Identification of HIF-1α/VEGFA signaling pathway and transcription factors in Kashin-Beck disease by integrated bioinformatics analysis

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Abstract. Kashin-Beck disease (KBD) is a chronic and endemic osteoarthropathy. The pathogenesis of KBD has yet to be fully elucidated, although previous studies have shown that its etiology may be associated with low selenium abundance and high exposure to mycotoxins, such as T-2 toxin. In the present study, the Comparative Toxicogenomics Database was used to identify key genes associated with KBD, T-2 toxin and selenium. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were used to identify the biological processes and pathways that key genes may be associated with. By searching the Search Tool for the Retrieval of Interacting Genes database and the Molecular Complex Detection plug-in with Cytoscape, it was possible to construct a KBD-associated protein-protein interaction (PPI) network, and screen the core modules and genes. Western blot analysis was subsequently used to verify the expression levels of hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor A (VEGFA), two components that are associated with the HIF-1 signaling pathway in KBD disease. Via this approach, 10 core genes and 15 transcription factors were obtained. These results may help to clarify the pathogenesis of KBD, thereby providing further avenues for the therapeutic treatment of KBD.

Introduction

Kashin-Beck disease (KBD) is a chronic and endemic osteoarthropathy characterized by chondrocyte necrosis in growth plates and articular cartilage (1). The geographical distribution of KBD includes southeastern Siberia in Russia, the northern region of North Korea and a long narrow zone from northeast to southwest China (2). Patients with KBD often exhibit clinical features such as arthralgia, restricted mobility and an enlarged metaphysis (1). In severe cases, short stature and dwarfism may occur (3). The pathogenesis of KBD has yet to be fully elucidated, although it is generally considered that its cause is multifactorial, including such contributory factors as selenium deficiency, iodine deficiency, food contaminated with mycotoxins and drinking water contaminated with humic acid (4).

Mycotoxins are a group of toxic secondary metabolites produced by fungal species. According to present statistics, ~25% of the world’s food crops are contaminated with mycotoxins (5). T-2 toxin is a mycotoxin widely found in grains in the geographical regions where KBD is prevalent, and has been shown to induce apoptosis of human chondrocytes, oxidative stress and mitochondrial damage (6). When compared with normal subjects, the expression levels of apoptosis-associated molecules, including Bcl-2, Bax, Fas and inducible nitric oxide synthase, in the articular cartilage of patients with KBD have been shown to be elevated (7). In addition, T-2 toxin contamination and selenium deficiency have been shown to be widespread in drinking water and cereals in KBD-endemic areas (8). Selenium is an essential biological trace element that has previously been used in the treatment of KBD (9).
The Database for Annotation, Visualization and Integrated Gene Ontology (GO) and pathway enrichment analysis. The Database for Annotation, Visualization and Integrated Gene Ontology (GO) and pathway enrichment analysis. The database includes in excess of 38 million toxicogenomic relationships that may be explored further in terms of analytical investigations and the development of scientific hypotheses. In the present study, all key genes associated with KBD, T-2 toxin and selenium were predicted using CTD database that was updated to June 2020 (inference score, >3.07).

Materials and methods

Target identification using the CTD. The CTD (http://ctdbase.org) is a useful public resource featuring extensive information regarding exposure to numerous types of chemicals and human health (28). The database includes in excess of 38 million toxicogenomic relationships that may be explored further in terms of analytical investigations and the development of scientific hypotheses. In the present study, all key genes associated with KBD, T-2 toxin and selenium were predicted using CTD database that was updated to June 2020 (inference score, >3.07).

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PPI network analysis. Information regarding PPIs may be evaluated using an online tool, the Search Tool for the Retrieval of Interacting Genes (STRING; https://string-db.org) (29). To estimate the interactions of KBD-associated key genes, these genes were first analyzed by STRING, and subsequently Cytoscape software (version 3.6.1; https://cytoscape.org) was used to construct a PPI network. The Molecular Complex Detection (MCODE; http://apps.cytoscape.org/apps/mcode) plug-in for Cytoscape was used to investigate modules of the PPI network (degree cutoff=2; maximum depth=100; k-core=2; node score cutoff=0.2). Similarly, the STRING database and Cytoscape software were used to construct a PPI network of genes associated with the HIF-1 signaling pathway.

Chondrocyte culture and experimental protocol. Human C28/I2 normal chondrocytes were purchased from the BeNa Culture Collection and cultured in DMEM Nutrient Mixture F-12 (DMEM/F12; 1:1) (Gibco; Thermo Fisher Scientific) supplemented with 10% FBS (Hyclone; Cytiva) in a humidified incubator containing 5% CO₂ at 37°C. All cells were used for subsequent experiments between the fifth and tenth passages. Cells were cultured in 6-well plates and used for protein extraction only once the cell density had reached 6x10⁴ cells/well. The medium was replaced every other day. T-2 toxin was provided by MedChemExpress and dissolved in DMSO to make a working solution with a concentration of 100 µg/ml T-2 toxin. The cells were plated and incubated for 24 h to allow them to adhere prior to treatment with T-2 toxin. Subsequently, cells were exposed to fresh medium containing various doses of T-2 toxin (0, 0.001, 0.005, 0.01, 0.02 and 0.05 µg/ml). Proteins were then extracted by ultrasonic disruption after the C28/I2 chondrocytes had been incubated at 37°C with T-2 toxin for 3 days, as detailed in previous studies (30,31). Incubation with each concentration of T-2 toxin was repeated five times, and DMSO-treated cells were used as a control.

Western blot analysis. The protein concentration was determined by using the BCA method (Biyunian Biotechnology). SDS-PAGE loading buffer was added and the protein sample boiled in 100°C water. Protein samples of chondrocytes treated with different concentrations of T-2 toxin were separated using SDS-PAGE (10% gels) and transferred to PVDF membranes (EMD Millipore). After blocking with 5% skimmed milk diluted in TBS containing 0.1% Tween-20 (TBST) overnight at room temperature, the membranes were incubated with primary antibodies, as detailed below, and then incubated with the secondary antibodies conjugated with horseradish peroxidase. Blots were visualized by using a hypersensitivity ECL chemiluminescence detection kit (Biyunian Biotechnology).
The anti-HIF-1α (1:1,000; cat. no. ab82832) and anti-VEGFA (1:1,000; cat. no. ab46154) antibodies were purchased from Abcam, whereas the rabbit anti-β-actin (1:3,000; cat. no. bs-0061R) and goat anti-rabbit IgG (1:3,000; cat. no. bs-0295G-HRP) antibodies were purchased from BIOSS. The primary antibodies were incubated for 30 min at 37˚C and then overnight at 4˚C. The membrane was subsequently incubated with a secondary antibody for 1 h at room temperature after washing three times in TBST. The relative intensities of the blots featuring the target proteins of interest were calculated by normalizing against β-actin. Densitometric analysis of western blots was performed using ImageJ software (v1.52; National Institutes of Health).

Transcription factor prediction. iRegulon (http://apps.cytoscape.org/apps/iregulon) was developed using a genome-wide ranking-and-recovery approach as a Cytoscape plug-in for the purpose of detecting enriched transcription factor motifs and their optimal sets of direct targets (32). This technology was used to perform the enrichment of transcription factor motifs in target sequences with a position matrix method to identify transcription factors that were associated with HIF-1α, VEGFA and the HIF-1 signaling pathway. A minimum identity between orthologous genes was defined as 0.05, and the maximum false discovery rate (FDR) value of motif similarity was set to 0.001. Associations with the normalized enrichment score (NES) were used for further analysis.

Statistical analysis. The results are expressed as the mean ± SD for experiments performed in triplicate. Statistical analysis was performed using one-way ANOVA and the means were compared by Dunnett’s post hoc test using GraphPad Prism statistical software (version 8.01; GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

Identification of genes associated with KBD, T-2 toxin and selenium. Using the CTD database, 3,676 genes were identified to be associated with KBD, 2,299 to be associated with T-2 toxin and 1,669 to be associated with selenium. In order to obtain the KBD-associated key genes, the above genes were selected for intersection analysis. A set of 301 key genes were revealed to be held in common among the genes of the KBD, T-2 toxin and selenium groups, as shown by the Venn diagram in Fig. 1.

Functional enrichment analysis of KBD-associated key genes. The results revealed that the majority of key genes included in the GO enrichment analysis were associated with the BP component term ‘negative regulation of apoptotic process’ (GO: 0043066; Fig. 2A). The BP terms ‘response to hypoxia’ and ‘response to reactive oxygen species’ were also in the top 10 results of the GO enrichment analysis. The majority of key genes were associated with the CC parameter term ‘cytosol’ (GO: 0005829) and MF parameter term ‘protein binding’ (GO: 0005515). Furthermore, KEGG enrichment analysis of the key genes found the term ‘pathways in cancer’ (hsa05200) to be most significantly enriched (Fig. 2B). In addition, signaling pathways known to be associated with KBD were identified among the top 15 enrichment terms, including ‘apoptosis’ (hsa04210), and the ‘TNF signaling pathway’ (hsa04668), ‘PI3K-AKT signaling pathway’ (hsa04151) and ‘HIF-1 signaling pathway’ (hsa04066).

Construction of the PPI network for KBD-associated key genes. A PPI network of key genes was constructed in the online database STRING, and contained 299 nodes and 2,830 edges. Subsequently, the interaction pairs were entered into Cytoscape software to construct multiple PPI networks. The core network module was then selected using the Cytoscape MCODE plug-in. The first-ranked module was extracted under the default parameters and contained 42 nodes and 716 edges (Fig. 3). The top 10 genes with MCODE scores in this module were revealed to be BCL2L1, MMP9, CASP8, HSP90AA1, IL10, HSPA4, CXCL8, CASP9, MTOR and TNF.

Downregulation of HIF-1α and VEGFA in T-2 toxin-treated chondrocytes. In order to study the expression of HIF-1α and VEGFA in KBD, different concentrations of T-2 toxin were used to treat chondrocytes for 3 days. Western blotting results revealed that the expression levels of HIF-1α and VEGFA in the T-2 toxin-treated group were significantly reduced in comparison with the control group. As the concentration of T-2 toxin was increased, the expression levels of HIF-1α and VEGFA were gradually reduced. However, when the concentration of the T-2 toxin added was 0.01 and 0.02 µg/ml, the expression level of HIF-1α showed a tendency to increase (Fig. 4).

Construction of the PPI network associated with the HIF-1α signaling pathway and prediction of transcription factors. Including HIF-1α and VEGFA, all molecules of the HIF-1α signaling pathway identified by KEGG enrichment analysis were used to construct a PPI network (Fig. 5). Subsequently,
the constructed network was imported into Cytoscape, and the iRegulon plug-in was used to predict the transcription factors that may regulate these target genes (NES=7.152). The results obtained showed that 7 targets (NOS3, VEGFA, IL6, RELA, TIMP1, GAPDH and HMOX1) in the PPI network composed of 17 genes were regulated by the predicted 15 transcription factors (MAFK, MAFG, NFE2, NFE2L2, MAFF, BACH1, MAFA, JUNB, FOS, JUND, FOSLI1, JUN, FOSB, MAF and DBP).

Discussion

KBD is a chronic and severe progressive bone and joint degenerative disease of unknown etiology. An elevated prevalence of KBD has been shown in populations living in geographic areas with low selenium abundance and high exposure to mycotoxins (8). An accumulating body of evidence, together with recent scientific discoveries, have indicated that mycotoxins, including T-2 toxin, have the potential to trigger cell hypoxia (9,10).
In the present study, the target genes associated with KBD, T-2 toxin and selenium were first obtained from the CTD database. These targets were associated with the GO BP terms of ‘apoptosis’ and ‘hypoxia’, as well as the ‘TNF signaling pathway’, ‘PI3K-AKT signaling pathway’ and ‘HIF-1 signaling pathway’. These results suggested that TNF-α was associated with inflammation and apoptosis in KBD; this is in agreement with previous studies that indicated the levels of TNF-α in the serum and cartilage of patients with KBD were markedly higher compared with those of healthy controls (33,34).

PI3K-AKT is the main signaling pathway for chondrocyte survival and apoptosis, and the core hub for transmitting external signals (35). A previous study indicated that oxidative stress-induced chondrocyte apoptosis may be mediated via upregulation of the PI3K-AKT signaling pathway (36). An additional study indicated that the regulation of HIF-1α by components of the PI3K-AKT signaling pathway may directly regulate the stability of HIF-1α protein via its downstream effects (37).

In addition to the PI3K-AKT/HIF-1α pathway, HIF-1 signaling also includes the MAPK/HIF-1α signaling pathway and the HIF-1α/VEGFA signaling pathway (38). A previous study revealed that excessive apoptosis of chondrocytes and oxidative stress served a crucial role in the pathophysiology of KBD (39). Earlier research also indicated that under hypoxic or normoxic conditions, the level of apoptosis of HIF-1α-deficient chondrocytes was significantly increased in osteoarthritis (40,41). Therefore, it was hypothesized that T-2 toxin-induced chondrocyte apoptosis was associated with HIF-1α, and experiments were devised to assess the expression of HIF-1α in KBD. The results indicated that HIF-1α expression was reduced in chondrocytes treated with different concentrations of T-2 toxin in a dose-dependent manner. By contrast, VEGF-A expression was shown to be reduced in a dose-dependent manner following treatment with different concentrations of T-2 toxin. However, the precise mechanism via which HIF-1α regulates VEGFA in KBD requires further exploration.

Through the PPI network constructed by the key genes associated with KBD and the MCODE plug-in of Cytoscape, 10 molecules were screened out that may be associated with KBD (42‑44). Using Cytoscape software and its plugin to analyze the HIF-1 signaling pathway led to the prediction of 15 transcription factors and 7 target genes. Of these, the genes CASP8 and MTOR have been previously associated with KBD (42‑44). Using Cytoscape software and its plugin to analyze the HIF-1 signaling pathway led to the prediction of 15 transcription factors and 7 target genes. Of these, the genes IL6 (45), RELA (46), TIMP1 (47) and HMOX1 (48), as well as...
the transcription factors NFE2L2 (48), JUNB (49), FOS (50), JUND (49) and JUN (47), have been reported to be associated with KBD.

There are several limitations of the present study that should be acknowledged. Follow-up experiments on selenium deficiency were not performed. Whether selenium deficiency or selenium deficiency combined with T-2 toxin is able to affect the expression of HIF-1α and VEGFA following treatment of the chondrocytes requires further experimental verification. In addition, further experiments, such as inhibitor studies or

Figure 4. Effects of T-2 toxin on the expression levels of proteins associated with the HIF-1α/VEGFA signaling pathway. (A) C28/I2 chondrocytes were pretreated with the indicated concentrations (0.001, 0.005, 0.01, 0.02 and 0.05 µg/ml) of T-2 toxin for 3 days, followed by western blotting analysis to examine the protein expression levels of HIF-1α and VEGF-A. β-actin was used as the loading control. Ratios of (B) HIF-1α and (C) VEGFA protein expression values normalized to β-actin expression. Data are shown as the mean ± SD from three independent experiments. **P<0.01 vs. control group (0 µg/ml T-2 toxin). HIF-1α, hypoxia-inducible factor/1α; VEGFA, vascular endothelial growth factor A.

Figure 5. Regulatory networks of transcription factors and genes associated with HIF-1α, VEGFA and the HIF-1 signaling pathway. Circles represent genes or target genes, and green ‘V’ shapes represent transcription factors. HIF-1α, hypoxia-inducible factor/1α; VEGFA, vascular endothelial growth factor A.
transfection experiments, are required to determine the association between HIF-1α and VEGFA.

In conclusion, a total of 10 core genes and 15 transcription factors associated with KBD were identified in the present study. The results also indicated that the expression levels of HIF-1α and VEGFA in T-2 toxin-treated chondrocytes were downregulated. Therefore, the results of the present study suggested that the HIF-1α/VEGFA signaling pathway is involved in KBD, and this knowledge may help to both further elucidate the pathogenesis of KBD and provide possible avenues for treatment of KBD in the future.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WL and GW conceived and designed the experiments, performed the experiments, analyzed the data, contributed materials and analytical tools, prepared the figures and authored and reviewed drafts of the paper. BX and HH analyzed the data and authored and reviewed drafts of the paper. All authors have read and approved the final manuscript. BX and HH analyzed materials and analytical tools, prepared the figures and authored and reviewed drafts of the paper. WL and GW conceived and designed the experiments, and this knowledge may help to both further elucidate the pathogenesis of KBD and provide possible avenues for treatment of KBD in the future.

References

1. Xiong G: Diagnostic, clinical and radiological characteristics of Kashin-Beck disease in Shaanxi Province, PR China. Int Orthop 25: 147-150, 2001.
2. Wang K, Yu J, Liu H, Liu Y, Liu N, Cao Y, Zhang X and Sun D: Endemic Kashin-Beck disease: A food-sourced osteoarthropathy. Semin Arthritis Rheum 50: 366-372, 2020.
3. Yang L, Zhang J, Li X, Xu C, Wang X and Guo X: Expression profiles of selenium-related genes in human chondrocytes exposed to T-2 toxin and deoxynivalenol. Biol Trace Elem Res 190: 295-302, 2019.
4. Sudre P and Mathieu F: Kashin-Beck disease: From etiology to prevention or from prevention to etiology? Int Orthop 25: 175-179, 2001.
5. Gutleb AC, Morrison E and Mark AJ: Cytotoxicity assays for mycotoxins produced by Fusarium strains: A review. Environ Toxicol Pharmacol 11: 309-320, 2002.
6. Lei Y, Guanghui Z, Xi W, Yingting W, Xialu L, Fangfang Y, Goldring MB, Xiong G and Lammi MJ: Cellular responses to T-2 toxin and/or deoxynivalenol that induce cartilage damage are not specific to chondrocytes. Sci Rep 7: 2231, 2017.
7. Yang Q, Guo X, Zuo H, Zhang YG, Xu P, Ping ZG, Zhang Z and Geng D: Chondrocyte apoptosis and expression of Bcl-2, Bax, Fas, and iNOS in articular cartilage in patients with Kashin-Beck disease. J Rheumatol 33: 615-619, 2006.
8. Lei R, Jiang N, Zhang Q, Hu S, Dennis BS, He S and Guo X: Prevalence of selenium, T-2 toxin, and deoxynivalenol in kashin-beck disease areas in qinghai province, Northwest China. Biol Trace Elem Res 171: 34-40, 2016.
9. Jirong Y, Huiyun P, Zhongze Y, Birong D, Weimin L, Ming Y and Yi S: Sodium selenite for treatment of Kashin-Beck disease in children: A systematic review of randomized controlled trials. Osteoarthritis Cartilage 20: 605-613, 2012.
10. Wu Q, Wu W and Kuca K: From hypoxia and hypoxia-inducible factors (HIF) to oxidative stress: A new understanding of the toxic mechanism of mycotoxins. Food Chem Toxicol 135: 110968, 2020.
11. Huang da W, Sherman BT and Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4: 44-57, 2009.
12. Zhao F, Guo X, Wang X, Yan H and Li C: Genome-wide gene expression analysis suggests an important role of hypoxia in the pathogenesis of endemic osteochondropathy Kashin-Beck disease. PLoS One 6: e22983, 2011.
13. Wu W, He A, Wen Y, Xiao X, Hao J, Zhang F and Guo X: Comparison of microRNA expression profiles of Kashin-Beck disease, osteoarthritis and rheumatoid arthritis. Sci Rep 7: 540, 2017.
14. Majmundar AJ, Wong WJ and Simon MC: Hypoxia-inducible factors and the response to hypoxic stress. Mol Cell 40: 294-309, 2010.
15. Schönberger MJ, Krek W and Kovacs WJ: EPAS1/HIF-2α is a driver of mammalian pexophagy. Autophagy 11: 967-969, 2015.
16. Guo RY, Wang M, Liu Q, Feng D, Wen Y, Xia Y, Colgan SP, Ebert H, HK and J C: Hypoxia-inducible factor-2α reprograms liver macrophages to protect against acute liver injury through the production of interleukin-6. Hepatology 71: 2105-2117, 2020.
17. Tian J, Yan J, Wang W, Zhong N, Tian L, Sun J, Min Z, Ma J and Liu S: T-2 toxin enhances catabolic activity of hypertrophic chondrocytes through ROS-NF-κB-HIF-2α pathway. Toxicol In Vitro 26: 1106-1113, 2012.
18. Xu J, Jiang C, Zhu W, Wang B, Yan J, Min Z, Geng M, Han Y, Ning Q, Zhang F, et al: NOD2 pathway via RIPK2 and TBK1 is involved in the aberrant catabolism induced by T-2 toxin in chondrocytes. Osteoarthritis Cartilage 23: 1578-1585, 2015.
19. Shi M, He Y, Zhang Y, Guo X, Lin J, Wang W and Chen J: LncRNA MIAT regulated by selenium and T-2 toxin increases disease. Hum Exp Toxicol 40: 869‑881, 2021.
20. Zhang T, Zhu X, Wu H, Jiang K and Zhao G: Targeting the ROS/P13K/AKT/HIF-1α/HK2 axis of breast cancer cells: Combined administration of polydatin and 2-Deoxy-d-glucose. J Cell Mol Med 23: 3711-3723, 2019.
21. Guan R, Wang J, Li Z, Ding M, Li D, Xu G, Wang T, Chen Y, Yang Q, Long Z, et al: Sodium tanshinone IIA suppresses cigarette smoke-induced inflammation and oxidative stress via blocking the activation of MAPK/HIF-1α signaling pathway. Front Pharmacol 9: 263, 2018.
22. Zhang Z, Deng M, Huang J, Wu J, Li Z, Xing M, Wang J, Guo Q and Zhu W: Microglial annexin A3 downregulation alleviates breast cancer-induced pain through inhibiting the Hif-1α/vascular endothelial growth factor signaling pathway. Pain 161: 2750-2762, 2020.
23. Carmeliet P: VEGF as a key mediator of angiogenesis in cancer. Oncology 69 (Suppl 3): S4-S10, 2005.
24. Guo X, Zuo H, Cao CX, Zhang Y, Geng D, Zhang ZT, Zhang YG, von der Mark K and von der Mark H: Abnormal expression of Col X, TPH4, TGF-beta, bFGF, and VEGF in cartilage with Kashin-Beck disease. J Bone Miner Metab 24: 319-328, 2006.
25. Zhang F, Guo X, Duan C, Wu S, Yu H and Lammi M: Identification of differentially expressed genes and pathways between primary osteoarthritis and endemic osteoarthritis (Kashin-Beck disease). Scand J Rheumatol 42: 71-79, 2013.

26. Balamurugan K: HIF-1 at the crossroads of hypoxia, inflammation, and cancer. Int J Cancer 138: 1058-1066, 2016.

27. Maes C, Carmeliet G and Schipani E: Hypoxia-driven pathways in bone development, regeneration and disease. Nat Rev Rheumatol 8: 358-366, 2012.

28. Davis AP, Grondin CJ, Johnson RJ, Sciaky D, McMorran R, Wiegers J, Wiegers TC and Mattingly CJ: The comparative toxicogenomics database: Update 2019. Nucleic Acids Res 47: D948-D954, 2019.

29. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, et al: STRING v10: Protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 43: D447-D452, 2015.

30. Wang X, Ying Y, Zhang P, Yang L, Wang Y and Guo X: Chondrocytes damage induced by T-2 toxin via Wnt/β-catenin signaling pathway is involved in the pathogenesis of an endemic osteoarthropathy, Kashin-Beck disease. Exp Cell Res 361: 141-148, 2017.

31. Liu YN, Jiang ZC, Li SY, Li ZZ, Wang H, Liu Y, Liao YC, Han J and Chen JH: Integrin α2β1 is involved in T-2 toxin-induced decrease of type II collagen in C28/I2 chondrocytes. Toxicon 186: 12-18, 2020.

32. Janky R, Verfaillie A, Imrichova H, Van de Sande B, Standaert L, Christiaens V, Hulselmann G, Herten K, Naval Sanchez M, Potier D, et al: iRegulon: From a gene list to a gene regulatory network using large motif and track collections. PLoS Comput Biol 10: e1003731, 2014.

33. Wang WZ, Guo X, Duan C, Ma WJ, Zhang YG, Xu P, Gao QZ, Wang ZF, Yan H, Zhang YF, et al: Comparative analysis of gene expression profiles between the normal human cartilage and the one with endemic osteoarthritis. Osteoarthritis Cartilage 17: 83-90, 2009.

34. Duan C, Guo X, Zhang XD, Yu HJ, Yan H, Gao Y, Ma WJ, Gao ZQ, Xu P and Lammi M: Comparative analysis of gene expression profiles between primary knee osteoarthritis and an osteoarthritis endemic to Northwestern China, Kashin-Beck disease. Arthritis Rheum 62: 771-780, 2010.

35. Wu Q, Qin Z, Kuca K, You L, Zhao Y, Liu A, Musilek K, Kusak P, et al: An update on T-2 toxin and its modified forms: Metabolism, immunotoxicity and molecular features between Kashin-Beck disease and primary osteoarthritis. Arthritis Res Ther 20: 41, 2018.

36. Wu C, Liu H, Zhang F, Shao W, Yang L, Ning Y, Wang S, Zhao G, Lee BJ, Lammi M and Guo X: Long noncoding RNA expression profile reveals IncRNAs signature associated with extracellular matrix degradation in kashin-beck disease. Sci Rep 7: 17553, 2017.

37. Ling Y, Wang X, Lammi MJ and Guo X: Changes in the NF-κB signaling pathway in juvenile and adult patients with Kashin-Beck disease. Exp Cell Res 379: 140-149, 2019.

38. Xiong YM, Mo XY, Zou ZX, Song RX, Sun WY, Lu W, Chen Q, Yu YX and Zang WJ: Association study between polymorphisms in selenoprotein genes and susceptibility to Kashin-Beck disease. Osteoarthritis Cartilage 18: 817-824, 2010.

39. Chang Y, Wang X, Sun Z, Jin Z, Chen M, Wang X, Lammi MJ and Guo X: Inflammatory cytokine of IL-1β is involved in T-2 toxin-triggered chondrocyte injury and metabolism imbalance by the activation of Wnt/β-catenin signaling. Mol Immunol 91: 195-201, 2017.

40. Li Y, Mo X and Xiong Y: The study on polymorphism of TrxR and Nrf2/HO-1 signaling pathway in Kashin-Beck disease. Biol Trace Elem Res 190: 303-308, 2019.

41. Wu R, Zhang R, Xiong Y, Sun W, Li Y, Yang X, Liu J, Jiang Y, Guo H, Mo X and Cao J: The study on polymorphisms of Sep15 and TrxR2 and the expression of AP-1 signaling pathway in Kashin-Beck disease. Bone 120: 239-245, 2019.

42. Han L, Yang X, Sun W, Li Z, Ren H, Li B, Zhang R, Zhang D, Shi Z, Liu J, et al: The study of GPX3 methylation in patients with Kashin-Beck disease and its mechanism in chondrocyte apoptosis. Bone 117: 15-22, 2018.

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