VEGF, MMP-1, and MMP-3 were significantly increased in both cell types during hypoxia, while VCAM, TIMP-1, and TIMP-2 were decreased [22].

(2) Genes, gene-targeted drugs, and gene-mediated signaling pathways associated with anabolism in discs.

IGF1: PInsulin-like growth factor 1 (IGF-1) and its receptor (insulin-like growth factor 1 receptor, IGF1R) can regulate the extracellular matrix synthesis and play a crucial role in maintaining the normal functions of the intervertebral disc. Li et al. showed that reduced expression of IGF1R leads to accelerated intervertebral disc degeneration in mice [23]. Furthermore, Liu et al. demonstrated that IGF-1 activates PI3k/Akt signaling to antagonize lumbar disc degeneration [24].

ACAN: This gene is a member of the aggrecan/versican proteoglycan family. The encoded protein is an integral part of the extracellular matrix in cartilagenous tissue and it withstands compression in cartilage. Chen et al. showed that metformin was shown to promote the expression of anabolic genes such as Col2a1 and Acan expression while inhibiting the expression of catabolic genes such as Mmp3 and Adamts5 in nucleus pulposus cells [25].

SPARC: SPARC is a glycoprotein that has an important role in modulating interactions between cells and matrix. It influences remodeling, collagen fibrillogenesis, metalloproteinase expression, and cytokine expression. Gruber et al. showed that decreased presence of SPARC in disc cells of older subjects with disc degeneration and point to the importance of future studies designed to elucidate the unrecognized role of SPARC in disc remodeling, aging, and degeneration [26].

In conclusion, the above studies have shown that Lumbar disc degeneration (LDD) is a multi-factorial process characterized by phenotypic and genotypic changes. Prolonged imbalance between anabolism

and catabolism in discs alters their composition resulting in progressive loss of proteoglycans and hydration leading to LDD. However, activation of autophagy could regulate this imbalance to inhibit LDD.

Genes, gene-targeted drugs, and gene-mediated signaling pathways associated with the inflammatory-associated factors in LDD

The inflammatory-associated factors interleukin-1β(IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α(TNF-α) are widely reported to be associated with lumbar disc degeneration (LDD)

IL1B: The protein encoded by this gene is a member of the interleukin 1 cytokine family. This cytokine is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1 (CASP1/ICE). This cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. The induction of cyclooxygenase-2 (PTGS2/COX2) by this cytokine in the central nervous system (CNS) is found to contribute to inflammatory pain hypersensitivity. Zhang et al. showed that melatonin modulates IL-1β-induced extracellular matrix remodeling in human nucleus pulposus cells and attenuates rat intervertebral disc degeneration and inflammation [27]. In terms of candidate drugs targeting genes, canakinumab and gallium nitratel are special inhibitors of L1B.

Tumor Necrosis Factor-α(TNF-α): This gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. This cytokine is mainly secreted by macrophages. It can bind to, and thus functions through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. This cytokine is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. Wang et al showed that TNF is a key contributor to intervertebral disc degeneration [28]. Furthermore, Zuo et al. found that the inflammatory cytokine tumor necrosis factor-α (TNF-α) increased the level of intracellular reactive oxygen species (ROS) and caused cell senescence and osteogenic differentiation of cartilage endplate stem cells (CESCs), whereas rapamycin-induced autophagy protected CESCs from TNF-α-induced oxidative stress and cell senescence [29]. In terms of candidate drugs targeting genes, certolizumab pegol, etanercept, golimumab, inamrinone, infliximab, lenalidomide, pirfenidone, pomalidomide, and thalidomide are special inhibitor of TNF. Besides, pentoxifylline is a special antibody of this gene.

IL6: This gene encodes a cytokine that functions in inflammation and the maturation of B cells. In addition, the encoded protein has been shown to be an endogenous pyrogen capable of inducing fever in people with autoimmune diseases or infections. The protein is primarily produced at sites of acute and chronic inflammation, where it is secreted into the serum and induces a transcriptional inflammatory response through interleukin 6 receptor, alpha. Lin et al. showed that the expression of microRNA-21 is abnormally high in the nerve root pain of the lumbar intervertebral disc, which can increase the IL-6 inflammatory response and reduce the capacity of cell autophagy [30]. In terms of candidate drugs targeting genes, siltuximab is a specoial inhibitor of IL6.

IL-1a: The protein encoded by this gene is a member of the interleukin 1 cytokine family. This cytokine is a pleiotropic cytokine involved in various immune responses, inflammatory processes, and hematopoiesis. This cytokine is produced by monocytes and macrophages as a proprotein, which is proteolytically processed and released in response to cell injury, and thus induces apoptosis. Chen et al. showed that Interleukin-1 α (IL-1α) was thought to be involved in the pathogenesis of disc degeneration by increasing the production of extracellular matrix degradation enzymes and by inhibiting extracellular matrix synthesis [31]. Furthermore, Li et al. showed that the levels of IL-1α and MMP-9 in degenerated lumbar disc tissues are higher than normal levels, and the increasing levels are positively correlated with the disease condition [32]. In terms of candidate drugs targeting genes, rilonacept is special binder of IL1A and IL1B.

The anti-inflammatory cytokines IL-4 and IL-10：Hou et al. found that shDNMT1 significantly reduced levels of the pro-inflammatory cytokines TNFα, IL-1β and IL-6, significantly increased levels of the anti-inflammatory cytokines IL-4 and IL-10, significantly increased M2 macrophage polarization, significantly reduced cell apoptosis in the disc degeneration zone and significantly reduced LDD-associated pain [33]. Furthermore, Hanaei et al. showed that the IL-10 SNPs were significantly associated with LDD in Iranian population, which proposes that genomic alterations of anti-inflammatory cytokines could lead to homeostasis imbalance in intervertebral discs and degenerative changes [34].

STAT3：The protein encoded by STAT3 is a member of the STAT protein family. In response to cytokines and growth factors including IFNs, EGF, IL5, IL6, HGF, LIF and BMP2, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein mediates the expression of a variety of genes in response to cell stimuli, and thus plays a crucial role in many cellular processes such as cell growth and apoptosis. In terms of candidate drugs targeting genes, acitretin is a specific inhibitor of this gene.

In conclusion, the inflammatory-associated factors could accelerate disc degeneration by inducing MMPs to degrade the extracellular matrix, but activation of autophagy could reduce inflammation.

Four significant signaling pathways are associated with autophagy in LDD.

(1) Three significant signaling pathways, which reduce apoptosis and neuroinflammation by enhancing autophagy, are associated with autophagy in LDD

AMPK signaling pathway:

Jiang et al. showed that activation of autophagy via ca(2+)-dependent AMPK/mTOR pathway in rat notochordal cells is a cellular adaptation under hyperosmotic stress [35]. In addition, Wang et al. showed that resveratrol attenuated TNF-α-induced MMP-3 expression in human nucleus pulposus cells by activating autophagy via AMPK/SIRT1 signaling pathway [36]. What's more, Zhang et al. showed that naringin could reduce the incidence of oxidative stress-induced apoptosis in nucleus pulposus cells and

promoted the expression of autophagy markers LC3-II/I and beclin-1. Further study showed that autophagy regulation of naringin may be related to AMPK signaling [37].

mTOR signaling pathway:

Jiang et al. showed that glucosamine can activate autophagy via the mTOR-dependent pathway to protect nucleus pulposus (NP) cells treated with IL-1βor hydrogen peroxide (100um H_2O_2) [38]. Furthermore, Yurube et al. showed that resident disc cells may utilize autophagy and mTOR signaling to cope with harsh low-nutrient conditions, such as low glucose, low oxygen, and low pH [39].

PI3K-Akt signaling pathway:

Li et al. showed that compression stress Induces nucleus pulposus cell autophagy by inhibition of the PI3K/AKT/mTOR pathway and activation of the JNK pathway [40]. Besides, Gao et al. showed that resveratrol enhances matrix biosynthesis of nucleus pulposus cells through activating autophagy via the PI3K/Akt pathway under oxidative damage（100um H_2O_2）[41]. Furthermore, Guo et al. showed that Moracin M inhibits lipopolysaccharide-induced inflammatory responses in nucleus pulposus cells via regulating PI3K/Akt/mTOR phosphorylation [42].

In conclusion, nutrient deprivation, hyperosmotic stress, compression stress, inflammatory-associated factors, and oxidative stress can activate autophagy through different pathways, which can play a positive role after activation. These positive effects include reducing apoptosis, reducing catabolism by inhibition of matrix metalloproteinases, enhancing the matrix biosynthesis of nucleus pulposus cells, and inhibiting inflammatory responses in nucleus pulposus cells. Three significant genes, *AKT1*, *PIK3CA*, and *TNFSF10, are involved* in these signaling pathways.

AKT1: In the developing nervous system AKT is a critical mediator of growth factor-induced neuronal survival. Survival factors can suppress apoptosis in a transcription-independent manner by activating the serine/threonine kinase AKT1, which then phosphorylates and inactivates components of the apoptotic machinery. In terms of candidate drugs targeting genes, arsenic trioxide is a special inducer of AKT1, but everolimus and nelfinavir are its inhibitor.

PIK3CA: Phosphatidylinositol 3-kinase is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The protein encoded by this gene represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2. In terms of candidate drugs targeting genes, candicidin, idelalisib, oxazepam, phenmetrazine, yohimbine are special inhibitors of PIK3CA.

TNFSF10: The protein encoded by this gene is a cytokine that belongs to the tumor necrosis factor (TNF) ligand family. The binding of this protein to its receptors has been shown to trigger the activation of MAPK8/JNK, caspase 8, and caspase 3.

ERK signaling pathway:

Three significant genes, FASLG, TP53 and BDNF*, are involved in this pathway.*

FASLG: This gene is a member of the tumor necrosis factor superfamily. The primary function of the encoded transmembrane protein is the induction of apoptosis triggered by binding to FAS.

TP53: This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. In terms of candidate drugs targeting genes, bortezomib is a special inhibitor of TP53.

BDNF: This gene encodes a member of the nerve growth factor family of proteins. Alternative splicing results in multiple transcript variants, at least one of which encodes a preproprotein that is proteolytically processed to generate the mature protein. Binding of this protein to its cognate receptor promotes neuronal survival in the adult brain.

Many types of research have demonstrated that the ERK signaling pathway is closely associated with LDD. Li et al. showed that autophagy attenuates compression-Induced apoptosis of human nucleus pulposus cells via MEK/ERK/NRF1/Atg7 signaling pathways during intervertebral disc degeneration [43]. However, Chen et al. showed H_2O_2 stimulated an early autophagy response through the ERK/m-TOR signaling pathway. Autophagy inhibition significantly decreased the apoptosis incidence in the cells insulted by H_2O_2 (400UM) [44]. Besides, Ni et al. showed that TGF-β1 reduces the oxidative stress-induced autophagy and apoptosis in rat annulus fibrosus cells through the ERK signaling pathway [45].

In conclusion, oxidative stress (H_2O_2) could activate autophagy through different signaling pathways. In Chen's research, 400-μM H_2O_2 was used and caused autophagy that promoted apoptosis. By contrast, Jiang and Gao used 100-μM H_2O_2, which caused autophagy that inhibited apoptosis. From the previous studies, we can conclude that different levels of oxidative stress could lead to autophagy that play different roles.

Therefore, we can speculate the relationships between autophagy, apoptosis, and necrosis. Autophagy is bidirectional in that it can both inhibit and induce apoptosis. However, in our opinion, autophagy-induced apoptosis is not necessarily unfavorable. For severely damaged cells that cannot be saved, autophagy can accelerate their programmed apoptosis to avoid greater damage response caused by cell necrosis while reserving more energy to save the less damaged cells. In general, autophagy is a positive physiological process.

There are two limitations in the our study: Firstly, the information on the functions or roles of the final list of 22 genes have not been verified through experiments but via databases used. Thus, further molecular biological experiments are required to confirm the function of these identified genes. Secondly, not all existing gene interactions are known for a given drug. Therefore, it is possible that drugs which could

potentially be useful were missed or ignored because their gene interactions have not yet been fully elucidated.

Conclusion

In conclusion, we presented a method to explore the potential key genes, molecular pathways and candidate drugs associated with LDD and autophagy. As a result, in this method, we identified a total of 22 potential genes, four significant pathways and 32 candidate drugs, which could provides a basis for new trials and the development of novel targeted therapies as potential treatments for LDD. However, further molecular biological experiments are required to confirm the function of these identified genes, molecular pathways and candidate drugs in LDD and autophagy.

Abbreviations

LDD: Lumbar disc degeneration; BP: Biological processes; CC: Cellular components; MF: Molecular function; PPI:Signal pathways and protein–protein interaction; GO: Gene ontology; KEGG: Kyoto encyclopaedia of genes and genomes; STRING: Search tool for the retrieval of interacting genes; MCODE:Molecular complex detection; DGIdb: Drug gene interaction database; MMPs: Matrix metalloproteinases; ECM: Extracellular matrix; TIMPs: Tissue inhibitors of metalloproteinases; IGF-1: PInsulin-like growth factor 1; IL-1β: Interleukin-1β; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α; ROS: Reactive oxygen species; CESCs: Cartilage endplate stem cells; NP: Nucleus pulposus

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors read the final manuscript and approved for publication

Availability of data and materials

All data generated or analyzed during this study are included in this published article

Competing interests

The authors declare that they have no competing interests.

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Author contributions

Both authors contributed to the preparation of the manuscript and approved the final manuscript.

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Tables

Table 1. 72 common genes were identified

The common genes					
ACAN	CASP9	FASLG	IL6	MSTN	TIMP1
ADAMTS5	CCL5	FGFR1	IL6ST	NOS2	TLR4
ADIPOQ	CDKN2A	FGFR3	LEP	NOS3	TNF
AKT1	COL1A1	GSR	LIF	PCBD1	TNFRSF10A
AQP3	COL2A1	HSPA8	MAPK1	PIK3CA	TNFRSF11B
BAX	CSF1	IGF1	MIR100	PPM1D	TNFSF10
BCL2	CSF1R	IGF1R	MIR146A	PRIMA1	TP53
BDNF	CTGF	IL10	MMP1	PTH	TRPC6
BGLAP	CX3CL1	IL1A	MMP13	PTK2B	TRPV4
BMPR2	CX3CR1	IL1B	MMP2	SOX9	TSLP
CALCA	CXCL12	IL1RN	MMP3	SPARC	VDR
CASP3	FAS	IL4	MMP9	STAT3	VEGFA

Table 2. The top six pathways in GO and KEGG enrichment analysis of the common genes

Category	Term	Count	PValue
GOTERM_BP_FAT	GO:0042127~regulation of cell proliferation	46	2.12E-30
GOTERM_BP_FAT	GO:0010562~positive regulation of phosphorus metabolic process	40	1.70E-29
GOTERM_BP_FAT	GO:0045937~positive regulation of phosphate metabolic process	40	1.70E-29
GOTERM_BP_FAT	GO:0008219~cell death	48	1.79E-28
GOTERM_BP_FAT	GO:0042981~regulation of apoptotic process	43	1.85E-28
GOTERM_BP_FAT	GO:0010941~regulation of cell death	44	2.20E-28
GOTERM_CC_FAT	GO:0005615~extracellular space	37	5.19E-19
GOTERM_CC_FAT	GO:0044421~extracellular region part	44	2.75E-10
GOTERM_CC_FAT	GO:0005576~extracellular region	47	1.36E-09
GOTERM_CC_FAT	GO:0005578~proteinaceous extracellular matrix	14	1.01E-08
GOTERM_CC_FAT	GO:0009986~cell surface	18	5.52E-08
GOTERM_CC_FAT	GO:0031012~extracellular matrix	15	1.25E-07
GOTERM_MF_FAT	GO:0005125~cytokine activity	20	7.59E-20
GOTERM_MF_FAT	GO:0005102~receptor binding	36	1.05E-18
GOTERM_MF_FAT	GO:0005126~cytokine receptor binding	20	3.81E-18
GOTERM_MF_FAT	GO:0008083~growth factor activity	13	5.03E-12
GOTERM_MF_FAT	GO:0042802~identical protein binding	23	3.69E-08
GOTERM_MF_FAT	GO:0019838~growth factor binding	9	8.56E-08
KEGG_PATHWAY	hsa04060:Cytokine-cytokine receptor interaction	22	3.02E-16

KEGG_PATHWAY	hsa05200:Pathways in cancer	24	4.51E-14
KEGG_PATHWAY	hsa05161:Hepatitis B	16	8.20E-13
KEGG_PATHWAY	hsa04210:Apoptosis	12	3.07E-12
KEGG_PATHWAY	hsa04668:TNF signaling pathway	14	4.18E-12
KEGG_PATHWAY	hsa04066:HIF-1 signaling pathway	13	2.18E-11

GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table 3. Functional and Pathway Enrichment of Module 1 Genes

Category	Term	Count	PValue	Genes
GOTERM_BP_FAT	GO:1903426~regulation of reactive oxygen species biosynthetic process	6	6.31E-12	IL4, IL6, TNF, IL1B, IL10, STAT3
GOTERM_BP_FAT	GO:0032642~regulation of chemokine production	6	7.31E-12	IL4, IL6, TNF, IL1B, IL10, IL1A
GOTERM_BP_FAT	GO:0032602~chemokine production	6	1.04E-11	IL4, IL6, TNF, IL1B, IL10, IL1A
GOTERM_CC_FAT	GO:0005615~extracellular space	6	5.31E-05	IL4, IL6, TNF, IL1B, IL10, IL1A
GOTERM_CC_FAT	GO:0009897~external side of plasma membrane	3	0.004095871	IL4, IL6, TNF
GOTERM_CC_FAT	GO:0044421~extracellular region part	6	0.006314367	IL4, IL6, TNF, IL1B, IL10, IL1A
GOTERM_MF_FAT	GO:0005126~cytokine receptor binding	7	2.85E-11	IL4, IL6, TNF, IL1B, IL10, IL1A, STAT3
GOTERM_MF_FAT	GO:0005125~cytokine activity	6	3.44E-09	IL4, IL6, TNF, IL1B, IL10, IL1A
GOTERM_MF_FAT	GO:0070851~growth factor receptor binding	5	6.61E-08	IL4, IL6, IL1B, IL10, IL1A
KEGG_PATHWAY	hsa04630:Jak-STAT signaling pathway	4	1.75E-04	IL4, IL6, IL10, STAT3
KEGG_PATHWAY	hsa04668:TNF signaling pathway	3	0.00345185	IL6, TNF, IL1B

KEGG_PATHWAY	hsa04010:MAPK signaling pathway	3	0.01832436	TNF, IL1B, IL1A

Table 4. Functional and Pathway Enrichment of Module 2 Genes

Category	Term	Count	PValue	Genes
GOTERM_BP_FAT	GO:0022617~extracellular matrix disassembly	7	5.09E-13	MMP9, ACAN, MMP3, MMP13, MMP2, MMP1, TIMP1
GOTERM_BP_FAT	GO:0030198~extracellular matrix organization	8	9.66E-12	MMP9, ACAN, SPARC, MMP3, MMP13, MMP2, MMP1, TIMP1
GOTERM_BP_FAT	GO:0043062~extracellular structure organization	8	9.86E-12	MMP9, ACAN, SPARC, MMP3, MMP13, MMP2, MMP1, TIMP1
GOTERM_CC_FAT	GO:0005578~proteinaceous extracellular matrix	8	4.33E-11	MMP9, ACAN, SPARC, MMP3, MMP13, MMP2, MMP1, TIMP1
GOTERM_CC_FAT	GO:0031012~extracellular matrix	8	6.42E-10	MMP9, ACAN, SPARC, MMP3, MMP13, MMP2, MMP1, TIMP1
GOTERM_CC_FAT	GO:0005615~extracellular space	7	2.24E-05	MMP9, IGF1, SPARC, MMP3, MMP13, MMP2, TIMP1
GOTERM_MF_FAT	GO:0004222~metalloendopeptidase activity	5	1.84E-07	MMP9, MMP3, MMP13, MMP2, MMP1
GOTERM_MF_FAT	GO:0008237~metallopeptidase activity	5	1.48E-06	MMP9, MMP3, MMP13, MMP2, MMP1

GOTERM_MF_FAT	GO:0004252~serine-type endopeptidase activity	5	4.78E-06	MMP9, MMP3, MMP13, MMP2, MMP1
KEGG_PATHWAY	hsa04066:HIF-1 signaling pathway	2	0.067876196	IGF1, TIMP1
KEGG_PATHWAY	hsa04915:Estrogen signaling pathway	2	0.069936289	MMP9, MMP2
KEGG_PATHWAY	hsa04668:TNF signaling pathway	2	0.075412069	MMP9, MMP3

Table 5. Functional and Pathway Enrichment of Module 3 Genes

Category	Term	Count	PValue	Genes
GOTERM_BP_FAT	GO:1901214~regulation of neuron death	5	2.74E-07	AKT1, BDNF, TP53, PIK3CA, FASLG
GOTERM_BP_FAT	GO:0043066~negative regulation of apoptotic process	6	2.83E-07	AKT1, TNFSF10, BDNF, TP53, PIK3CA, FASLG
GOTERM_BP_FAT	GO:0043069~negative regulation of programmed cell death	6	3.02E-07	AKT1, TNFSF10, BDNF, TP53, PIK3CA, FASLG
GOTERM_CC_FAT	GO:0061695~transferase complex, transferring phosphorus-containing groups	2	0.084109658	TP53, PIK3CA
GOTERM_MF_FAT	GO:0005126~cytokine receptor binding	3	0.002992627	TNFSF10, BDNF, FASLG
GOTERM_MF_FAT	GO:0005102~receptor binding	4	0.007267044	TNFSF10, BDNF, TP53, FASLG
GOTERM_MF_FAT	GO:0051721~protein phosphatase 2A binding	2	0.0086928	AKT1, TP53
KEGG_PATHWAY	hsa04010:MAPK signaling pathway	4	4.65E-04	AKT1, BDNF, TP53, FASLG
KEGG_PATHWAY	hsa04151:PI3K-Akt signaling pathway	4	0.001159592	AKT1, TP53, PIK3CA, FASLG
KEGG_PATHWAY	hsa04150:mTOR signaling pathway	2	0.041464218	AKT1, PIK3CA

Table 5 Candidate drugs targeting genes

Number	Drug	Gene	Interaction type	Score	Approved?	Reference (PubMed ID)
1	Acitretin	*STAT3*	inhibitor	1	Yes	None found
2	Adalimumab	*TNF*	inhibitor	12	Yes	12044041
3	Arsenic trioxide	*AKT1*	inducer	6	Yes	12472888
4	Aspirin	*TP53*	acetylation	2	Yes	21475861
5	Bortezomib	*TP53*	inhibitor	1	Yes	None found
6	Canakinumab	*IL1B*	inhibitor	7	Yes	19169963
7	Candicidin	*PIK3CA*	inhibitor	3	Yes	26839307
8	Captopril	*MMP2, MMP9*	inhibitor	7	Yes	12381651
9	Certolizumab pegol	*TNF*	inhibitor	6	Yes	22917017
10	Doxycycline calcium	*MMP1, MMP13*	inhibitor	1	Yes	None found
11	Doxycycline hyclate	*MMP1, MMP13*	inhibitor	1	Yes	None found
12	Doxycycline hydrate	*MMP1, MMP13*	inhibitor	1	Yes	None found
13	Etanercept	*TNF*	inhibitor	12	Yes	10375846
14	Everolimus	*AKT1*	inhibitor	3	Yes	None found
15	Gallium nitrate	*IL1B*	inhibitor	3	Yes	16122880
16	Glucosamine	*MMP9*	antagonist	6	Yes	12405690
17	Golimumab	*TNF*	inhibitor	6	Yes	21079302
18	Idelalisib	*PIK3CA*	inhibitor	5	Yes	26466009
19	Inamrinone	*TNF*	Inhibitor	6	Yes	11805217
20	Infliximab	*TNF*	inhibitor	17	Yes	16456024
21	Lenalidomide	*TNF*	inhibitor	2	Yes	None found
22	Nelfinavir	*AKT1*	inhibitor	1	Yes	None found
23	Oxazepam	*PIK3CA*	inhibitor	1	Yes	None found
24	Pentoxifylline	*TNF*	antibody	1	Yes	None found
25	Phenmetrazine	*PIK3CA*	inhibitor	33	Yes	27672108

26	Pirfenidone	*TNF*	inhibitor	1	Yes	None found
27	Pomalidomide	*TNF*	inhibitor	2	Yes	22917017
28	Rilonacept	*IL1A, IL1B*	binder	5	Yes	23319019
29	Siltuximab	*IL6*	inhibitor	4	Yes	8823310
30	Thalidomide	*TNF*	inhibitor	11	Yes	8755512
31	Tiludronic acid	*MMP2*	inhibitor	1	Yes	None found
32	Yohimbine	*PIK3CA*	inhibitor	35	Yes	27672108

Figures

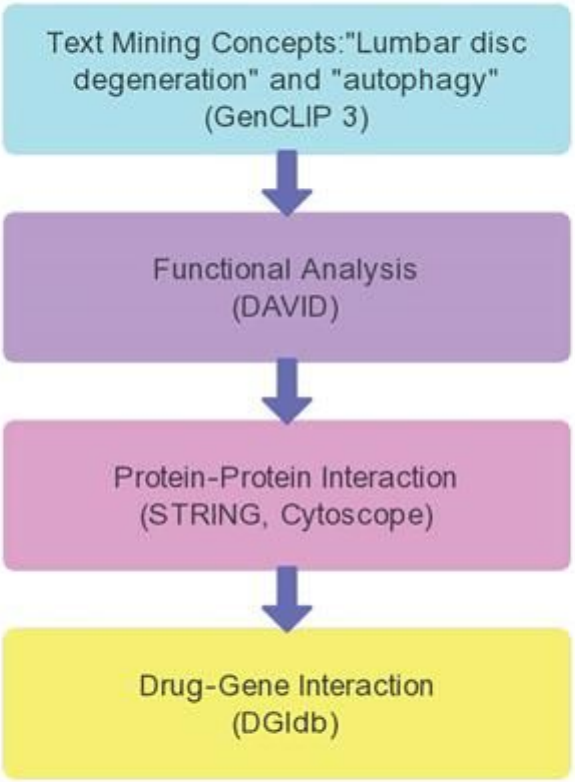

Figure 1

Overall data mining strategy

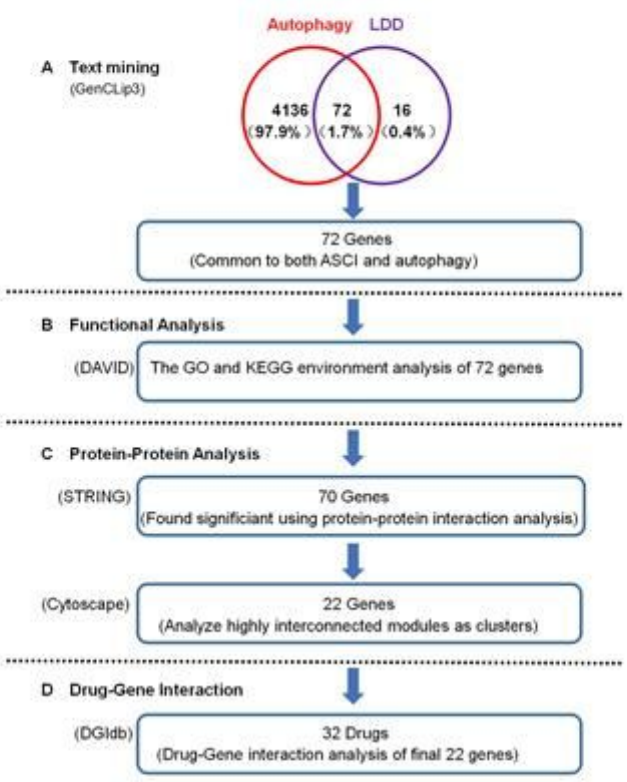

Figure 2

Summary of data mining results

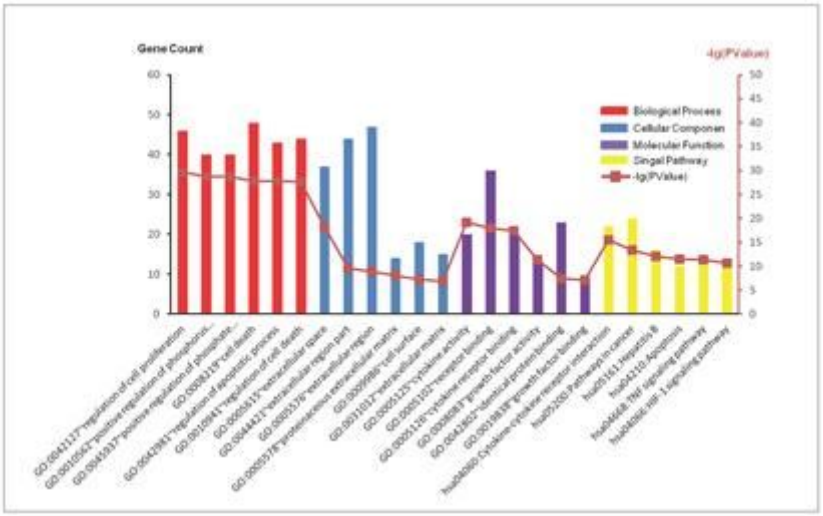

Figure 3

GO term sand KEGG pathways of the common genes

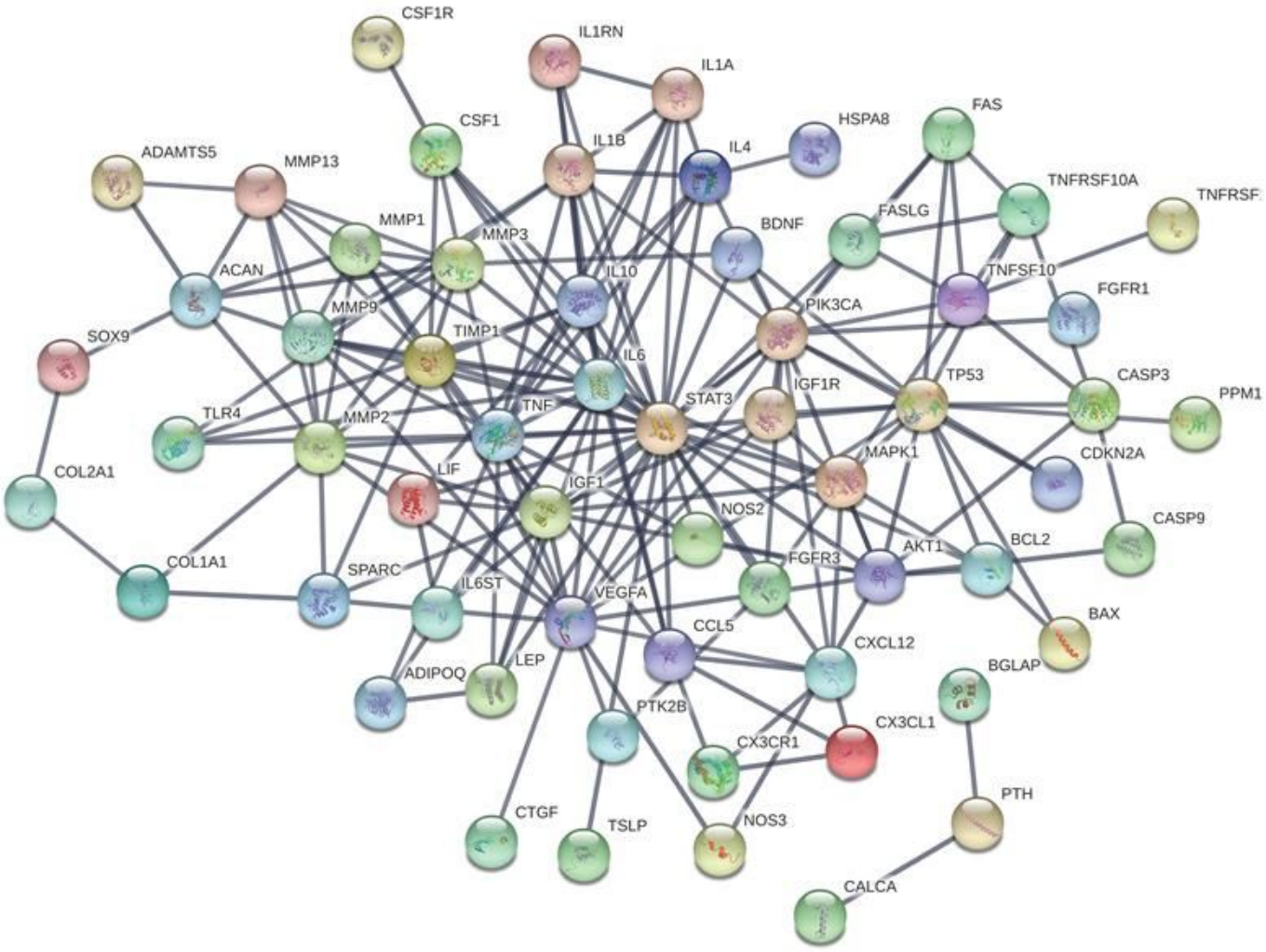

Figure 4

genes were filtere din to the PP Inetwork

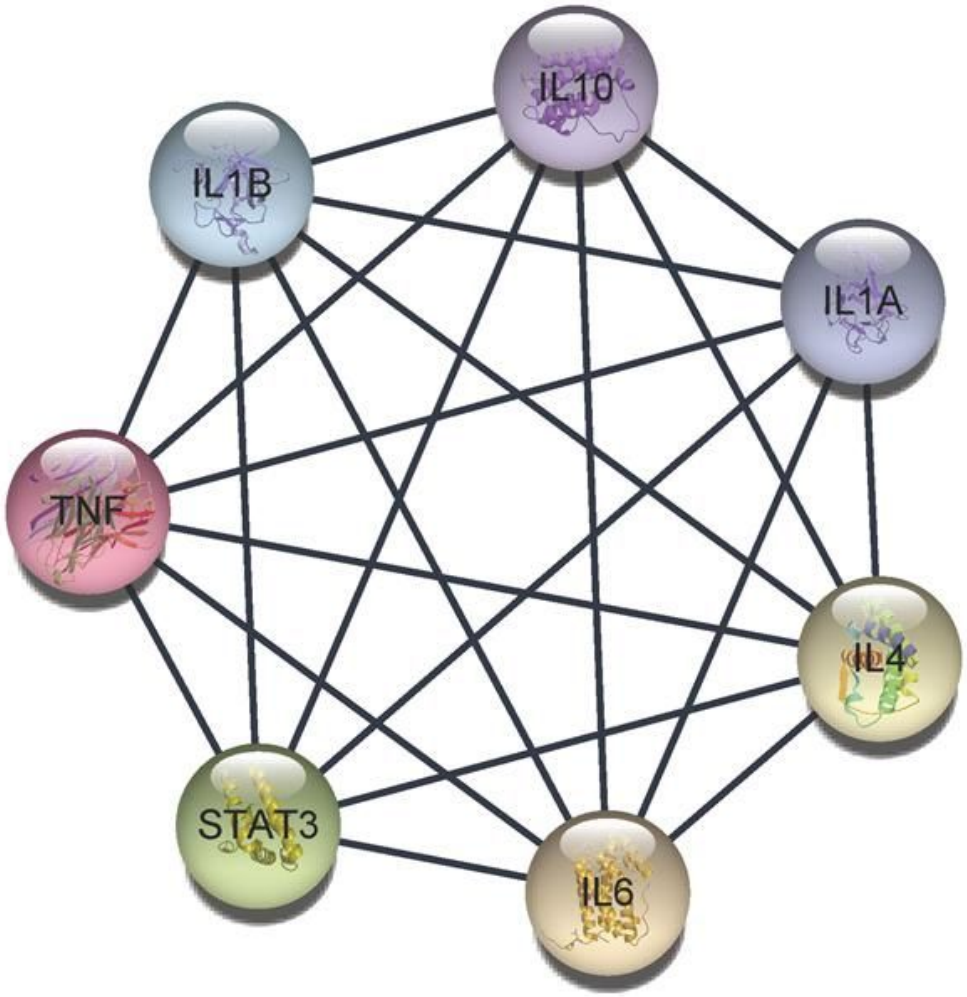

Figure 5

The most significant module 1 from the PP Inetwork

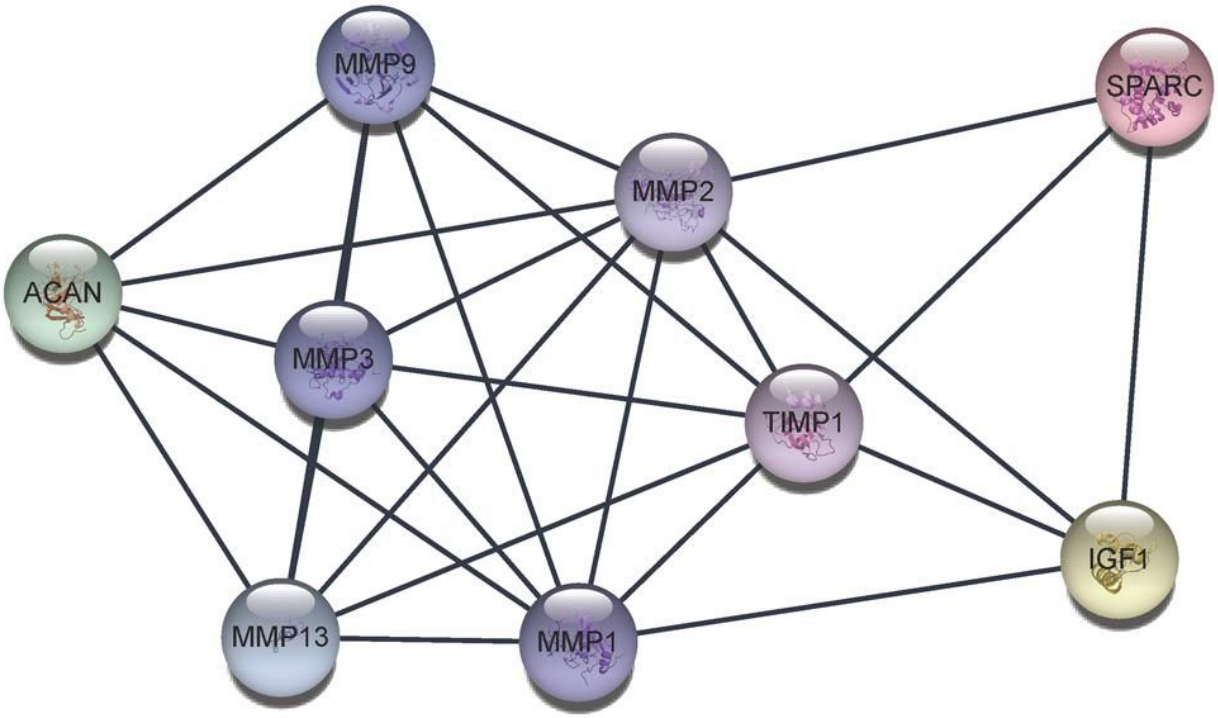

Figure 6

The second significant module 2 from the PP Inetwork

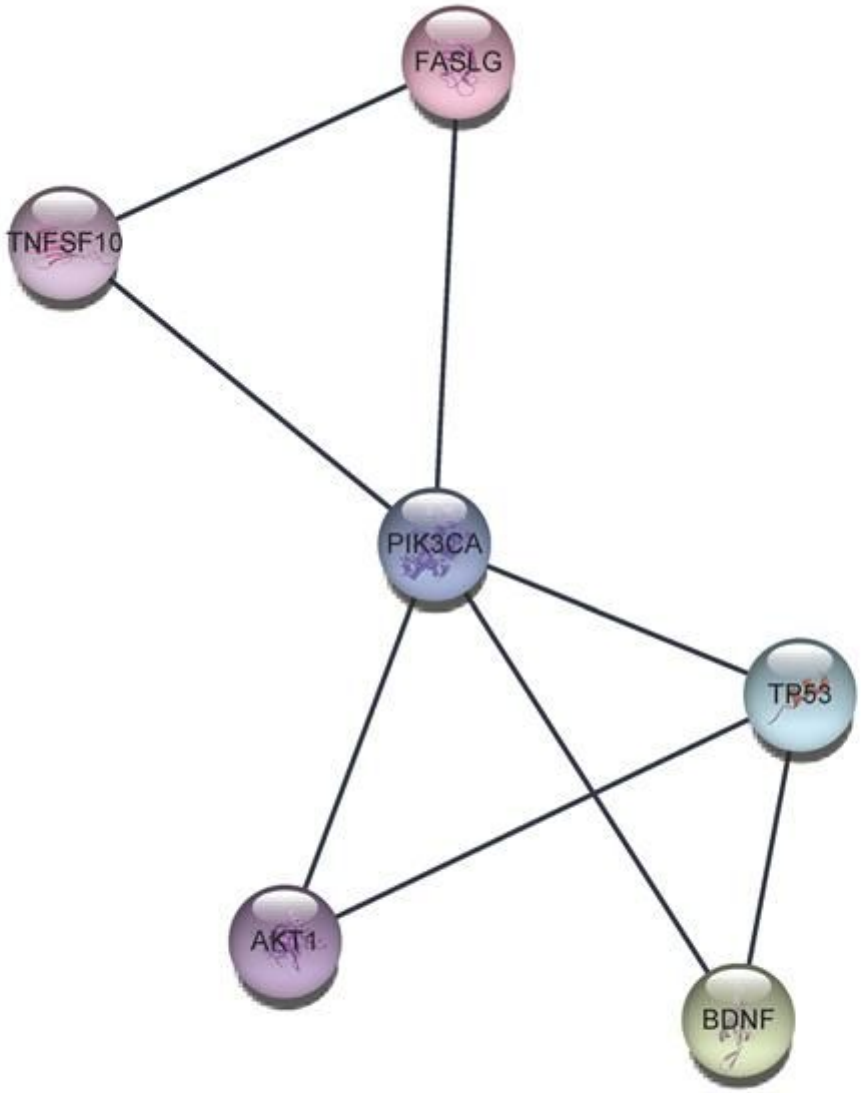

Figure 7

The third significant module 3 from the PP Inetwork