Identification of potential crucial genes in sarcopenia: a bioinformatic analysis

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Abstract

Background: Sarcopenia is a clinical syndrome characterized by continuous loss of skeletal muscle mass, muscle strength or function. Sarcopenia, which increases the risk of falls and fractures, is also closely associated with metabolic diseases and cognitive dysfunction, resulting in serious adverse effects on people's quality of life and inflicting a heavy socio-economic burden. In the present study, we aim to identify potential crucial genes associated with sarcopenia through bioinformatic analyses of public datasets.

Methods: The gene expression profiles (GSE1428) were downloaded from the Gene Expression Omnibus (GEO) database. The difference expression genes (DEGs) between sarcopenia samples and normal samples were screened. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of the DEGs were performed. Protein-protein interaction (PPI) network was constructed using the STRING database. Cytoscape with CytoHubba were used to identify the hub genes.

Results: Totally, 314 up-regulated genes and 289 own-regulated genes were identified. In addition, the most significantly enriched pathway was Cell cycle, Human T-cell leukemia virus 1 infection, Natural killer cell mediated cytotoxicity, T cell receptor signaling pathway, Signaling pathways regulating pluripotency of stem cells. In combination with the results of the protein–protein interaction (PPI) network and CytoHubba, 10 hub genes including CD4、PLK1、CHEK2、LCP2、NANOG、PTPN11、RUNX2、ANXA5、BUB1B、CCR5 were selected.

Conclusions: The 10 potential crucial genes may be associated with risk of sarcopenia. Our study provided new insights of sarcopenia into genetics, molecular pathogenesis and new therapeutic targets. It also will contribute to identification of potential biomarkers and novel strategies for sarcopenia.

Introduction

Sarcopenia involves a progressive age-related decline of skeletal muscle mass and strength/function, with an increased risk of adverse outcomes such as disability, metabolic dysfunction, poor quality of life, and mortality[1, 2]. In general, the skeletal muscle area and muscle strength of elderly individuals decreases by 2530% and 3040%, respectively, compared with those in their 20s, and muscle mass decreases by 12% each year after the age of 50[3]. While sarcopenia is mainly observed in the elderly, it can also develop in young adults[4]. Agerelated sarcopenia is termed primary sarcopenia, and diseaserelated sarcopenia is termed secondary sarcopenia[5].Among the processes contributing to age-related muscle wasting, altered hormonal status, chronic inflammation, redox imbalance, loss of α-motor neurons, muscular mitochondrial dysfunction, altered myocyte autophagy, accelerated apoptosis of myonuclei, and impaired satellite cell function are believed to be major factors[6]. Sarcopenia is a universal phenomenon with a complex and multifactorial etiology[7]. At the molecular level, sarcopenia results from a disproportionate increase in muscle protein breakdown and/or decrease in muscle protein

synthesis[8], but the mechanisms of sarcopenia are not clearly defined. Increasing morbidity and limited treatment of sarcopenia result in a huge public health and economic burden. Therefore, it is necessary to improve our understanding of sarcopenia pathogenesis and to develop better screening methods for sarcopenia.

Microarray techniques and bioinformatics analysis have been widely used to screen for genome-level differences involved in sarcopenia, enabling the identification of differentially expressed genes (DEGs) and functional pathways associated with sarcopenia. In this study, a microarray data sets were downloaded from Gene Expression Omnibus (GEO) for analysis to identify DEGs between the sarcopenia group and the normal group. We then carried out Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and protein-protein interaction (PPI) network analysis to identify the potential crucial genes.

Material And Methods

Data Source

GEO (http://www.ncbi.nlm.nih.gov/geo) [9]is a public database containing a large number of high-throughput sequencing and microarray data sets submitted by research institutes worldwide. We searched for related gene expression datasets using sarcopenia as keywords and the test specimens included should be from humans. Finally, GSE1428 microarray dataset [10] was downloaded from it (Affymetrix GPL96 platform, Affymetrix Human Genome U133 Array). The dataset contains 12 sarcopenia samples and 10 normal samples.

Identification of differentially expressed genes (DEGs)

To assess differential expression, using the “limma” package of R software, a linear model was fitted and a simple empirical Bayes model was used to moderate standard errors. [11]A oderated t-statistic and a logodds of differential expression was computed for each contrast for each gene. The Benjamini and Hochberg (BH) method was performed to adjust P value to reduce the false positive error. A gene was defined as a DEG between the sarcopenia and normal sample, when the P value was < 0.05 and $|\log2\ FC| \geq 0.5$,

Functional and pathway enrichment analyses of DEGs

Metascape *https://metascape.org/gp/index.html#/main/step1)*[12] is an analytical website that combines functional enrichment, interactome analysis, gene annotation and membership search to leverage over 40 independent knowledgebases within one integrated portal. KEGG is a database resource for elucidating high-level functions and effects of the biological system.[13, 14] GO is a major bioinformatics initiative that for high-quality functional gene annotation and analyzing gene biological processes (BP), molecular functions (MF) and cellular components (CC).[15] Metascape was used for

analyzing the function of DEGs. Min overlap = 3 and Min Enrichment = 1.5 were the screening conditions. $P < .01$ was considered statistically significant.

PPI network construction and selection of hub genes

The PPI network was analyzed using the Search Tool for the Retrieval of Interacting Genes (STRING; http://string-db.org). Analysis of functional interactions between proteins was performed in order to elucidate the mechanisms of osteogenesis and development[16]. An interaction with a combined score > 0.4 was selected and used to construct a PPI network with Cytoscape software. Cytoscape (version 3.9.1) is an open source bioinformatics software platform for visualizing molecular interaction networks.[17, 18] The top 10 genes were obtained by Degree algorithm with Cytoscape's plug-in cytoHubba. “ggplot” packages of R software was applied to generate box plots for hub genes.

Functional and pathway enrichment analyses of hub genes

To further explore the potential biological functions of the hub genes had a high correlation with disease statue, we performed Gene Ontology (GO) terms enrichment analysis by the clusterProfiler and DOSE package in R [19, 20]. A *P* value of less than 0.05 was considered statistically significant. KEGG Orthology Based Annotation System (KOBAS) (http://kobas. cbi.pku.edu.cn) [21]is a Web server for gene/protein functional annotation and functional enrichment developed by Peking University, which collects 4325 species functional annotation information. The enrichment analysis results of KEGG Pathway were obtained from the KOBAS 3.0 database. Adjusted P-value < 0.05 was considered significant.

Results

Identification of DEGs

Gene expression levels of GEO series that have been adjusted batch effects were standardized and the results of pre- and post- standardized were presented in Fig. 1A□B. 603 of DEGs with $|\log2\ FC| \geq 0.5$ in sarcopenia samples compared with normal samples was identified, including 314 up-regulated genes and 289 down-regulated genes. Volcano plot of 603 DEGs enrolled in subsequent analyses was showed in Fig. 1C.

Analysis of the Functional Characteristics of DEGs

In order to analyze the biological functions and pathways involved in the 603 DEGs, GO and KEGG Pathway enrichment analysis were performed. GO analysis results show that these genes were mainly enriched in protein binding, nucles, cytosol, cytoplasm, nucleoplasm(Fig. 2A). In terms of KEGG Pathway, the three enrichment pathways are gulation of actin cytoskeleton, MP signaling pathway, protein interaction with cytokine and cytokine receptor. (Fig. 2B).

PPI network construction and selection of hub genes

The PPI network of DEGs and most dense connected regions (470 nodes, 1096 edges) were obtained by Cytoscape (Fig. 3A). Through the Degree algorithms of plug-in cytoHubba, we have calculated the top 10 hub genes, including CD4,PLK1,CHEK2,LCP2,NANOG,PTPN11,RUNX2,ANXA5,BUB1B,CCR5(Fig. 3B). Boxplots for the 10 hub genes were shown in Fig. 4. Their full names and related functions were showed in Table 1.

Table 1
Ten hub genes and their functions

Gene symbol	Description	Function
CD4	CD4 molecule	This gene encodes the CD4 membrane glycoprotein of T lymphocytes. The CD4 antigen acts as a coreceptor with the T-cell receptor on the T lymphocyte to recognize antigens displayed by an antigen presenting cell in the context of class II MHC molecules.
PLK1	polo like kinase 1	The Ser/Thr protein kinase encoded by this gene belongs to the CDC5/Polo subfamily. It is highly expressed during mitosis
PTPN11	protein tyrosine phosphatase non-receptor type 11	The protein encoded by this gene is a member of the protein tyrosine phosphatase (PTP) family. PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation.
NANOG	Nanog homeobox	The protein encoded by this gene is a DNA binding homeobox transcription factor involved in embryonic stem (ES) cell proliferation, renewal, and pluripotency.
CHEK2	checkpoint kinase 2	In response to DNA damage and replication blocks, cell cycle progression is halted through the control of critical cell cycle regulators. This nuclear protein is a member of the CDS1 subfamily of serine/threonine protein kinases.
LCP2	lymphocyte cytosolic protein 2	This gene encodes an adapter protein that acts as a substrate of the T cell antigen receptor (TCR)-activated protein tyrosine kinase pathway. The encoded protein associates with growth factor receptor bound protein 2, and is thought to play a role TCR-mediated intracellular signal transduction.
ANXA5	annexin A5	The protein encoded by this gene belongs to the annexin family of calcium-dependent phospholipid binding proteins some of which have been implicated in membrane-related events along exocytotic and endocytotic pathways.
BUB1B	BUB1 mitotic checkpoint serine/threonine kinase B	This gene encodes a kinase involved in spindle checkpoint function. The protein has been localized to the kinetochore and plays a role in the inhibition of the anaphase-promoting complex/cyclosome (APC/C), delaying the onset of anaphase and ensuring proper chromosome segregation.
CCR5	C-C motif chemokine receptor 5	This gene encodes a member of the beta chemokine receptor family, which is predicted to be a seven transmembrane protein similar to G protein-coupled receptors.
RUNX2	RUNX family transcription factor 2	This gene is a member of the RUNX family of transcription factors and encodes a nuclear protein with an Runt DNA-binding domain. This protein is essential for osteoblastic differentiation and skeletal morphogenesis and acts as a scaffold for nucleic acids and regulatory factors involved in skeletal gene expression.

Functional and pathway enrichment analyses of hub genes

GO analysis showed that these genes are mainly involved in cell cycle checkpoint signaling, negative regulation of cell cycle phase transition, negative regulation of cell cycle process, negative regulation of cell cycle, regulation of cell cycle phase transition, regulation of mitotic cell cycle(Fig. 5A). In addition, KEGG Pathway analysis showed that they are mainly involved in Cell cycle, Human T-cell leukemia virus 1 infection, Natural killer cell mediated cytotoxicity, T cell receptor signaling pathway, Signaling pathways regulating pluripotency of stem cells(Fig. 5B).

Discussion

In 1989, Rosenberg emphasized the importance of the loss of skeletal muscle mass that occurs with aging and coined the term 'sarcopenia'[22]. Since then, sarcopenia has attracted considerable attention due to the aging population. This muscle loss is associated with increased risk of adverse health outcomes, including falls, morbidity, loss of independence, disability, and mortality[2]. Thus, it is necessary to screen the key genes and pathways related to the progression of sarcopenia. In the present study, we integrated gene expression profiles of 12 sarcopenia samples and 10 normal samples from a GEO dataset (GSE1428) and analyzed the data using bioinformatics tools. A total of 603 DEGs with $|\log2 FC| \geq 0.5$ in sarcopenia compared with normal samples were selected. Furthermore, 10 potential crucial genes (CD4,PLK1,CHEK2,LCP2,NANOG,PTPN11,RUNX2,ANXA5,BUB1B,CCR5), and several important pathways, which were associated with sarcopenia risk, were identified, suggesting these may play important role in the mechanism of sarcopenia.

T lymphocytes are essential for skeletal muscle regeneration and repair[23]. Lymphocytes and their secreted proteins play a crucial role in muscle repair and regeneration, considered as a potential control switch for this process[24]. T Lymphocytes can also affect the proliferation and migration of muscle satellite cells[25], which may be associated with reduced muscle mass in patients with sarcopenia[26]. CD4 can regulate the sensitivity and output of T cell responses and most critically contribute to the T cell function in *vivo* and thus are known as coreceptors of T-cell receptors(TCR).It also contribute to T-cell development, homeostasis and antigenic response. It has extremely low affinity for its ligand but which is also essential in T-cell development and in the removal of pathogens during T cell-dependent immune responses.[27] Bharath et al indicated that aging compromises autophagy in CD4 + T lymphocytes to stimulate the secretion of several pro-inflammatory interleukins, thus contributing to inflammaging[28]. Lymphocyte cytosolic protein 2 (LCP2) is one of the SLP-76 family of adapters, which are critical intermediates in signal cascades downstream of several receptors. LCP2 regulates immunoreceptor signaling (such as T-cell receptors) and it is also required for integrin signaling in neutrophils and platelets[29]. It plays an important role in NK-cell mediated recognition of missing-self targets10 and positively regulates antigen-induced mast cell activation by recruiting BCR[30]. Quantitative reductions of LCP2 trigger immune dysregulation with the excessive production of proinflammatory cytokines and autoantibodies[31]. These studies indicate that LCP2 deeply participates in immune responses through the regulation of immune cells[29]. We found that CD4 and LCP2 were enriched in KEGG term of T cell receptor signaling pathway. These results indicated that CD4 and LCP2 might be the potential biomarkers of sarcopenia.

In our study,CD4 and C-C motif chemokine receptor 5(CCR5)were enriched in KEGG term of Cytokine-cytokine receptor interaction and Human immunodeficiency virus 1 infection.CCR5 and its ligands may play a role in various inflammatory diseases, as cellular activation of CCR5 normally happens through chemokine binding, which then regulate intracellular trafficking and protective cellular and humoral responses. Indeed, the migration of lymphocytes to inflammatory areas is controlled by chemokine gradients[32]. CCR5 is expressed in inflammatory cells infiltrating the central nervous system in *vivo*[33]. It is also expressed on immune cells within inflammatory lesions in multiple sclerosis(MS) and may contribute to recruitment of these cells to the inflamed tissue or to their activation.

Polo-like kinase 1 (PLK1) is a conserved mitotic serine-threonine protein kinase, functions as a regulatory protein, and is involved in the progression of the mitotic cycle. It plays important roles in the regulation of cell division, maintenance of genome stability, in spindle assembly,mitosis, and DNA-damage response.[34] A role for PLK1 has been recently described in diverse immune disorders including Graft versus Host Disease, where it contributes to immunological responses of alloreactive T cells.[35] Also, in neurological disorders such as Huntington's disease and Alzheimer's disease, activity of PLK1 is altered in affected neurons.[36] checkpoint kinase 2(CHEK2)kinase was reported to be involved in the processes of DNA damage repair (DDR) and cell cycle regulation.It also has been linked to cell protection via autophagy in response to oxidative stress.High levels of reactive oxygen species (ROS) and hypoxia were reported to trigger the ATM-CHK2 axis and the phosphorylation of Beclin 1[37]. BUB1 mitotic checkpoint serine/threonine kinase B (BUB1B) is a conserved multifunctional protein that is vital for the function of mitotic spindle checkpoint and correcting kinetochore-microtubule attachments[38]. Our study revealed that the PLK1、CHEK2、BUB1B expression level was down-regulated in sarcopenia samples compared to normal samples. Enrichment analyses in the present study indicated that PLK1、CHEK2and BUB1B were enriched in KEGG term of cell cycle. Yin J et al. indicated that the differentiation of satellite cells is a fundamental process for the maintenance of muscle trophism[39]. The multi-signal pathway cascade regulates the cell cycle process, and plays a crucial role in promoting myoblasts growth[40]. Thus, downregulation of PLK1, CHEK2and BUB1B may inhibit the growth of satellite cells, leading to sarcopenia.

Nanog homeobox(NANOG) is a stem cell transcription factor that plays a major role in regulation of human development. It is involved in cell fate determination, proliferation, and apoptosis.[41] Notably, ectopic expression of NANOG preserved the morphology and restored the myogenic differentiation capacity of late passage myoblasts, possibly by restoring the expression level of myogenic factors[42].So, NANOG might be the a potential biomarker of sarcopenia.

It have also confirmed that testosterone can promote the number of stellate cells in a dose-dependent manner and is the main regulator of their function in vitro[43].Moreover, testosterone levels in men decrease by about 1% a year with age, which plays an important role in the development of sarcopenia[44].ANXA5, also known as Annexin A5, is a member of the annexin family of proteins,which can bind to calcium and phospholipid, acting as an endogenous regulator of various physiological processes[45] .Moreover, Yao et al. found ANXA5 was highly expressed in rat Leydig and Sertoli cells and

could involve in the regulation of testosterone synthesis and secretion[46]. Gidrol et al. found that ANXA5 might exert antioxidant function in some cases[47]. Ewing et al. found that ANXA5 been believed closely related to inflammatory response and cell apoptosis[48, 49].Similarly, Protein tyrosine phosphatase non-receptor type 11(PTPN11) mutations induce metabolic changes that increase the ability of mutated cells to utilize multiple energy sources.[50] It is involved in numerous signal transduction functions important for normal hematopoiesis, including proliferation,differentiation, and apoptosis[51]. In Leydig cells, PTPN11 supports mitochondrial fusion and the expression of acyl-CoA synthetase (ACSL4) needed for the production of steroids including testosterone[52].

Bones also affect muscles through mechanical and chemical processes. Osteoblasts or factors secreted by osteocytes, such as osteocalcin, osteostin and fibroblast growth factor – 23, may regulate muscle. Osteocalcin may also affect glucose, lipid and energy metabolism, thereby affecting muscle function[53].Runt-related transcription factor 2 (RUNX2) was first described as an essential regulator of skeletal development. Post-translational regulation modulates the activity, stability and function of many proteins including transcription factors. Phosphorylation, acetylation, and ubiquitination are the most representative post-translational modifications and also phosphorylation-directed conformational changes by enzymes play key roles in regulating protein activity. [54]

In current study, we have discussed that 10 potential crucial genes are involved in the currence and development of sarcopenia, suggesting these genes may serve as potential biomarkers and therapeutic targets for sarcopenia. However, the limitations of this study should be considered. First of all, this is a retrospective study that requires external verification to verify our findings; Secondly, the function of the hub gene needs to be further verified in an in vitro model, which will be the focus of our future work.

Conclusion

Our study identified 9 potential crucial genes (CD4,PLK1,CHEK2,LCP2,NANOG,PTPN11,RUNX2,ANXA5,BUB1B,CCR5), and pathways using bioinformatic analyses. The exploration of potential crucial genes of sarcopenia may provide some potential aid in further identification of new biomarkers for the susceptibility of sarcopenia and useful treatment targets.

Abbreviations

PKP : percutaneous kyphoplasty; PVP :percutaneous vertebroplasty;

PCKP :percutaneous kyphoplasty; PMMA :polymethyl methacrylate; OVCF :osteoporotic vertebral compressive fracture; DR□digital radiography; MRI: magnetic resonance imaging; CT: computed tomography; DSA :digital subtraction angiography.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

GSE1428 dataset for this study is openly available in Gene Expression Omnibus database at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE1428.

Competing interest

The authors declare that they have no competing interests.

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Authors' contributions

YS collected the data and wrote the report. NL had the initial idea for the research and is guarantor. JX assisted with the writing of the report . RC had contributed in conception. SG had contributed in revising the manuscript. YS has contributed in acquisition of data. XQ,YL and XM took part in the discussion of the paper. All authors read and approved the final manuscript.

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Figures

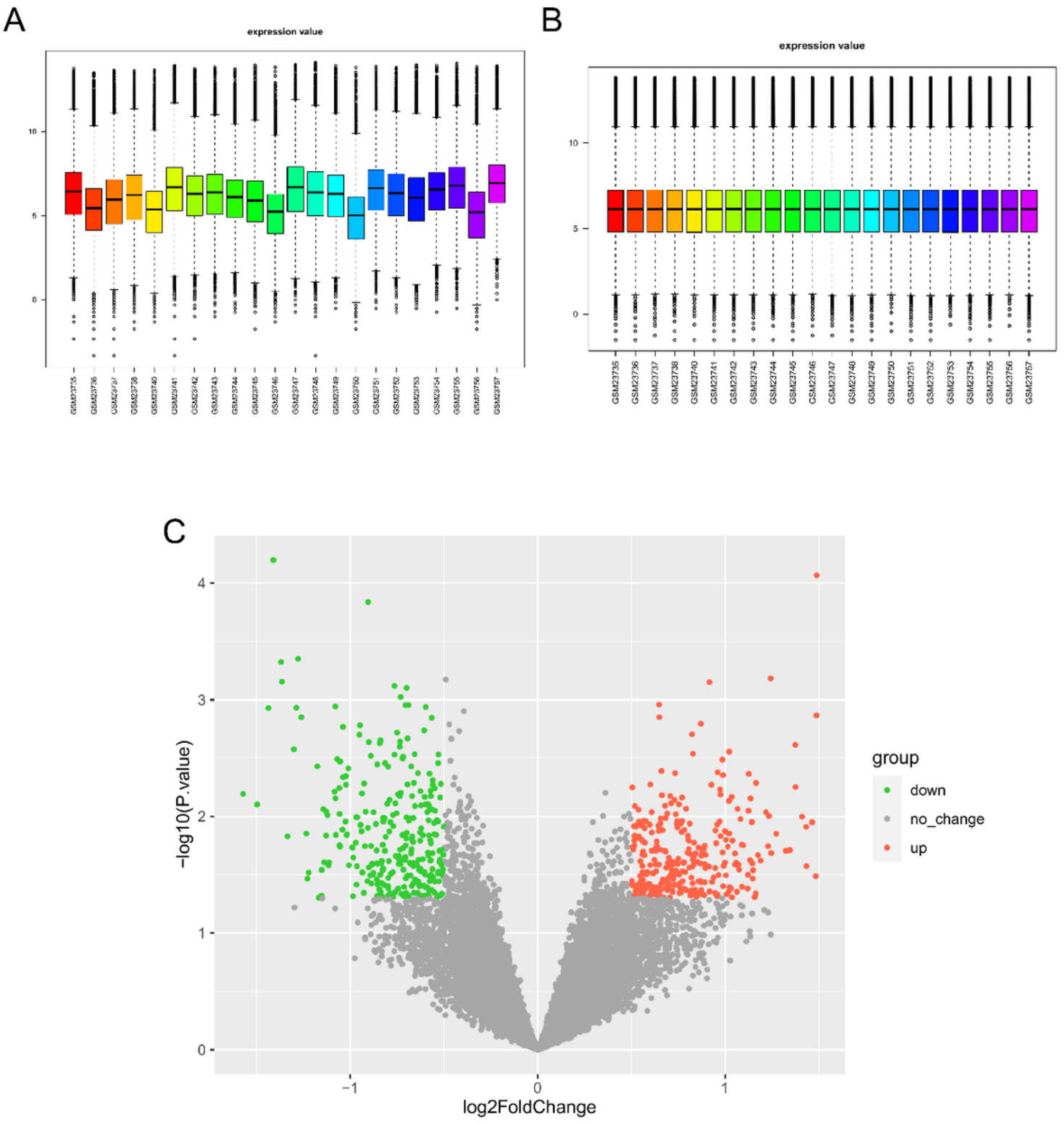

Figure 1

The results of pre- standardized (A) and post- standardized(B) gene expression levels. A volcano plot (C) showing the 603 differentially expressed genes. Red colour indicates up-regulated genes, and green indicates down-regulated genes.

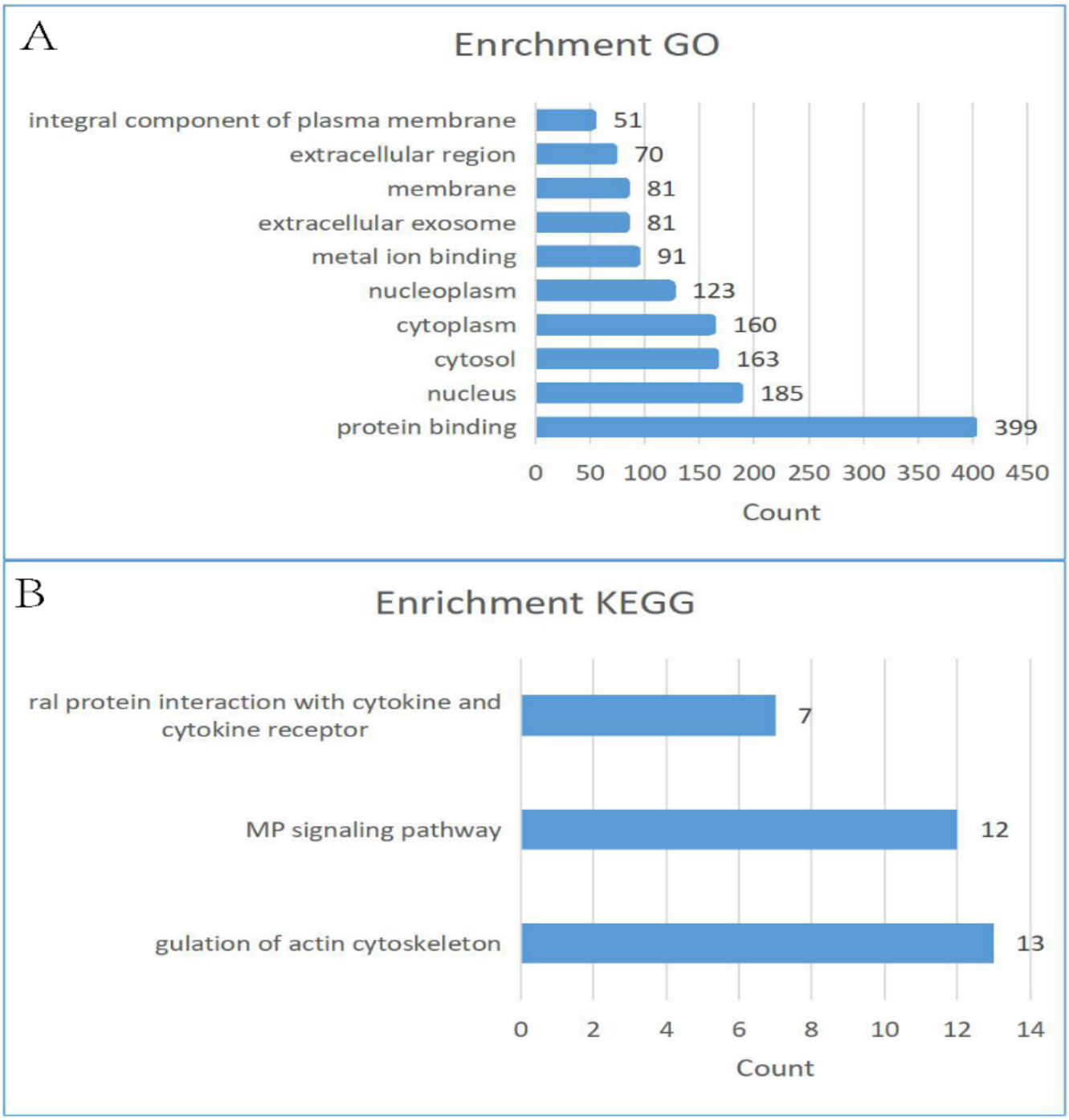

Figure 2

Common DEGs enrichment analysis results. (A, B) The enrichment analysis results of GO and KEGG Pathway. Adjusted P-value < 0.05 was considered significant.

Figure 3

PPI network diagram and hub genes. A, The greater the difference in expression, the darker the colour. The size of nodes represents the difference in expression; the larger the size, the more significant the P value. B, Ten hub genes were identified in the densest connected regions with Degree algorithm, using cytoHubba. The score is indicated in red colour. Darker colour indicates a higher score.

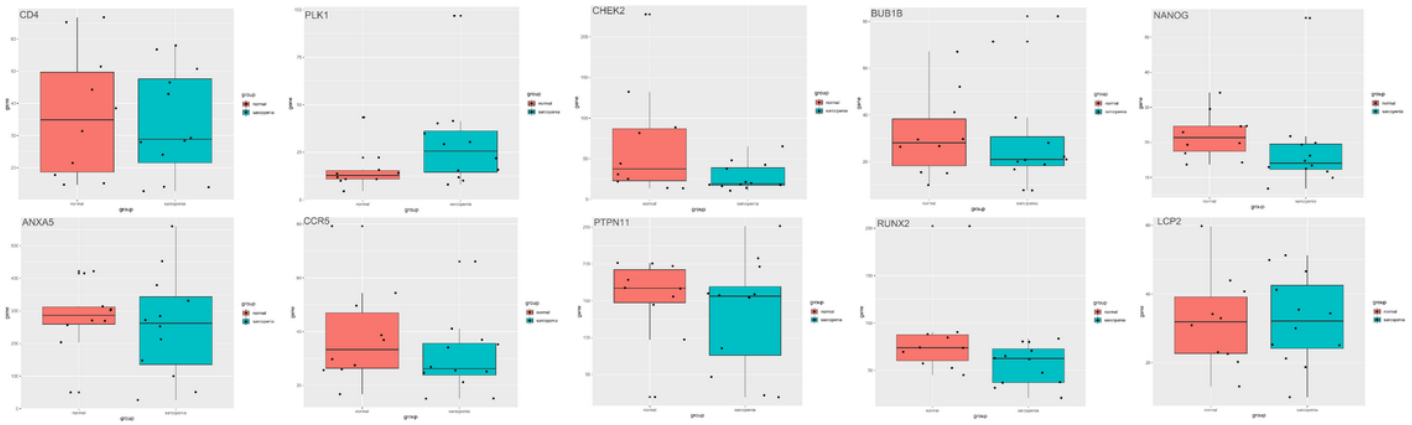

Figure 4

Boxplots of gene expressions for 10 hub genes.

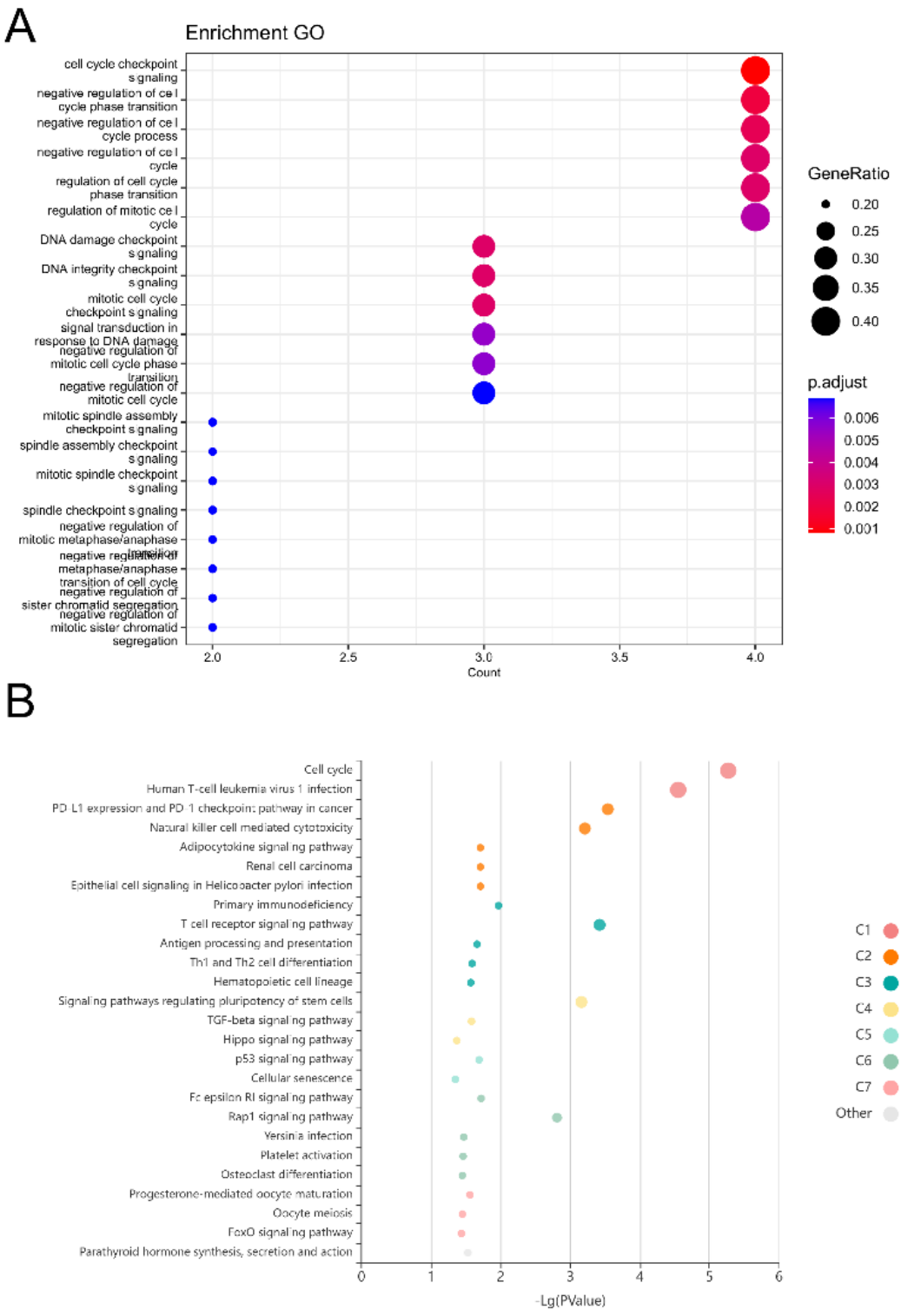

Figure 5

GO and KEGG enrichment analysis of the hub genes. The size of the circle represents the number of genes involved.