

## Towards elucidating the role of SirT1 in osteoarthritis

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## 1. ABSTRACT

Osteoarthritis (OA) is a degenerative joint disease particularly affecting the elderly population. Although several genetic features have been characterized as risk factors for OA susceptibility, a growing body of evidence indicates that epigenetic effectors may also modulate gene expression and thus contribute to OA pathology. One such epigenetic regulator of particular relevance to OA is Silent Information Regulator 2 type 1 (SirT1) which has been linked to aging and caloric intake. Consistently, SirT1 has been also connected with various age-associated diseases such as diabetes type II, Alzheimers and osteoporosis. Recent reports show that OA is linked to changes in SirT1 activity or levels within cartilage. In human chondrocytes, SirT1 plays a role in cartilage extracellular matrix (ECM) synthesis and promotes cell survival, even under proinflammatory stress. It appears that SirT1 fine tunes many cellular biochemical processes through its capacity to interact and modify various histone and non-histone proteins. Taken together these investigations demonstrate that SirT1 is involved in cartilage biology and could potentially serve as novel drug target in treating OA even at its premature stages, thereby possibly reversing mechanical-stress induced cartilage degeneration.

## 2. INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease particularly affecting the elderly population. It causes severe joint pain and malfunction, leading to major impairment in quality of life. The precise etiology of OA remains obscure and no satisfactory chondroprotective therapy has been yet offered to OA patients. The impact of OA on health systems worldwide is expected to be further accentuated by the continuous increase in life expectancy. OA commonly appears in joints such as the knee and hip, is often the consequence of excessive loading which compromises the resilience of the articular cartilage (AC), and thus is common in overweight individuals. As OA progresses, AC is severely degraded leading to restricted mobility and inflicting severe pain.

The prevalence of symptomatic OA increases with age and has been reported to affect approximately 10% of men and 20% of women over 60 years of age, worldwide (1-5). This disease impacts not only the individual, but also the entire society, since these patients are unable to work and require long-term expenditure including pain-control drugs, surgical procedures and lengthy physical therapy.

The unique structure of the AC extracellular matrix (ECM) plays a key role in its load bearing capacity. It is composed of collagen fibers, the most prominent of which is collagen type II, which provide tensile force to the tissue (3,5). In addition, proteoglycans predominantly aggrecan, provide a hydrated gel-like medium enabling AC resilience to load-induced deformation (6,7,8). Aggrecan is composed of a large protein core with branched and charged glycosaminoglycans which enable the AC to draw water molecules thus providing the tissue with its capacity to deform (7,8). In OA, aggrecan and other ECM constituents are degraded thereby reducing cartilage lubrication and ability to withstand compressive loads (6).

Although several genetic features have been characterized as risk factors for OA susceptibility (9-12), a growing body of evidence indicates that epigenetic effectors may also modulate gene expression and thus contribute to OA pathology (13-19). These observations turned the spotlight towards novel regulatory epigenetic mechanisms in OA, cartilage biology and development. In fact, recent emerging discoveries link OA pathology with various epigenetic regulatory paths mediated by microRNAs, DNA methylation and histone modifications (20,21). This intriguing aspect insinuates that joint tissues such as cartilage are responsive to environmental conditions and subsequently undergo nuclear reprogramming. Evidently these nuclear alterations lead to changes in gene expression patterns and may thus lead to cartilage degeneration as seen in OA.

One such epigenetic regulator of particular relevance to OA is Silent Information Regulator 2 type 1 (SirT1) which has been linked to aging and caloric intake. Given that obesity and ageing are known risk factors for OA development, SirT1 could be related to OA development and possibly constitute a potential therapeutic target for the disease. This is especially true since levels of SirT1 are altered during OA progression, as discussed in detail below.

This review will explore the various attributes of SirT1 in the physiology, biology and degeneration of cartilage, with a special emphasis on OA. The review will first introduce the basic biochemical theory of epigenetic regulation via histone acetylation/deacetylation and the relevant chromatin modifying enzymes involved in these processes, including the class III histone deacetylases also referred to as sirtuins. The latest discoveries linking SirT1, a sirtuin member, to cartilage biology will be discussed with respect to its potential implications in basic research, diagnostics and therapy of cartilage degeneration and OA.

### 2.1. Introduction to epigenetics and sirtuins

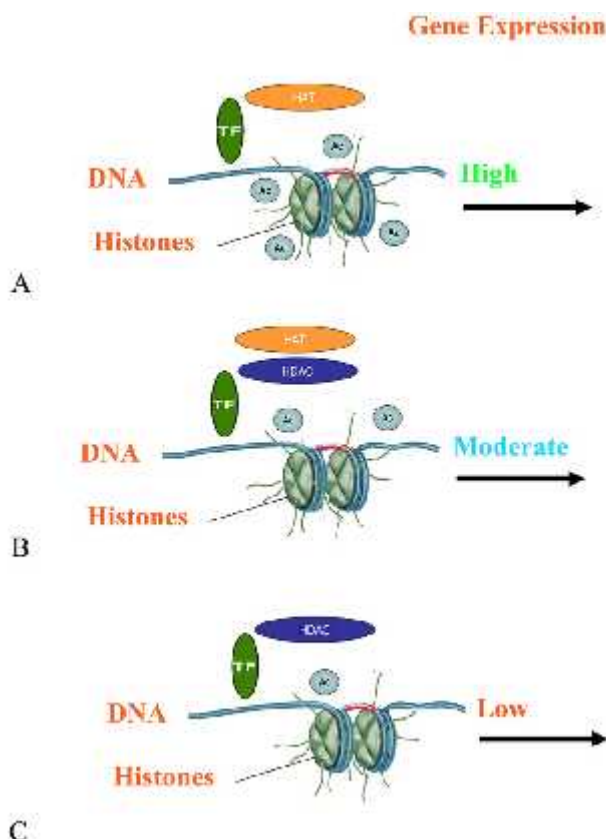
Epigenetic regulation is defined as changes that govern gene expression patterns and cellular phenotype, which are not dependent on the gene sequence. The epigenome is a superior instance controlling the genome in that it can determine which information within a given region of the genome is induced or suppressed. Epigenetic-mediated modifications include DNA methylation, micro-RNA regulation and histone modifications (22-27).

Enzymes responsible for histone modifications are termed "histone-modifying enzymes" and are responsible for post-translational variations of histone tails that regulate the degree of chromatin compaction and subsequently gene transcription (22-27) (Figure 1). Histone modifications include acetylation, methylation, ubiquitination and phosphorylation, which mostly involve the N-terminal histone tails perturbing from the nucleosomal unit. In general, histone modifying enzymes are unable to bind to chromatic regions without their association to transcription factors (23-27). Therefore, these enzymes often form complexes with various coactivators and transcription factors to modulate the expression of their gene targets (23-27), as illustrated in Figure 1.

The nucleosomal unit is a histone octamer consisting of four dimers (H2A, H2B, H3, and H4), and 147 base pairs of DNA coiled around it (Figure 1). The most common and dynamic modification of histones is acetylation, which is carried out by histone acetyltransferases (HATs) and encourages DNA relaxation (i.e. euchromatin) by reducing the electrostatic binding of histones to DNA. This leads to loosening of DNA and subsequently to enhanced gene transcription through recruitment of the transcriptional machinery (23-27). On the other hand, histone deacetylation, carried out by histone deacetylase enzymes (HDACs), promotes chromatin condensation (i.e. heterochromatin), rendering the DNA less accessible to associate with the transcriptional machinery, leading to repressed gene expression (Figure 1).

Histone deacetylases are classified into four classes; class I (HDACs 1, 2, 3 & 8), class II (HDACs 4, 5, 6, 7, 9 & 10), Class III which are also referred to as sirtuins (SirT1-7), and Class IV (HDAC11). HDACs are classified according to homology of their enzymatic domain with yeast HDAC orthologs. Sirtuins are classified as class III HDACs, perform deacetylation through an NAD-dependent mechanism (27) and are characterized by their highly conserved NAD<sup>+</sup> binding and catalytic domain (22). Sirtuins range in size from <40kD to more than 100 kD and share a 300 amino-acid long conserved catalytic domain. Depending on the isoforms, the cellular localization differs from mainly nuclear for SirT1, 6 and 7, while SirT2 is cytoplasmic, and SirT 3, 4 & 5 mitochondrial (22). A growing body of evidence shows that the predominantly nuclear SirT1 can localize to the cell cytoplasm in various cells or under stress conditions. As summarized in Table 1, SirT1 transport could target various cytoplasmic proteins and facilitate various cellular activities such as survival under stress conditions (28-33).

SirT1, as other enzymatically active sirtuins, binds acetyl-lysine to its catalytic groove only in the presence of NAD (14,34). Following lysine deacetylation and cleavage of NAD, O-acetyl-ADP-ribose and NAM are produced. The resulting NAM molecule inhibits SirT1 activity in a noncompetitive manner, possibly via its association with a different binding region than that of NAD within the SirT1 protein (22). Thus, while abundance of NAD drives SirT1 activity, accumulation of NAM



**Figure 1.** Regulation of gene expression via histone acetylation. The nucleosomal octamer composed of 147bp DNA wrapped around 8 histones (i.e H3, H4, H2A, H2B dimers) forms a barrel like structure, wherein the N-terminal histone tails are perturbing and subject to post-translational modifications. This scheme illustrates acetylation (Ac) of histone tails. (A) According to the accepted dogma, enhanced acetylation is facilitated by enrichment of HATS/TF complex rendering a relaxed state of chromatin which exhibits augmented gene expression. (B) Moderate gene expression levels, due to complex formation of HAT/HDAC/TFs which promote reduced acetylation of histone tails as compared to (A). (C) Local enrichment of HDAC/TFs on regulatory gene sites which significantly reduce histone tail acetylation, thereby condensing chromatin and shutting off gene expression. HAT- Histone acetyl transferase, HDAC histone deacetylase, TF transcription factor.

through enhanced deacetylation, acts as a negative feedback loop by preventing SirT1 activity (Figure 2A).

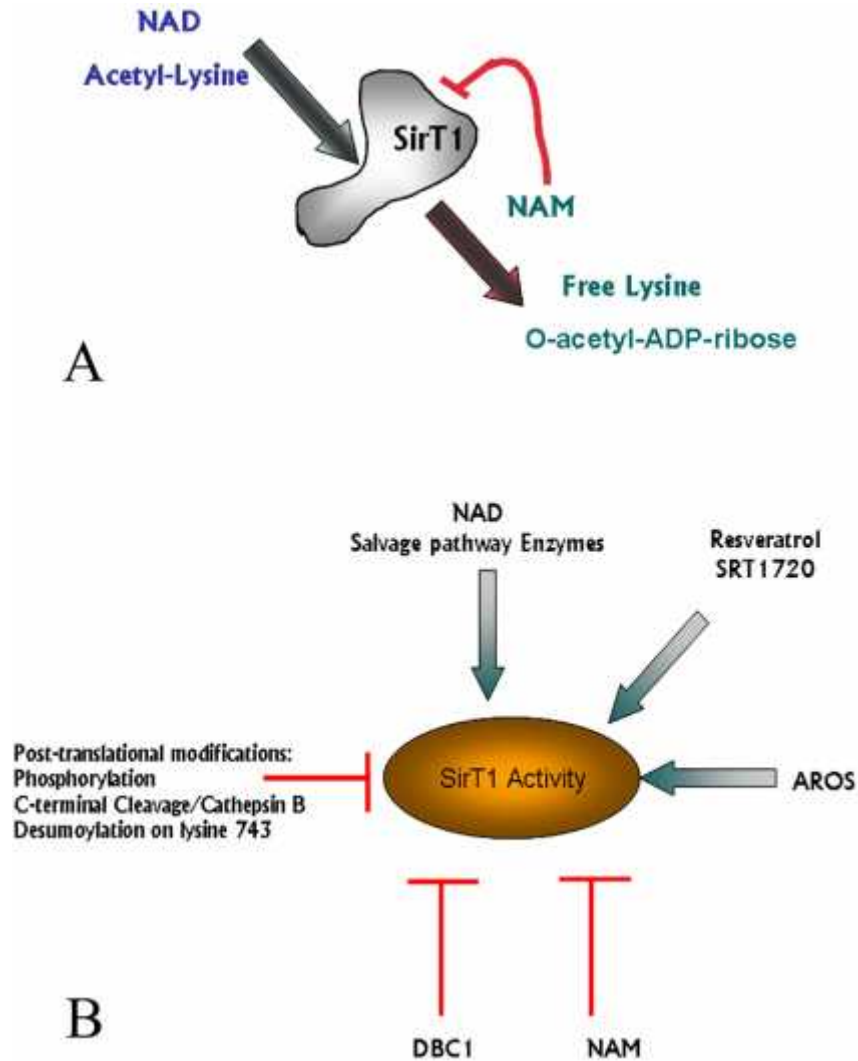
### 3. BIOCHEMICAL ATTRIBUTES OF SIRT1

Since the enzymatic activity of SirT1 is modulated by the bioavailability of its cofactor NAD (Figure 2), many studies have aimed to elucidate the dynamics between SirT1 and the salvage pathway enzymes nicotinamide phosphoribosyltransferase (NAMPT) and nicotinamide mononucleotide adenylyltransferase (NMNAT), which are known to generate the intracellular NAD depot (14,35,36). The importance of the salvage pathway enzymes is especially relevant in cartilage biology since oxidative phosphorylation is limited under the hypoxic conditions of avascularized cartilage tissue. Salvage pathway enzymes are highly expressed in chondrocytes and most probably the key source of the NAD depot within cartilage tissue, enabling the enzymatic activity of SirT1 (14,37). Since chondrocytes are long-lived and seldom replicate in adult articular surfaces, cumulative biochemical attributes of aging could be

manifested through reduced bioavailability of NAD, resulting in an enzymatic impairment of SirT1 (see Figure 2A) and possibly altered ECM expression.

Yet, not only NAD bio-availability is crucial for SirT1 activity, but recent evidence shows that SirT1 enzymatic activity may be regulated by its post-translational modification (Figure 2B). For example, phosphorylated SirT1 is enzymatically inactive (38). Similarly, desumoylation on lysine 743 of SirT1 by SENP1 (SUMO/Sentrin-specific protease 1) causes a reduction in SirT1's activity (39). Additional reports identified protein DBC1 (deleted in breast cancer 1) (40) as a repressor of SirT1, whereas AROS (active regulator of SirT1) enhances SirT1 activity (41). On a post-transcriptional level, recent data demonstrate that the SirT1 RNA transcript can undergo alternate splicing on exon 8, rendering a minor effect in its deacetylase capacity (42).

Overall, cumulative data presented in this section and summarized in Figure 2 indicate that as well as the availability of NAD, SirT1 activity depends on various



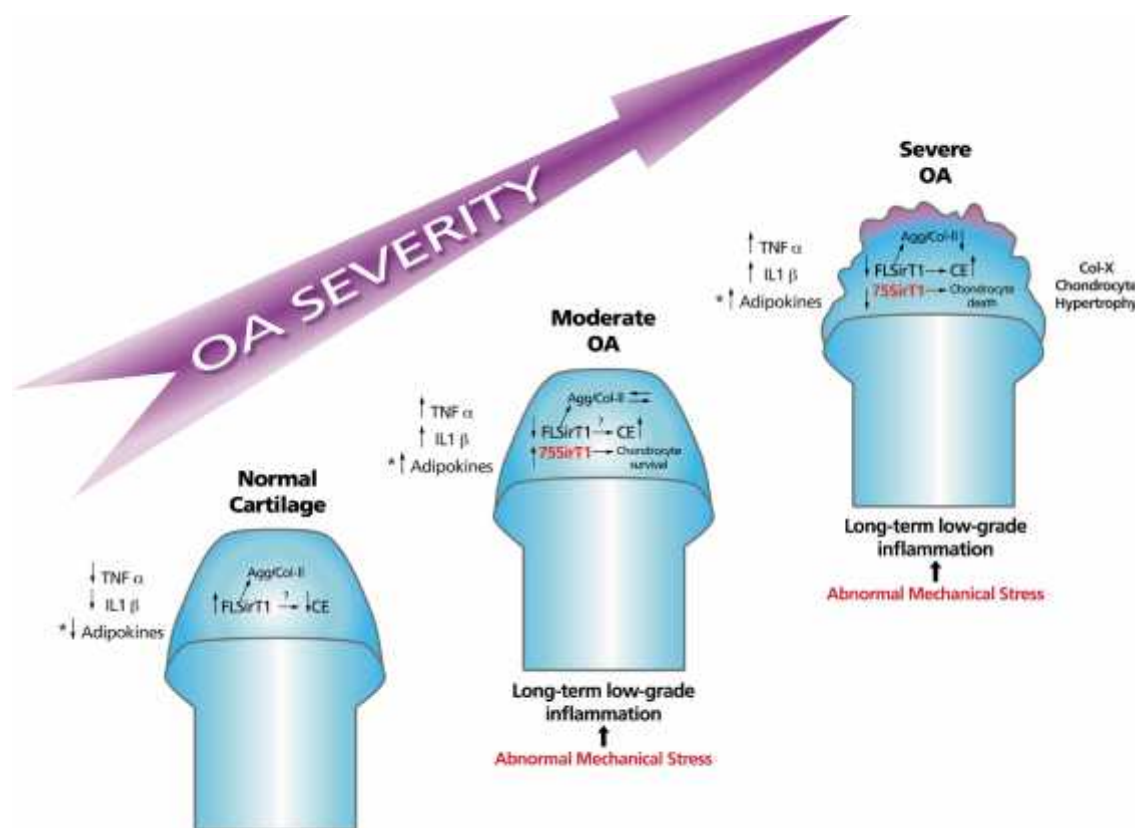
**Figure 2.** SirT1 activity and its influencing factors. (A). schematic illustration depicting the catalytic lysine deacetylase activity of SirT1. (B) The effect of various chemical, pharmaceutical agents and biochemical processes on SirT1 activity. Association of proteins as DBC1 and AROS are known to modulate SirT1 activity. Post-translational modifications as cleavage, phosphorylation, desumoylation are known regulators of SirT1 activity. In chondrocytes (32,44,78) SirT1 is cleaved by cathepsin B to form a 75kD inactive fragment during proinflammatory stimuli. Biochemical and pharmaceutical agents (i.e. NAM, NAD, resveratrol, SRT1720) may affect SirT1 activity in a dose dependent manner. NAM is generated following sirtuin lysine deacetylation and transformed into NAD by the salvage pathway enzymes (i.e. NAMPT and NMNAT). Therefore active NAMPT and NMNAT render increased NAD levels and enhanced SirT1 activity.

post-transcriptional and post-translational modifications. Thus, the activity of SirT1 could be fine-tuned through various extracellular circuits and regulators, including biochemical and pharmacological factors as resveratrol and SRT1720, respectively.

#### 4. SIRT1 BIOLOGY IN HEALTHY AND OA CARTILAGE

Figure 3 presents a model for SirT1 involvement in OA, based on the evidence obtained from the literature. During normal loading and non-inflammatory conditions SirT1 is expressed and healthy cartilage homeostasis

maintained. As abnormal loading conditions develop in articular surfaces, short-term exposure of chondrocytes to inflammatory cytokines renders SirT1 a protective effect which enhances chondrocyte survival and reduces cartilage-specific ECM expression, possibly through enhanced export of SirT1 to the cytoplasm (32). Long-term, low-dose exposure to proinflammatory cytokines confers severe OA, wherein SirT1 is barely detected (14,32,43), and correlates with reduced collagen type II and aggrecan expression as well as augmented chondrocyte death (14,44). Additional increased Collagen type X (Col-X) expression insinuates that chondrocyte hypertrophy occurs under these conditions (43). The enzyme NAMPT (or



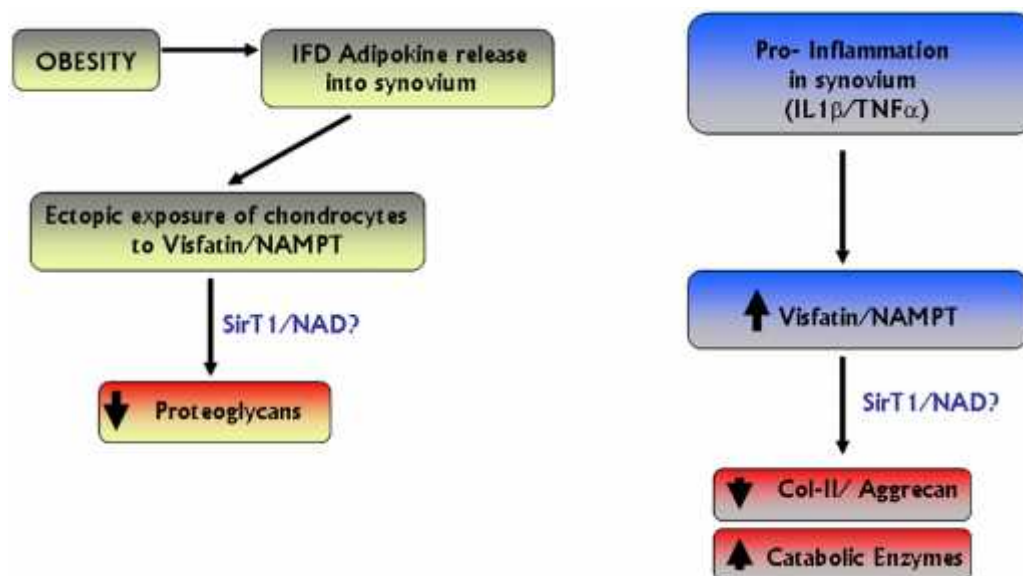
**Figure 3.** Model for SirT1 involvement in OA. Lower left joint scheme illustrates non-inflammatory conditions wherein Full-length SirT1 (FLSirT1) levels are elevated and correlate with enhanced collagen type II (Col-II) and aggrecan (Agg) expression as well as chondrocyte viability. CE denotes catabolic enzymes, which are reduced when FLSirT1 is elevated. Middle scheme illustrates occurrences in early OA, wherein cartilage is exposed to increasing loads and short-term low-grade inflammation and adipokines. Under these conditions FLSirT1 is cleaved to form 75SirT1 which promotes chondrocyte survival at the expense of collagen type II and aggrecan expression. Upper right scheme illustrates severe OA, wherein articular cartilage has been exposed to increased loads and low-grade inflammation for long-term periods. Under these conditions FLSirT1 is barely detected (14, 78, 32), causing enhanced CE, reduced collagen type II and aggrecan expression. The significant reduction of FLSirT1 is concomitant with reduced 75SirT1, leading to augmented chondrocyte death and characteristic morphologic features of OA. Additional increased Collagen type X (Col-X) expression insinuates chondrocyte hypertrophy occurs under these conditions. The scheme is based on reports from references 14,32,43,54,71,70,78. \* Adipokines have not been linked to SirT1 levels in cartilage, but have been shown to be increased in OA and correlate with matrix degradation (50-55).

visfatin) is one adipokine (i.e. cell signaling protein secreted by adipose tissue) which has been linked to SirT1 activity in cartilage (14,37), however so far other adipokines have not been shown to modulate SirT1 activity in cartilage. In this section, relevant data will be discussed in detail with respect to the model proposed in Figure 3.

The first work correlating SirT1 to human OA through its capacity to regulate chondrocyte-specific gene expression was reported by Dvir-Ginzberg *et al.*, (2008). Stable and transient overexpression of SirT1 in human OA chondrocytes resulted in increased RNA levels of aggrecan and collagen type II, dependent on the activity of NAMPT and NAD availability (14). A further mechanistic insight regarding SirT1's capacity to enhance collagen type II, was found by chromatin immunoprecipitation assays, revealing that SirT1 is able to recruit various coactivators and HATs to regulatory gene sites, which is also consistent with

Furumatsu *et al.*, (2005) and Kawakami *et al.*, (2005), (45,46). Sox9 was shown to be a deacetylase target for SirT1, although this action did not enhance Sox9 binding capacity to collagen type II chromatic enhancer region. Consistent with these observations, Fujita *et al.* also established a link between OA and reduced SirT1, and correlated impaired SirT1 levels with reduced aggrecan expression, as well as increased Col-X and ADAMTS5 expression (43). Fujita and colleagues indicated that impaired SirT1 levels could contribute to cartilage hypertrophy and loss of ECM.

Despite these observations,  $IL1\beta$ -induced rabbit-derived articular chondrocytes showed reduced collagen type II expression while NAMPT expression and SirT1 activity were enhanced (37). Additional observations in nucleus pulposus (NP) chondrocytes derived from intervertebral disc (IVD) of human subjects, showed that



**Figure 4.** The influence of the adipokine visfatin (i.e. NAMPT) on cartilage homeostasis. (A) hypothetical scheme which depicts increased adipokine secretion into synovium, with increased weight. "IFD" denotes infrapatellar fat pad. One such adipokine relevant to SirT1 activity (Figure 2B) is NAMPT/visfatin. Ectopic exposure to NAMPT/visfatin correlates with reduced proteoglycan production. (B) Chondrocytes exposed to proinflammatory cytokines may possess enhanced NAMPT/visfatin levels, which correlate with enhanced levels of catabolic enzymes (CE) and reduced aggrecan and collagen type II (Col-II) levels.

Sirt1 levels were elevated in early stages of IVD degeneration (47). These results demonstrated that Sirt1 reduced anabolic gene expression (i.e. for aggrecan and collagen type 2), while increasing NP proliferative capacity. Contradicting data regarding regulation of ECM-encoding cartilage genes by Sirt1 possibly derive from the variations in chondrocyte sources. Such variations in gene expression profiles are also possible due to altered Sirt1 enzymatic activity (Figure 2B) or variations in the capacity of Sirt1 to associate with other contributing coactivators such as p300, GCN5, PGC $\alpha$  and Sox9 (14,45,46,34). In fact when Sirt1 is cleaved and inactivated during TNF $\alpha$ -dependent stimuli of human chondrocytes, Sirt1, Sox9 and PGC1 $\alpha$  exhibit reduced association to the enhancer of collagen type II, resulting in an abrogated expression of collagen type II (44). More detailed mechanistic insight as to Sirt1's association with regulatory gene loci of repressed or activated cartilage ECM genes could provide informative insight as to the diverse mode of Sirt1-mediated regulation in anabolic and catabolic gene expression. The use of sophisticated molecular techniques as chromatin immunoprecipitation protocol (ChIP) and sequential-ChIP will shed new light onto the exact regulatory mechanisms and protein complexes controlling gene expression, which might be dependent or independent on Sirt1.

Under hypoxic stress Sirt1 promotes hypoxia-inducible factor 2 $\alpha$  (HIF2 $\alpha$ ) transcriptional activity in cells other than chondrocytes (48). The finding that HIF2 $\alpha$  encourages cartilage catabolic gene expression in human chondrocytes (49), implies that an indirect Sirt1/HIF2 $\alpha$  regulatory axis may cause augmented catabolic gene expression in chondrocytes. Still, the mechanism by which

Sirt1 may regulate catabolic gene expression during inflammation and whether this mode of regulation is directly upon the gene loci or indirect via association with other regulatory factors remains to be determined.

It is also known that adipokines as well as cytokines play a significant role in OA and cartilage homeostasis (Figure 4A) (50). This is especially relevant since obesity is a major risk factor in OA development. In obese or overweight individuals cartilage is subjected to increased load as well as enhanced exposure to adipose-derived adipokines or cytokines, either systemically or locally through the joint infrapatellar fat pad (51, 52). Enhanced adipokine levels may regulate Sirt1 activity in the surrounding articular surfaces. Of particular interest in connection with Sirt1 regulation is the adipokine visfatin (i.e. NAMPT), an NAD salvage pathway enzyme which is released from the infrapatellar fat pad into the synovium where it correlates with augmented biomarkers of cartilage degradation (52,53). However, it remains to be determined whether enhanced NAMPT/visfatin in the joint milieu is capable of entering chondrocytes and thus potentially modulating Sirt1 activity through enhanced NAD levels.

Recently Yammani and Loeser reported that NAMPT/visfatin inhibits Insulin-like growth factor 1 function by activating an independent ERK/MAPK pathway, which correlates with reduced proteoglycan production (54). In line with these observations IL1 $\beta$ -stimulated articular chondrocytes displayed enhanced NAMPT/visfatin expression and Sirt1 activity which results in reduced collagen type II expression (37). Interestingly, it appears that intracellular levels of NAMPT/visfatin could be modulated by external



inflammatory cues as illustrated for human chondrocytes by Gosset *et al.*, (55) and additionally supported by Hong *et al.*, (37). Gosset and colleagues reported that IL1 $\beta$  increased intracellular NAMPT/visfatin levels in chondrocyte cultures together with an elevation in catabolic gene expression and a reduction in aggrecan expression (55). Elevated NAMPT/visfatin levels may not necessarily elicit enhanced intracellular NAD levels since these also depend on NMNAT levels, which is another member of the NAD salvage pathway (see Figure 2B). Further, modulation of SirT1 activity is not only dependent on NAD availability but also on post-translational modifications of SirT1, as previously summarized in Figure 2B. Notwithstanding, the recent advances in the field of adipokines provide promise in understanding the pathogenesis of OA. With respect to SirT1 activity and its role in OA, the adipokine NAMPT/visfatin is of particular interest since it elicits cartilage degeneration (Figure 4A) even under pro-inflammatory stimuli (Figure 4B). Therefore future evidence regarding SirT1 and NAMPT, should confirm that enhanced NAMPT/visfatin levels are accompanied with enhanced SirT1 activity due to increasing NAD levels and not as a result of post-translational modifications (Figure 2B).

### 5. LINKING SIRT1 AND CARTILAGE BIOMECHANICS

Even though it is well recognized that OA is a multifactorial disease in which mechanical factors play an important role, the nature of the complex biomechanical interactions with epigenetic changes are largely unknown. Mechanical factors are involved in maintaining a healthy cartilage as well as triggering OA by for instance abnormal patterns of mechanical loading (Figure 3). Regardless, the specific mechanisms by which chondrocytes sense mechanical signals and convert them to an intracellular biochemical response is poorly understood. Yet, the final metabolic response of mechanically stimulated chondrocytes appears to be the result of signals received by several signal transduction pathways including integrin-mediated pathways, stretch activated or inactivated ion channels, involvement of the cytoskeleton, decreased pH, electrical streaming potential, nitric oxide or second messenger systems (56-58).

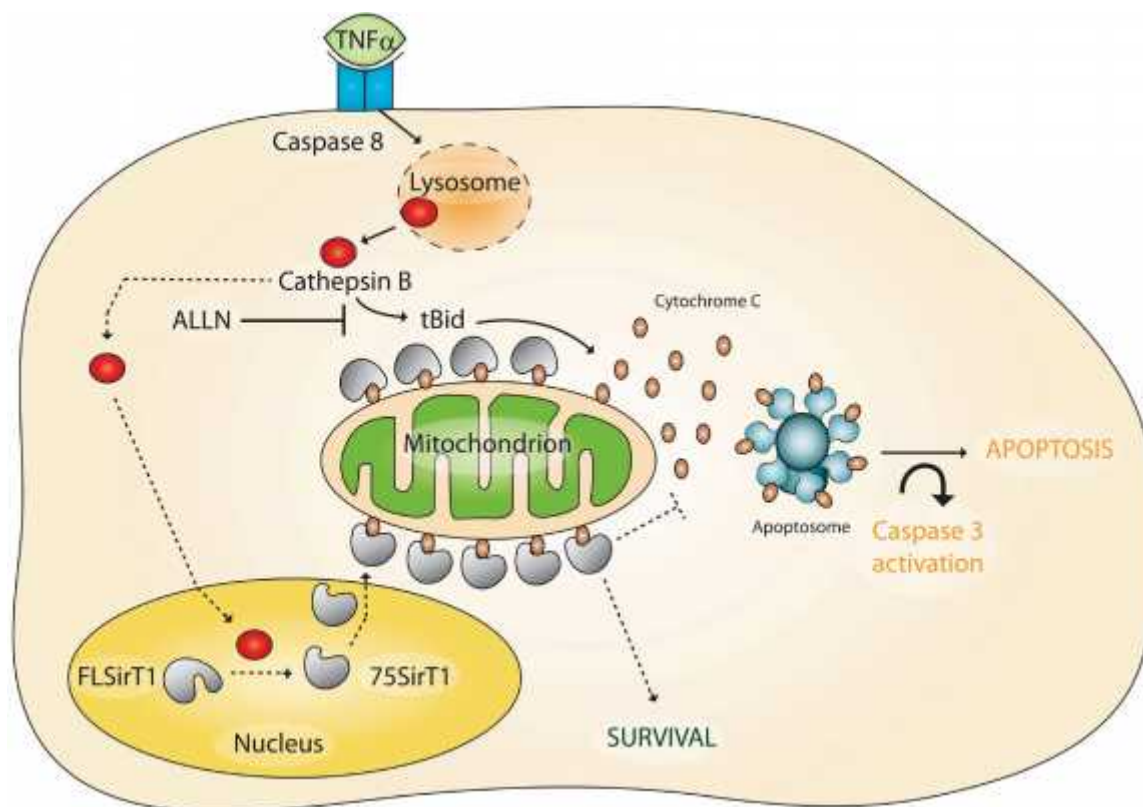
Further evidence pointing to the role of epigenetic changes in OA stems from human OA chondrocytes presenting higher levels of HDAC1, HDAC2 and HDAC7 (17,59) as well as decreased levels of SirT1 (illustrated in Figure 3). Recent *in vitro* and *in vivo* studies using cartilage from rats, rabbits and steers revealed that certain mechanical stimuli are able to antagonize both the IL-1 $\beta$  induced increased expression of iNOS, COX-2, MMP-9 and MMP-13 as well as the IL-1 $\beta$  induced decreased aggrecan production (59-63). This anti-inflammatory effect of certain mechanical stimuli seems to be mediated by the NF- $\kappa$ B-pathway (63,64). This pathway is also modulated by SirT1 since its overexpression results not only in an inhibition of p53 but also in reduced transcription of the p65 NF- $\kappa$ B subunit by promoting its acetylated state and thereby preventing expression of

inflammatory-responsive genes (65,66). However, in the biomechanical studies mentioned above cartilage from young animals was used, making the extrapolation to the pathophysiology of elder human patients quite difficult. Along this line, several studies already showed that age, species, severity of disease and even the joint sample investigated have a tremendous impact on the signal transduction pathways used and thus on the resulting metabolic response (67-70). Therefore, evaluation of any possible interactions between biomechanical factors and epigenetic changes should finally also include human OA cartilage, if possibly from elderly or obese subjects.

### 6. SIRT1, CELL SURVIVAL AND INFLAMMATION

It is well established that SirT1 regulates cell survival and gene expression under stress through its capacity to interact with various non-histone proteins such as p53, forkhead transcription factor, Ku70 and RelA/p65 (22). In the joint, SirT1 seems to play a role in promoting chondrocyte survival, especially under inflammatory conditions. Takayama *et al.*, suggested that in chondrocytes, SirT1 possesses the capacity to modulate mitochondrial levels of Bax and Bcl2 in nitric oxide (NO)-induced apoptosis (71). In addition, Gagarina *et al.*, reported that the proapoptotic Protein Tyrosine Phosphatase 1B (PTP1B) is elevated in OA cartilage and that SirT1 is capable of downregulating its level to achieve enhanced chondrocyte survival (72). Also Lei *et al.* showed that in rat articular chondrocytes resveratrol, a chemical activator of SirT1, inhibits IL-1 $\beta$ -induced nitric oxide synthase expression via impaired NF- $\kappa$ B transcriptional activity, and thus further supports its role in promoting chondrocyte survival under proinflammatory conditions (73). Additional studies using Rheumatoid Arthritis (RA)-derived synovial cells show that TNF $\alpha$  enhances SirT1 expression, which in turn promotes cytokine production while inhibiting apoptosis (74). Overall, these studies strongly demonstrate that SirT1 promotes cell survival in the joint, even under pro-inflammatory conditions.

Human chondrocytes stimulated with TNF $\alpha$  present a moderate increase in SirT1 protein levels, however a portion of endogenous SirT1 is cleaved by Cathepsin B to generate an inactive 75kD fragment (75SirT1) (44). This fragment is partially translocated to the cytoplasm and mitochondria (44,32; Table 1 and Figure 5), where it inhibits apoptosis. Similar observations were made in TNF $\alpha$  stimulated HeLa cells, showing that caspases cleave SirT1, induce its transport to the cytoplasm, enhancing apoptosis (33; Table 1). In endothelial progenitors, Cathepsin B was also shown to cleave SirT1 and generate a 75kDa fragment during stress conditions, although its enzymatic activity and cellular trafficking were not reported (75). In chondrocytes, Oppenheimer *et al.*, found that 75SirT1 fragment is capable to interact with the mitochondrial membrane and to block Cytochrome C release from the mitochondria and subsequent assembly with the apoptosome complex, resulting in enhanced chondrocyte survival under proinflammatory stress (32). Figure 5 illustrates the cellular occurrences involving endogenous SirT1 cleavage and



**Figure 5.** Intracellular trafficking of 75SirT1 in chondrocytes and its role in survival. Following  $\text{TNF}\alpha$  stimulation, caspase -8 dependent lysosomal permeability occurs, releasing cathepsin B into the cell cytoplasm and nucleus. Active cathepsin B cleaves nuclear FLSirT1 to generate an inactive stable 75SirT1 fragment, which is exported via CRM1 to the cytoplasm (broken arrows). While in the cytoplasm, 75SirT1 interacts with cytochrome C on the mitochondrial membrane to prevent downstream apoptosome assembly (broken arrows). Solid arrows indicate the  $\text{TNF}\alpha$ –cathepsin B pathways generated through activation of truncated Bid (tBid). ALLN, a cathepsin B inhibitor, will block both Bid cleavage and 75SirT1 generation. The model may be relevant *in vivo* for articular chondrocytes prone to OA development. Reproduced with permission from John Wiley & Sons (32).

trafficking of the cleaved 75SirT1 fragment under  $\text{TNF}\alpha$  induction of human articular chondrocytes (32). Intact cartilage of OA patients exhibited elevated levels of 75SirT1 and no full-length SirT1 (FLSirT1), whereas normal cartilage showed only FLSirT1 (32). This raised the question whether in the cytoplasm SirT1 is present only in its cleaved form, and whether this enzymatically inactive C-terminally truncated SirT1 form (i.e. 75SirT1) carries out an additional unknown regulatory role. Kang *et al.*, (2011) reported that a special region within the C-terminus of SirT1 (76) is necessary for the catalytic activity. This result is similar to our own study reporting that 75SirT1 lacks the C-terminal end and is thus enzymatically inactive (44). In light of these observations, it has yet to be determined whether SirT1 possesses regulatory capacities that are independent of its enzymatic aptitude.

## 7. SIRT1 AND “INFLAMM-AGING”

Aging increases susceptibility to a variety of diseases such as OA, cardiovascular and neurodegenerative diseases, cancer, diabetes and inflammation (22). Many age-related diseases resulting in tissue degeneration are

caused by chronic inflammation and are thus termed “Inflamm-aging”. Age-related diseases may also involve regulation by SirT1. It may be that part of the anti-aging function of SirT1 resides in its ability to inhibit inflammation and its devastating effects (73, 77,65). However, little is known about the effects of long-term chronic inflammation on the expression and activity of SirT1. When examined in a cartilage context, proinflammatory cytokines attenuate the functions of SirT1, resulting in a decreased cartilage specific gene expression *in-vitro* and *in-vivo* (32,44,78). Our *in-vitro* study indicates that 75SirT1 plays a role in prolonging chondrocyte survival under proinflammatory conditions (Figure 5), which was further verified in an animal model using 129/J mice (32, 77). Gabay and colleagues found that 9-month old 129/J mice develop mild OA, which was enhanced in severity in haploinsufficient SirT1 129/J mice compared to age-matched wildtype (w.t.) controls (78). Interestingly, a lesser extent of chondrocyte death was observed in w.t. 129/J mice, which positively correlates with enhanced 75SirT1 in w.t. vs. haploinsufficient SirT1 mice (77). These *in-vivo* data indicate that SirT1 could play multiple roles in various conditions involving “inflamm-aging” as seen in OA, by regulating various cellular



**Table 1.** Role of cytoplasmic SirT1 in cell physiology

SirT1 protein	Cell target	Role in cytoplasm	Culture conditions	Reference
Full-length SirT1	PC12 (pheochromocytoma) cells	Enhanced Neurite outgrowth	NGF induction	28
Full-length SirT1, SirT1 with truncated NLS	HeLa	Enhanced apoptosis in cytoplasm	H <sub>2</sub> O <sub>2</sub> induction	29
Full-length murine SirT1	Cardiomyocytes from adult heart, murine tissues and C2C12 myoblast lines	Enhanced differentiation and inhibited cell death.	* LY294002 ¶ Leptomycin B # Antimycin A	30 * LY294002 inhibited nuclear SirT1 localization. ¶ Leptomycin B inhibited nuclear export by CRM1. # Nuclear SirT1 suppressed Antimycin A-induced apoptosis.
Full-length SirT1	Prostate cancer cells, normal prostate and cell lines	Elevated mitotic activity and PI3K/IGF-1R signaling in cancer cells	Normal vs. cancer cells	31
75kDa-SirT1‡	Primary Chondrocytes	Enhanced chondrocyte survival via mitochondrial association	TNFα stimuli	32 ‡ 75kDa SirT1 generated by Cathepsin B cleavage.
Cleaved SirT1†	Hela Cells	Enhanced cell death	TNFα stimuli	33 † Caspase mediated cleavage of SirT1

processes affecting gene expression and survival, depending on the inflammatory, biomechanical and even pharmacological environment.

## 8. CONCLUDING REMARKS

To conclude, recent data indicate that SirT1 is necessary for healthy cartilage homeostasis and is reduced or inactivated during systemic occurrences involving OA like aging, adipokine release and inflammation. The reduction or inactivation of SirT1 is correlated with reduced ECM expression, enhanced expression of cartilage degrading enzymes and even cartilage hypertrophy. More pronounced proinflammatory cues that arise later in the pathophysiology of OA, render SirT1 inactivation via cathepsin B specific cleavage of the C-terminal end of SirT1 (Figure 3,5), causing its export from the nucleus to the cytoplasm, where it acts as a pro-survival factor (Figure 5). This process appears to correlate with loss of cartilage ECM and with maturation (44,77).

As we progress towards the post-genomic era, we find increasing evidence for the potential of nuclear reprogramming in cellular phenotypes and its relevance in disease. Age-related diseases such as OA are in the focus of epigenetic research initiatives since their prevalence due to the general aging of human population increase and thus have an enormous impact on both the individual patient as well as the health systems of our societies. The involvement of SirT1 in a myriad of age-related diseases and its regulation by controlled caloric intake lead several research avenues to examine its role in OA. This review focused on recent advances in understanding the role of SirT1 in OA, which is one of many regulators that may contribute to joint destruction. So far, reports support that SirT1 is required for normal cartilage phenotype and is altered during OA and other pathologies involving cartilage degeneration (summarized in Figures 3,4,5).

In future, more detailed molecular observations could lead to a better understanding of the regulatory functions of SirT1, either locally within cartilage-specific gene loci or

systemically affecting intracellular biochemical processes of chondrocytes under various stress conditions. Additional evaluations of *in-vivo* models involving SirT1 in aging and chronic inflammatory diseases will provide insight as to its involvement in OA on a whole-organism level. OA can be triggered by prolonged increased loads, and cartilage destruction is further enhanced by abnormal stress. However, we have yet to determine how these phenomena affect SirT1 and understand how it in turn disrupts cartilage homeostasis and chondrocyte survival. Elucidating the role of SirT1 in OA will expectedly provide novel biomarkers to monitor OA susceptibility as well as identify potential drug targets e.g. sirtuin activators to combat cartilage destruction in OA, which is a debilitating degenerative joint disease with increasing prevalence in the general population.

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**Abbreviations:** HIF: hypoxia inducible factor, MMP: matrix metalloproteinase, ChIP: Chromatin immunoprecipitation, CR: caloric restriction, ECM: extracellular matrix, OA: osteoarthritis, AC: articular cartilage, SirT1: Silent Information Regulator 2 type 1, HDAC: histone deacetylase enzyme, HAT: histone acetyl transferase, TF: Transcription factor, IVD: intervertebral disc, (IVD), ADAMTS: A Disintegrin And Metalloproteinase with Thrombospondin Motifs, NAD: Nicotinamide adenine dinucleotide, NAM: nicotinamide, NMNAT: Nicotinamide mononucleotide adenylyltransferase 1, NAMPT: Nicotinamide phosphoribosyltransferase (also referred as visfatin), TNFalpha: Tumor necrosis factors alpha, IL1beta: interleukin beta, BMAL1: brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)-like, CLOCK: Circadian Locomotor Output Cycles Kaput; DBC: Deleted in breast cancer 1; AROS: Active regulator of SirT1; IFD: infrapatellar fat pad.

**Key Words:** Ageing, osteoarthritis, OA, Histone Modifying Enzymes, Silent Information Regulator 2 Type 1, Sirt1, Inflammation, Review

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