Potent Role Of Sirt6 In The Crosstalk Between Metabolic Syndrome And Osteoarthritis.

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Introduction: Osteoarthritis (OA) is a chronic degenerative joint disorder characterized by articular cartilage destruction and osteophyte formation, and is prevalent in society as a major cause of disability. A number of cohort studies have demonstrated that obesity is an independent risk factor for hand OA; however, mechanical stress cannot explain such a correlation. Therefore, it has been hypothesized that one or more systemic factors are responsible for the correlation between obesity and OA. Recent studies have revealed that a metabolic syndrome rather than obesity itself has the greatest impact on the initiation and severity of OA. The reasons why there is such a correlation between metabolic syndrome and OA remains elusive.

The infrapatellar fat pad (IPFP), is an unique fat depot that is closely contact with articular cartilage. Recently, the role of the IPFP in the initiation and progression of OA has been paid more attention as the adipose tissue is a highly active metabolic and endocrine organ. Ageing is also a pivotal risk factor for the osteoarthritis. The stress-response and chromatin-silencing factor Sir2, a yeast sirtuin, is a NAD+-dependent histone deacetylase and is involved in controlling ageing. In mammals the sirtuin family contains seven genes (SIRT1-SIRT7), which encodes different types of sirtuin proteins, in tissue specificity, subcellular localization, enzymatic activity and targets. Among them, SIRT6 is localized to the nucleus and is involved in transcriptional silencing, genome stability, and longevity. Recently, SIRT6 overexpression is reported to associate with significantly less visceral fat, LDL-cholesterol, and triglycerides in mice. We previously disclosed Sirt6 is expressed in chondrocytes and its deficiency is associated with inhibition of proliferation and differentiation of chondrocytes through the regulation of Indian hedgehog signaling (1). These facts suggest potential roles of Sirt6 in the metabolic syndrome and/or joint cartilage metabolism. However, the role of Sirt6 in OA pathogenesis is poorly understood. This study sought to investigate the involvement of Sirt6 in the ageing of articular tissues and in the development of high fat diet (HFD)-induced OA by using a murine HFD-induced osteoarthritis model and limb specific Sirt6 conditional knock out mice.

Methods: To determine the role of Sirt6 in the spontaneous progression of OA and in the metabolic syndrome, six-month-old Sirt6+/− mice in 129X1/SvJ strains and wild-type (WT) littermates were fed normal diet (ND) or HFD for three months. All mice were sacrificed at twelve weeks after the diet and evaluated serum levels of adipokines and inflammatory cytokines using the Bio-Plex Pro assay kit ((Bio-Rad Laboratories, Hercules, CA, USA). The IPFP, the visceral fat and the subcutaneous fat were isolated and analyzed by real-time RT-PCR. To evaluate limb specific Sirt6 function, Prx1cre;Sirt6f/f mice were obtained and the knee joints were analyzed at five months of age. The knee joints of all the mice were scored for OA severity and evaluated by immunohistochemistry and TUNEL staining.
**Results:** Body weights of Sirt6+/-mice were comparable to WT littermates at six and nine months of age. Mice fed the HFD weighed 20% more by twelve weeks both in WT and Sirt6+/- mice. Body weights of Prx-1Cre;Sirt6f/f mice were also comparable to the controls at five months of age. HFD was associated with the elevation of serum Leptin level independent of Sirt6 deficiency. The concentration of Adiponectin, TNF-α and IL-6 were not affected by HFD or Sirt6 deficiency. Next, we monitored the mRNA expression levels of inflammatory cytokines/adipokines of various fat tissues, such as visceral fat, subcutaneous fat. Real-time RT-PCR analysis demonstrated spontaneously elevated mRNA expression of TNF-α and IL-6 in the IPFP, visceral fat and subcutaneous fat of ND Sirt6+/- mice. HFD further enhanced the expression of these cytokines in the IPFP and the visceral fat, but not in subcutaneous fat. The enhancement of inflammatory cytokines were not observed in the IPFP and the visceral fat of Prx-1Cre+;Sirt6f/f mice, in which Sirt6 expression were normal in these fat depot. Histological investigation of the knee joint cartilage revealed promoted irregularity at the surface of the joint cartilage in Sirt6+/- mice. When HFD was applied to Sirt6+/- mice and the littermates, the histological features of OA, such as attenuated Safranin-O intensity and cartilage surface irregularity, were observed both in WT and Sirt+/- mice, although the histological alteration was more apparent in Sirt6+/- mice. The OA severity based on OARSI score indicated Sirt6+/- mice were scored higher compared to WT littermates. On the other hand, Prx-1Cre;Sirt6f/f mice did not show obvious alteration at the surface of the knee joint cartilage compared to control mice. In association with the synovial hypertrophy, osteophyte formation was accelerated in Sirt6+/- mice. The thickness of the synovium and the number of synovial cells were spontaneously increased in the nine-month-old Sirt6+/- ND group compared to age-matched WT ND group. Osteophyte formation and synovium hypertrophy were significantly enhanced by HFD in Sirt6+/- mice. Immunohistological analysis indicated the expression of OA marker proteins, such as MMP-13 and ColX, were increased in the articular cartilage of Sirt6+/- mice. Conversely, in Prx-1Cre+;Sirt6f/f mice, the expression of these OA markers were reduced in association with significant reduction in the number of hypertrophic chondrocyte at the deep layer of the joint cartilage. The expression of senescence marker Plasminogen Activator Inhibitor -1(PAI-1) was enhanced in Sirt6+/- mice and Prx1cre;Sirt6f/f mice. TUNEL assay indicated apoptotic cells were increased by HFD and Sirt6 haploinsufficiency.

**Discussion:** Here we revealed the roles of Sirt6 in the etiology of OA. First, Sirt6 haploinsufficiency in aged mice promoted the expression of the inflammatory cytokines in the IPFP as well as the visceral fat in association with promoted OA change. These enhancements were further augmented by HFD. Macrophage is implicated in the inflammation of the fat tissue triggered by HFD. Macrophages derived from the bone marrow of Sirt6 deficient mice exhibit increased IL-6, and TNFα expression levels and are hypersensitive to LPS stimulation through activation of c-JUN signaling. NF-kB is also a target of Sirt6 and is involved in the regulation of inflammation. Sirt6 inhibits inflammation by interacting with the p65 subunit of NF-kB and by deacetylating H3K9 at target promoters. Furthermore, SIRT6 is reported to directly promote the secretion of TNF-α by removing the fatty acyl modification of TNF-α. Combined with our data, Sirt6 may function as an anti-inflammatory factor in the course of HFD-induced OA development through the action on the IPFP. On the other hand, inflammation of the IPFP and other fat depot were not observed in Prx1cre;Sirt6f/f mice, in which the expression of OA markers, ColX and MMP-13, were both reduced. This data was consistent with our previous paper (1). Considering these facts, the increased ColX, MMP-13 expression of the chondrocyte in Sirt6+/- mice were triggered by the
enhancement of inflammation of the IPFP, but not the result of chondrocyte cell autonomous regulation through Sirt6 signaling.

**Significance:** This study indicates that Sirt6 in the fat depot has a protective effect against metabolic syndrome, ageing related fat inflammation and metabolic OA progression, and also supports a potential therapeutic application of SIRT6 agonist at the IPFP in human OA.

*ORS 2015 Annual Meeting*

*Poster No: 1304*