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Activation of JNK signaling in osteoblasts is inversely correlated with collagen synthesis in age-related osteoporosis

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ABSTRACT

The age-related reduction in the function of osteoblasts plays a central role in the pathogenesis of bone loss and osteoporosis. Collagen synthesis is a primary function of differentiated osteoblasts, however, the mechanisms for age-related changes in collagen synthesis in human osteoblasts remain elusive.

We use Gene Ontology (GO) analysis and Gene Set Enrichment Analysis (GSEA) analysis to exploit the transcriptional profiles of osteoblasts from young and old donors. A panel of collagen members was downregulated in aged osteoblasts, including COL12A1, COL5A1, COL5A3, COL8A1 and COL8A2. Co-expression analysis followed by GO analysis revealed that oxidoreductase activity and kinase activity were inversely correlated with collagen synthesis in osteoblasts. GESA analysis further showed that JNK signaling was upregulated in aged osteoblasts. Consistently, MAP3K4 and MAP4K2, upstream of JNK, were also increased in aged osteoblasts. Moreover, expression levels of MAP3K4 were significantly inversely correlated with levels of the collagen genes. Those transcriptomic results were further verified by examining clinical specimens of osteoporosis by immunohistochemistry.

These results provide transcriptomic evidence that deregulated JNK signaling may impair collagen synthesis in osteoblasts and imply a therapeutic value of JNK inhibitors for treating osteoporosis and preventing skeletal aging by counteracting the age-related reduction in the function of osteoblasts.

1. Introduction

Bone fragility is a silent condition that increases the risk of bone fracture [1,2]. Chronic pain and a decreased ability to carry out normal activities may occur following a broken bone [3]. Bone fragility can be enhanced by low bone mass and microarchitecture deterioration of bone tissue that lead to osteoporosis [4,5]. As an age-related metabolic bone disease, osteoporosis itself has no symptoms, but it can cause the gradual loss of bone density and strength which may increase the risk of bone fracture [6]. Central to our understanding of osteoporosis is the idea that bone homeostasis, an imbalance between bone resorption and bone formation, is dyregulated. In recent years, much attention has been paid to the relations between bone resorption and osteoporotic fractures [7–10]. In contrast, little is known about the molecular mechanisms of bone formation in impaired bone cells. Due to the high but elusive morbidity, prevention and treatment of bone fragility become an urgent medical issue. Rather than considering bone fragility as being the result of a reduced amount of bone, we recognize that bone fragility is the result of changes in the material and structural properties of bone.

Collagen is the main structural protein in the extracellular space of various connective tissues in human bodies [11,12]. The synthesis of collagen is a major function of osteoblasts, but the underlying mechanisms tend to be complicated. Disorders of collagen are found have relations with bone fragility [4]. Consequently, treatment concentrated on regulating collagen synthesis is regarded as a promising therapeutic direction for bone fragility in elderly people. However, however, the mechanisms for age-related changes in collagen synthesis in human osteoblasts remain elusive.

c-Jun N-terminal kinases (JNKs), including JNK1, JNK2 and JNK3,

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were originally identified as kinases that bind and phosphorylate c-Jun on Ser-63 and Ser-73 within its transcriptional activation domain [13]. JNKs belong to mitogen-activated protein kinase (MAPK) family, and can be activated by stress stimuli, such as cytokines, ultraviolet irradiation, heat shock and osmotic shock [14,15]. The JNK signaling pathway is involved in regulation of many cellular events, including differentiation and apoptosis [16–18]. Accumulating evidence shows that the activation of JNK signaling has relations with aging and various degenerative diseases [15,19]. However, whether JNK signaling is involved in osteoblast aging remains elusive.

Advances of next-generation sequencing technique enable characterization of biological processes in a more comprehensive manner, facilitating generation of new therapeutic hypotheses. In the present study, we interrogated RNA-sequencing data of osteoblasts from young and old donors and found that expression of a series of collagen genes declined in aged osteoblasts. Co-expression analysis and pathway analysis revealed that JNK signaling inversely correlated with expression of these collagen genes. These results indicated that JNK signaling pathway may be a possible therapeutic target for prevention and treatment of bone fragility.

2. Materials and methods

2.1. Gene expression analysis of the RNA-seq data

RNA-seq raw reads were downloaded from ArrayExpress (http://www.ebi.ac.uk/arrayexpress/) under accession number E-MTAB-4879. The 8 osteoblast samples subjected to RNA-Seq were derived from four young donors (20–25 years old) and four old donors (54–74 years old) without any clinical syndrome or medication, with an attempt to identify age-related common pathway alterations. Reads were mapped against the hg19 genome using TopHat v2.0.13. Read counts per gene were counted using HTSeq and the ENSEMBL annotation. Subsequent analysis was done with the limma software package. Counts were transformed to $\log_2$(counts per million + 1). Differential expression between osteoblasts from young and those from aged individuals was assessed using empirical Bayes moderated *t*-statistics with robust estimation of prior parameters.

2.2. Co-expression analysis, GO analysis and GO connectivity network

For co-expression analysis, log2(counts per million + 1) values were used. Genes co-expressed with the 5 collagen genes identified in Fig. 1 were selected based on the Spearman rank correlation coefficient with a threshold of 0.5 for positive correlation and −0.5 for negative correlation, respectively. Functional annotation of the negatively co-expressed genes was done with GO analysis using FGNet package in R [20]. Terms with P values < 0.05 were determined as enriched. GO connectivity network was generated using the same package.

2.3. GSEA analysis

Gene Set Enrichment Analysis (GSEA), using the Broad Institute algorithm v2.1.0 [21], was conducted on the ranked gene lists. The msigdb. v6.0. symbols.gmt gene set was used for running GSEA and 1000 permutations were used to calculate the P value.

2.4. Patient samples and ethical aspects

Bone fragment specimens were collected from patients who had undergone surgical debridement at the Ninth People's Hospital of Wuxi City, Jiangsu, China. All of the patients gave written, informed consent, and the Ethics Committee of the Ninth People's Hospital of Wuxi City approved the data and tissue collection (no. 201702377).

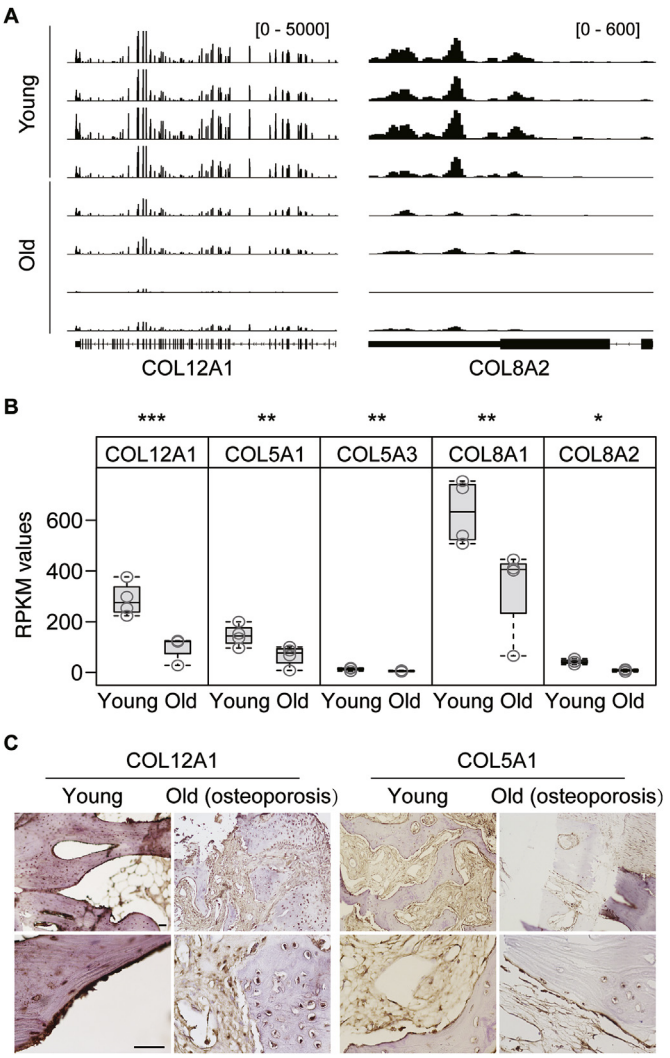

Fig. 1. Reduced expression of collagen in aged osteoblasts.
(A) Normalized RNA-seq signal for COL12A1 and COL8A2 in aged osteoblasts compared with osteoblasts from young individuals.
(B) RPKM (Reads Per Kilobase Million) values for indicated genes in aged osteoblasts compared with osteoblasts from young individuals.
(C) COL12A1 and COL5A1 IHC in bone fragment specimens. Scale bar: 50 μm.

2.5. Immunohistochemistry

Paraffin sections (3 μm) were used for routine immunohistochemistry staining using the Dako DAB detection kit (Cat. K5007). The following primary antibodies were used: rabbit anti-COL12A1 (1:100 dilution, OmnimAbs, Cat. OM123326); rabbit anti-COL5A1 (1:100 dilution, OmnimAbs, Cat. OM247743); rabbit anti-c-Jun (1:50 dilution, OmnimAbs, Cat. OM123213).

2.6. Statistical analysis

The results were considered statistically significant when p values were <0.05. All P values were indicated in the figures.

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