The human sirtuin family: Evolutionary divergences and functions

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Date received (in revised form): 9th June 2011

Abstract

The sirtuin family of proteins is categorised as class III histone deacetylases that play complex and important roles in ageing-related pathological conditions such as cancer and the deregulation of metabolism. There are seven members in humans, divided into four classes, and evolutionarily conserved orthologues can be found in most forms of life, including both eukaryotes and prokaryotes. The highly conserved catalytic core domain composed of a large oxidised nicotinamide adenine dinucleotide (NAD^+)-binding Rossmann fold subunit suggests that these proteins belong to a family of nutrient-sensing regulators. Along with their function in regulating cellular metabolism in response to stressful conditions, they are implicated in modifying a wide variety of substrates; this increases the complexity of unravelling the interplay of sirtuins and their partners. Over the past few years, all of these new findings have attracted the interest of researchers exploring potential therapeutic implications related to the function of sirtuins. It remains to be elucidated whether, indeed, sirtuins can serve as molecular targets for the treatment of human illnesses.

Keywords: *Evolution, histone deacetylases, human diseases, metabolism, sirtuins*

Introduction

Epigenetic modifications of protein, histone and chromatin play an important role in regulating gene expression, cancer formation and life span. Acetylation is a major player in epigenetic modifications, resulting in open chromatin structures and, hence, permissive conditions for transcription-factor recruitment to the promoters, followed by initiation of transcription. By contrast, histone deacetylases (HDACs) oppose the activity of histone acetyltransferases by removing the acetyl groups from lysine residues within specific promoters, leading to gene silencing.[1] In addition, many non-histone proteins have been identified as substrates of HDACs, implicating acetylation as a post-translational modification that affects various aspects of cell physiology.[2] There are two protein families having HDAC activity: the classical HDAC family, which consists of two different phylogenetic classes (class I and class II); and the sirtuin family of proteins, which requires the co-factor nicotinamide adenine dinucleotide (NAD) for its deacetylase activity.[3,4]

The sirtuin family

The sirtuin family of proteins is highly conserved, both functionally and structurally. Its members are integrated into most forms of life, including eubacteria, archaea and eukaryotes, and therefore predate both histone and chromatin formation.[5] Sirtuins

have been involved in metabolic and chromatin regulation throughout evolution, dating back to the first examples of chromatin-like organisation of DNA in archaea.[6,7] The silent information regulator 2 gene (*Sir2*) was first discovered in *Saccharomyces cerevisiae* and was named after its ability to relieve gene silencing.[8] Once discovered, sirtuins were rapidly characterised in yeast, bacteria, plants and mammals.

Sirtuins belong to the deoxyhypusine synthase (DHS)-like NAD/flavin adenine dinucleotide (FAD)-binding domain clan and all members contain the Rossmann fold structural motif, which can be found in proteins that bind nucleotides. The other members of this clan sharing evolutionary relatedness are the CO dehydrogenase β-subunit/acetyl-coenzyme A (CoA) synthase ε-subunit, DHS, electron transfer flavoprotein FAD-binding domain, NAD(P) transhydrogenase β-subunit and thiamine pyrophosphate enzyme. The mammalian family of sirtuins is categorised as class III histone deacetylases and consists of seven members which are present in nearly all subcellular compartments. SIRT1, SIRT6 and SIRT7 are predominantly localised in the nucleus; SIRT3, SIRT4 and SIRT5 reside within the mitochondria; and SIRT2 is limited to the cytoplasm (Table 1). The highly conserved structure of sirtuins, as well as their NAD^+ dependence, suggests that these enzymes are a family of nutrient-sensing regulators cooperating in semi-redundancy to direct cellular metabolism in response to altered nutrition or stress.[9] Whereas this enzyme family is associated with HDACs because of their related nuclear function, the majority of their substrates are non-core histone proteins. Chemically, deacetylation primarily occurs on *N*-ε-lysine residues and typically regulates protein function. Each isoform maintains a highly conserved catalytic core comprising a large NAD^+-binding Rossmann Fold subunit and a small zinc-binding domain.[9] The majority of sirtuins function as deacetylases (SIRT1, -2, -3, -5, -6 and -7), where enzymatic activity results in the removal of an acetyl group from *N*-ε-lysine residues and generates *O*-acetyl-ADP-ribose and nicotinamide. SIRT4 and -6, however, have also been reported to display mono-adenosine diphosphate (ADP)-ribosyl transferase activity.[10,11] An increased awareness concerning the regulatory function of sirtuins has resulted in an explosion of research on this family of proteins. The majority of research characterising the functional targets of sirtuins has focused on nuclear SIRT1; however, the other family members are becoming more widely studied.

Phylogenetic analysis of sirtuins from a variety of prokaryotes and eukaryotes has divided the family into five different classes (Table 2).[5,9,12] SIRT1, together with Sir2, homologue of Sir Two (Hst) 1, SIR-2.1 and *Drosophila melanogaster* Sir2 (D.mel1) orthologues belong to subclass Ia. SIRT2 and SIRT3 share subclass Ib with Hst2, D.mel2 and other fungi and protozoa sirtuins. SIRT4 is in class II and SIRT5 in class III, and both classes include sirtuins from bacteria, archea, nematodes and protozoans. Class IV contains SIRT6 and SIRT7, and can be distinguished from the two previously mentioned classes because only sirtuins from eukaryotes can be found in this category. Finally, class U consists of bacterial Sir2 homologues with undifferentiated motifs. According to this classification of sirtuins, it is believed that sirtuins from classes II, III and U appeared earliest in evolution; the early eukaryotes possessed all four types of sirtuins, whereas later in evolution some eukaryotes lost a few classes, which explains the variable distribution of sirtuins in different organisms.[5] The phylogenesis of all isoforms of human sirtuins is summarised as a dendrogram (Figure 1), demonstrating the splitting of the proteins into distinctly separate branches. This analysis confirms the categorisation of the members in different classes and provides a better assessment of evolutionary divergence.

SIRT1

The *SIRT1* human gene is located at chromosome (Chr) 10q21.3.[13] The role of SIRT1 has been the most extensively studied among the sirtuin family members. The first substrate to be identified for SIRT1 was the TATA binding protein-associated factor 168 (TAFI68), a transcription factor necessary for regulating the RNA

Table 1. List of the members of the sirtuin family in humans. For each member, cellular localisation, major functional activity, molecular weight (MW), total number of amino acids (AA), active domain and chromosomal location is listed.

Gene name	Intracellular location	Functional activity	MW (kDa)	AA	Active domain (AA)	Chromosomal location
SIRT1	Nucleus and cytoplasm	Deacetylase	81.7	747	244–498	10q21.3
SIRT2	Cytoplasm	Deacetylase	41.5	389	65–340	19q13.3
SIRT3	Mitochondria	Deacetylase	43.6	399	126–382	11p15.5
SIRT4	Mitochondria	ADP-rlbosyl transferase	35.2	314	45–314	12q
SIRT5	Mitochondria	Deacetylase	33.9	310	41–309	6p23
SIRT6	Nucleus	Deacetylase/ ADP-ribosyl transferase	39.1	355	35–274	19p13.1
SRT7	Nucleolus	Deacetylase	44.9	400	90–331	17q25

polymerase I transcriptional complex,[14] where it was shown that deacetylation inhibits transcriptional initiation *in vitro*. The list of SIRT1 substrates and targets is continuously growing and includes several transcription factors and proteins, implicating it in a variety of cellular functions. For example, p53 was the first non-histone substrate identified for the histone acetyltransferase. It was demonstrated that acetylation activates the DNA-binding activity and

Table 2. The mammalian sirtuins classification by phylogeny. The mammalian sirtuins (bold) and their orthologues in other organisms are classified into five classes according to phylogenetic analysis.[5]

Class I a	**SIRT1** *D. melanogaster D. mel1* *C. elegans* SIR-2.1 *S. cerevisiae* Hst1
Class I b	**SIRT2** **SIRT3** *D. melanogaster* *D. mel2* *S. cerevisiae* Hst2 Protozoans
Class I c	*S. cerevisiae* Hst3 *S. cerevisiae* Hst4 Fungi
Class II	**SIRT4** Bacteria Protozoans
Class III	**SIRT5** Archeans Bacteria Nematodes
Class IV a	**SIRT6** *D. melanogaster* *D. mel4* Protozoans Plants
Class IV b	**SIRT7** *D. melanogaster D. mel5*
Class U	Bacteria

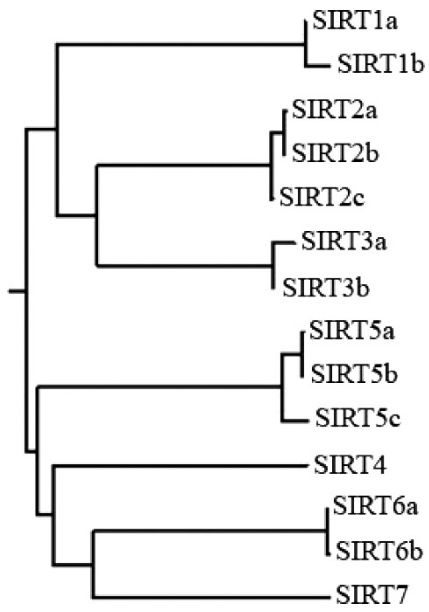

Figure 1. Phylogenetic analysis of human sirtuin family members. Known peptide sequences for all isoforms of SIRT1-7 from the National Center for Biotechnology Information (NCBI)'s GenBank Database were aligned using ClustalW alignment software, and a dendogram with branch lengths was constructed.

target gene expression, as well as increasing its stability.[15] SIRT1-mediated reversal of this post-transcriptional modification can modulate p53 function, weakening the biological effects of acetylation described earlier by repressing p53-dependent transcription.[16,17] Moreover, it was found that SIRT1-dependent deacetylation regulates localisation of p53, controlling cell fate decisions. For example, in SIRT1-deficient mouse embryonic stem (mES) cells, translocation of the protein to the nucleus is blocked, resulting in cytoplasmic accumulation, passage to mitochondria and induction of the transcription-independent apoptotic pathway following oxidative stress.[18] Consistent with SIRT1 inhibition of p53 function, *Sirt1*$^{(-/-)}$ knockout mice exhibit p53 hyperacetylation and increased radiation-induced apoptosis, raising the possibility that SIRT1 can facilitate tumour growth by antagonism of p53.[19] This, together with evidence that sirtinol, an inhibitor of SIRT1, induces a senescence-like cell growth arrest,[20] led to the suggestion that SIRT1 may act as an oncogene. By contrast, other studies[21] demonstrated that SIRT1 seems to act as a tumour suppressor, as *Sirt1*$^{(+/-)}$ – *Trp53*$^{(+/-)}$ mice exhibit a higher incidence of tumours, compared with *Trp53*$^{(+/-)}$ mice.

In white adipose tissue, it was reported that the nuclear peroxisome proliferator-activated receptor gamma (PPARγ) belongs to the category of SIRT1-regulated proteins, as activation of SIRT1 represses it, leading to reduced fat accumulation, whereas its inhibition results in triglyceride accumulation.[22,23] In the liver, SIRT1 is also involved in nutrient control of glucose homeostasis by modulating the activity of the metabolic co-regulator PPARγ coactivator-1a (PGC-1a), which regulates gluconeogenesis, glycolysis and fatty-acid β-oxidation. Specifically, SIRT1-mediated deacetylation increases hepatic glucose output and use of fat for energy under fasting conditions,[24,25] whereas SIRT1 was also found to activate PGC-1a, resulting in enhanced mitochondrial function and protection against metabolic disease.[26]

Another important group of substrates for SIRT1 are members of the forkhead box factors (FOXO) family of proteins, which are regulated by insulin/AKT. After the finding that longevity in *Caenorhabditis elegans* depends on the coordinated function of *sir-2.1* and the decay accelerating factor 16 gene (*daf-16*) (the orthologues of FOXO proteins),[27] Brunet *et al.* were the first to demonstrate that SIRT1 can deacetylate FOXO3 when they are in a complex in response to oxidative stress.[28] In addition, they found that SIRT1 deacetylates *FoxO3*, tipping FOXO-dependent responses away from apoptosis and towards cell cycle arrest and cellular stress resistance. The beneficial effect of SIRT1-dependent deacetylation of FOXOs was further confirmed by the oxidative stress-resistant phenotype in the heart[29] and the starvation-induced autophagy in cardiac myocytes.[30] Besides the above-mentioned well-characterised targets, several studies have identified more striking partners of SIRT1. Tau protein is deacetylated by SIRT1, affecting the stability of the protein and implying that SIRT1 activators may have therapeutic implications by reducing tau-mediated neurodegeneration.[31] Recently, Guarani *et al.* demonstrated that SIRT1 associates with the Notch1 intracellular domain (NICD) and controls stabilisation of the protein,[32] increasing the number and variety of target proteins for SIRT1. Finally, in another recent study, Kim *et al.* demonstrated that SIRT1 binds to the promoter of *SIRT6* and positively regulates its expression, suggesting that different members of the sirtuin family can function collaboratively in maintaining cellular homeostasis.[33]

Several studies over the past few years have used gene-targeted mutagenesis experiments in mice to examine the consequences of expressing a mutant SIRT1 protein lacking part of the catalytic domain or deleting the *Sirt1* gene completely.[19,21,34] McBurney *et al.* showed that animals homozygous for a null allele of *Sirt1* are born at only half the expected frequency, suggesting prenatal lethality.[34] Moreover, they showed that homozygous embryos and pups are smaller than their wild-type and heterozygous littermates and have developmental defects of the eyes, lungs and pancreas. Even under conditions in which *Sirt1*-null animals survive to adulthood, both sexes are sterile because of a failure of females to ovulate and inefficient

spermatogenesis in males. Similar results were obtained in a second study,[19] in which mice were generated either lacking *Sirt1* or expressing a mutant *Sirt1* gene. Both types of mutant mice were smaller than their wild-type and heterozygous littermates and had developmental defects in the retina and heart, and most died postnatally. Even more severe embryonic lethality was also observed in another *Sirt1* mutant mouse model, in which exons 5 and 6 were deleted, suggesting that *Sirt1* is essential for normal embryogenesis.[21]

Since SIRT1 is involved in many different cellular processes, as already described, several studies have focused primarily on genetic variation of the *SIRT1* gene. Studies have attempted to elucidate whether there are any associations between *SIRT1* genetic variation and pathological conditions in humans. For example, evidence to date suggests that there might be an association between some aspects of *SIRT1* genetic variation and risk for obesity, as well as the response to lifestyle interventions for obesity.[35–38] In addition, mortality in type 2 diabetes mellitus (T2DM) in the presence of factors such as smoking, niacin intake[39] and cardiovascular disease[40] has been linked to *SIRT1* genetic variations. Moreover, a recent study reports that four single nucleotide polymorphisms (SNPs) in *SIRT1* were associated with diabetic nephropathy in Japanese subjects with T2DM.[41] No association between exceptional longevity and *SIRT1* genetic variation was found in this study,[42] however, although life expansion was one of the first phenotypes linked with the role of sirtuins.

SIRT2

Human *SIRT2* is located at Chr 19q13.3.[13] The SIRT2 protein is similar in sequence to yeast Hst2p and both proteins are located in the cytoplasm,[43] making it the first cytoplasmic sirtuin found. SIRT2 co-localises with the microtubule network and deacetylates Lys40 of tubulin, which was the first identified substrate for this protein.[44] SIRT2 also associates with another deacetylase, HDAC6, along microtubules; these two proteins together regulate the level of tubulin acetylation. SIRT2 activity is increased before mitosis[45] and localises to chromosomes, serving as a histone deacetylase during mitosis,[46,47] suggesting a role for this protein in chromosome condensation. Recent findings showed that, during the cell cycle, SIRT2 is associated with the centrosome, the mitotic spindle and the midbody, further emphasising its role in completion of mitosis.[48]

SIRT2 also has been found to interact with FOXO1, as is true for SIRT1. In particular, Jing *et al*. showed that SIRT2 affects adipocyte differentiation through modulation of both acetylation and phosphorylation of FOXO1.[49] Recently, SIRT2 was also involved in cellular processes such as neuroprotection and the inflammatory response. It was found that either genetic or pharmacological inhibition of SIRT2 plays a neuroprotective role by reducing sterol levels, which are increased in Huntington's disease.[50] By contrast SIRT2 inhibitors rescue α-synuclein-mediated cytotoxicity in models of Parkinson's disease.[51] Regarding the immune system, hyperacetylated p65 in *Sirt2*$^{(-/-)}$ cells results in increased expression of a subset of nuclear factor (NF)-κB-dependent target genes.[52]

Unfortunately, SIRT2 is the only sirtuin so far that has not been ablated in mice by gene targeting, which is a limitation in the elucidation of its role and its relation to human diseases. Hiratsuka *et al*. recently reported a role for SIRT2 in cancer pathogenesis by using a proteomic approach.[53] As previously mentioned, the *SIRT2* gene is located at Chr 19q13.3 and lies within a region that is frequently deleted in human gliomas; levels of SIRT2 mRNA and protein expression are severely reduced in a large fraction of human glioma cell lines.[53] Moreover, ectopic *SIRT2* expression in these cell lines suppresses colony formation and modifies the microtubule network.[53] There have been no studies on genetic variation of the *SIRT2* gene so far, due probably to the limited evidence for any association between SIRT2 and human diseases, .

SIRT3

Human *SIRT3* is located at Chr 11p15.5[13] and is located primarily in the mitochondrial matrix,[54,55]

where it is proteolytically processed after entry into the mitochondria. Interestingly, the unprocessed protein is enzymatically inactive *in vitro* and becomes enzymatically active only after proteolytic processing by 1-methyl-4-phenylpyridine (MPP).[54] In accordance with its localisation, human acetyl-CoA synthetase-2 was the first substrate to be shown to be deacetylated by SIRT3, thereby activating the acetyl-CoA synthetase activity, linking acetylation with metabolic regulation.[56] Later, it was confirmed that SIRT3 regulates global mitochondrial lysine acetylation, although no metabolic change was observed in *Sirt3*$^{(-/-)}$ knockout mice.[57] Scher *et al*., however, demonstrated that in humans SIRT3 can be found not only in mitochondria, but also in the nucleus under normal conditions, where it can deacetylate H4-K16 when recruited to a gene.[58] In support of the nuclear localisation of SIRT3, it was further demonstrated that co-expression of SIRT5 shifts SIRT3 to the nucleus,[59] challenging the concept that mitochondria are the only point of localisation for SIRT3. Focusing again on the mitochondrial role of SIRT3, it was found that SIRT3 mediates cell survival during genotoxic stress by increasing mitochondrial NAD^+;[60] SIRT3 is also a major regulator of energy homeostasis, controlling ATP production by direct interaction with complex I.[61]

Like SIRT1, SIRT3 has been found to interact with the FOXO family, and has been implicated in the genetic regulation of longevity. SIRT3 interacts with FOXO3a and deacetylation induces FOXO3a-dependent gene expression via an unknown mechanism,[62] as well as blocking hypertrophy in the heart by suppressing cellular levels of reactive oxygen species (ROS).[63] The role of SIRT3 in oxidative stress has been further highlighted after finding that it regulates enzymes that play a key role in the antioxidant defence mechanism. Two recent studies demonstrated that SIRT3 regulates both manganese superoxide dismutase (MnSOD) activity (by mediating the deacetylation of lysine residues[64,65]) and isocitrate dehydrogenase 2 (IDH2) activity, thereby increasing the ratio of reduced-to-oxidized glutathione.[66] SIRT3 recently stepped out of the shadow of SIRT1 when several studies demonstrated that SIRT3 regulates cellular processes related to metabolism under both normal and stressful conditions. More specifically, the long-chain acyl-CoA dehydrogenase (LCAD) is hyperacetylated in the absence of SIRT3, resulting in reduced enzymatic activity and decreased levels of fatty acid oxidation during fasting.[67] By contrast, SIRT3 seems to regulate both ketone body production, by affecting mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2 (HMGCS2),[68] and the urea cycle through ornithine transcarbamolase (OTC)[69] during caloric restriction. Interestingly, Kim *et al*. showed for the first time that SIRT3 maintains mitochondrial integrity and oxidative metabolism, which is known to function as a barrier against tumorigenesis as loss of SIRT3 results in a cellular environment that favours carcinogenesis.[70] In accordance with these data, other studies found that SIRT3-mediated regulation of hypoxia-inducible factor (HIF) further induces reprogramming of cancer cell metabolism,[71,72] emphasising the interplay between ROS and metabolism specifically in cancer cells.

Gene-targeting mutagenesis has contributed to the elucidation of various effects of SIRT3 in pathological conditions. Lombard *et al*. first inactivated the *Sirt3* gene by homologous recombination, deleting exons 2 and 3, which encode the translational start site plus a portion of the catalytic domain. In contrast to *Sirt1*, *Sirt3*-deficient mice were born with a normal Mendelian ratio and without any morphological or functional defects.[57] No embryolethality was observed in another *Sirt3* mutant mouse model,[61] suggesting that the SIRT3 protein is not essential for normal embryogenesis. This finding has allowed the use of these mouse models further to unravel the role of SIRT3 in the various cellular processes described earlier.

Despite growing evidence that SIRT3 is directly involved in many pathological conditions, only few studies have focused on genetic variation of the *SIRT3* gene. One study reported an association between a genetic variant and survival in elderly subjects. Specifically, the TT and GT genotypes relevant to the G477T marker of SIRT3 were

associated with increased versus decreased survival in the elderly, respectively.[73] It was also found that the *SIRT3* gene shares an intronic enhancer with the *PSMD13* gene, which encodes a regulator subunit of the 26S proteasome that has been linked to ageing.[74] Intriguingly, it was reported recently that at least one copy of the *SIRT3* gene is deleted in 20 per cent of all human cancers and 40 per cent of breast and ovarian cancers; this is similar to the deletion frequency of the well-known breast cancer tumour suppressor genes, breast cancer susceptibility gene1 (*BRCA1*) and 2 (*BRCA2*).[72]

SIRT4

The human *SIRT4* gene is located at Chr 12q,[13] and the encoded SIRT4 protein is localised within the mitochondria and has no detectable deacetylase activity. Therefore, it is solely an NAD^+-dependent protein ADP-ribosyl transferase.[11,75] SIRT4 has been detected in a number of tissues — including brain, kidney, pancreas, liver, thyroid, vascular smooth muscle and striated muscle — which suggests a role in global metabolic function. One of the first reported interactions of SIRT4 identified its co-localisation with insulin-degrading enzyme and the ADP/ATP carrier proteins, adenine nucleotide translocase (ANT) 2 and ANT3.[75] Recently, SIRT4 has been implicated in the regulation of fatty acid oxidation and mitochondrial gene expression in liver and muscle cells.[76] Interestingly, SIRT4 knockdown results in an increase in SIRT1 mRNA and protein levels, suggesting a redundant interplay of metabolic regulation among sirtuins. SIRT4 was previously thought to act secondarily to SIRT3 as a minor regulator of mitochondrial function; however, this study indicates that SIRT4 may have a more important role in mitochondrial metabolism, as SIRT4 inhibition increases fat oxidative capacity in liver and mitochondrial function in muscle.[76] These findings may prove beneficial in developing therapeutics for numerous diseases, such as T2DM, in which SIRT1 and SIRT4 have recently been reported to play a role in T2DM pathogenesis.[77]

Another known target of SIRT4 activity includes mitochondrial glutamate dehydrogenase (GLUD1), as ADP ribosylation inhibits GLUD1 enzyme activity and opposes the effects of caloric restriction in pancreatic β-cells.[11] SIRT4 has also been linked to non-alcoholic fatty liver disease, in which its location in the cytoplasm was suggested to play a role in the development of insulin resistance.[78] Whereas SIRT4 remains one of the less-studied sirtuins, an increasing body of work supports its involvement in critical metabolic pathways and disease pathologies.

SIRT5

The human *SIRT5* gene is located at Chr 6p23,[13] and the gene product functions as a mitochondrial NAD^+-dependent deacetylase with well-defined substrates. Supporting a model of conserved evolution, the crystal structures of the core domains of SIRT3 and SIRT5 reveal remarkable structural conservation with other sirtuins, especially human SIRT2 and the ancestral yeast protein.[79–81] Initial characterisation of $Sirt5^{(-/-)}$ knockout mice found no defects in basal glucose, insulin or lipid homeostasis.[57,82] As fasting increases SIRT5 mRNA levels, however, transgenic mice overexpressing SIRT5 exhibited a novel target of SIRT5 deacetylase activity — carbamoyl phosphate synthetase-1 (CPS1).[82,83] CPS1 is a key enzyme within the urea cycle that catalyses the condensation of ammonia and bicarbonate, yielding carbamoyl phosphate. These findings support a protective role for SIRT5 because the conversion of ammonia to non-toxic urea appears to be regulated tightly by this enzyme through CPS1 deacetylation and activation. SIRT5 was also found to deacetylate and regulate the activity of cytochrome C, a protein regulating oxidative metabolism and apoptosis.[80] It was determined that SIRT5 translocates into the mitochondrial inter-membrane space from the matrix, indicating that varying localisation by means of altered cellular signalling may contribute to SIRT5 regulation and substrate selection.

Two isoforms of SIRT5 have been identified (SIRT5iso1 and SIRT5iso2); the mechanism of

subcellular localisation for these two has been examined.[84] The C-termini of the two isoforms differ slightly, whereas each contains a cleavable mitochondrial-targeting sequence at its N-terminus. The cleaved SIRT5iso2 was found localised within the mitochondria, while cleaved SIRT5iso1 was localised in both mitochondria and cytoplasm. Another form, SIRT5DC, contains only the common domain and was found within the mitochondria, similarly to SIRT5iso2. This report suggests that the cytoplasmic localisation of cleaved SIRT5iso1 is regulated by altering the C-terminal sequence. Additionally, it was found that the C-terminus of SIRT5iso2 is rich in hydrophobic amino acid residues and, consequently, functions as a mitochondrial membrane-insertion signal. In sum, these results demonstrate that SIRT5 plays a role in controlling various cellular functions via two isoforms with different intracellular localisations or stabilities.[84]

SIRT6

The human *SIRT6* gene is located at Chr 19p13.3.[85] Initial investigation into SIRT6 activity supported a lack of NAD^+-dependent protein deacetylase activity and showed that it is a broadly expressed nuclear ADP-ribosyltransferase.[10] These studies were later contradicted, however, revealing that SIRT6 does in fact contain deacetylase activity.[86] The complete genetic characterisation of the human *SIRT6* gene showed eight exons, ranging in size from 60 base pairs (bp) (exon 4) to 838 bp (exon 8), encoding a 355-amino acid protein (39.1 kDa).[85] Another report identified novel functions for the N- and C-terminal domains of SIRT6; the authors were able to demonstrate that the C-terminal extension of SIRT6 directs nuclear localisation, but that it is not required for activity. By contrast, the N-terminal extension of SIRT6 is required for chromatin regulation and catalytic activity.[87]

SIRT6 is predominantly a nuclear chromatin-associated protein which aids in the protection of DNA damage and suppresses genomic instability through association with base-excision repair and DNA-end resection.[88,89] Phenotypically, *Sirt6*-deficient mice are under-sized and develop a number of abnormalities by two to three weeks of age — including lymphopenia, fat depletion and severe metabolic defects, resulting in death by four weeks. These findings clearly demonstrate a major role for SIRT6 in genome stability and longevity-related processes; however, specific mechanisms of interaction remain elusive.

A recent study provided exceptional insight into the mechanism of SIRT6-related chromatin regulation.[86] This study outlined a role for SIRT6 in the attenuation of NF-κB-signalling via its interaction with the NF-κB RelA subunit. SIRT6 directly deacetylates histone H3 lysine 9 (H3K9) at NF-κB target promoters. Interestingly, RelA insufficiency rescues the *Sirt6*-deficient phenotype in the knockout mouse. Additional data indicate that SIRT6 also deacetylates H3 K56 to modulate genomic stability.[90] Given the implied importance of SIRT6 in genomic stability and overall longevity, it is no surprise that *Sirt6* deficiency causes the most extreme phenotype among all sirtuin-knockout animal models, generating severely retarded growth rates and lethality.

Sirt6 gene overexpression in adipose tissue resulted in a highly positive outcome, in which *Sirt6* expression was related to peroxisome proliferator-activated receptor-responsive genes, and genes associated with the regulation of lipid storage and transport.[91] Additional findings further present a case for metabolic regulation through genetic regulation of the expression of multiple glycolytic genes.[92] More specifically, SIRT6 functions as a co-repressor of HIF1α, regulating nutrient stress-response pathways. *Sirt6*-deficient cells displayed an increase in HIF1α activity, increased glucose uptake, upregulation of glycolysis and reduced mitochondrial respiration. These results imply that SIRT6 may serve as a 'master regulator' of glucose homeostasis and provide a compelling target for therapeutic approaches to metabolic diseases.

A recent report provided critical data in support of tight regulation and interaction of the sirtuin protein family. The authors determined that SIRT1 forms a complex with FOXO3a and nuclear respiratory factor 1 (NRF1) on the *SIRT6*

promoter and positively regulates *SIRT6* expression. Consequently, this action negatively regulates glycolysis, triglyceride synthesis and fat metabolism through the known deacetylation of H3 K9.[33] As a result of a liver-specific *Sirt6* deletion in mice, alterations in gene expression occur, leading to increases in a host of metabolic pathways, including glycolysis and β-oxidation. Taken together, it seems clear that SIRT6 plays a critical role in glucose and lipid metabolism and is a likely therapeutic target for treating a number of metabolic-related fatty liver diseases.[93]

SIRT7

The human *SIRT7* gene is located at Chr 17q25.[94] The exact mechanism of SIRT7 deacetylase activity remains undefined; however, localised within the nucleolus, it is known to play a significant role in cell growth and proliferation. The *SIRT7* genomic sequence spans a region of 6.2 kilobases (kb)[94] and the *SIRT7* gene comprises ten exons and encodes a 400-amino acid protein (44.9 kDa).

SIRT7 is associated with a number of active rRNA genes (rDNA), including RNA polymerase I (Pol I) and histones.[95] SIRT7 overexpression increases Pol I-mediated transcription, whereas SIRT7 knockdown results in decreased association of Pol I with rDNA and reduced Pol I transcription. Interestingly, silencing of SIRT7 expression stops cell proliferation and triggers apoptosis. SIRT7 also directly interacts with the rDNA transcription factor, upstream binding factor (UBF). Moreover, SIRT7 is phosphorylated via the cyclin-dependent kinase 1 (CDK1)–cyclin B pathway during mitosis, and these phosphorylation events are known to induce conformational modifications to the C-terminal region of SIRT7. This conformational change is likely to alter SIRT7 activity and results in the regulation of rDNA transcription.[96] Recently, it was found that *Sirt7*-deficient mice experience a reduction in mean and maximum lifespan and develop heart complications, such as fibrosis and inflammatory cardiomyopathy.[97] A noteworthy observation with $Sirt7^{(-/-)}$ knockout mice was an association between hyperacetylated p53 and increased rates of apoptosis in the myocardium.[97] Additional knockout data in primary cardiomyocytes demonstrated a twofold increase in apoptosis and increased susceptibility to oxidative and genotoxic stress. These data suggest a critical role for SIRT7 in stress responses and cell survival in the heart. Furthermore, recent evidence demonstrates that SIRT7 expression inversely correlates with the tumorigenesis potential of numerous mouse cell lines; SIRT7 probably enables cells to maintain a host of critical metabolic pathways through the inhibition of cell growth under severely stressful situations.[98]

Future directions/conclusions

The sirtuin genes encode an important and complex family of proteins that participate in a wide spectrum of physiological processes. In several species, caloric restriction has been shown to increase lifespan and decrease spontaneous rates of illness, such as insulin resistance, neurodegenerative disease and cancer. Because caloric restriction activates specific cellular signalling networks, including sirtuin protein deacetylase activity, it has long been thought that chemical agents that induce sirtuin activity would have similar beneficial therapeutic effects to caloric restriction. This idea is well justified by results demonstrating that several of the sirtuin knockout mouse models develop illnesses similar to those observed in humans. Thus, it seems reasonable to propose that the sirtuin protein family will prove to represent novel molecular targets for the treatment of human diseases having a strong association with increasing age. As such, these novel findings for the potential role of sirtuins in human illnesses should allow for the identification of potential molecular targets and biomarkers to determine risk and the development of agents that may be chemopreventive.

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