Loss of SirT1 impairs autophagy in chondrocytes and is associated with accelerated cartilage aging and experimental osteoarthritis

Sacitharan P.K., Zarebska J, Nechyporuk-Zloy V, Stott B, Curtinha M; Parisi I, Bou Gharios G, Edwards J, Vincent TL.

Purpose: The cellular process of autophagy, which identifies, degrades and recycles unwanted proteins and cellular debris, is defective in aging cartilage and osteoarthritis (OA). However, the regulation of autophagy in chondrocytes is largely unknown. The SirT1 longevity factor has been shown to control lifespan and decreases with age. Here we hypothesize that SirT1 regulates autophagy in chondrocytes, and loss of SirT1 impairs autophagy which dysregulates chondrocytes and predisposes to OA.

Methods: Gene and protein expression and protein binding profiles were examined by q-PCR, western blotting, FACS or immunoprecipitation in HTB-94 chondrocyte cell line. Cells were either treated with a pharmacological inhibitor of SirT1 (EX-527; 100nM), SirT1 siRNA, or a pharmacological activator of SirT1 (SRT1720; 500nM). Novel cartilage specific inducible SirT1 knockout mice were generated by crossing $SirT1^{fl/fl}$ x Aggrecan-$CreER^{T2}$ ($SirT1^{fl/fl}$; Agg-$CreER^{T2}$). Young cartilaginous hips and micro-dissected cartilage from naïve (unoperated) mice were analysed by protein and gene expression, electron microscopy and LC3 immunohistochemistry. 10 week old male mice were subjected to either sham or destabilisation of medial meniscus (DMM) surgeries. Chondropathy scores and LC3 immunohistochemistry were analysed 8 and 12 weeks post-surgery. Chondropathy scores and LC3 immunohistochemistry were also analysed in 2, 6 and 12 month aged $SirT1^{fl/fl}$; Agg-$CreER^{T2}$ and control ($SirT1^{fl/fl}$) mice. Young cartilaginous hips from transgenic LC3-GFP mice were also used to quantify autophagy in the presence of a SirT1 inhibitor or activator.

Results: Pharmacological inhibition and siRNA knockdown of SirT1 decreased gene expression of COL2A1, ACAN and SOX-9 ($p<0.01$), whilst pharmacological activation of SirT1 stimulated the expression of COL2A1, ACAN and SOX-9 ($p<0.01$) in HTB-94 chondrocyte cells. In addition, pharmacological activation of SirT1

positively regulated gene expression of key autophagy markers (BECN1, ULK-1 and LC3 ($p<0.01$)) and LC3 conversion by western blot and FACS. Furthermore, immunoprecipitation studies in HTB-94 chondrocyte cells revealed SirT1 to bind directly to ATG5, ATG7, Beclin-1 and LC3 but not ULK1.

Young cartilaginous hips from transgenic LC3-GFP mice treated with the SirT1 activator displayed increased percentage of cells with GFP punctate compared with control hips ($p<0.001$). Conversely, a decrease of the percentage of cells with GFP punctate was observed when hips were treated with the SirT1 inhibitor ($p<0.01$). Microdissected cartilage from naïve joints (unoperated) $SirT1^{fl/fl}$; Agg-$CreER^{T2}$ mice showed decreased expression of ACAN and COL2A1 ($p<0.001$) and of BECN1, ULK-1, LC3, ATG5, ATG9, ATG7, ATG10, and ATG13 ($p<0.01$) compared with control ($SirT1^{fl/fl}$) mice. Protein expression of LC3 in young cartilaginous hips and the number of autophagosomes in microdissected knee cartilage was decreased ($p<0.05$) in $SirT1^{fl/fl}$; Agg-$CreER^{T2}$ (naïve unoperated) mice compared with controls. A decrease in LC3 immunohistochemical staining ($p<0.001$) and increased chondropathy scores were observed in $SirT1^{fl/fl}$; Agg-$CreER^{T2}$ mice compared with control mice post DMM ($p<0.001$) and in aged mice ($p<0.0001$).

Conclusion: This data suggests SirT1 directly regulates autophagy in chondrocytes. The loss of SirT1 impairs autophagy; dysregulating normal chondrocyte biology and contributes to accelerated cartilage aging and experimental OA. Future studies will access if therapeutic targeting of the SirT1-autophagy pathway could be beneficial for treating OA.