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Review Article

Sirtuins, Bioageing, and Cancer

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The Sirtuins are a family of orthologues of yeast Sir2 found in a wide range of organisms from bacteria to man. They display a high degree of conservation between species, in both sequence and function, indicative of their key biochemical roles. Sirtuins are heavily implicated in cell cycle, cell division, transcription regulation, and metabolism, which places the various family members at critical junctures in cellular metabolism. Typically, Sirtuins have been implicated in the preservation of genomic stability and in the prolongation of lifespan though many of their target interactions remain unknown. Sirtuins play key roles in tumourigenesis, as some have tumour-suppressor functions and others influence tumours through their control of the metabolic state of the cell. Their links to ageing have also highlighted involvement in various age-related and degenerative diseases. Here, we discuss the current understanding of the role of Sirtuins in age-related diseases while taking a closer look at their roles and functions in maintaining genomic stability and their influence on telomerase and telomere function.

1. Sirtuins

Sirtuins are a highly conserved family of proteins found in all organisms from yeast to mammals. All are orthologues of the yeast protein, silent information regulator 2 (Sir2) [1] and their primary targets are acetylated lysines of various peptides and proteins, including histones. Along with sequence homology, they also share functional similarities although the functions performed in mammals are more complex than in yeast, as reflected in the number of distinct orthologous forms. These play key roles in cellular stress and ageing, and as such, their function has been linked to diseases associated with ageing, including Alzheimer's [2], Parkinson's Disease [3], cancer [4], type II diabetes [5], and atherosclerosis [6].

Every member of the family contains a highly conserved core domain consisting of a NAD^+-binding site and a catalytic domain [7]. Sirtuin function is tied to cellular energy production through nicotinamide adenine dinucleotide-(NAD^+-) dependent deacetylation reactions, as well as o-ADP ribosylation, in response to changes in the cellular NAD^+/NADPH ratio. Sirtuins appear to be involved in the extension of life span and health promotion in several species including yeast, nematodes and flies [8]. Pertinent to this is the observation that Sirtuins can be activated through caloric restriction, stress, or by pharmacological agents [9]. Sirtuins have a pivotal role in the expansion of lifespan in lower organisms via caloric restriction [10–15]. This phenomenon is also believed to occur in higher mammals, and ongoing studies in monkey models have demonstrated promising results in proving this connection [16]. Additionally, some small-scale studies with centigenarians have demonstrated that allelic variants of some Sirtuin genes are linked to longevity in humans [17–19]. Despite this, the involvement of Sirtuins in enhanced human health and lifespan is still the subject of great debate. There is, however, increasing corroborative evidence of their links to cancer processes, genomic instability and other diseases of ageing.

Central to such associations is the observation that Sirtuin activity is directly correlated with the metabolic state of the cell [20]. Sirtuins act as substrate-specific type III protein lysine deacetylases, in contrast to the classic deacetylases, which facilitates a link between cell metabolism and

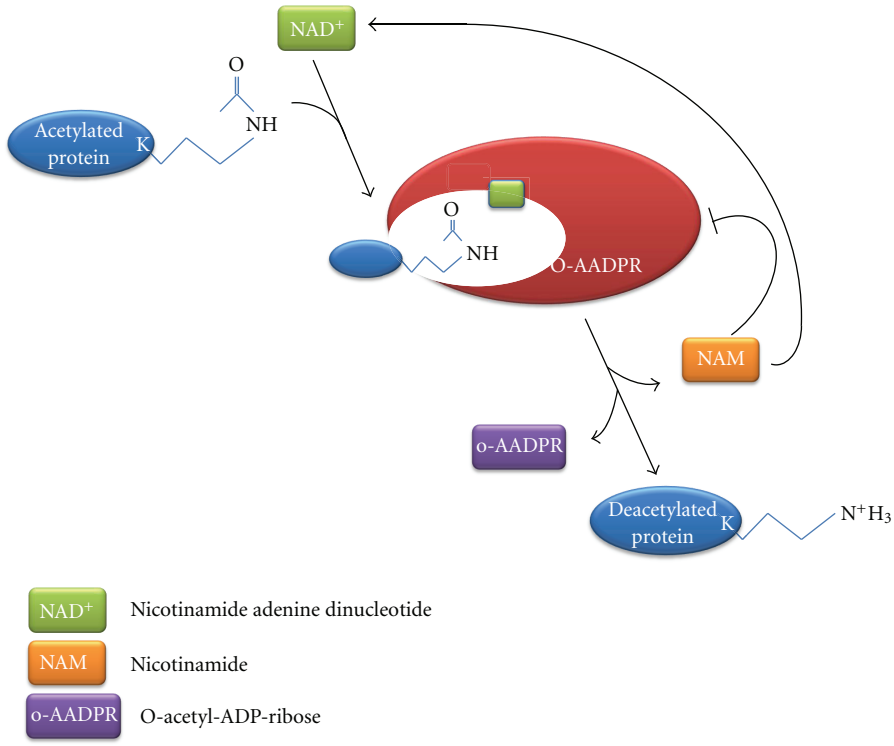

Figure 1: Protein deacetylation by Sirtuins. Sirtuins deacetylate lysine (K) residues of target proteins using cofactor—Nicotinamide-adenine-dinucleotide (NAD^+) and releasing Nicotinamide (NAM). 2′-O-acetyl-ADP ribose is generated as a result of transfer of the acetyl group of K onto ADP-ribose residue. Deacetylation is inhibited by NAM, which can also reverse the reaction to reproduce NAD^+.

control of transcription. Briefly, the deacetylation involves a unique enzymatic NAD^+-dependent reaction which begins with amide cleavage from NAD^+ leading to the formation of Nicotinamide (NAM) and a covalent ADP-ribose peptide-imidate intermediate (ADPR). This intermediate is transformed to O-acetyl-ADP-ribose and the deacetylated protein is released from the complex (Figure 1, [21]). Due to the reliance of Sirtuin deacetylation activity on NAD^+, it is hardly surprising that evidence suggesting NAD^+ and NAD^+ generating pathways are directly involved in the regulation of Sirtuin activity is mounting rapidly. This is supported by the observation that the Nicotinamide (NAM) product site can be occupied in the presence of substrates and reaction intermediates [22, 23]. Bound NAM is able to inhibit the enzymatic activities of Sirtuins and can, in some cases, reverse the reaction, thus regenerating NAD^+ and the acetylated substrate. Sirtuins, together with other NAD^+ consumers (ADP-ribosyltransferases and cAMP ribose synthetase), have also been implicated in the salvage/elimination of NAM, thus playing a vital role in the homeostatic maintenance of NAD^+ metabolism [7, 24].

In humans, seven Sirtuins have been identified (Sirt1–7) [25, 26], all with unique characteristics, functions, and localisations (Table 1).

Sirt1, Sirt6, and Sirt7 are localised mainly in the nucleus, whereas Sirtuins 3–5 are found mainly in the mitochondria [27]. Conversely, Sirt2 has a predominantly cytoplasmic localisation [28, 29].

The Sirtuins contain nuclear localisation signals (NLSs) as well as nuclear export signals (NESs) and their intracellular localisation is determined by cell/tissue type and physiological conditions. Sirt6 and Sirt7 contain a single NLS, while Sirt1 contains 2 NLS and 2 NES domains [29, 30]. The presence of an N-terminal mitochondrial targeting sequence ensures Sirt3–5 localisation within the mitochondrial matrix [31, 32], whereupon the signal sequence is cleaved, activating the enzymatic function of the proteins. Interestingly, it has been recently suggested that under specific conditions (stress), Sirt3 can translocate from mitochondria to the nucleus [33, 34].

Sirtuins are actively involved in the regulation of gene expression, principally due to their histone deacetylase activity and the consequential ability to influence the activity of a wide range of transcription factors. It has been shown that all Sirtuins, with the exception of Sirt4 and Sirt7, have histone deacetylase activity (HDAC). Sirt1 can affect core histones (H1, H2, H3, and H4), but it preferentially deacetylates H3 (K9, K14 and K56 residues), H4 (K16) and H1 (K26) [35–37]. The specific deacetylation of lysine residues at H3K9/H4K16 and H1K26 by Sirt1 has been linked to gene silencing and chromatin remodelling. Additionally, histone deacetylation can facilitate the methylation of histones, for example, di/tri-methylation of H3 on the K9 residue and H4 on the K20 residue. These modifications have been linked to global transcriptional repression and are characteristic for facultative heterochromatin [38]. This reaction can be

Table 1: The mammalian Sirtuins.

	Enzymatic activity	Localisation	Substrates/targets	Function
SIRT1	Deacetylase	Nuclear/cytoplamic	p53, FOXO, NFκB, MyoD, Ku70, LXR, PPARγ, p300, Tat, PCAF, ERα, AR, SMAD7, PCAF, p73, Sox9, HES1, PGC1α, HEY2, NcoR/SMRT, E2F1, RelA/p65	Glucose metabolism, fatty-acid and cholesterol metabolism, differentiation, insulin secretion, and neuroprotection
SIRT2	Deacetylase	Nuclear/cytoplamic	α-tubulin, FOXO	Cell-cycle control, tubulin deacetylation
SIRT3	Deacetylase	Mitochondrial	AceCS2, GDH complex1	ATP production, regulation of mitochondrial proteins deacetylation, and fatty-acid oxidation
SIRT4	ADP-ribosylotransferase		GDH, IDE, ANT	Insulin secretion
SIRT5	Deacetylase		CPS1	Urea cycle
SIRT6	Deacetylase ADP-ribosylotransferase	Nuclear	NFκB, Hif1α, helicase, DNA polymeraseβ	Telomeres and telomeric functions, DNA repair
SIRT7	Deacetylase	Nuclear	RNA polymerase type I, E1A, SMAD6	RNA polymerase I transcription

further enhanced by Sirtuins; for example, H3K9 methylation is enhanced by Sirt1. Sirt1 binds to the histone methyltransferase Suv39H1 (suppressor of variegation 3–9 homolog 1) and facilitates binding of this protein to chromatin. Sirt1 is then activated by deacetylation of Suv39H1 [36, 39]. There are additional regulation mechanisms involved in the chromatin silencing mediated by Sirt1-Suv39H1 complex, it has recently been shown that deleted in breast cancer 1 (DBC1), not only inhibits both Sirt1 and Suv39H1 activity, but also disrupts the interaction between these two molecules leading to the increased methylation of H3K9 [40]. The other interesting aspect of Sirt1 involvement in the epigenetic regulation of gene expression is its association with aberrant expression of the methylated genes that can be facilitated by its interaction with Dnmt3b [41] or Dmnt1 [42]. Overall, the ability of Sirt1 to remodel chromatin, together with the ability of this enzyme to deacetylate and/or interact with a broad range of transcription factors (i.e, p300, NFκB, FOXO, E2F1, and Smad7 (Table 1)) suggest that Sirt1 may be a major player in the regulation of organism homeostasis, stress responses, endocrine signalling, and cell metabolism.

Acetylated lysine residues on Histone 4 (H4K16) and Histone H3 (H3K9) are targets for Sirt2 deacetylation when the nuclear envelope disassembles during the mitotic process [43]. This makes Sirt2 a regulator of the cell cycle, involved in the promotion of chromatin condensation. Similarly, Sirt3 deacetylates H4K16 and H3K9 *in vitro* although the importance of this process under *in vivo* conditions remains somewhat controversial [34].

Finally, Sirt6 deacetylates H3K9 at telomeres, indicating that this particular molecule may be a modulator of cellular senescence and ageing induced chromosomal abnormalities [44].

Sirtuins are also involved in the regulation of RNA Polymerase II transcribed genes although their involvement in the formation of the transcription initiation complex has not been proven to date [45]. Interestingly, Sirtuins affect transcription of ribosomal RNA also. Sirt1 and Sirt7 have opposing effects on rRNA transcription; Sirt1 deacetylates TATA box-binding protein associated factor (TAF_I68) leading to the inhibition of Polymerase I [46], while Sirt7 directly binds to polymerase I and induces enzyme activity [47].

A search for Sirtuin-binding sites revealed many putative targets. However, all of these targets follow on a common theme in Sirtuin function, namely, cellular stress responses [48]. These incorporate cell death responses, senescence, stress-related transcription regulation, cell-cycle control, cell metabolism, genomic stability and formation, and maintenance and control of telomeric function (Table 1). These activities for Sirtuins reinforce the link between key features of cellular bioage and disease, centred on telomere stability and cellular lifespan. Extrapolating Sirtuin activity to longevity, already established in lower organisms is thus intuitive for higher animals though it remains unproven. The increasing evidence for Sirtuin involvement in age related diseases is a key link to their function in the control of the cell lifespan and genomic stability [49–51]. This involvement in age-related disease further supports the link between Sirtuin function and longevity, possibly making Sirtuins the key to unlocking the causes and treatments for many age related diseases.

2. Sirtuins and Genomic Instability

The involvement of Sirtuin function in disease is typified by Sirt1, which is rapidly emerging as a tumour keystone,

providing both tumour suppressor and tumour promoter functionality [52]. Sirt1 has been shown to be overexpressed in several cancers, including prostate [53], acute myeloid leukaemia [54], colon cancer [55], and some nonmelanoma skin cancers [56]. Sirt1 has also been observed to be repressed in many other cancers, including glioblastoma, bladder, ovarian, and prostate cancers [57]. This duality of purpose indicates the pivotal role this Sirtuin exerts in the cell. Overexpression of Sirt1 can lead to deacetylation of p53 [58, 59] and reduction of many tumour suppressor genes, thus promoting genomic instability by reducing the cell's ability to respond to DNA damage and stress. Conversely, it can also deacetylate B-catenin causing the oncogenic form of this protein to translocate to the cytoplasm, thus reducing the growth of tumours [60]. More recently, Oberdoerffer et al. have demonstrated that redistribution of Sirt1 in a mouse model of genomic instability results in improved survival rates and transcription profiles similar to those found in the ageing process, particularly involved in repairing DNA breaks and other forms of genomic instability [61]. Further investigation into Sirt1 involvement in genomic instability has been hampered by an inability to produce a viable null model, as Sirt1 knockout mice die during the mid-gestation stage although it was determined that these mice showed histone modifications and impaired DNA-damage repair. Additionally, Sirt1 and p53 heterozygotes showed an increase in tumour formation in multiple tissues, a phenotype that could be at least partially rescued by activation of Sirt1 using Resveratrol [57]. It is still undetermined whether this Sirtuin acts as a tumour keystone with suppressor or promoter functions.

Sirt2, which acts as a G2 checkpoint mitotic regulator, appears to have a similar dichotomous role in both the formation and prevention of gliomas [62]. Increased expression of Sirt2 has been linked to a prolonged cell-cycle, with severe delays in cell cycle progression, suggesting a tumour suppressor role [63]. Furthermore, its role as a mitotic checkpoint protein helps prevent chromosome instability and the development of hyperploidy [64]. Downregulation of Sirt2 has been shown to interfere with cell cycle progression and in some cases can induce cell-cycle arrest [65], while overexpression has been shown to cause a prolongation of the mitotic phase of the cell cycle [63] resulting in multinucleated cells [28, 65]. The induction of multiploidy phenotypes indicates that Sirt2 plays a role in chromosomal stability by controlling the cell division associated separation of recently replicated chromosomes. Sirt2 directly deacetylates α-tubulin, providing it with a further mechanism for the control over mitosis and its ability to ensure single ploidy cells [64], thus ensuring genomic stability during mitosis.

Sirt3 may play a role in mitochondrial redox regulation [27] though data on its role is equivocal. Two independent studies have demonstrated that Sirt3 null mice have no associated phenotype, with normal development and fertility [66, 67]. Conversely, another Sirt3 null model, using mouse embryonic fibroblasts, has demonstrated abnormal mitochondrial function, including an increase in stress-induced ROS and genomic instability [68]. In this model, expression of a single oncogene (c-myc or ras) was sufficient for neoplastic transformation of the cells, which could be reversed by introducing superoxide dismutase to counteract the increase in ROS. It has now been shown, by the same group, that this effect is dependent upon deacetylation of mnSOD [69]. Mice with Sirt3 knockouts also developed oestrogen receptor $(ER)^+$ and Progesterone receptor $(PR)^+$ mammary tumours, suggesting that Sirt3 is a mitochondrially localised tumour suppressor. However, there is still some debate over the localisation of Sirt3, with the majority of studies claiming that Sirt3 is exclusively mitochondrial [70, 71]. Notably, two distinct forms (long and short) of Sirt3 have been reported [72–74] with the short version lacking a mitochondrial localisation signal peptide, indicating it may be localised elsewhere. This may account for the equivocal reports that Sirt3 can be localised to the nucleus. There are also reports that suggest Sirt3 translocates to the nucleus upon overexpression of Sirt5 or oxidative challenge to the cell [33, 34]. These reports did not investigate whether this was accomplished by translocation of Sirt3 from mitochondria to the nucleus, *de novo* synthesis, or expression of the short version of Sirt3.

Sirt4 shows no discernable NAD^+-dependent deacetylase activity *in vitro* [75, 76], confirmed by a lack of mitochondrial protein acetylation variation in a null mouse model [67]. Like Sirt3 null mice, Sirt4 null mice demonstrate an overtly normal phenotype [50]. Sirt4 is associated with insulin secretion by pancreatic β-cells, which may link it to type II diabetes, an age-related disorder. There is no direct evidence to date, however, that Sirt4 has any direct affect on genomic stability, through either over- or underexpression. Recently it has been suggested that Sirt3 and Sirt4 activities are antiapoptotic in response to DNA damage when extremely low levels of NAD^+ are present [77].

Sirt5 localises to the mitochondrial matrix, where its N-terminus is cleaved. Sirt5 appears to operate exclusively in the mitochondria and one of its major targets is carbamoyl phosphate synthase 1 (CPS1) [78], which is responsible for converting ammonia to urea. It also regulates the entry of ammonia into the urea cycle. Therefore, it would appear that the major function of Sirt5 *in vivo* is to enhance the body's reaction to the breakdown of amino acids during calorie restriction via CPS1 and Cytochrome C [79, 80]. Very little else is known about the function of Sirt5 other than that it has been demonstrated that Sirt5 plays a role in the localisation of Sirt3 [34]. Sirt3 is ordinarily present in the mitochondria; however, overexpression of Sirt5 causes Sirt3 to localise to the nucleus this phenomenon has also been shown as part of the cell stress response [33]. Whether this is due to increased expression of Sirt3 Short has yet to be established. This indicates that Sirt5 may contribute, in part, to the cellular response to stress, or that it is produced as a result of the stress response. Given the dependence of Sirtuins on NAD^+ for their action, it is feasible that Sirt5 is part of the sensing apparatus to initiate the stress response and would then activate it's deacetylation functions to affect other transcription factors, thus initiating the cell wide stress reaction, which may include sending Sirt3 to the nucleus. Therefore, Sirt5 may exert an influence over genomic stability via the action of Sirt3.

The role of Sirt6 has been established as being a key component of base excision repair (BER), as part of

intra-cellular DNA-damage responses. Sirt6 directly stabilises DNA-dependant protein kinase at the site of dsDNA breaks, allowing the formation of the DNA repair complex and the initiation of repairs [81]. Sirt6 also associates directly with chromatin, demonstrated by its association with chromatin enriched cellular fractions [82]. Sirt6 has also been shown to localise to the promoter regions of NF-κB activated proteins, whereupon it deacetylates the associated H3 histone at Lysine 9, thereby silencing the recently activated genes [65]. Sirt6 deficiency is associated with shortened lifespan and accelerated ageing phenotypes. In fact, mice with Sirt6 knockouts have been shown to have a progeroid phenotype, with extreme hypoglycaemia and are unable to survive beyond 4 weeks. The lethal hypoglycaemia observed in Sirt6 deficient mice is a direct result of its H3K9 deacetylase function which controls the expression of glycolytic genes [83]. Furthermore, knockout mice demonstrate a very high level of genomic instability and hypersensitivity to DNA damage [82, 84], confirming Sirt6's key role in DNA damage repair and also demonstrating its close relationship with the original Sir protein in yeast, Sir2. It was also noted that the increased sensitivity to DNA damage did not appear to be a function of impaired cell-cycle checkpoints, nor the dsDNA break repair mechanism. Deletion of Sirt6 results in chromosomal abnormalities including breaks and fusions, as well as a breakdown in BER, a phenotype that can be rescued by introduction of a fragment of Polymerase β (Polb), which has been determined as a target for Sirt6 [85]. The deacetylation of histones by Sirt6 is likely to have a stabilising effect on the genome, for example, H3K56 [86] although a direct link has yet to be established.

Sirt7 directly interacts with RNA polymerase I (Pol I) and histones, giving a direct link between this Sirtuin and genomic stability [87]. This link is demonstrated by increasing Sirt7 levels directly increasing Pol I function and inhibition of Sirt7 leading to decreased Pol I activity [47]. Complete depletion of Sirt7 results in cell death, after a complete halt to cell proliferation; it is believed that this direct linkage allows Sirt7 to regulate Pol I function with regard to NAD^+ levels, tying it to cell metabolism and energy levels in keeping with the original postulate of Shiels and Davies (2003) [48]. They argue that cellular responses to stress and damage centre on how much damage has been accrued, how much energy the cell needs to effect any repair, and how much fuel it must burn to achieve this. If the damage is too great; the cell will effect death, however, if the damage is not critical, then cellular energy metabolism is regulated to allow repair, and ribosome biogenesis is modulated to facilitate this [48, 88].

All cells have an in built mitotic clock associated with telomeres [89], this clock is continually reset in germ line cells by telomerase, and it also appears to be modified, turned off, or reset in cancer to allow tumours to grow unabated. It has been well established that ageing is associated with the degradation of telomeres, which ultimately leads to cell senescence and apoptosis when the cell has reached the end of its useful life [48]. The system of telomeric instability associating with age is an essential checkpoint in the control of life and disease, in particular cancer. Sirtuins are rapidly emerging as the key link between ageing, disease, metabolism and cellular stress.

3. Sirtuins and the Regulation of Cellular Stress Responses

The intricate role Sirtuins play in the control of the cell metabolism is mediated through their dependence on NAD^+; this control inextricably links their function with the metabolic status of the cell. It also provides a sensing platform for the response to cellular stress.

p53 tumour suppressor is involved in the regulation of apoptosis and its reactivity is tightly regulated. Under physiological condition, this molecule is maintained at very low levels in the cells, but its expression is rapidly increased in response to stress in order to fulfil its regulatory functions [90]. It has been documented that p53 activity can be modulated by SIRT1 in particular; overexpression of SIRT1 not only abrogated p53 dependent apoptosis in response to oxidative stress, DNA damage, and ionizing radiation, but also sensitised cells to apoptosis induced by these factors [58, 59]. Various studies have demonstrated that SIRT1 plays a critical role in the regulation of both p53 dependent and p53 independent apoptosis in response to oxidative stress. This regulation occurs via the deacetylation of p53 which leads to its retention in the cytoplasm and enhances passage of p53 into the mitochondria [91, 92]. The ability to modulate p53 acetylation establishes SIRT1 in the inhibition of cell senescence in response to oxidative stress. In this case, SIRT1 is recruited to the PML bodies and p53, where it blocks p53-dependent transactivation; this phenomenon has been observed in human endothelial cells, where Downregulation of SIRT1 led to increased acetylation of p53 and development of a premature senescence phenotype [93, 94]. In contrast to SIRT1, SIRT2 overexpression promotes neurodegeneration and affects the ability of cells to recover after cellular stress, mainly due to Downregulation of 14-3-3ζ [95, 96].

Another mechanism by which Sirt1 can regulate the cellular response to stress is the ability of Sirt1 to regulate members of the FOXO (Forkhead box class O) transcription factor family. Sirtuin 1 deacetylates 3 members of FOXO family, Foxo1, Foxo3a, and Foxo4 [97, 98]. Sirt1 regulation of Foxo3a function in mammalian cells reduces apoptosis in response to cellular stress, but also increases the expression of genes involved in DNA repair and cell-cycle check points [97]. SIRT1 activates Foxo1 and Foxo4 which are involved in the promotion of cell-cycle arrest by induction of $p27^{Kip1}$ and in enhancing cellular defences against oxidative stress through the regulation of manganese superoxide dismutase, catalase, and GADD45 (growth arrest and DNA damage inducible α) [98, 99]. It has been demonstrated that Sirt2 under oxidative stress deacetylates Foxo3a, and thus enhances the expression of Foxo-regulated genes and reduces ROS levels in cells [100]. Similarly to Sirt1, Sirt7 depletion in mice leads to a specific phenotype, characterised by p53 hyperacetylation and lack of resistance to the oxidative or genotoxic stress [101].

4. Sirtuins, Telomeres, and Telomerase

TNFα has been shown to induce telomerase activity in lymphocytes [102], this proinflammatory cytokine is controlled by NF-κB which in turn is influenced by Sirt1. Therefore, Sirt1 has direct influence over TNFα activation of telomerase activity. Whether this activation can be achieved in cells other than lymphocytes or whether it can contribute to the immortalisation of tumour cells has yet to be elucidated. Inhibition of Sirt1 has also been associated with increased telomerase activity in human cells [103].

Sirt2 is predominantly cytoplasmic and is unlikely to play any role in telomere biology. Sirt3–5 are mitochondrial and to date have no information linking them to telomeric sites, telomerase, or mitotic division. However, Sirt6 is absolutely essential for dsDNA repair, playing an active role in the recruitment of other factors to the site of dsDNA breaks [81]. Sirt6 also appears to be extremely important in the maintenance of telomeres and telomeric function. Recent studies have demonstrated that reduction or removal of Sirt6 results in telomere dysfunction and end-to-end chromosomal fusions. This absence of Sirt6 is similar in symptoms to Werner's syndrome, which is a disease characterised by premature ageing. It is an extremely rare, autosomal recessive disorder caused by a mutation in the *WRN* gene encoding DNA helicase [44]. This results in genomic instability and telomeric attrition, the process by which this occurs is unknown. It is believed that Sirt6 is essential for proper telomere maintenance and function. Sirt6-deficient cells have been shown to have an increased susceptibility to genotoxic DNA damage resulting in the accumulation of chromosomal abnormalities resulting in genomic instability. Sirt6-deficient mice exhibit an accelerated ageing phenotype; however, the researchers were unable to determine any cellular lifespan change [82]. In another study using Sirt6 null mice it was demonstrated that these mice have a progeroid like syndrome, profound hypoglycaemia, and premature death at around the 4-week stage [50]. This appears to indicate that Sirt6 does, in fact, have a major impact on organismal lifespan control.

Very little is known about Sirt7, and although it is localised to the nucleolus, there has been no evidence presented that suggests any involvement with telomere function, formation, or stability.

5. The Association of Sirtuins with Diseases of Ageing

Sirt1 is heavily implicated in several diseases associated with ageing, as well as with ageing itself. This Sirtuin has been shown to protect axons from damage in animal models of the Wallerian degenerative disease (Parkinson's disease) [104]. Furthermore, the use of resveratrol (a Sirt1 activator) in models of Huntington's disease shows that Sirt1 is able to reduce cell death by inhibition of NF-κB signalling [105]. Alzheimer's disease has also been linked to Sirt1 function and calorie restriction in monkeys [106]. A recent study has demonstrated that Sirt1 overexpression in the brain of mice directly reduces β-Amyloid production and the formation of plaques [107]. Another study demonstrated that induction of Sirt1 function also reduced macular degeneration by protecting retinal ganglial cells [108]. Furthermore, it has been shown that Sirt1 has a direct influence on the pancreatic β cell production of insulin. Along with Sirt3, altered expression of these Sirtuins has been implicated in the development of Type II Diabetes [8]; however, no links with Type I diabetes have yet been established. This activity is believed to occur through acetyl coenzyme A synthetase (AceCS) upon which both Sirt1 and 3 act to produce acetate. The production of acetate has been shown to be disrupted in diabetes as well as in ageing. Sirt4 has also been shown to be downregulated in pancreatic b cells in response to calorie restriction implicating it in diabetes although no links have yet been demonstrated.

Although Sirt2 is associated mainly with the brain, there have been no links made between this Sirtuin and neurodegenerative diseases. The limited amount of information available on Sirt5 makes it very difficult to make any connections between this Sirtuin and diseases of ageing; however, its heavy involvement in the mitochondria leads to speculation that it may be related to metabolic disorders.

Sirt3 has been linked to overall longevity in humans, although the studies conducted were small scale. The first study linked a polymorphism in Sirt3 to increased longevity in males [19], and the authors also determined that the chromosomal location of Sirt3 is also home to four other proteins associated with longevity (tyrosine hydroxylase, proinsulin, IGF2, and HRAS1). A subsequent study confirmed this observation but went further to suggest that decreased levels of Sirt3 was detrimental to longevity in males [17]. Furthermore, Lescai et al., (2009) [109] linked a Sirt3 SNP to longevity in centenarians from Italy, France, and Germany. Recently, Sirt3 has been directly linked to age-related hearing loss [110].

Sirt6 is heavily associated with DNA damage, telomeres, and cancer. Another link to degenerative disease exists with the association between Sirt6 and WRN which is implicated in premature ageing like Werner Syndrome [111]. Furthermore, Sirt6 actively represses genes associated with age-related cellular senescence and it is, therefore, highly likely that more associations will be discovered and that Sirt6 will become a key player and target in the research and treatment of cancer and other age-related diseases. There is also a suggestion that it may play a key role in the maintenance of organ integrity particularly associated with ageing [8]. Another key mediator in age-related diseases is inflammation, which in this context is generally induced by age-related increases in NF-κB activity. This activity is directly opposed by both Sirt1 and Sirt6, where Sirt1 acts directly on the RelA subunit causing deacetylation and reducing its action. Sirt6 is also sequestered to NF-κB activated targets and shuts them down at the transcription level. Thus, both Sirtuins may be active in age-related inflammatory disorders. Although no direct causal links between these Sirtuins and inflammatory disorders have been made, the level of circumstantial evidence suggest that formal demonstration may be a matter of time and research.

Sirt1 and Sirt7 are associated with age-related cardiovascular disease through their interactions with p53, Fox01 and nitric oxide synthetase (NOS). Sirt1 has also been shown to improve the regeneration of vascular endothelia and smooth muscle cells [112].

6. Sirtuins and Cancer

Cancer is now established as a disease of ageing. Consequently it was inevitable that Sirtuins would play a vital role in tumourigenesis. The roles played by Sirtuins at key points in the cell are also highly indicative of their roles in modulating the aberrant survival and replication of tumour cells. The most obvious involvement for Sirtuins in cancer comes from Sirt1 and Sirt7 mediation of p53-function, which is well established as a focal point in many cancers. Sirt1 (and Sirt7) deacetylates p53 reducing its influence over cell cycle control during stress and in response to DNA damage. Thus overexpression of Sirt1 deactivates p53 and disrupts p53 dependent pathways and this results in a large reduction in the cell's ability to respond to stress and DNA damage [58, 59]. This has lead to many researchers describing Sirt1 as a tumour promoter, a suggestion that has now been supported by several studies. These identify increased levels of Sirt1 associated with various cancers including prostate [53], AML [54], primary colon [55] and several nonmalignant skin cancers [56]. Overexpression studies resulted in lowered production and or action of several key tumour suppressors including FOXO family members [113], p73 [114], RB [115], and several others [116–119]. However, the story for Sirt1 is not so simple. Several studies have reported decreased levels in cancer, for example glioma, bladder, prostate, and ovarian cancers [57]. Several studies have reinforced this connection demonstrating a reduced level of Sirt1 associated with tumourigenesis [118, 120–122]. In fact, Sirt1 acts as a tumour keystone, where its level and action maintain a fine and delicate balance between suppression and promotion of oncogenesis. Based on the available evidence, it is plausible that Sirt1 acts as a suppressor and then a promoter (or vice versa) depending on the stage and situation of tumourigenesis.

Control of cell-cycle progression by Sirt2 has been shown to be essential in the prevention of tumours, as it is suppressed in gliomas [62]. Sirt3 is the only mitochondrial Sirtuin to have a demonstrated role in tumourigenesis to date and its reduction in several cancers leads to an increase in ROS which results in enhanced tumour growth [68]. Interestingly, Sirt5 overexpression has been implicated in a study of pancreatic cancer [123].

The role of Sirt6 in controlling NF-κB function and DNA damage repair also indicate a key role in tumourigenesis although very little information is available on specific correlations with cancer to date some studies have been conducted which demonstrate a link through interaction with GCIP in colon tumours [124]. Our group has previously demonstrated that Sirtuins 3–7 are elevated in some forms of breast cancer [49] and mRNA levels of Sirt7 have been inversely correlated with the ability to undergo tumourigenesis in mouse cell lines [125]. Sirtuin influence and types of cancer where associations have been shown are summarised in Table 2.

Table 2: Cancers associated with Sirtuins and their proposed mechanism of involvement.

	Association with cancer
Sirt1	Acute myeloid leukemia, colon, nonmalignant skin, bladder, prostate ovarian cancers, and glioma—mediates p53 function
Sirt2	Glioma—control of cell cycle progression
Sirt3	Breast cancer—decrease in levels is associated with a general increase in tumour growth due to increase in ROI
Sirt4	Breast cancer—metabolic
Sirt5	Pancreatic, breast cancers—metabolic
Sirt6	Colon, breast cancers—mediates NFκB and GCIP function
Sirt7	Breast cancer—mediates p53 function

An interesting link between Sirtuin levels and circadian rhythm has also been reported [126]. This is noteworthy given the understood disruption of circadian rhythm in cancer [127]. This opens the possibility of the use of chronotherapy using Sirtuin regulators at specific times to target tumours [126].

It is obvious that the Sirtuins, in line with the function of Sir2 in yeast, play critical roles in the maintenance of the genome in all organisms. These vital roles have led to speculation that these molecules are heavily involved in two key areas, tumourigenesis and ageing. Further, evidence for these proteins in such crucial roles is accumulating at an accelerating rate. As this area of molecular science consolidates and advances, the Sirtuin family of proteins are gaining significance in human biology and disease. This group show strong potential to become valuable predictive and prognostic markers for disease and as therapeutic targets for the management of a variety of cancer types and other age-related diseases.

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