Comprehensive network analysis reveals genes related to immune infiltration, metabolism, and progression in steroid-induced osteonecrosis of the femoral head

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INTRODUCTION: Steroid-induced osteonecrosis of the femoral head (SONFH) is a serious adverse effect of glucocorticoids excessive use with impairment of immune and metabolism. The exact mechanisms that contribute to SONFH remain unknown. Here, by using RNA sequencing technique followed by bioinformatic analysis, we aim to identify potential genes/pathways related to SONFH. Although originally considered "noise" or "junk" RNAs, noncoding RNAs (ncRNAs) are now widely recognized as important regulators of gene expression. Hence, we hypothesized that ncRNAs play an important role in development and progression of SONFH via regulating immune and metabolism.

METHODS: A total of 68 patients were included with either SONFH or femoral head fracture (FNF) in the present study. Subchondral bone samples were collected during surgery to extract total RNA and for high-throughput sequencing. Differential expression analysis, functional enrichment, gene set enrichment analysis (GSEA) and gene set variation analysis were utilized to identify significant differential expressed genes (DEGs) and functionally involved pathways. The immune infiltration and metabolism were quantified by single sample GSEA. The protein-protein interaction (PPI) and competing endogenous RNA (ceRNA) network was used to study miRNA-mRNA and miRNA-lncRNA/pseudogene relationship and to identify potential hub genes and ceRNA axis. Machine learning models, including logistic regression, least absolute shrinkage and selection operator regression and random forest, were used to identify genes associated with immune infiltration or metabolism in SONFH. To explore the potential mechanism of transcription of hub genes, transcription factors (TFs) and binding site were investigated.

RESULTS: Through deep RNA sequencing, a total of 1567 mRNAs, 665 lncRNAs, 5 miRNAs, 278 pseudogene, 21 IGgene and 4 TRgene were differentially expressed in SONFH when compared to controls **(Fig.1).** To infer involved diseases, biological functions, and associated pathways of DEGs, immune and metabolism related terms were highly enriched. Regarding immune infiltration and metabolism level associated with SONFH, 9 immune cells and 24 metabolisms were significantly different, such as Macrophages M2, T cells regulatory, prostaglandin biosynthesis. To better understand the interactions among the identified DEGs, we constructed PPI network and IL1B found to be the hub gene and TFs to have regulatory potential of IL1B including CEBPB, FOS, MYC, RXRG. Based on machine learning models, FOS, Macrophages M2, Prostaglandin biosynthesis, Ubiquinone and other Terpenoid Quinone biosynthesis were found to be significant risk factors of SONFH progression **(Fig. 2).** To identify the potential pathways related to FOS, GSEA was performed based on the median value of FOS. 8 pathways were determined, including IL-17 signaling pathway, TNF signaling pathway. Interestingly, IL1B, were significantly enriched in 4 pathways. According to the evidence we found above, FOS is a TF of IL1B in several kinds of cells. After retrieving the TF motif, we detected 3 binding sites of FOS on the promoter of IL1B, -205, -1431 and -1613, respectively **(Fig. 3).**

DISCUSSION: The results of the present study demonstrate that C20orf197-MIR3606-FOS-IL1B axis play a role in the development and progression of SONFH. The underlying mechanism involved immune cells and metabolism regulated by IL1B, such as Macrophages M2, Prostaglandin biosynthesis, and Ubiquinone and other Terpenoid Quinone biosynthesis. Taken together, IL1B, FOS, and C20orf197-MIR3606-FOS axis play an important role in development and progression of SONFH, with potential mechanism of affecting immune infiltration and metabolism.
The most important limitation of our study is that no age-matched control could be obtained because of the absence of a surgical indication for hip arthroplasty in young patients with FNF.

SIGNIFICANCE/CLINICAL RELEVANCE: The present investigations provide new insights into development and progression of SONFH and potential novel therapeutic targets of the disease.

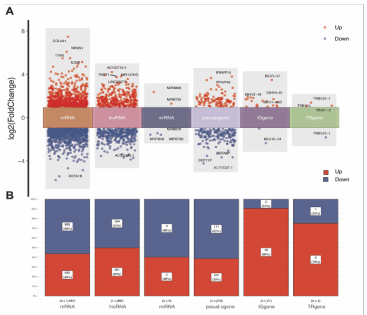

Fig. 1 Differentially expressed genes in SONFH.

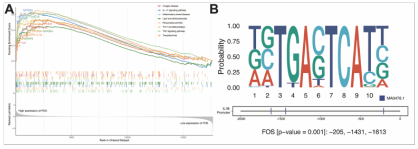

Fig. 3 Potential pathways related to FOS and binding sites of FOS on the promoter of IL1B.

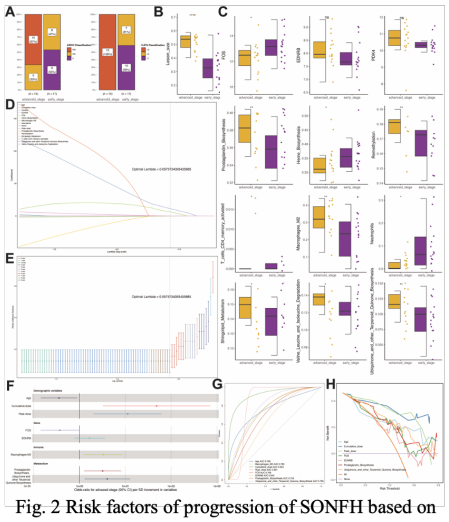

Fig. 2 Risk factors of progression of SONFH based on machine learning models.