SirT1 blocks apoptosis in human chondrocytes via activation of the IGF-1 Receptor/Akt pathway

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Introduction.
IGF-1 (insulin-like growth factor-1)/Akt signaling is known to affect the growth and development of cartilage, proteoglycan synthesis and regulation of cell chondrocyte survival. The IGF-1/Akt pathway integrates signals arising from the IGF-1Receptor, IGFBPs (IGF Binding Proteins) and growth factor availability. This pathway is not activated efficiently in osteoarthritis chondrocytes because of decreased IGF-1 synthesis and IGF-1 sequestration by abnormally high levels of IGFBP3. SirT1 is a member of Sirtuin family of NAD(+) dependent histone deacetylases and can prolong organism life-span. We investigated a role of SirT1 in the regulation of apoptosis in human OA chondrocytes (OA hChs) and found that it works through activation of the IGF-1/Akt/p53 network. SirT1 protects human OA chondrocytes from apoptosis through activation/phosphorylation of the IGF-1 Receptor, PI3Kinase, PDK1, Akt and MDM2. In part this is mediated by elevation in IGFBP5 and a reduction in IGFBP3 and PTP1B.

Methods. Human chondrocytes were isolated from the cartilage of OA patients and were grown in monolayer culture. SirT1 was overexpressed in human chondrocytes by AMAXA transfection. Apoptotic levels were assessed by Flow Cytometry. Genes expression was confirmed by real-time RT-PCR. Protein extracts were generated and immunoblotted (WB) or immunopreceptipated (IP) by standard procedures. Confocal microscopy was used for imaging.

Results. To explore the function of SirT1 we expressed the gene in hChs. We found that SirT1 overexpression (Fig.1A) decreased the background % of apoptotic cells. However, using the SirT1 inhibitor nicotininamide (NAM) completely reversed this effect (Fig.1B). We also found that SirT1 blocked the ability of TNFalpha to induce apoptosis in these chondrocytes (data not shown).

Discussion. Our results indicate that SirT1 protects OA human chondrocytes against apoptosis through multiple coordinated mechanisms. SirT1 activates the IGF-1 Receptor by repression of PTP1B elevation of IGFBP5 and a reduction in IGFBP3. Signaling through the IGF Receptor then leads to activation of Akt, which phosphorylates MDM2, thereby inhibiting p53. Additionally, SirT1 appears to directly deacetylate p53. Thus in human chondrocytes, SirT1 is a powerful regulator of survival, via activation of the IGF-1/Akt pathway. Our preliminary data indicates that SirT1 levels are downregulated in the chondrocytes from OA patients compared to normal. If this is indeed the case, the reduction in SirT1 may be an important contributor chondrocyte apoptosis evident in osteoarthritis. This would suggests that treatment of OA patients with Resveratrol may elevate SirT1 activity and block apoptosis in this disease.

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Figure 1. Survival effect of SirT1 in OA hChs. OA Amaxa transfected hChs with a vector control or SirT1 expressing plasmid. (A)Cell extracts used in immunoblot assays for the indicated antibodies. (B) After transfection, the cells were treated with or without 10nM NAM for 24 hours and analyzed by Flow Cytometry.

To identify the mechanisms by which SirT1 protects cells from apoptosis we analyzed the components of IGF-1/Akt pathway. The IGF-1R was phosphorylated at Tyr1135/1136 in the presence of SirT1 (Fig.2A). The protein tyrosine phosphatase PTP1B, a known negative regulator of the IGF-1R activation, was repressed by SirT1 at the RNA and protein level (Fig.2B).

Figure 2. SirT1 activated IGF-1Receptor and repressed PTP1B. Cells were Amaxa transfected with either a vector control or SirT1 expressing plasmid. Extracts were generated and used in an immunoblot with the indicated antibodies (A). PTP1B RNA and protein levels were assessed by real-time RT-PCR and immunoblotting (B). SirT1 did not affect levels of IGF-I or IGF-II in hChs and so focused on the IGFBPs. SirT1 led to a downregulation of IGFBP3 and upregulation of IGFBP5 (Fig.3A). To test whether IGFBP5 was critical for survival, cells were cotransfected with both SirT1 and an IGFBP5siRNA. As shown in Figure 3B, reducing IGFBP5 led to a reduction in survival.

Figure 3. SirT1 regulates IGFBP5 level. hChs transfected as in Fig.1 were processed for immunoblots with the indicated antibodies (A). Immunofluorescence staining with indicated fluorescent Abs and DAPI confirmed that IGFBP5 was markedly elevated in SirT1 transfected cells (B) and IGFBP3 was decreased (C). Flow cytometry analysis of SirT1 vs. control transfected cells treated with IGFBP5siRNA displayed a significant difference in apoptotic cells.

To better understand the downstream events that protect cells from apoptosis we analyzed the intermediate steps in the IGF-1R kinase cascade. Expression of SirT1 led to a higher level of phosho Phosphatidylinositol 3-kinase (pPI3K), which in turn led to activation of PDK1 and Akt (Fig.4A). Akt was fully activated due to phosphorylation on both Ser473 and Thr308 (Fig.4B). Furthermore, activated pAkt led to phosphorylation of MDM2 (Fig.4C) leading to decreased p53 activity (Fig.4D). Additionally SirT1 was able to inactivate p53 directly by deacetylation on Lysine 382 (data not shown).