High expression of MAPK-14 promoting the death of chondrocytes is an important signal of osteoarthritis process

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Background Osteoarthritis (OA) is one of the most common degenerative diseases worldwide. Many researchers are studying the pathogenesis of OA, however, it is still unclear. **Methods** Screening and validation of OA relevant hub genes are an important part of exploring their potential molecular mechanism. Therefore, this study aims to explore and verify the mechanisms of hub genes in the OA by bioinformatics, qPCR, fluorescence and propidium iodide staining. **Results** Microarray datasets GSE43923, GSE55457 and GSE12021 were collected in the Gene Expression Omnibus (GEO), including 45 samples, which divided into 23 osteoarthritis knee joint samples and 22 samples of normal knee joint. Thereafter, 265 differentiallyexpressedgenes (DEGs) were identified in all, which divided into 199 upregulated genes and 66 downregulated genes. The hub genes MAPK-14, PTPRC, PTPN12 were upregulated, while B9D1 was downregulated. In order to further confirm the expression of screening differential genes in human chondrocytes, the human chondrocytes were extracted from a joint replacement surgery and stained with toluidine blue for identification. Compared with normal chondrocytes, OA chondrocytes had high expression of COL I protein and low expression of COL II protein. The expression levels of MAPK-14, PTPRC and PTPN12 in OA chondrocytes were significantly higher than the expression levels of B9D1 in normal chondrocytes. Moreover, the inflammatory necrosis of OA chondrocytes was increased compared with the normal chondrocytes by propidiumiodide staining. **Conclusions** The high expression of MAPK-14 works as a promoter of chondrocytes death and an important signal of the osteoarthritis process.

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3   **of osteoarthritis process**

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Abstract

24   **Background** Osteoarthritis (OA) is one of the most common degenerative diseases worldwide.

25   Many researchers are studying the pathogenesis of OA, however, it is still unclear.

26 **Methods** Screening and validation of OA relevant hub genes are an important part of exploring

27 their potential molecular mechanism. Therefore, this study aims to explore and verify the

28 mechanisms of hub genes in the OA by bioinformatics, qPCR, fluorescence and propidium

29 iodide staining.

30 **Results** Microarray datasets GSE43923, GSE55457 and GSE12021 were collected in the Gene

31 Expression Omnibus (GEO), including 45 samples, which divided into 23 osteoarthritis knee

32 joint samples and 22 samples of normal knee joint. Thereafter, 265 differentially expressed genes

33 (DEGs) were identified in all, which divided into 199 upregulated genes and 66 downregulated

34 genes. The hub genes MAPK-14, PTPRC, PTPN12 were upregulated, while B9D1 was

35 downregulated. In order to further confirm the expression of screening differential genes in

36 human chondrocytes, the human chondrocytes were extracted from a joint replacement surgery

37 and stained with toluidine blue for identification. Compared with normal chondrocytes, OA

38 chondrocytes had high expression of COL I protein and low expression of COL II protein. The

39 expression levels of MAPK-14, PTPRC and PTPN12 in OA chondrocytes were significantly

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41 necrosis of OA chondrocytes was increased compared with the normal chondrocytes by

42 propidium iodide staining.

43 **Conclusions** The high expression of MAPK-14 works as a promoter of chondrocytes death and

44 an important signal of the osteoarthritis process.

45

46 **Key words:** Osteoarthritis; MAPK-14, chondrocytes, differentially expressed genes; protein-

47 protein interaction; Gene Ontology;

48

1 Introduction

Osteoarthritis is the most serious chronic bone disease and can cause severe pain and even disability in the elderly(Song et al. 2006). OA not only brings suffering to patients but also aggravates their economic burden. Nearly half of the elderly patients are receiving treatment, and most of them are still suffering or disabled(McKenzie & Torkington 2010). About one-third of elderly over 60 years old are supposed to be diagnosed with OA by 2030(Croft 2005). One of the most important risks in OA development is the pathogenic changes of hub genes (Hochberg et al. 2013). Heritability of osteoarthritis reached approximately 50% (Cibrian Uhalte et al. 2017). However, the molecular mechanism genetic changes in OA remains unknown.

However, current researches showed some disadvantages such as using small amount of genetic samples and the lack of relevant experimental verification. Therefore, the expanding of the number of screening sample size on the basis of the predecessors, optimizing the relevant screening conditions and carrying out the relevant verification through cell lines experiments could contribute to further understanding and enhances further study of the internal mechanism regarding the occurrence and development of osteoarthritis. Recently, microarray technology and bioinformatics have made significant advances which contribute to understand differentially expressed genes (DEGs) in OA and enable us to do further studies. Here, we got 3 datasets from Gene Expression Omnibus (GEO) for analysis and obtain DEGs between OA knee joint samples and normal knee joint samples. Based on this, we haven't only analyzed Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of DEGs, but also produced protein-protein interaction (PPI) network to help us unlock some of the unstudied parts of the molecular mechanisms of OA. The purpose of this research was to explore regarding underlying mechanisms of OA, so as to promote the further development of OA treatment.

2 Materials & Methods

2.1. Microarray Data Information

The Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo) is a genomic database for all users, from which users can get all kinds of data free of charge(Barrett et al. 2013).The expression profiles of GSE43923, GSE55457 and GSE12021 were all searched and got from the GEO. GSE43923 include 3 OA knee joint samples and 3 normal knee joint samples. GSE12021 include 10 OA knee joint samples and 9 normal knee joint samples, and GSE55457 include 10 OA knee joint samples and 10 normal knee joint samples.

2.2. Identification of DEGs

GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r), a free public network platform, was used to process the DEGs between OA knee joint samples and normal knee joint samples. Using GEO2R, we have integrated and analyzed multiple datasets for identifying DEGs. |logFC| >1 and P-value <0.05 were considered the cutoff criterion and have statistically significant.

2.3. GO and KEGG enrichment analyses of DEGs

DAVID(http://david.ncifcrf.gov), a free public online biological information database was used to provide biological function analysis for a large number of genes and can visualize the results(Huang et al. 2007). GO is a biological information analysis tool that can induce and analyze a large number of genes and visually export corresponding results, including BP, CC, and MF(Shannon et al. 2003). KEGG is a utility database resource for understanding advanced functions and biological systems (such as cells, organisms, and ecosystems), generating genomic sequencing and other high-throughput experimental technologies from molecular level information, especially large molecular datasets(Szklarczyk et al. 2011). Deep analyses were

97   implemented by using the DAVID online database. P<0.05 was considered statistically

98   significant.

99

100 **2.4. PPI network construction and module analysis.**

101   STRING(https://string-db.org/cgi/input.pl), a free public online search platform was used to

102 elucidate the relationship between interacting genes(Franceschini et al. 2013). DEGs previously

103 screened in this research were imported into STRING to product the PPI network, and

104 interaction with a confidence score >0.4 was considered significant. Cytoscape, a free and public

105 source bioinformatics application that we imported the data into it. The built-in APP Molecular

106 Complex Detection (MCODE) of Cytoscape is convenient to select the most significant modules

107 in all DEGs and identify the most prominent gene in each module(Bader & Hogue 2003).

108

109 **2.5 Isolation and culture of chondrocytes**

110   Cartilage specimens of the fracture patients' femoral head and the knee joint of patients with

111 OA in Shenzhen second people's hospital were collected to isolate and cultivate chondrocytes.

112 The adherent cells were cultured in the chondrocyte medium, which contains the DMEM-

113 LG(Gibco), 10%FBS(Gibco),1% penicillin-streptomycin (Gibco), 1%HEPES, 1% vitamin

114 solution, 1% proline solution and 1% non-essential amino acid NEAA. These adherent cells are

115 what we call chondrocytes. chondrocytes were cultured in at 37℃ and 5% CO_2. The study was

116 reviewed and approved by the First Affiliated Hospital of Shenzhen University

117 Health Science Center Research Ethics Committee. All patients signed a written informed

118 consent before participating in this study.

119

120 **2.6 Toluidine blue staining of chondrocytes**

121   First, we washed the Petri dishes with PBS for 2 times and fixed the cells with 4%

122   paraformaldehyde directly for about 0.5 h (4℃). Then wash with tap water for 15 minutes, then

123   with distilled water for 5 minutes, then add 1% toluidine blue for 2h. Finally, remove excess dye

124   and filter impurities. The dyeing condition was observed under the microscope, dried after

125   satisfaction, and sealed with neutral gum.

126

127   **2.7 Immunofluorescence staining of chondrocytes**

128   PBS washed the cells twice. The cells were fixed with paraformaldehyde for 15 min and

129   washed with PBS for 6 times / 5min. 0.5% Triton-X100 was incubated and drilled for 15 min.

130   Wash PBS 6 times / 5min.The cells were sealed with 5% BSA at room temperature for 2 h and

131   cleaned with 0.5% BSA for 6 times / 5min. The primary antibody (Rabbit Anti-Collagen II/FITC

132   Conjugated antibody (bs-10589R-FITC) and Rabbit Anti-Collagen I/Cy3 Conjugated antibody

133   (bs-10423R-Cy3), Bioss, Beijing, China) were added and incubated at room temperature for 2h

134   and washed with PBS for 6 times / 5min. DAPI staining was added for 5min. Photos were taken

135   using a confocal microscope ZEIZZ LSM800, Germany.

136

137   **2.8 Western Blot**

138   OA chondrocytes and normal control were cultured for three days, and then it was taken out of

139   the CO_2 incubator, cells were washed twice with PBS. Total protein was obtained by lysis

140   buffer (Beyotime, China), and then placed on ice for 30 minutes. Centrifuged the sample at

141   12000g for 25 min at 4°C. The supernatants were transferred to a new EP tube. The protein

142   concentration was measured by BCA quantitative method. Protein samples were electrophoresed

143   on 10% SDS-PAGE gel. After that, protein samples were transferred on PVDF membranes

144   according to the three-character sandwich method. Use 5% skimmed milk powder to block for

145   2h. Use the primary antibody containing COL I (1:1000, boss, China), COL II (1:1000, Thermo

146   Scientific, USA) and GAPDH (1:1000, abcam, USA) to co-incubate with the sample overnight at

147   4 ℃. TBST cleaning, 5 times/10 min. Then use the second antibody containing goat anti-rabbit

148   incubation bath for two hours at room temperature. After washing the unbound antibody, use

149   chemiluminescence to take pictures in the two-color laser and chemiluminescence imaging

150   system (Odyssey FC, Gene Company Limited, China).

151

152   **2.9 Apoptosis assay of chondrocytes**

153   The chondrocytes were isolated from the cartilage tissue, washed and then resuspended, and

154   the precipitate was taken. About 50uL cell suspension was added into each flap-throwing tank

155   and centrifuged in a flap-throwing centrifuge (1500 g, 10 min). The chondrocytes can be

156   attached to a slide after the surrounding water is absorbed by the filter paper. Then wash with

157   PBS for 3 times and fix with 4% paraformaldehyde for 15min. Wash again with PBS for 3 times,

158   add PI immunofluorescence staining and incubate in a wet tank for 30 min. After washing with

159   PBS again for 3 times, fluorescence microscope observation and image collection were

160   performed.

161

162   **2.10 qRT-PCR Validation and Statistical Analysis**

163   After previous research, we've found four hub genes, including MAPK14, PTPRC, B9D1,

164   PTPN12. We searched the primers of the four hub genes through the PrimerBank

165   (https://pga.mgh.harvard.edu/primerbank/) and ordered the relevant primers through the Sangon

166   Biotech. Quantitative real-time polymerase chain reaction primers are detailed in Table 1. Total

167   RNA was extracted from chondrocytes using the RNeasy mini kit (Takara), according to the

168   manufacturer's instructions. The RNA was reversely transcribed into cDNA by iScriptTM cDNA

169   Synthesis Kit (Takara) and qRT-PCR was performed with a Real-time quantitative PCR

170   instrument (Life Technologies).

171

172   **2.11 Statistical analysis**

173   Use SigmaPlot 10.0 to draw. Use IBM SPSS Statistics 19 to conduct statistical analysis, data

174   are expressed as mean ± std. A significant statistical difference was $P < 0.05$.

175

176   ## 3 Results

177   **3.1. Identification of DEGs in Osteoarthritis**

178   A total of 23 OA knee joint samples and 22 matched normal knee joint samples were

179   analyzed; taking $P < 0.05$ and $|logFC| > 1$ as a threshold, DEGs (2555 in GSE43923, 1347 in

180   GSE55457 and 1398 in GSE11201) were identified. First, we made a pairwise comparison and

181   got three Venn graphs (Figure 1A, B, C). Then we comprehensively compared and plotted the

182   final Venn diagram of the differentially expressed gene (Figure 1 D). A total of 265 common

183   differential genes were screened, including 66 down-regulated genes and 199 up-regulated

184   genes.

185

186   **3.2 KEGG and GO enrichment analyses of DEGs**

187   By using DAVID, the DEGs functions were classified into 3 main categories, including BP,

188   MF, and CC (Figure 2). We can see that otherness in BP were significantly enriched in

189   anatomical structure formation involved in morphogenesis, cell adhesion, vasculature

190   development, blood vessel development (Figure 3A). Otherness in MF was mainly enriched in

191   calcium channel inhibitor activity, signaling receptor binding, growth factor activity, cytoskeletal

192   protein binding and adenylate cyclase activator activity (Figure 3B). Otherness in CC was mainly

193   enriched in the cytoplasmic part, cytoplasm and extracellular region (Figure 3C).

194

195   **3.3 PPI network construction and module analysis**

196   We use the data obtained through STRING and then build the PPI network through

197   Cytoscape, containing 140 nodes and 356 edges (Figure 4). Next, we identified 4 hub genes,

198   including MAPK14, PTPN12, PTPRC and B9D1. Then we analyzed the 4 modules using

199   MCODE (the plug-in for Cytoscape), the four modules were shown in (Figure 5). Functional

200   enrichment analyses were also carried out for these modules. Pathway enrichment analysis

201   showed that Module 1 is the signaling pathways regulating pluripotency of stem cells and mainly

202   associated with leukocyte transendothelial migration. Module 2 is mainly associated with the

203   regulation of actin polymerization or depolymerization. Module 3 is mainly associated with the

204   positive regulation of response to external stimulus and leukocyte migration. It also can response

205   to lipopolysaccharide. Module 4 is mainly associated with the docking of the ciliary basal body-

206   plasma membrane (Table 2).

207

208   **3.4 Morphological observation and authenticate of normal chondrocytes and OA**

209   **chondrocytes**

210   When chondrocytes are completely adherent to the dish, they are mainly flat and polygonal.

211   The nuclei are located in the center of the cell, with 2-3 nucleoli. The morphology of OA

212   chondrocytes was more irregular, with rare cell community aggregation (Figure 6A).

213   The cytoplasm of chondrocytes stained with toluidine blue was blue, and 1-3 purplish blue

214   nucleoli were observed (Figure 6B). The results showed that all the cells we cultured were

215   chondrocytes.

216

217   **3.5 Biological identification of OA and control chondrocytes**

218   COL I, COL II and DAPI immunohistochemical tested were carried out on the Chondrocytes.

219   And then we looked at it through a fluorescence microscope, the nuclei are dyed blue, collagen

220   type I dyed red, collagen type II dyed green. Results showed that OA chondrocytes compared

221   with normal chondrocytes, the COL I expression increased (Figure 6 C-H and O), and the

222   expression of COL II reduced (Figure 6 I-N and O) detected by immunofluorescence and

223   westernbolt. This indicates that OA chondrocytes mainly express collagen type I, which means

224   that fibrochondrocytes are newly formed on the surface of cartilage, and the spontaneous

225   depolymerization of fibrochondrocytes further accelerates the death of chondrocytes.

226

227   **3.6 Validation of Hub Gene**

228   4 hub genes have been found. Their details are shown in Table 3. We validated our results by

229   measuring the relative expression of our hub genes using qRT-PCR. As can be seen, the relative

230   expression of MAPK-14, PTPN12 and PTPRC in OA chondrocytes were significantly increased,

231   while the expression of B9D1 were significantly decreased (P< 0.05) (Figure 7A-D), which was

232   consistent with our expected results.

233

234   **3.7 Apoptosis test of chondrocytes**

235   The chondrocytes were stained with iodide propidium (PI) and examined by fluorescence

236   microscope. Results showed that compared with normal chondrocytes, a large number of OA

237   chondrocytes were surrounded by pathological vesicles and showed intracellular red staining,

238   which was the PI entering the cells (Figure 7E-J). It suggested that the cell membrane was

239   incomplete and the cells death of OA chondrocytes was increased.

240

241   **4 Discussion**

242   The incidence of OA is a second after heart diseases in the statistical reports of some

243   countries. OA, cardiovascular, cerebrovascular diseases and cancer are also called "three major

244   killers" threatening human health. The most immediate symptom of OA is joint pain (Torres et

245   al. 2006). A large number of epidemiological studies have confirmed that osteoarthritis is a

246   complex disease involving multiple factors (Neogi & Zhang 2013). Therefore, the molecular

247   mechanisms of OA are the most important direction we can study. It can help us to make early

248   diagnosis and treatment of OA.

249   In previous researches on bioinformatic analysis, many of them are only limited in two cohort

250   studies or two genetic events. This study makes up for the deficiency in this aspect, we used 3

251   datasets to obtain DEGs between OA knee joint samples and normal knee joint samples. This can

252   reduce the contingency of analysis. 265 DEGs were identified in all, which divided into 199

253   upregulated genes and 66 downregulated genes.

254   GO and KEGG enrichment analyses were performed found that the upregulated genes were

255   mainly enriched in calcium channel inhibitor activity, cytoskeletal protein binding, adenylate

256   cyclase activator activity, while the downregulated genes were mainly enriched in vasculature

257   development, and blood vessel development. According to previous studies, calcium channels

258   have been shown to mediate the OA pain pathway (Rahman et al. 2015). In addition, some

259   studies have confirmed that the cytoskeletal structure is incomplete and diseased in cartilage

260   chondrocytes of OA(Blain 2009). Other researches had indicated a protective role for pituitary

261   adenylate cyclase-activating polypeptide (PACAP) in chondrocyte differentiation and

262   metabolism(Strange-Vognsen et al. 1997),(Juhasz et al. 2014),(Juhasz et al. 2015),(Juhasz et al.

263   2015). In addition, some studies have pointed out that one of the most important members of

264   osteogenesis is the skeletal vasculature and distinct blood vessel subtypes play different roles in

265   osteogenesis (Sivan et al. 2019),(Cui et al. 2016). Moreover, the enriched KEGG pathways of

266   DEGs and modules analysis included NF-kappa B signaling pathway, TNF signaling pathway,

267   PI3K-Akt signaling pathway and MAPK signaling pathway. Previous studies showed that PI3K-

268   Akt,NF-kappa B and TNF signaling pathways are involved in synovial hyperplasia and

269   osteoarthritis cartilage degeneration(Fu et al. 2016),(Lai et al. 2014) and changes in the

270   expression of cytokines, biological factors and enzymes on chondrocytes in human osteoarthritis

271   may be caused by the p38-MAPK signaling pathway(Feng et al. 2017). These theories are also

272   consistent with our results.

273   In this study, normal chondrocytes and OA chondrocytes were extracted from the collected

274   cartilage specimens of the knee joint of patients with femoral head and OA. The cytoplasm of

275   chondrocytes stained with toluidine blue was purplish blue, and 1-3 purplish blue nucleoli were

276   observed. we found that the expression levels of MAPK-14, PTPN12 and PTPRC in OA

277   chondrocytes were significantly increased, while the expression levels of B9D1 were

278   significantly decreased by qPCR test. This indicates that the changes in the expression level of

279   the potential key genes in the development of OA are consistent with the changes in the

280   expression level of OA chondrocytes, which is consistent with our conjecture.

281   However, does the high expression of MAPK-14 predict chondrocyte death? The chondrocyte

282   apoptosis experiment showed that the cell membrane of OA chondrocytes was more incomplete

283   than that of normal chondrocytes, and pathological vesicles appeared, indicating that the amount

284   of necrosis of OA chondrocytes was significantly increased. It has been proved that chondrocytes

285   are doomed to die in the development of OA, while high expression of MAPK-14 plays a role in

286   inducing the autophagy of cells.

287   MAPK14 is one of the representatives of the MAPK family. Furthermore, the MAPK

288   signaling pathway is the most important signaling system to mediate osteoarthritic cartilage

289   damage (Chowdhury et al. 2008). Studies have shown that the major pathological changes of

290   osteoarthritis are degradation of the extracellular matrix, leading to progressive loss of cartilage

291   components and destruction of chondrocyte structure and function(Stoddart et al. 2009).

292   Inflammatory factors and growth factors specifically bind to receptors on cell membranes to

293   activate the intracellular MAPK signaling pathway, causing an increase in matrix

294   metalloproteinases (MMPs) expression, chondrocyte apoptosis and cartilage destruction. MMPs

295   can degrade almost all the extracellular matrix of chondrocytes and has been shown involved in

296   the pathogenesis of OA(Murphy et al. 2002). In addition, MAPK signaling pathway also

297   participates in and regulates chondrocyte enlargement, calcification and proliferation in the

298   process of OA pathology (Huang et al. 2008). Therefore, the MAPK signaling pathway can be

299   used as an effective target for the study of new drugs.

300   Other DEGs PRPRC and PTPN12 are members of the PTP family. On the basis of previous

301   studies, an important part of the pathogenesis of OA is the immune system(Goldring & Otero

302   2011). The proliferation of T lymphocytes can regulate the immune response, and the

303   biochemical activities of human articular cartilage can directly affect the proliferation of T

304   lymphocytes(Pereira et al. 2016). T lymphocytes are lymphoid stem cells derived from bone

305   marrow and distributed to immune systems throughout the body through lymph and blood

306   circulation (Moro-Garcia et al. 2018). On the other hand, dendritic cells(DCs) as a crucial

307   component in triggering an immune response, as well as in inflammatory and autoimmune

308   diseases(Ganguly et al. 2013). Furthermore, the PTP family is important in the activation of DCs

309   ability to induce T cell activation and function (Rhee et al. 2014). Therefore, the PTP family and

310   its related pathways may provide a new idea to treat OA.

311   The involvement of B9D1 in OA has not been well studied, but in recent research, mutations

312   of B9D1 result in common skeleton defects(Yuan & Yang 2015). RB9D1 and its related

313   pathways can be used as a supplement to the current traditional treatment of OA.

314   In brief, four potential key genes in the pathogenesis of OA were identified through

315   bioinformatics screening, including MAPK-14, PTPRC, PTPN12 and B9D1. The imbalance of

316   matrix synthesis and catabolism of OA chondrocytes and the increase of cell membrane

317   destruction may be related to the high expression of MAPK-14 promoting the death of OA

318   chondrocytes. The regulation of the expression of these key genes can provide a potential

319   reference target for the treatment of OA.

320

321   **Conclusions**

322   The hub genes MAPK-14, PTPRC, PTPN12 were upregulated while B9D1 was

323   downregulated after screening from the 265 differentially expressed genes. OA chondrocytes had

324   high expression of COL I protein and low expression of COL II protein. The inflammatory

325   necrosis of OA chondrocytes was increased compared with the normal chondrocytes. The

326   expression levels of MAPK-14, PTPRC and PTPN12 in OA chondrocytes were significantly

327   higher, while the expression levels of B9D1 were significantly lower than that in normal

328   chondrocytes. The MAPK-14 among the four potential key genes has been proved to play an

329   important role in the treatment of OA.

330

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342

Data Availability

344   The following information was supplied regarding data availability:

345 Raw data are available in the Supplemental Files.

346

Author Contributions

348 Review conception and design: WL and DW.

349 Drafting of the article: ZD, XH and MA.

350 Drawing the schematic: ZD, XH, and MA.

351 Revising the manuscript: JL, MA, ZZ, XC, GW, JX, and DL.

352 Final approval of the article: All authors.

353

Ethics approval and consent to participate

355 The study was reviewed and approved by the First Affiliated Hospital of Shenzhen University

356 Health Science Center Research Ethics Committee.

357

Patient consent for publication

359 All patients signed informed consent forms for publication.

360

Competing interests

362 The authors declare that they have no competing interests.

363

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470

Figure 1

Figure 1. Venn diagram.

A. GSE43923 and GSE55457 B. GSE11201 and GSE55457 C. GSE43923 and GSE11201 D. GSE43923, GSE55457 and GSE11201.
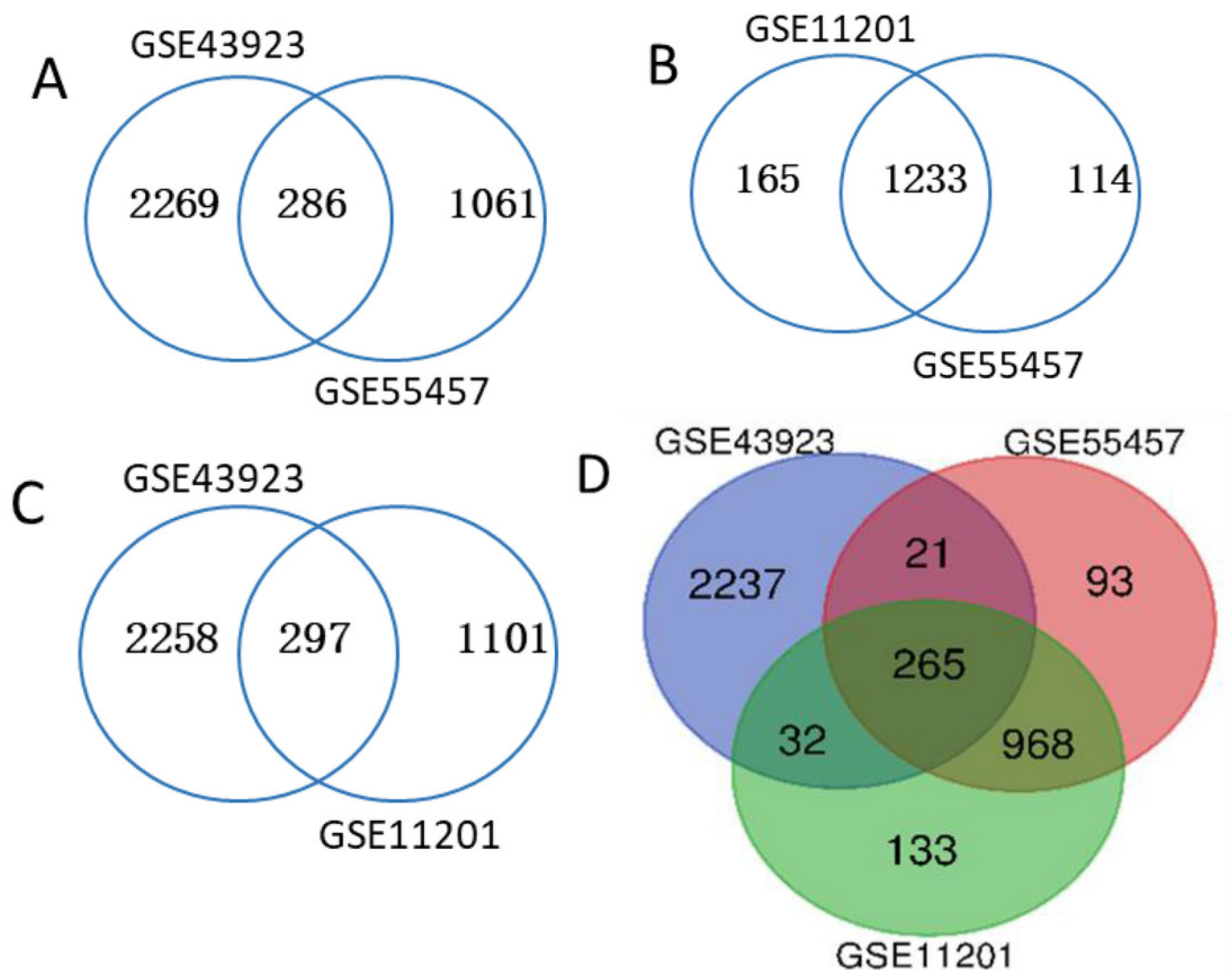
Figure 2

Figure 2. Gene Ontology (GO) analysis classified the DEGs into 3 groups: molecular function, biological process, and cellular component.
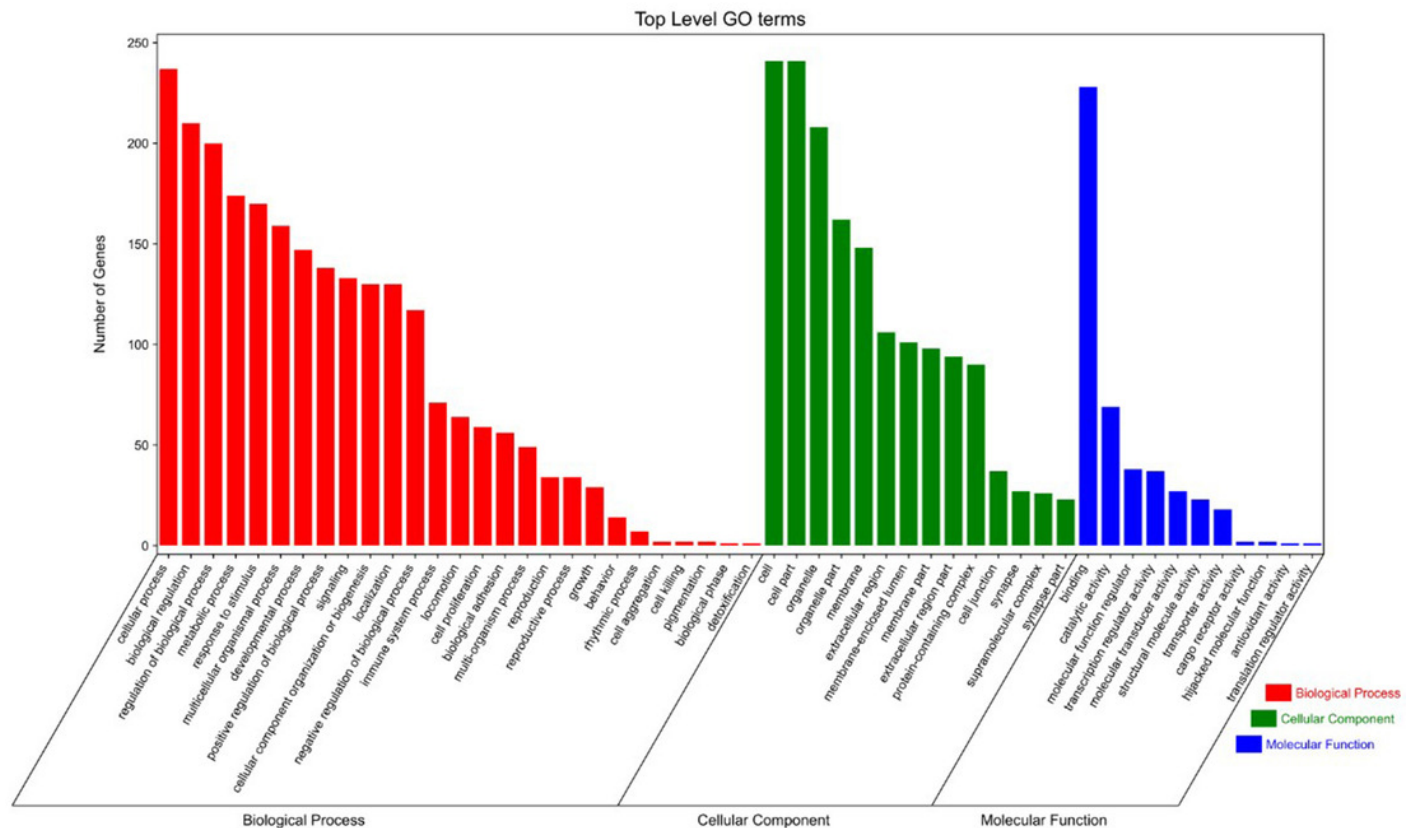
Figure 3

Figure 3. Gene Ontology (GO) enrichment analysis.

A Enrichment analysis of biological processes (BP). *B* Enrichment analysis of molecular function (MF). *C* Enrichment analysis of cellular component (CC). The gradual color represents the P value; the size of the black spots represents the gene number.
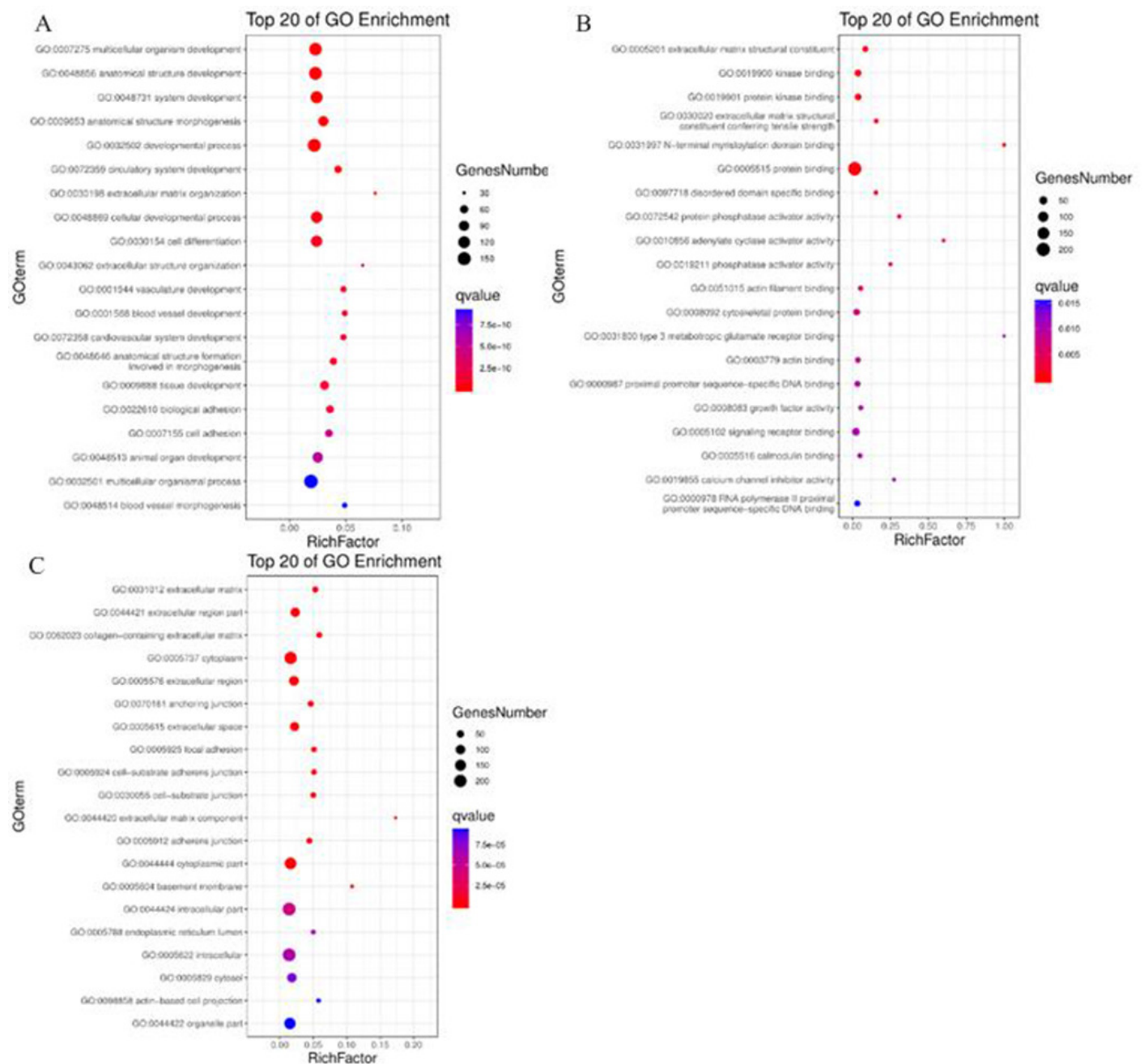
Figure 4

Fig 4. The PPI network of DEGs.

(Upregulated genes are marked in red; downregulated genes are marked in blue).
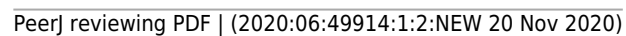
Figure 5

Figure 5. The 4 significant modules were obtained from PPI network.

Upregulated genes are marked in red; downregulated genes are marked in blue. **A**. MAPK14 module. **B**. PTPN12 module. **C**. PTPRC module. D. B9D1 module.
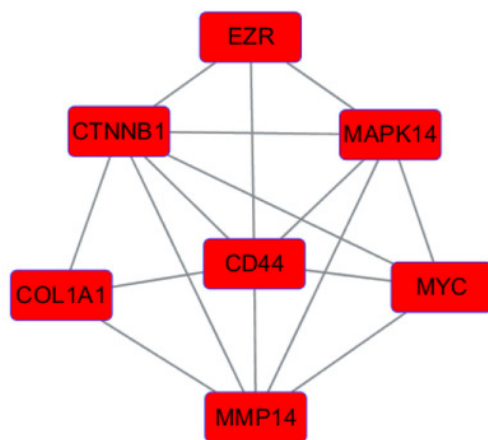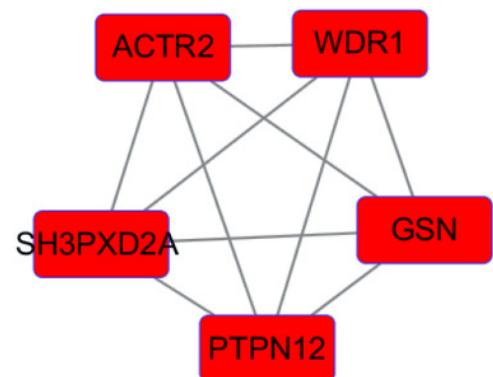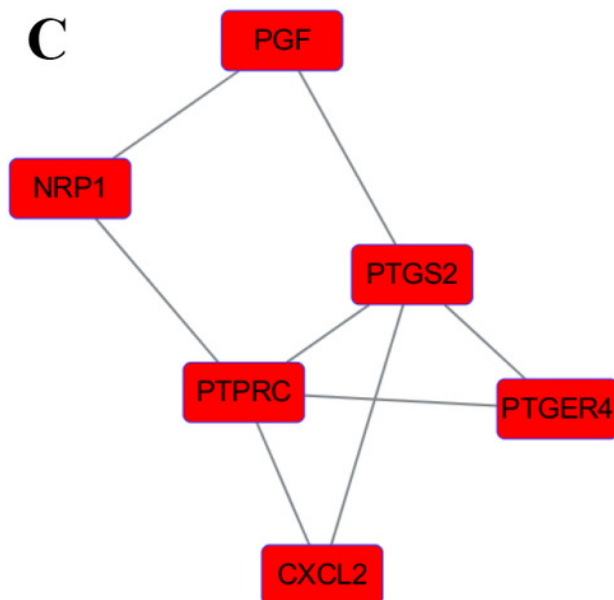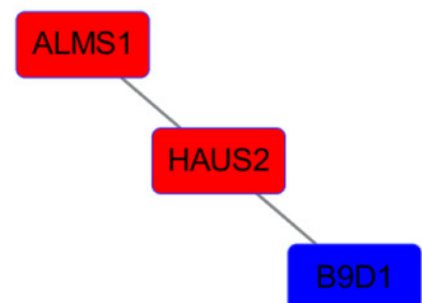
Figure 6

Figure 6. Cell biology detection.

A. Morphological observation of chondrocytes. **B**. Microscopic observation of toluidine blue staining of chondrocytes. **C.** - **N.** are the expression of COL I and COL II by immunofluorescence staining in human chondrocytes. **O.** is the expression of COL I and COL II by fluorescence western blot in human chondrocytes.
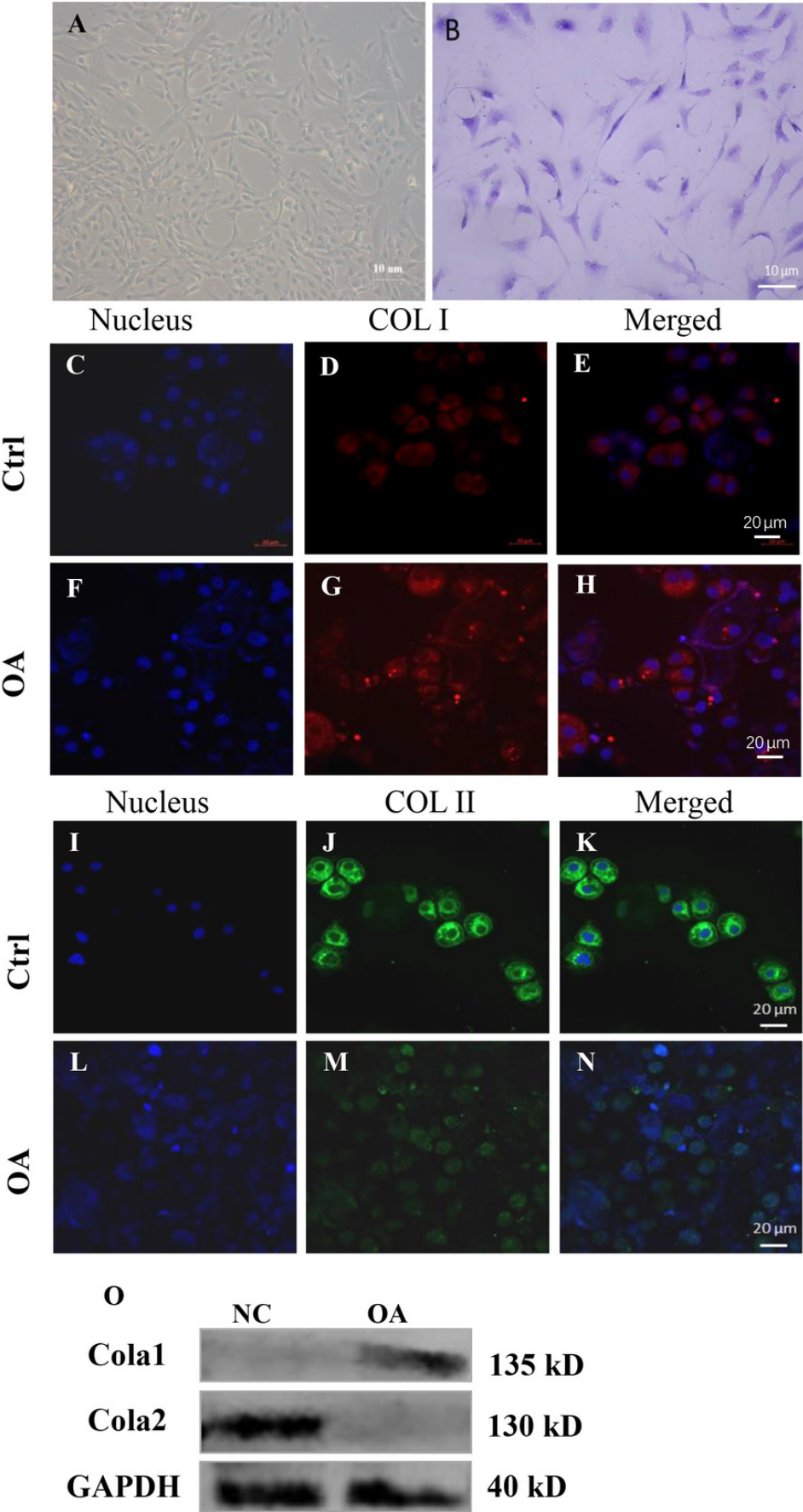
Figure 7

Figure 7. Comparison of expression of potential key genes and Apoptosis test in chondrocytes.

(**A**. MAPK-14; **B**. PTPRC; **C**. PTPN12; **D**. B9D1). All samples were normalized to the expression of GAPDH, and the relative expression levels of each gene were analyzed using the $2^{\wedge}\Delta\Delta C\tau$ method. **E.-J.** Apoptosis test of chondrocytes (DIC: bright field imaging; PI: piridine iodide staining imaging; Merged: composite imaging). The red arrow refers to cells that are stained or not by PI.
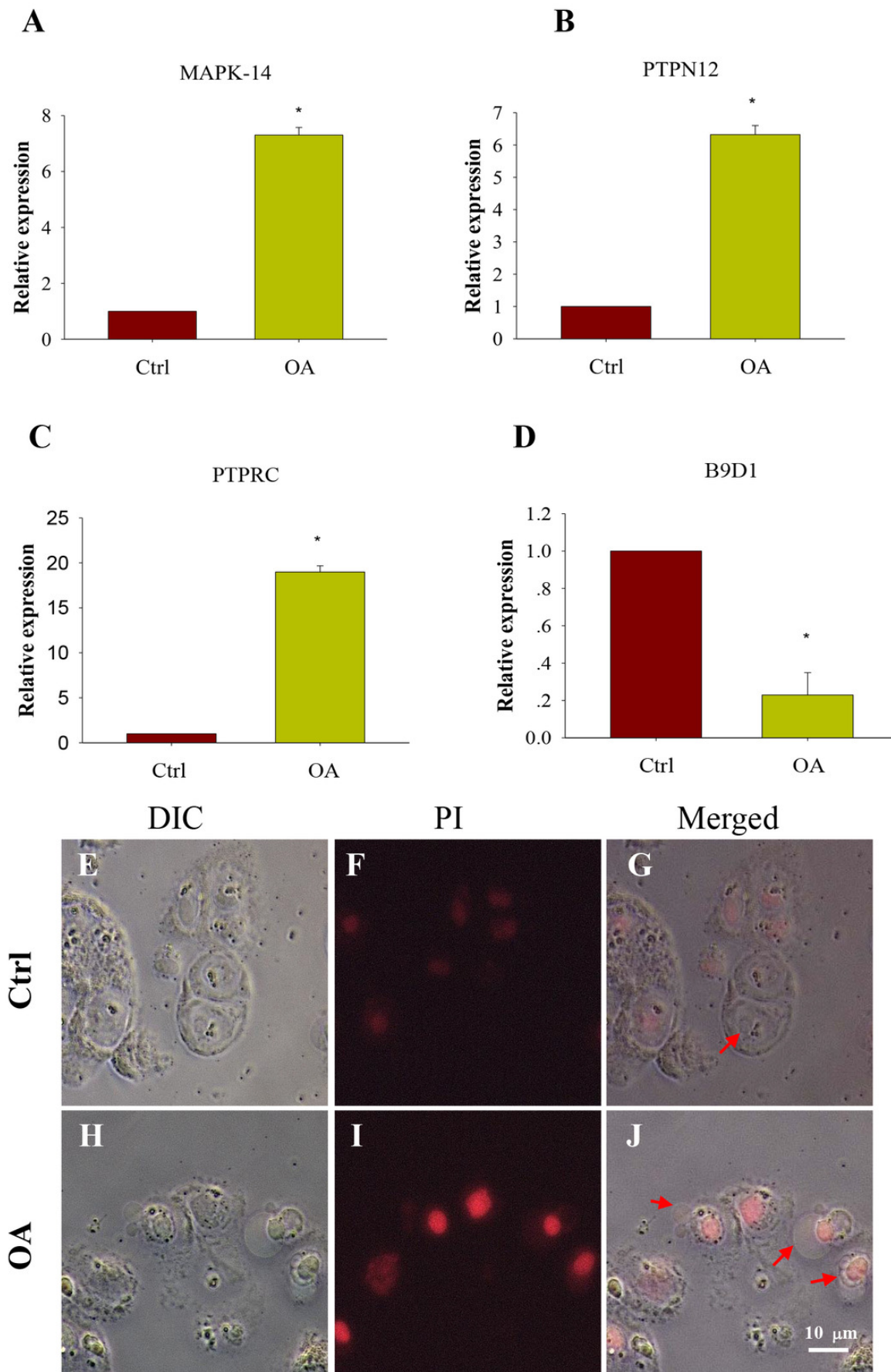
Table 1: The primers of 4 hub genes

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Gene	Primer sequence	Annealing Temperature (°C)	Length	PrimerBank ID
☐	☐			
MAPK14	Fwd 5′- CCCGAGCGTTACCAGAACC -3′	62.4	19	194578904c1
	Rev 5′- TCGCATGAATGATGGACTGAAAT-3′	60.4	23	
PTPRC	Fwd 5′- ACCACAAGTTTACTAACGCAAGT-3′	60.4	23	18641362a1
	Rev 5′- TTTGAGGGGGATTCCAGGTAAT-3	60.5	22	
B9D1	Fwd 5′- TGGAGGAGGGGATCTCACAG-3′	61.9	20	343478278c1
	Rev 5′- CCGTAGGGGTTGGTGCTTTT-3	62.4	20	
PTPN12	Fwd 5′- AGTTGCCTTGTTGAAGGGGAT-3′	61.6	21	196114950c1
	Rev 5′ - AGAAGGTGTCAAGATGGGTGG-3	61.4	21	☐

Table 2 Signaling pathway enrichment analysis of hub DEGs function in OA

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Expression	Term	Description	Gene Count	LogP
UP-DEGs	hsa04550	Signaling pathways regulating pluripotency of stem cells	3	-5.43
	hsa04670	Leukocyte transendothelial migration	3	-5.69
	GO:0008064	regulation of actin polymerization or depolymerization	3	-5.4
	GO:0032103	positive regulation of response to external stimulus	3	-6.33
	GO:0097529	myeloid leukocyte migration	3	-4.88
☐	GO:0032496	response to lipopolysaccharide	3	-4.31
DOWN-DEGs	GO:0097711	ciliary basal body-plasma membrane docking	3	-7.23

Table 3. Functional roles of 4 hub genes

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No.	Gene symbol	Full name	Function
1	MAPK-14	mitogen-activated protein kinase 14	Play an important role in the cascades of cellular responses evoked and inhibit the lysosomal degradation pathway of autophagy
2	PTPRC	protein tyrosine phosphatase receptor type C	High expression of PTPRC is associated with progression of PTP family
3	PTPN12	protein tyrosine phosphatase non-receptor type 12	High expression of PTPN12 is associated with progression of PTP family
4	B9D1	B9 domain containing 1	Component of the tectonic-like complex and acting as a barrier that prevents diffusion of transmembrane proteins between the cilia and plasma membranes