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RESEARCH ARTICLE

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Putative functional variants of PI3K/AKT/mTOR pathway are associated with knee osteoarthritis susceptibility

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Abstract

Background: Osteoarthritis (OA) is a degenerative musculoskeletal disease which causes joint deformity and pain and finally leads to limb dysfunction. Knee osteoarthritis (KOA) has the highest incidence among all kinds of OA. Strong evidence leads to the understanding that *P13K/AKT/mTOR* signaling is very important in cartilage degeneration.

Methods: This research sought to understand the association between genetic variation of *PI3K/AKT/mTOR* genes and KOA susceptibility among Chinese population. All the genetic variants of *PI3K/AKT/mTOR* pathway were graded and selected using RegulomeDB database, and then, an association study including 278 osteoarthritis patients and 289 controls was conducted.

Results: Finally, eight SNPs' genotypes' distributions and susceptibility to KOA were presented. *AKT1* rs2498789 was associated with KOA susceptibility in dominate genetic model (AA + GA vs GG) after adjusted for BMI, age, and gender: OR = 1.46, 95% CI: 1.03-2.05, *P* = .03. *PIK3CA* rs7646409 was also associated with KOA susceptibility (TC vs TT) after adjusted for BMI, age, and gender: OR = 0.58, 95% CI: 0.36-0.93, *P* = .02. *PIK3CA* rs7646409 (TC vs TT) with KOA risk was more significant in age < 60 group (*P* for heterogeneity was .03). Risk score showed significant association with KOA susceptibility after cumulative analysis (OR = 2.45, 95% CI: 1.35-4.45, *P* = .003).

Conclusions: This study shows that genetic variation of *PI3K/AKT/mTOR* is associated with KOA susceptibility in Chinese Han population, indicating that *PI3K/AKT/mTOR* is very important in KOA pathogenesis.

KEYWORDS

osteoarthritis, *PI3K/AKT/mTOR*, polymorphism, regulomeDB

Abbreviations: ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; BMI, body mass index; CIs, confidence intervals; ECM, extracellular matrix; HWE, Hardy-Weinberg equilibrium; KOA, knee osteoarthritis; MMP, matrix metalloproteinase; ORs, odd ratios; SNP, single nucleotide polymorphisms.

Kejie Wang and Minjie Chu are contributed equally to this work.

1 | INTRODUCTION

Osteoarthritis (OA) is a kind of musculoskeletal disease which influence the bone, synovial tissues, and articular cartilage with no obvious regional and racial difference.[1] Over million people are affected by OA worldwide, and it is an important reason for long-term immortality in China.[2,3] OA commonly affects various human joints, and knee joints are most vulnerable. Pathology characteristics of knee osteoarthritis (KOA) are cartilage degeneration, cartilage extracellular matrix (ECM) deterioration, subchondral bone sclerosis, and synovitis.[4,5] The clinical manifestations of KOA are joint swelling, pain, malformation, and lose of motion.[6] KOA brings huge burden to family and society all over the world.[7] Thus, it is urgent to explore detail pathophysiology and molecular biology mechanism of OA for early diagnosis and treatment.

The etiology of osteoarthritis has not been fully understood. Risk factors such as obesity, genetics, family susceptibility, local biomechanics, age, and previous trauma may lead to OA occurrence and progression.[8] As the sole cell type in joint cartilage, the imbalance between apoptosis and proliferation of chondrocytes is very important for OA.[9] Therefore, keeping the homeostasis between apoptosis and proliferation of chondrocytes may facilitate cartilage repair and symptoms relieve of OA *PI3K/AKT/mTOR* pathway has received substantial attentions because it plays a crucial role during several characteristic alterations of cartilage such as expression of matrix metalloproteinase (MMP) or a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) which will finally lead to the apoptosis of chondrocytes.[10] *PI3K/AKT/mTOR* pathway belongs to serine/threonine protein kinase family. Inhibiting *PI3K/AKT/mTOR* pathway could relieve inflammation response in rats with OA which means that *PI3K/AKT/mTOR* pathway participates in the pathology of OA.[11]

RegulomeDB, which is a database integrating data from Encyclopedia of DNA Elements project, illuminates regulatory variants' function in human genes. Based on the functional confidence of variants, RegulomeDB provides a scoring system, which ranges from 1 to 6. A variant will possibly affect gene expression or transcription factor binding if its score is low. In summary, RegulomeDB is a powerful tool for genetic association study.

In this research, we assumed that *PI3K/AKT/mTOR* genetic variants are potential pivotal targets for diagnosis or treatment of OA. To verify it, an association study was conducted between *PI3K/AKT/mTOR* genetic variants identified by RegulomeDB and KOA susceptibility.

2 | MATERIALS AND METHODS

In this research, patients were diagnosed with KOA according to American College of Rheumatology (ACR) classification of KOA from 2013 to 2017 at the Department of Orthopaedics, Changzhou First People's Hospital, Jiangsu, China.[12] Anteroposterior weight-bearing radiographs of every patient's affected knees were taken. A group of orthopedist and radiologist evaluated the X-ray of knee to give a 0-4 score based on the Kellgren-Lawrence (KL) classification of KOA.[13] Posttraumatic arthritis, postseptic arthritis, inflammatory arthritis (autoimmune disease, rheumatoid arthritis, or septic arthritis), developmental dysplasia, and other etiologies of knee were excluded. At the same hospital during the same period, age- and sex-matched healthy volunteers were recruited. All controls reported no history of KOA and other joint disease.

TABLE 1 Distributions of select variables in OA cases and controls

Variables	Case n = 278 (%)	Control n = 289 (%)	*P*
Age, y (mean ± SD)	62.00 ± 10.55	61.13 ± 10.92	.34
Gender			
Male	82(29.5)	87(30.1)	.87
Female	196(70.5)	202(69.9)	
BMI	24.97 ± 3.26	23.84 ± 2.96	**<.01**
BMI < 25	150(54.0)	189(66.5)	**<.01**
BMI ≥ 25	128(46.0)	95(33.5)	
KL classification			
1-2	135		
3-4	143		

Note: Bold font presents $P < .05$.

Two trained interviewers inquired each case and control subject to collect demographic information. Every participant written a consent, and then, 5 mL peripheral blood was taken. Each participant's weight and height were measured accurate to 0.1 kg and 1 cm. Body mass index (BMI) was calculated. This research was agreed by Changzhou First People's Hospital's Human Research Ethics Committees.

This study tries to explore whether potential functional variations were associated with knee osteoarthritis of Chinese Han population. We select seven important genes (*mTOR*, *ULK1*, *ULK2*, *ATG13*, *AKT1*, *AKT2*, *and PIK3CA*) in *PI3K/AKT/mTOR* pathway.[14,15] The lower RegulomeDB score is, the more possible these variants will have functional significance. With this principle, 90 SNPs whose RegulomeDB scores range from 1 to 2b were selected. And also according to the information from UCSC database (GRCh37/hg19), linkage disequilibrium (LD) <0.8 and minor allele frequency (MAF) >0.05, 12 potentially functional genetic variants of *PI3K/AKT/mTOR* were finally singled out.

Genomic DNA was extracted according to the method described before.[16] Genotyping was performed with Sequenom's MassARRAY® iPLEX assay following instructions of the manufacturer. Genotyping of three SNPs was failure because of probe design. Finally, 9 SNPs were genotyped successfully with over 95% call rate. We randomly selected more than 10% samples to test again for quality control with over 99% consistency. We used Student's *t* tests and chi-square test to detect demographic data or genotypes' distribution difference between different groups for continuous

TABLE 2 Logistic regression analysis of associations between selected polymorphisms and KOA risk

Gene	Genotype	Case	Control	OR (95% CI)	*P*	OR (95% CI)[a]	*P*[a]
ATG13 rs10838610	AA	199 (72%)	197 (68%)				
	AG	75 (27%)	82 (28%)	0.91 (0.63-1.31)	.6	0.94 (0.64-1.37)	.74
	GG	3 (1%)	10 (4%)	0.30 (0.08-1.1)	.07	0.30 (0.08-1.13)	.08
	GG + AG vs.AA			0.84 (0.59-1.20)	.34	0.87 (0.60-1.25)	.44
	GG vs.AG + AA			0.31 (0.08-1.12)	.07	0.31 (0.08-1.15)	.08
	ADD			0.79 (0.58-1.1)	.16	0.81 (0.59-1.13)	.22
ULK1 rs1134574	AA	243 (88%)	247 (86%)				
	GA	34 (12%)	41 (14%)				
	GG	0 (0%)	0 (0%)				
	GG + GA vs.AA			0.84 (0.52-1.37)	.49	0.75 (0.45-1.23)	.25
	GG vs.GA + AA						
	ADD			0.84 (0.52-1.37)	.49	0.75 (0.45-1.23)	.25
ULK2 rs157389	TT	168 (61%)	163 (56%)				
	TC	89 (33%)	110 (38%)	0.79 (0.55-1.12)	.18	0.81 (0.56-1.16)	.25
	CC	17 (6%)	16 (6%)	1.03 (0.5-2.11)	.93	0.89 (0.42-1.87)	.76
	CC + TC vs.TT			0.82 (0.58-1.14)	.24	0.82 (0.58-1.16)	.26
	CC vs.TC + TT			1.13 (0.56-2.28)	.74	0.96 (0.46-2.00)	.92
	ADD			0.89 (0.68-1.17)	.41	0.87 (0.66-1.15)	.34
AKT1 rs2498789	GG	119 (44%)	148 (52%)				
	GA	126 (46%)	106 (37%)	1.48 (1.04-2.11)	.03	1.58 (1.10-2.28)	**.01**
	AA	26 (10%)	30 (11%)	1.08 (0.60-1.92)	.8	1.07 (0.59-1.91)	.83
	AA + GA vs.GG	152 (56)	136 (48)	1.39 (1.00-1.94)	.05	1.46 (1.03-2.05)	**.03**
	AA vs.GA + GG			0.90 (0.52-1.56)	.7	0.87 (0.49-1.52)	.62
	ADD			1.18 (0.92-1.52)	.2	1.20 (0.93-1.55)	.16
AKT1 rs3001371	TT	106 (39%)	117 (41%)				
	CT	127 (46%)	137 (47%)	1.02 (0.72-1.46)	.9	1.01 (0.70-1.46)	.96
	CC	41 (15%)	34 (12%)	1.33 (0.79-2.25)	.29	1.39 (0.81-2.39)	.23
	CC + CT vs.TT			1.08 (0.77-1.52)	.64	1.08 (0.76-1.53)	.66
	CC vs.CT + TT			1.32 (0.81-2.14)	.27	1.38 (0.84-2.28)	.2
	ADD			1.12 (0.88-1.43)	.37	1.13 (0.88-1.45)	.34
AKT2 rs3730051	TT	134 (49%)	132 (46%)				
	TC	114 (41%)	121 (42%)	0.93 (0.65-1.32)	.68	1.02 (0.71-1.46)	.91
	CC	28 (10%)	35 (12%)	0.79 (0.45-1.37)	.4	0.86 (0.49-1.52)	.61
	CC + TC vs.TT			0.9 (0.64-1.25)	.52	0.98 (0.70-1.38)	.93
	CC vs.TC + TT			0.82 (0.48-1.38)	.45	0.85 (0.50-1.47)	.57
	ADD			0.9 (0.7-1.15)	.41	0.96 (0.74-1.23)	.74
AKT2 rs7247515	CC	247 (90%)	258 (89%)				
	CT	27 (10%)	31 (11%)	0.91 (0.53-1.57)	.73	0.96 (0.55-1.68)	.9
	TT	1 (0%)	0 (0%)				
	TT + CT vs. CC			0.94 (0.55-1.62)	.83	0.99 (0.57-1.72)	.98
	TT vs. CT + CC						
	ADD			0.98 (0.58-1.66)	.95	1.02 (0.6-1.75)	.93

(Continues)

TABLE 2 (Continued)

Gene	Genotype	Case	Control	OR (95% CI)	*P*	OR (95% CI)[a]	*P*[a]
PIK3CA rs7646409	TT	237 (87%)	225 (81%)				
	TC	35 (13%)	53 (19%)	0.63 (0.39-1.00)	.05	0.58 (0.36-0.93)	**.02**
	CC	0 (0%)	0 (0%)				
	CC + TC vs. TT						
	CC vs. TC + TT						
	ADD						

Notes: Bold font presents $P < .05$.

[a]Adjusted for age, gender and BMI.

and categorical variables. Hardy-Weinberg equilibrium (HWE) was calculated for each SNP in controls. Logistic regression was adopted to estimate 95% confidence intervals (CIs) or odd ratios (ORs) as an evaluation of association with the KOA susceptibility, adjusted for BMI, gender, and age. Corresponding subgroups' heterogeneity was detected with chi-square-based Q test. Cumulative effects of all genotyped SNPs were also evaluated with a risk score analysis using a linear of genotypes (coded as 0, 1, and 2). SPSS Statistics Version 18.0 software was used to finish above calculation.

3 | RESULTS

The clinical variable distribution between controls and cases is demonstrated in Table 1. Gender and age of two groups were comparable ($P > .05$). All of them have over 95% for success rates of genotyping. One variant rs892119 was not in accordance with HWE. Genotypes' distributions and susceptibility to KOA of the remaining eight SNPs are presented in Table 2.

AKT1 rs2498789 shown borderline association with KOA susceptibility in dominate genetic model (AA + GA vs GG): OR = 1.39, 95% CI: 1.00-1.94, $P = .05$. The association remained after adjusted for BMI, age, and gender in dominate model: OR = 1.46, 95% CI: 1.03-2.05, $P = .03$. *PIK3CA* rs7646409 also shown borderline association with increased risk of KOA susceptibility (TC vs TT): OR = 0.63, 95% CI: 0.39-1.00, $P = .05$. After adjusted for BMI, gender, and age, the results were more significant: OR = 0.58, 95% CI: 0.36-0.93, $P = .02$.

Stratified analyses (Table 3) were preceded in positive results mentioned above. Stratified analyses showed that *PIK3CA* rs7646409 (TC vs TT) with KOA susceptibility was more obvious in age <60 group (P for heterogeneity test was .03). A risk score analysis was used to calculated cumulative effects of the remaining 8 genetic variants (Table 4). The mean cumulative risk score of controls (−0.03 ± 0.28) was lower than that of cases (−0.11 ± 0.31) (P for T test < .05). Logistic regression analyses indicated that cumulative risk score was associated with KOA susceptibility (Table 4).

TABLE 3 Stratified analyses of rs2498789 and rs7646409 genotypes associated with patients of KOA by selected variables

	rs2498789AA + GA vs. GG					rs7646409TC vs. TT				
	Case	Control	OR (95% CI)[a]	*P*[a]	P_{het}[b]	Case	Control	OR (95% CI)[a]	*P*[a]	P_{het}[b]
Age										
Age < 60	62/49	69/69	1.38 (0.82-2.32)	.23	.77	11/100	30/107	0.31 (0.14-0.69)	.00	**.03**
Age ≥ 60	90/70	67/79	1.53 (0.96-2.43)	.07		24/137	23/118	0.95 (0.50-1.78)	.87	
Gender										
Male	43/36	39/48	1.59 (0.84-2.99)	.15	.74	7/72	16/70	0.45 (0.17-1.17)	.10	.55
Female	109/83	97/100	1.40 (0.93-2.11)	.11		28/165	37/155	0.63 (0.36-1.09)	.10	
BMI										
BMI < 25	88/59	88/97	1.67 (1.08-2.60)	.02	.29	18/130	33/149	0.62 (0.33-1.16)	.14	.75
BMI ≥ 25	64/60	45/49	1.15 (0.67-1.97)	.62		17/107	20/71	0.53 (0.26-1.11)	.09	
KL										
KL12	76/52	136/148	1.59 (1.03-2.45)	.03	.58	21/112	53/225	0.76 (0.43-1.33)	.33	.17
KL34	74/65	136/148	1.34 (0.87-2.05)	.18		13/122	53/225	0.41 (0.21-0.81)	.01	

Note: Bold font presents $P < .05$.

[a]Adjusted for age, gender, and BMI.

[b]P value for heterogeneity test.

TABLE 4 Cumulative risk scores of eight SNPs on the risk of KOA

	Case	Control	P^a	OR (95% CI)	P^b
Risk score	−0.11 ± 0.31	−0.03 ± 0.28	**.03**	2.45 (1.35-4.45)	**.003**

Note: Bold font presents $P < .05$.

[a]*P* for *t* test.

[b]Adjusted for age, gender, and BMI.

4 | DISCUSSION

In this article, an association study was conducted between putative functional variants of *PI3K/AKT/mTOR* pathway and KOA. Our results show that *AKT1* rs2498789 and *PIK3CA* rs7646409 were associated with KOA susceptibility in Chinese Han population. Different age-group of *PIK3CA* rs7646409 showed heterogeneity in our research, which means gene-environment interaction exists between genetic variants of *PIK3CA* rs7646409 and age.

The *PI3K/AKT/mTOR* pathway is a pivotal axis in various cellular processes including apoptosis, metabolism, angiogenesis, transcription, and cell cycle.[17] And it is found abnormal expressed in many cancers.[18] Previous studies have indicated that *PI3K/AKT/mTOR* is very important during pathogenesis of OA.[15,19] Inhibition of *PI3K/AKT/mTOR* increases articular chondrocyte autophagy and ameliorates the progression of osteoarthritis in rats.[11,20] Other research reported that *PI3K/AKT/mTOR* pathway's activation can promote chondrocyte autophagy and protect against cartilage injury.[21] Progression of osteoarthritis is affected by inflammatory cytokines. *PI3K/AKT/mTOR* signaling pathway could mediate TNF-α mRNA expression and NF-κB activation in osteoblasts.[22] Another research reported that inhibition of *PI3K/AKT/mTOR* could protect chondrocytes against inflammation in osteoarthritis.[23] *PI3K/AKT/mTOR* activation is essential for the chondrocyte proliferation and protection of chondrocytes from apoptosis.[24,25] Thus, the detail mechanism of *PI3K/AKT/mTOR* in OA remains unclear.

Rs7646409 of *PIK3CA* is associated with knee osteoarthritis susceptibility. Rs7646409 was also proved to be associated with osteosarcoma.[26] *PIK3CA* encoded human p110α protein and participates in a complex interaction with tumor microenvironment. Its mutations appear in more than one-third of breast cancer patients.[27] The RegulomeDB score of *PIK3CA* rs7646409 is 2a. ChIP-seq data showed that rs7646409 could affect *EP300*, *JUND*, *FOS*, *FOSL2*, and *FOSL1*'s expression. *EP300* directly incorporated with autophagy-related proteins and enhanced their activity and acetylation status in chondrocytes.[28] *EP300* gene and protein expression are increased in human OA chondrocytes following spermidine treatment, compared with controls.[28] It is reported that *JUND*, *FOSL2*, and *FOS* are suppressed in OA chondrocytes.[29] Activator protein (AP-1) complex is composed of c-FOS and JUND. AP-1 is crucial for expression increase of collagenase-3. It is also required for stimulation of TGF-beta for optimal response in human osteoarthritis chondrocytes.[30] Based on the above evidence, we may hypothesize that *PIK3CA* is very important in the pathology of OA. So any factor that influences expression of *PIK3CA* may have effect directly or indirectly on the pathology of OA.

In this study, we found *AKT1* rs2498789 was associated with KOA *AKT1* which involved in protein synthesis and cellular survival pathway is very important for muscle or skeletal hypertrophy and general tissue growth. *AKT1* knockout mouse reveals increased spontaneous apoptosis and growth retardation in tissues such as thymus and testes.[31] *AKT1* has also been shown connection with kinds of cancer since it could block apoptosis and therefore increase cell survival.[32] Beyond that *AKT1* is an important signaling factor in insulin signaling pathway.[33] The RegulomeDB score of *AKT1* rs2498789 is 2b. ChIP-seq data showed that rs2498789 could influence *IKZF1* expression. *IKZF1* encodes Ikaros, which is a zinc-finger transcription factor related with cytokine expression maintenance of mature lymphocytes.[34] Further researches need to be done to clarify how *AKT1* participates in the pathology of OA.

However, there are several limitations in our study. First, the samples in our study are relatively small. Further independent studies with large sample sizes are necessary to confirm our findings. Second functional studies should be conducted to unravel the molecular mechanisms underlying this association. But it is very difficult to collect the cartilage from normal people due to limited donors. So it is feasible to compare degenerative cartilage and relative "normal" cartilage from patients who underwent total knee joint replacement in future. Third, our study finds the association in Chinese Han population, and multicenter association should be conducted between different races to confirm our findings in future.

5 | CONCLUSION

This research indicated that potential functional genetic variation in *PI3K/AKT/mTOR* pathway is associated with KOA susceptibility. Further functional studies are essential to illuminate the molecular pathology of KOA.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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REFERENCES

1. Hussain SM, Neilly DW, Baliga S, Patil S, Meek R. Knee osteoarthritis: a review of management options. *Scott Med J*. 2016;61(1):7-16.
2. Bhatia D, Bejarano T, Novo M. Current interventions in the management of knee osteoarthritis. *J Pharm Bioallied Sci*. 2013;5(1):30-38.
3. Yu H, Wang Y, Guo Y, Wang H, Chen B, Zhao X. Quality assessment of randomized controlled trials reporting on knee osteoarthritis treated with warming needle moxibustion. *J Tradit Chin Med*. 2014;34(5):621-626.
4. Brandt KD, Fife RS. Ageing in relation to the pathogenesis of osteoarthritis. *Clin Rheum Dis*. 1986;12(1):117-130.
5. Loeser RF, Collins JA, Diekman BO. Ageing and the pathogenesis of osteoarthritis. *Nat Rev Rheumatol*. 2016;12(7):412-420.
6. Mobasheri A. Role of chondrocyte death and hypocellularity in ageing human articular cartilage and the pathogenesis of osteoarthritis. *Med Hypotheses*. 2002;58(3):193-197.
7. Robertson C, Archibald D, Avenell A, et al. Systematic reviews of and integrated report on the quantitative, qualitative and economic evidence base for the management of obesity in men. *Health Technol Assess*. 2014;18(35):v–vi, xxiii-xxix, 1-424.
8. Rayegani SM, Raeissadat SA, Heidari S, Moradi-Joo M. Safety and effectiveness of low-level laser therapy in patients with knee osteoarthritis: a systematic review and meta-analysis. *J Lasers Med Sci*. 2017;8(suppl 1):S12-S19.
9. Sezgin M, Barlas IO, Yildir S, et al. Apoptosis-related Fas and FasL gene polymorphisms' associations with knee osteoarthritis. *Rheumatol Int*. 2013;33(8):2039-2043.
10. Huang CY, Lin HJ, Chen HS, Cheng SY, Hsu HC, Tang CH. Thrombin promotes matrix metalloproteinase-13 expression through the PKCdelta c-Src/EGFR/PI3K/Akt/AP-1 signaling pathway in human chondrocytes. *Mediators Inflamm*. 2013;2013:326041.
11. Xue JF, Shi ZM, Zou J, Li XL. Inhibition of PI3K/AKT/mTOR signaling pathway promotes autophagy of articular chondrocytes and attenuates inflammatory response in rats with osteoarthritis. *Biomed Pharmacother*. 2017;89:1252-1261.
12. Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum*. 1986;29(8):1039-1049.
13. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Ann Rheum Dis*. 1957;16(4):494-502.
14. Ramos YF, Bos SD, Lakenberg N, et al. Genes expressed in blood link osteoarthritis with apoptotic pathways. *Ann Rheum Dis*. 2014;73(10):1844-1853.
15. Zhang Y, Vasheghani F, Li YH, et al. Cartilage-specific deletion of mTOR upregulates autophagy and protects mice from osteoarthritis. *Ann Rheum Dis*. 2015;74(7):1432-1440.
16. Zhang CW, Zhang XL, Xia YJ, et al. Association between polymorphisms of the CYP11A1 gene and polycystic ovary syndrome in Chinese women. *Mol Biol Rep*. 2012;39(8):8379-8385.
17. Malemud CJ. The PI3K/Akt/PTEN/mTOR pathway: a fruitful target for inducing cell death in rheumatoid arthritis? *Future Med Chem*. 2015;7(9):1137-1147.
18. Aziz AUR, Farid S, Qin K, Wang H, Liu B. PIM kinases and their relevance to the PI3K/AKT/mTOR pathway in the regulation of ovarian cancer. *Biomolecules*. 2018;8(1):7.
19. Cravero JD, Carlson CS, Im HJ, Yammani RR, Long D, Loeser RF. Increased expression of the Akt/PKB inhibitor TRB3 in osteoarthritic chondrocytes inhibits insulin-like growth factor 1-mediated cell survival and proteoglycan synthesis. *Arthritis Rheum*. 2009;60(2):492-500.
20. Hu ZC, Gong LF, Li XB, et al. Inhibition of PI3K/Akt/NF-kappaB signaling with leonurine for ameliorating the progression of osteoarthritis: in vitro and in vivo studies. *J Cell Physiol*. 2019;234(5):6940-6950.
21. Zhang Q, Lai S, Hou X, Cao W, Zhang Y, Zhang Z. Protective effects of PI3K/Akt signal pathway induced cell autophagy in rat knee joint cartilage injury. *Am J Transl Res*. 2018;10(3):762-770.
22. Qiu L, Zhang L, Zhu L, et al. PI3K/Akt mediates expression of TNF-alpha mRNA and activation of NF-kappaB in calyculin A-treated primary osteoblasts. *Oral Dis*. 2008;14(8):727-733.
23. Chen HW, Lin AH, Chu HC, et al. Inhibition of TNF-alpha-Induced Inflammation by andrographolide via down-regulation of the PI3K/Akt signaling pathway. *J Nat Prod*. 2011;74(11):2408-2413.
24. Liang J, Xu L, Zhou F, et al. MALAT1/miR-127-5p regulates osteopontin (OPN)-mediated proliferation of human chondrocytes through PI3K/Akt pathway. *J Cell Biochem*. 2018;119(1):431-439.
25. Rao Z, Wang S, Wang J. Peroxiredoxin 4 inhibits IL-1beta-induced chondrocyte apoptosis via PI3K/AKT signaling. *Biomed Pharmacother*. 2017;90:414-420.
26. He ML, Wu Y, Zhao JM, Wang Z, Chen YB. PIK3CA and AKT gene polymorphisms in susceptibility to osteosarcoma in a Chinese population. *Asian Pac J Cancer Prev*. 2013;14(9):5117-5122.
27. Thirumal Kumar D, George Priya Doss C. Role of E542 and E545 missense mutations of PIK3CA in breast cancer: a comparative computational approach. *J Biomol Struct Dyn*. 2017;35(12):2745-2757.
28. Sacitharan PK, Lwin S, Gharios GB, Edwards JR. Spermidine restores dysregulated autophagy and polyamine synthesis in aged and osteoarthritic chondrocytes via EP300. *Exp Mol Med*. 2018;50(9):123.
29. Fisch KM, Gamini R, Alvarez-Garcia O, et al. Identification of transcription factors responsible for dysregulated networks in human osteoarthritis cartilage by global gene expression analysis. *Osteoarthritis Cartilage*. 2018;26(11):1531-1538.
30. Tardif G, Reboul P, Dupuis M, et al. Transforming growth factor-beta induced collagenase-3 production in human osteoarthritic chondrocytes is triggered by Smad proteins: cooperation between activator protein-1 and PEA-3 binding sites. *J Rheumatol*. 2001;28(7):1631-1639.
31. Chen WS, Xu PZ, Gottlob K, et al. Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene. *Genes Dev*. 2001;15(17):2203-2208.
32. Staal SP, Hartley JW, Rowe WP. Isolation of transforming murine leukemia viruses from mice with a high incidence of spontaneous lymphoma. *Proc Natl Acad Sci U S A*. 1977;74(7):3065-3067.
33. Garofalo RS, Orena SJ, Rafidi K, et al. Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB beta. *J Clin Invest*. 2003;112(2):197-208.
34. Swafford AD, Howson JM, Davison LJ, et al. An allele of IKZF1 (Ikaros) conferring susceptibility to childhood acute lymphoblastic leukemia protects against type 1 diabetes. *Diabetes*. 2011;60(3):1041-1044.

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