Int J Clin Exp Med 2015;8(1):1027-1034
www.ijcem.com /ISSN:1940-5901/IJCEM0003564

Original Article

Identification of B cells participated in the mechanism of postmenopausal women osteoporosis using microarray analysis

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Received November 3, 2014; Accepted January 7, 2015; Epub January 15, 2015; Published January 30, 2015

Abstract: To further understand the molecular mechanism of lymphocytes B cells in postmenopausal women osteoporosis. Microarray data (GSE7429) were downloaded from Gene Expression Omnibus, in which B cells were separated from the whole blood of postmenopausal women, including 10 with high bone mineral density (BMD) and 10 with low BMD. Differentially expressed genes (DEGs) between high and low BMD women were identified by Student's *t*-test, and $P < 0.01$ was used as the significant criterion. Functional enrichment analysis was performed for up- and down-regulated DEGs using KEGG, REACTOME, and Gene Ontology (GO) databases. Protein-protein interaction network (PPI) of up- and down-regulated DEGs was respectively constructed by Cytoscape software using the STRING data. Total of 169 up-regulated and 69 down-regulated DEGs were identified. Functional enrichment analysis indicated that the genes (*ITPA*, *ATIC*, *UMPS*, *HPRT1*, *COX10* and *COX15*) might participate in metabolic pathways, *MAP3K10* and *MAP3K9* might participate in the activation of JNKK activity, *COX10* and *COX15* might involve in mitochondrial electron transport, and *ATIC*, *UMPS* and *HPRT1* might involve in transferase activity. *MAPK3*, *ITPA*, *ATIC*, *UMPS* and *HPRT1* with a higher degree in PPI network were identified. *MAPK3*, *MAP3K10*, *MAP3K9*, *COX10*, *COX15*, *ATIC*, *UMPS* and *HPRT1* might participate in the pathogenesis of osteoporosis.

Keywords: Osteoporosis, function enrichment, pathway enrichment, PPI network

Introduction

Osteoporosis is a common disease for postmenopausal women and characterized by the reduction in the density of bone tissue, and subsequently the increase of bone fragility [1]. The old bone is replaced by new bone tissue through continuous remodeling dynamics, including bone resorption and bone formation [2]. The imbalance between bone resorption and formation may result in the decrease of bone density [3]. The activation of hematopoietic precursors to become osteoclast (OC) contributes to the bone remodeling in OC, osteoblast (OB) and osteocytes [4]. T cells, acted as a regulator of OC and OB formation, participate in the development of rheumatoid arthritis [5], bone metastasis [6] and osteoporosis [7]. T cells may contribute to osteoclastogenesis by the overexpression of *receptor activator of nuclear factor-kappa B ligand* (*RANKL*) and *tumor necrosis factor-alpha* (*TNF-alpha*) [8]. The expression of TNF increases the numbers of T cells, and induces the bone loss due to estrogen deficiency [9]. Furthermore, Onal *et al.* suggested that the expression of *RANKL* can be increased in T cells and B cells and may finally lead to the bone loss [10]. However, few studies showed that B cells participate in the development of osteoporosis. So it is an interesting study to identify the correlation of B cells and osteoporosis.

In 2008, Xiao *et al.* [11] analyzed the gene expression profile in B cells of postmenopausal osteoporosis patients and identified that down-regulation of *estrogen receptor 1* (*ESR1*) and *mitogen activated protein kinase 3* (*MAPK3*) in B cells regulates secretion of factors, resulting in increased osteoclastogenesis or decreased osteoblastogenesis. To obtain the more genes in B cells correlated with the osteoporosis, differentially expressed genes (DEGs) between high BMD (normal) and low BMD (osteoporosis)

Table 1. Enriched KEGG pathways for up- and down-regulated DEGs

DEGs	KEGG pathway	Gene counts	*P*-value	Gene
Up-regulated	Prion diseases	3	0.006874007	*C1QB*, *C7*, *MAPK3*
	p53 signaling pathway	4	0.006924881	*CHEK1*, *PIDD*, *TNFRSF10B*, *TP53I3*
Down-regulated	Metabolic pathways	15	0.000226044	*ATIC*, *CMAS*, *COX10*, *COX15*, *DPM1*, *GBE1*, *HPRT1*, *ITPA*, *ODC1*, *PIGC*, *PIGK*, *PIP5K1B*, *POLE3*, *SUCLG2*, *UMPS*
	Nucleotide excision repair	3	0.001525797	*GTF2H1*, *POLE3*, *RFC4*
	Drug Metabolism-other enzymes	3	0.002474417	*HPRT1*, *ITPA*, *UMPS*
	Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	2	0.007508316	*PIGC*, *PIGK*
	mRNA surveillance pathway	3	0.009212606	*CPSF6*, *CSTF2T*, *PPP2CA*
	Purine metabolism	4	0.009837667	*ATIC*, *HPRT1*, *ITPA*, *POLE3*

KEGG: Kyoto Encyclopedia of Genes and Genomes; DEGs: differentially expressed genes.

Table 2. Enriched REACTOME pathways for up- and down-regulated DEGs

DEGs	REACTOME pathway	Gene counts	*P*-value	Adjust *p*-value	Gene
Up-regulated	Complement cascade	3	0.00757477	1	*C1QB*, *C7*, *GZMM*
	Synthesis of PE	2	0.00772870	1	*CHKA*, *PISD*
Down-regulated	Post-translational modification	3	0.00025911	0.39591249	*DPM1*, *PIGC*, *PIGK*
	Metabolism of nucleotides	4	0.00058424	0.89271608	*ATIC*, *HPRT1*, *ITPA*, *UMPS*
	Heme biosynthesis	2	0.00124899	1	*COX10*, *COX15*
	Metabolism of porphyrins	2	0.00602279	1	*COX10*, *COX15*

DEGs: differentially expressed genes.

Table 3. Top five GO terms were respectively enriched in BP, CC and MF category for up-regulated DEGs

GO ID	Category	Term	Gene counts	*P*-value	Genes
GO: 0007256	BP	activation of JNKK activity	2	0.000916844	*MAP3K10*, *MAP3K9*
GO: 0006959	BP	humoral immune response	6	0.001738278	*C1QB*, *C7*, *FOXJ1*, *IFNG*, *TREM2*, *ZP3*
GO: 0035634	BP	response to stilbenoid	2	0.001900954	*FGL1*, *SLC22A7*
GO: 0048609	BP	multicellular organismal reproductive process	15	0.002142066	*DAZ1*, *DAZ2*, *DAZ3*, *DAZ4*, *EIF2B4*
GO: 0046683	BP	response to organophosphorus	5	0.002641967	*AKR1C1*, *ALDH3A1*, *AQP8*, *DMTN*, *VGF*
GO: 0034703	CC	cation channel complex	6	0.001553268	*CNGB1*, *KCNA6*, *KCNG1*, *KCNJ5*, *SCN4A*, *SCNN1B*
GO: 0005887	CC	integral to plasma membrane	21	0.003272558	*AGTR2*, *ABCC1*, *AQP8*, *ART3*, *C7*, *CNGB1*
GO: 0044459	CC	plasma membrane part	29	0.003560869	*MAPK3*, *ABCC1*, *AGTR2*, *AQP8*, *ART3*
GO: 0031226	CC	intrinsic to plasma membrane	21	0.005042495	*AGTR2*, *ABCC1*, *AQP8*, *ART3*, *C7*
GO: 0005796	CC	Golgi lumen	4	0.006999886	*MUC3A*, *MUC3B*, *MUC5AC*, *WNT7A*
GO: 0008106	MF	alcohol dehydrogenase (NADP +) activity	3	0.000150784	*AKR1C1*, *ALDH3A1*, *DHRS3*
GO: 0004706	MF	JUN kinase kinase kinase activity	2	0.000243013	*MAP3K10*, *MAP3K9*
GO: 0004033	MF	aldo-keto reductase (NADP) activity	3	0.000537427	*AKR1C1*, *ALDH3A1*, *DHRS3*
GO: 0022838	MF	substrate-specific channel activity	11	0.000700403	*AQP8*, *CNGB1*, *FXYD7*, *KCNA6*, *KCNG1*
GO: 0005261	MF	cation channel activity	9	0.000949791	*CNGB1*, *KCNA6*, *KCNG1*, *KCNJ5*, *KCNK7*

GO: Gene Ontology; BP: biological process; CC: cellular component; MF: molecular function; DEGs: differentially expressed genes.

were screened by the cut-off point of $P < 0.01$, but not the Benjamini and Hochberg (BH) adjusted $P \leq 0.05$ as the work of Xiao *et al.* [11]. Furthermore, we also explored the underlying function of DEGs by KEGG, REACTOME pathway and Gene Ontology (GO) enrichment analyses. Protein-protein interaction network (PPI) was also constructed to obtain the crucial genes that are involved in osteoporosis by regulating and influencing the other genes.

Table 4. Top five GO terms were respectively enriched in BP, CC and MF category for down-regulated DEGs

GO ID	Category	Term	Gene counts	*P*-value	Genes
GO: 0006123	BP	mitochondrial electron transport, cytochrome c to oxygen	2	1.66E-05	*COX10*, *COX15*
GO: 0006784	BP	heme a biosynthetic process	2	1.66E-05	*COX10*, *COX15*
GO: 0046160	BP	heme a metabolic process	2	1.66E-05	*COX10*, *COX15*
GO: 0006501	BP	C-terminal protein lipidation	3	0.000142394	*DPM1*, *PIGC*, *PIGK*
GO: 0018410	BP	C-terminal protein amino acid modification	3	0.000247707	*DPM1*, *PIGC*, *PIGK*
GO: 0005622	CC	intracellular	57	0.000964678	*ATIC*, *CPSF6*, *HPRT1*, *ITPA*, *POLE3*, *RFC4*, *UMPS*, *ADORA2A*, *ASH2L*, *ATP5S*
GO: 0044464	CC	cell part	62	0.001191597	*ATIC*, *CPSF6*, *HPRT1*, *ITPA*, *POLE3*, *RFC4*, *UMPS*, *ADORA2A*, *ASH2L*, *CCNE1*
GO: 0005623	CC	cell	62	0.001196158	*ATIC*, *CPSF6*, *HPRT1*, *ITPA*, *POLE3*, *RFC4*, *UMPS*, *ADORA2A*, *ASH2L*, *CCNE1*
GO: 0005671	CC	Ada2/Gcn5/Ada3 transcription activator complex	2	0.001560069	*POLE3*, *MBIP*
GO: 0044424	CC	intracellular part	56	0.001932115	*ATIC*, *CPSF6*, *HPRT1*, *ITPA*, *POLE3*, *RFC4*, *UMPS*, *ADORA2A*, *ASH2L*, *CD1A*
GO: 0016740	MF	transferase activity	17	0.000211892	*ATIC*, *HPRT1*, *POLE3*, *UMPS*, *ASH2L*, *CCNE1*, *CMAS*, *COX10*, *DPM1*, *EIF2B3*
GO: 0001664	MF	G-protein coupled receptor binding	5	0.000925208	*ADORA2A*, *CREB3*, *NPFF*, *ROR2*, *YARS*
GO: 0016757	MF	transferase activity, transferring glycosyl groups	5	0.003521176	*UMPS*, *DPM1*, *GBE1*, *HPRT1*, *PIGC*
GO: 0004129	MF	cytochrome-c oxidase activity	2	0.004120099	*COX10*, *COX15*
GO: 0015002	MF	heme-copper terminal oxidase activity	2	0.004120099	*COX10*, *COX15*

GO: Gene Ontology; BP: biological process; CC: cellular component; MF: molecular function; DEGs: differentially expressed genes.

Methods

Microarray data

Microarray data (GSE7429) [11] were downloaded from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/). A total of 20 samples were available. B cells were isolated from the whole blood of 20 unrelated postmenopausal women 54 to 60 years of age, including 10 with high BMD and 10 with low BMD. The microarray platform of GSE7429 was GPL96 [HG-U133A] Affymetrix Human Genome U133A Array.

Data preprocessing and identification of DEGs

The data were preprocessed by Affy package [12] in Bioconductor and Affymetrix annotation files from Brain Array Lab. The background correction, quartile data normalization and probe summarization were performed by the Robust Multiarray Average (RMA) algorithm [13] to obtain the gene expression matrix. DEGs were identified by Student's *t*-test. $P < 0.01$ was used as the significant criterion.

Functional enrichment of DEGs

Pathway enrichment analysis in KEGG database based on conserved sub-pathways information [14]. However, REACTOME database based on open-source open-data resource of human pathways and reactions [15]. So we performed the pathway enrichment analysis for up- and down-regulated DEGs using the two databases with the threshold of $P < 0.01$. In addition, DEGs were also enriched in the biological process (BP), cellular component (CC) and molecular function (MF) categories of GO [16] database, and $P < 0.01$ was chosen as cut-off criteria.

Construction of PPI network

PPI network of up- and down-regulated DEGs was respectively constructed using the STRING (Search Tool for the Retrieval of Interacting

Genes) data [17], and the confidence score > 0.4 was used as cut-off criterion. The PPI network was visualized by Cytoscape software [18].

Results

DEGs analysis

Total of 235 DEGs between the high BMD group and low BMD group were identified, of which 169 DEGs were up-regulated and 69 DEGs were down-regulated.

Pathway enrichment analysis

The two enriched KEGG pathway of up-regulated DEGs were prion diseases pathway (P = 0.006874007) and p53 signaling pathway (P = 0.006924881) (**Table 1**). *MAPK3* involved in the prion diseases pathway. The six enriched KEGG pathway of down-regulated DEGs included metabolic pathways (P = 0.000226044), nucleotide excision repair (P = 0.001525797), drug metabolism-other enzymes (P = 0.002474417), Glycosylphosphatidylinositol (GPI)-anchor biosynthesis (P = 0.007508316), mRNA surveillance pathway (P = 0.009212606) and purine metabolism (P = 0.009837667) (**Table 1**). The genes (*ITPA*, *ATIC*, *UMPS*, *HPRT1*, *COX10* and *COX15*) participated in metabolic pathways.

The two enriched REACTOME pathway of up-regulated DEGs were complement cascade (P = 0.00757477) and synthesis of PE (P = 0.00772870) (**Table 2**). The REACTOME pathway enrichment of down-regulated DEGs showed that the genes (*ATIC*, *HPRT1*, *ITPA* and *UMPS*) involved in metabolism of nucleotides (P = 0.00059424). The genes (*COX10* and *COX15*) participated in the two pathways, including heme biosynthesis (P = 0.00124899) and metabolism of porphyrins (P = 0.00602279) (**Table 2**).

GO enrichment analysis

GO enrichment analysis of up-regulated DEGs showed that 44, 6 and 32 terms were respectively enriched in BP, CC and MF category, and the top five terms in BP, CC and MF category were listed (**Table 3**). The top three enriched GO terms in BP category of up-regulated DEGs were the activation of JNKK activity (P = 0.000916844), humoral immune response (P = 0.001738278) and response to stilbenoid (P = 0.001900954) (**Table 3**). *MAP3K10* and *MAP3K9* involved in activation of JNKK activity. The top three enriched GO terms in MF category were alcohol dehydrogenase ($NADP^+$) activity (P = 0.000150784), JUN kinase kinase kinase activity (P = 0.000243013) and aldo-keto reductase (NADP) activity (P = 0.000537427) (**Table 3**). *MAP3K10* and *MAP3K9* involved in JUN kinase kinase kinase activity.

GO enrichment analysis of down-regulated DEGs showed that 67, 8 and 14 terms were respectively enriched in BP, CC and MF category, and the top five terms in BP, CC and MF category were listed (**Table 4**). The top three enriched GO terms in BP category showed that the genes (*COX10* and *COX15*) involved in the three GO terms, such as mitochondrial electron transport (P = 1.66E-05), heme a biosynthetic process (P = 1.66E-05) and heme a metabolic process (P = 1.66E-05) (**Table 4**). The enriched GO terms in MP category was transferase activity (P = 0.000211892) involving in *ATIC*, *HPRT1* and *UMPS*, cytochrome-c oxidase activity (P = 0.004120099) including in *COX10* and *COX15*, and heme-copper terminal oxidase activity (P = 0.004120099) involving in *COX10* and *COX15* (**Table 4**).

PPI network analysis

The genes/proteins with the degree in PPI network of up-regulated DEGs were *MAPK3* (degree = 3) and *AGTR2* (degree = 3) (**Figure 1**). The genes/proteins with the degree in PPI network of down-regulated DEGs were *ITPA* (degree = 4), *RFC4* (degree = 3), *ATIC* (degree = 3), *UMPS* (degree = 2) and *HPRT1* (degree = 2) (**Figure 2**).

Discussion

In this study, 235 DEGs between the high BMD group and low BMD group were identified, including 169 up-regulated DEGs and 69 down-regulated DEGs. Functional enrichment analysis showed that *MAPK3* involved in the prion diseases pathway, *MAP3K10* and *MAP3K9* involved in the activation of JNKK activity, *COX15* and *COX10* involved in mitochondrial electron transport and heme a biosynthetic process, and *ATIC*, *UMPS* and *HPRT1 involved in transferase activity.*

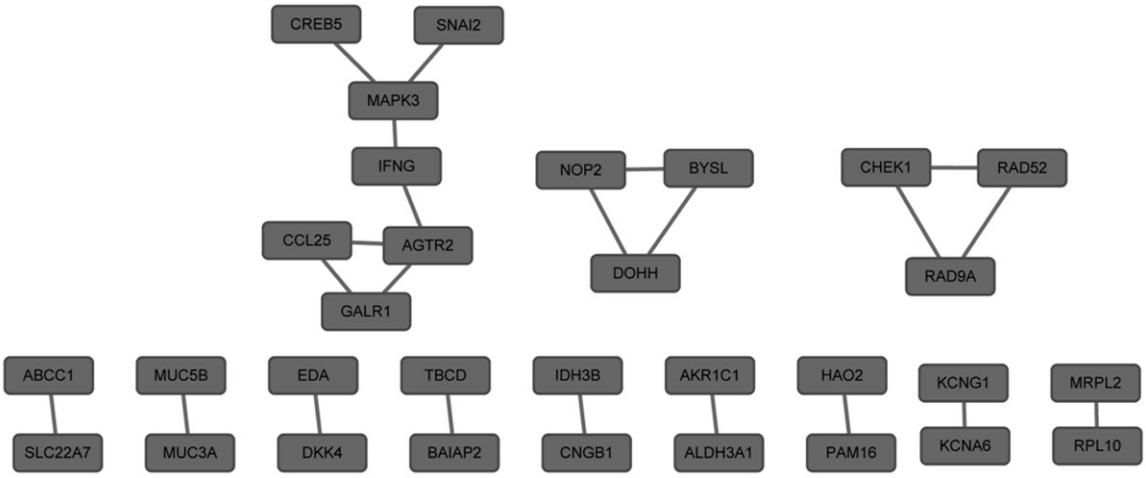

Figure 1. Protein-protein interaction network of up-regulated differentially expressed genes. The nodes represented up-regulated differentially expressed genes.

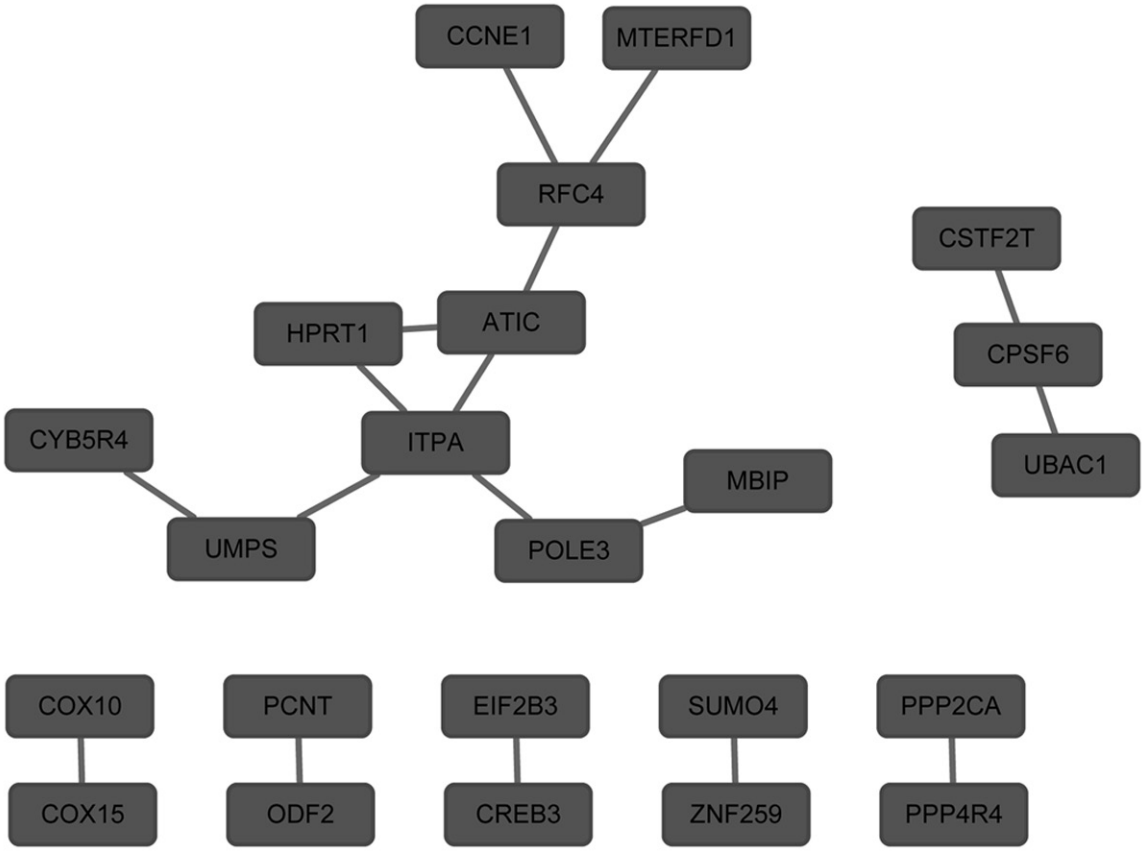

Figure 2. Protein-protein interaction network of down-regulated differentially expressed genes. The nodes represented down-regulated differentially expressed genes.

MAPK3 belongs to the Mitogen-Activated Protein Kinase (MAPK) family, which also known as the extracellular signal-regulated kinase (ERK) [19]. According to the work of Park *et al.*, hydrogen peroxide (H_2O_2)-induced cell apoptosis of osteoblasts may be mediated by the ERK signaling pathway [20]. Osteocyte apoptosis could be inhibited by estrogens via the activa-

tion of the ERKs signaling pathway [21]. ERK activity played an important role in the serum-induced osteoblast proliferation, and the up-regulated expression of *MKP-1*, acted as dual-specificity MAPK phosphatases, could be induced by the glucocorticoid which decreased the numbers of osteoblasts [22]. MAP*K3* may participate in the etiology of osteoporosis via the ERK/MAPK signaling pathway [11]. Although our findings were consistent with the previous results that *MAPK3* involved in the development of osteoporosis, *MAPK3* participated in the prion diseases in this study. Prion diseases and Alzheimer disease (AD) share similar pathogenic mechanisms, including generation of oxidative stress molecules and complement activation [23]. Reactive oxygen species (ROS) involve in the pathogenesis of osteoarthritis which are induced by pro-inflammatory cytokines, such as *interleukin-1* (*IL-1*) and *tumour necrosis factor alpha* (*TNFalpha*) [24, 25]. The results implied that *MAPK3* also involved in the development of osteoporosis via the prion diseases pathway, which was a new identified pathway in this study.

MAP3K10 (Mitogen-Activated Protein Kinase Kinase Kinase 10), MAP3K9 (Mitogen-Activated Protein Kinase Kinase Kinase 9) and MAP3K11 (Mitogen-Activated Protein Kinase Kinase Kinase 11) activate the JNK signaling cascade [26]. In addition, miR-155, targeting MAP3K10 (Mitogen-Activated Protein Kinase Kinase Kinase 10) [27, 28], involves in the regulation of MAPK pathway, which included extracellular signal-regulated kinases (ERKs) pathway, c-Jun N-terminal kinase (JNK) pathway, p38 MAPK pathway and ERK5 pathway [29]. miR-155 also regulates the release of IL-6 and TNF-α [30]. The production of cytokines, including IL-1β, IL-6 and TNF-α, are higher in osteoporotic postmenopausal women than in healthy women [31]. Based on these results, we could speculate that MAPK3, MAP3K10 and MAP3K9 participated in the etiology of osteoporosis through the MAPK pathway.

According to the above reports, ROS involves in the development of osteoporosis. The impairment of mitochondrial electron transport chain causes the increase of intracellular ROS, and finally results in the diseases related to mitochondria [32]. Guo *et al.* report that mitochondrial DNA participated in the pathogenesis of osteoporosis, age-related diseases [33]. According to the work of Petruzzella *et al.*, *COX15* and *COX10* involved in the formation of mitochondrial respiratory chain [34]. *COX15*, along with *SCO1*, involve in COX deficiency, and the expression of *COX15* increases heme A products and activates the COX [35]. *SCO1* and *COX10* are involved in the Cytochrome c oxidase (COX) assembly, and COX defects are determined in mitochondrial disorders [36]. Our results, which *COX15* and *COX10* involved in mitochondrial electron transport and heme a biosynthetic process, were consistent with the previous reports. So we could speculate that *COX15* and *COX10* involved in the pathology of osteoarthritis via mitochondrial respiratory chain.

In this study, the genes (*ATIC*, *UMPS* and *HPRT1*) involved in transferase activity. Gamma-glutamyl transferase contributes to the generation of free radical species, and oxidative stress has harmful effects on bone metabolism [37]. Isomura *et al.* also report that oxidative stress participated in the pathogenesis of osteoporosis by analysis of the correlation of oxidative stress and bone metabolism using bone metabolic markers and cytokines, including serum osteopontin and *TGF-β1* [38]. The distinct metabolic changes, including lipid, energy and amino acid metabolism changed, in osteoporotic ovariectomized rats have been determined, and altered metabolites participate in the oxidative defense system [39]. Based on these findings, we could speculate that *ATIC*, *UMPS* and *HPRT1* might regulate the bone metabolism to involve in the development of osteoporosis via the process of the transferase activity.

Conclusion

In this study, 235 DEGs between the high and low BMD group were identified, of which 169 DEGs were up-regulated and 69 DEGs were down-regulated. *MAPK3* and the genes (*MAP3K10* and *MAP3K9*) might involve in the pathogenesis of osteoporosis via the prion diseases pathway (or the MAPK signaling pathway) and the MAPK signaling pathway, respectively. *COX10* and *COX15* might participate in the pathogenesis of osteoporosis via the mitochondrial electron transport chain. *ATIC*, *UMPS* and *HPRT1* might involve in the development of osteoporosis via the process of the transferase activity. The results implied that B cells may be

participated in the mechanism of postmenopausal women osteoarthritis. However, the results need to be further confirmed by experiments.

Disclosure of conflict of interest

None.

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References

[1] Bonnick S, De Villiers T, Odio A, Palacios S, Chapurlat R, Dasilva C, Scott BB, Le Bailly De Tilleghem C, Leung AT, Gurner D. Effects of odanacatib on BMD and safety in the treatment of osteoporosis in postmenopausal women previously treated with alendronate: a randomized placebo-controlled trial. J Clin Endocrinol Metab 2013; 98: 4727-4735.

[2] Karsenty G, Oury F. The central regulation of bone mass, the first link between bone remodeling and energy metabolism. J Clin Endocrinol Metab 2010; 95: 4795-4801.

[3] Recker R, Lappe J, Davies KM, Heaney R. Bone remodeling increases substantially in the years after menopause and remains increased in older osteoporosis patients. J Bone Miner Res 2004; 19: 1628-1633.

[4] Faienza MF, Ventura A, Marzano F, Cavallo L. Postmenopausal osteoporosis: the role of immune system cells. Clinical and Developmental Immunology Clin Dev Immunol 2013; 2013: 575936.

[5] Kotake S, Udagawa N, Hakoda M, Mogi M, Yano K, Tsuda E, Takahashi K, Furuya T, Ishiyama S, Kim KJ. Activated human T cells directly induce osteoclastogenesis from human monocytes: possible role of T cells in bone destruction in rheumatoid arthritis patients. Arthritis Rheum 2001; 44: 1003-1012.

[6] Roato I, Grano M, Brunetti G, Colucci S, Mussa A, Bertetto O, Ferracini R. Mechanisms of spontaneous osteoclastogenesis in cancer with bone involvement. FASEB J 2005; 19: 228-230.

[7] D'amelio P, Grimaldi A, Di Bella S, Brianza SZ, Cristofaro MA, Tamone C, Giribaldi G, Ulliers D, Pescarmona GP, Isaia G. Estrogen deficiency increases osteoclastogenesis up-regulating T cells activity: a key mechanism in osteoporosis. Bone 2008; 43: 92-100.

[8] Brunetti G, Colucci S, Pignataro P, Coricciati M, Mori G, Cirulli N, Zallone A, Grassi FR, Grano M. T cells support osteoclastogenesis in an in vitro model derived from human periodontitis patients. J Periodontol 2005; 76: 1675-1680.

[9] Roggia C, Gao Y, Cenci S, Weitzmann MN, Toraldo G, Isaia G, Pacifici R. Up-regulation of TNF-producing T cells in the bone marrow: a key mechanism by which estrogen deficiency induces bone loss in vivo. Proc Natl Acad Sci U S A 2001; 98: 13960-13965.

[10] Onal M, Xiong J, Chen X, Thostenson JD, Almeida M, Manolagas SC, O'brien CA. Receptor activator of nuclear factor kappaB ligand (RANKL) protein expression by B lymphocytes contributes to ovariectomy-induced bone loss. J Biol Chem 2012; 287: 29851-29860.

[11] Xiao P, Chen Y, Jiang H, Liu YZ, Pan F, Yang TL, Tang ZH, Larsen JA, Lappe JM, Recker RR. In Vivo Genome-Wide Expression Study on Human Circulating B Cells Suggests a Novel ESR1 and MAPK3 Network for Postmenopausal Osteoporosis. J Bone Miner Res 2008; 23: 644-654.

[12] Gautier L, Cope L, Bolstad BM, Irizarry RA. affy-analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 2004; 20: 307-315.

[13] Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003; 4: 249-264.

[14] Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000; 28: 27-30.

[15] Croft D, Mundo AF, Haw R, Milacic M, Weiser J, Wu G, Caudy M, Garapati P, Gillespie M, Kamdar MR. The Reactome pathway knowledgebase. Nucleic Acids Res 2014; 42: D472-D477.

[16] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT. Gene Ontology: tool for the unification of biology. Nat Genet 2000; 25: 25-29.

[17] Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J, Minguez P, Bork P, Von Mering C. STRING v9. 1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res 2013; 41: D808-D815.

[18] Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics 2011; 27: 431-432.

[19] Dineley KT, Westerman M, Bui D, Bell K, Ashe KH, Sweatt JD. β-Amyloid activates the mitogen-activated protein kinase cascade via hippocampal α7 nicotinic acetylcholine receptors: in vitro and in vivo mechanisms related to Alzheimer's disease. J Neurosci 2001; 21: 4125-4133.

[20] Park BG, Yoo CI, Kim HT, Kwon CH, Kim YK. Role of mitogen-activated protein kinases in hydrogen peroxide-induced cell death in osteoblastic cells. Toxicology 2005; 215: 115-125.
[21] Plotkin LI, Aguirre JI, Kousteni S, Manolagas SC, Bellido T. Bisphosphonates and estrogens inhibit osteocyte apoptosis via distinct molecular mechanisms downstream of extracellular signal-regulated kinase activation. J Biol Chem 2005; 280: 7317-7325.
[22] Engelbrecht Y, De Wet H, Horsch K, Langeveldt C, Hough F, Hulley P. Glucocorticoids induce rapid up-regulation of mitogen-activated protein kinase phosphatase-1 and dephosphorylation of extracellular signal-regulated kinase and impair proliferation in human and mouse osteoblast cell lines. Endocrinology 2003; 144: 412-422.
[23] Unterberger U, Höftberger R, Gelpi E, Flicker H, Budka H, Voigtländer T. Endoplasmic reticulum stress features are prominent in Alzheimer disease but not in prion diseases in vivo. J Neuropathol Exp Neurol 2006; 65: 348-357.
[24] Wang M-X, Wei A, Yuan J, Trickett A, Knoops B, Murrell GA. Expression and regulation of peroxiredoxin 5 in human osteoarthritis. FEBS Lett 2002; 531: 359-362.
[25] Wu X, Kondragunta V, Kornman K, Wang H, Duff G, Renner J, Jordan J. IL-1 receptor antagonist gene as a predictive biomarker of progression of knee osteoarthritis in a population cohort. Osteoarthritis Cartilage 2013; 21: 930-938.
[26] Katoh M. WNT/PCP signaling pathway and human cancer (review). Oncol Rep 2005; 14: 1583.
[27] Zhu J, Chen T, Yang L, Li Z, Wong MM, Zheng X, Pan X, Zhang L, Yan H. Regulation of microRNA-155 in atherosclerotic inflammatory responses by targeting MAP3K10. PLoS One 2012; 7: e46551.
[28] Zhong J, Kyriakis JM. Dissection of a signaling pathway by which pathogen-associated molecular patterns recruit the JNK and p38 MAPKs and trigger cytokine release. J Biol Chem 2007; 282: 24246-24254.
[29] Bai XC, Lu D, Bai J, Zheng H, Ke ZY, Li XM, Luo SQ. Oxidative stress inhibits osteoblastic differentiation of bone cells by ERK and NF-κB. Biochem Biophys Res Commun 2004; 314: 197-207.
[30] Ceppi M, Pereira PM, Dunand-Sauthier I, Barras E, Reith W, Santos MA, Pierre P. MicroRNA-155 modulates the interleukin-1 signaling pathway in activated human monocyte-derived dendritic cells. Proc Natl Acad Sci U S A 2009; 106: 2735-2740.
[31] Zheng S, Vrindts Y, Lopez M, De Groote D, Zangerlé PF, Collette J, Franchimont N, Geenen V, Albert A, Reginster JY. Increase in cytokine production (IL-1β, IL-6, TNF-α but not IFN-γ, GM-CSF or LIF) by stimulated whole blood cells in postmenopausal osteoporosis. Maturitas 1997; 26: 63-71.
[32] Indo HP, Davidson M, Yen HC, Suenaga S, Tomita K, Nishii T, Higuchi M, Koga Y, Ozawa T, Majima HJ. Evidence of ROS generation by mitochondria in cells with impaired electron transport chain and mitochondrial DNA damage. Mitochondrion 2007; 7: 106-118.
[33] Guo Y, Yang TL, Liu YZ, Shen H, Lei SF, Yu N, Chen J, Xu T, Cheng Y, Tian Q. Mitochondria-Wide Association Study of Common Variants in Osteoporosis. Ann Hum Genet 2011; 75: 569-574.
[34] Petruzzella V, Tiranti V, Fernandez P, Ianna P, Carrozzo R, Zeviani M. Identification and characterization of human cDNAs specific to BCS1, PET112, SCO1, COX15, and COX11, five genes involved in the formation and function of the mitochondrial respiratory chain. Genomics 1998; 54: 494-504.
[35] Antonicka H, Mattman A, Carlson CG, Glerum DM, Hoffbuhr KC, Leary SC, Kennaway NG, Shoubridge EA. Mutations in< i> COX15 </i> Produce a Defect in the Mitochondrial Heme Biosynthetic Pathway, Causing Early-Onset Fatal Hypertrophic Cardiomyopathy. Am J Hum Genet 2003; 72: 101-114.
[36] Valnot I, Von Kleist-Retzow JC, Barrientos A, Gorbatyuk M, Taanman JW, Mehaye B, Rustin P, Tzagoloff A, Munnich A, Rötig A. A mutation in the human heme A: farnesyltransferase gene (COX10) causes cytochrome c oxidase deficiency. Hum Mol Genet 2000; 9: 1245-1249.
[37] Kim BJ, Baek S, Ahn SH, Kim SH, Jo MW, Bae SJ, Kim HK, Park GM, Kim YH, Lee SH. A higher serum gamma-glutamyl transferase level could be associated with an increased risk of incident osteoporotic fractures in Korean men aged 50 years or older. Endocr J 2014; 61: 257-263.
[38] Isomura H, Fujie K, Shibata K, Inoue N, Iizuka T, Takebe G, Takahashi K, Nishihira J, Izumi H, Sakamoto W. Bone metabolism and oxidative stress in postmenopausal rats with iron overload. Toxicology 2004; 197: 92-99.
[39] Xue L, Wang Y, Liu L, Zhao L, Han T, Zhang Q, Qin L. A 1HNMR-based metabonomics study of postmenopausal osteoporosis and intervention effects of er-xian decoction in ovariectomized rats. Int J Mol Sci 2011; 12: 7635-7651.