SIRT1: Linking Adaptive Cellular Responses to Aging-Associated Changes in Organismal Physiology

Sirtuins comprise a family of enzymes implicated in the determination of organismal life span in yeast and the nematode. The mammalian sirtuin SIRT1 has been shown to deacetylate several proteins in an NAD+-dependent manner. SIRT1 substrates are involved in the regulation of apoptosis/cell survival, endocrine signaling, differentiation, chromatin remodeling, and transcription. Thus SIRT1 provides a molecular link between nutrient availability and adaptive transcriptional responses. This review presents current evidence as to how SIRT1 functions are relevant to changes in tissue physiology that occur with ageing and its implications for future pharmacological intervention to alleviate such degenerative processes.

The sirtuin protein family comprises members with protein deacetylase and ADP-ribosyltransferase activity (4, 36, 46). Sirtuin deacetylases are also referred to as class III deacetylases, being distinct from class I and II enzymes in that their activity depends on NAD+ (oxidized nicotinamide adenine nucleotide) and is not sensitive to the broad deacetylase inhibitor trichostatin A (TSA) (22, 36). Thus, given the importance of histone deacetylation in the regulation of gene expression, sirtuins have been proposed to provide a molecular link between cellular metabolic status, as expressed by the NAD+/NADH levels, and adaptive transcriptional responses (7).

The founding member of the family S. cerevisiae Sir2p (silence information regulator 2) deacetylates histones and thus mediates transcriptional silencing at telomeric, rDNA (encoding ribosomal RNA), and mating type loci (7). In addition, overexpression of Sir2p extended the life span of yeast cells, supporting the hypothesis that Sir2p is a key mediator of yeast longevity. A similar finding was also reported for Sir2-1, its C. elegans ortholog (71). Furthermore, experimental evidence suggests that Sir2 may partially mediate the beneficial effects of caloric restriction on longevity, most likely as a modulator of the IGF (insulin-like growth factor) signaling pathway, another well-known mediator of organismal life span (7, 30, 66).

Based on such observations, the involvement of mammalian sirtuins in determining organismal life span has been the subject of extensive investigation. Seven sirtuins have been described in humans, named SIRT1–7 (4, 26). SIRT1, the best characterized among them, is a nuclear deacetylase whose substrates include proteins primarily but not exclusively involved in transcriptional regulation, thus influencing diverse aspects of organismal physiology such as differentiation, cell survival, and metabolism (FIGURE 1).

An overview of the sirtuin protein family has been provided in several excellent recent reviews (4, 22, 30). Thus here we will present, in an integrative manner, evidence supporting a role for SIRT1 in processes and tissues affected during ageing with the aim to highlight potential sites of therapeutic intervention.

SIRT1 and Cancer

Cancer is a term coined to describe a vastly heterogeneous set of diseases that are characterized by aberrant proliferation to the expense of physiological body functions. Although malignancies occur in younger individuals too, sporadic cancer incidence shows a striking correlation with age (23). Genetic and epigenetic processes have both been linked to age-induced cancer, and mouse models have provided extensive evidence that genes involved in the maintenance of genomic stability and cancer are also intimately linked to the development of ageing phenotypes (23, 47).

SIRT1 and Epigenetic Changes Occurring in Cancer

Several lines of evidence implicate SIRT1 in epigenetic regulation of gene expression in cancer cells (FIGURE 2). SIRT1 was identified as a component of the polycomb repressive complex 4 (PRC4), which harbors the SET domain histone methyltransferase Ezh2 (44). Four distinct PRC complexes have been identified to date that differ in their subunit composition and substrate specificity. In a mouse model of prostate cancer, the protein levels of all PRC4 components tested, including SIRT1, were upregulated (44). Concomitantly, expression of PRC4 target genes was accordingly modified in these tissues. Although there
is no evidence confirming a causal role for PRC4 in cancer initiation, it is possible that PRC4-mediated histone modifications contribute to cancer-specific epigenetic changes.

Furthermore, in the context of the PRC4 complex, SIRT1 deacetylates histone H1-K26 and promotes heterochromatin formation through spreading of hypomethylated histone H3-K79 (44, 74). Histone H1 is a linker histone that primarily has a structural role in maintaining chromatin structure and through this regulates genomic stability and ageing (34).

siRNA-mediated downregulation of SIRT1 leads to H4-K16 hyperacetylation and reduction in H3-TriMeK9 and H4-MeK20 in mammalian cells, whereas, in vitro, SIRT1 preferentially deacetylates H4-K16 (36, 74). Interestingly, Fraga et al. reported a consistent loss of H4-K16 acetylation and H4-K20 trimethylation in various tumors and tumor-derived cell lines, suggesting that these modifications constitute epigenetic hallmarks of cancer (25).

SIRT1 localizes specifically to the promoters of tumor suppressor genes whose DNA is hypermethylated and are silenced in many cancers (37, 63). Downregulation of SIRT1 levels and/or activity resulted in increased H4-K16 as well as H3-K9 acetylation in such promoters and sufficed to induce reexpression of the corresponding genes in breast and colon cancer cells (63).

Nonhistone Targets of SIRT1 in Tumor Development

Besides histone modifications (73), posttranslational modification of transcription factors, including acetylation (43), constitutes an additional level of transcriptional control. Such modifications can regulate various aspects of transcription factor function, including DNA binding, stability, subcellular localization or recruitment of co-factors (e.g., Refs. 6, 12). SIRT1 deacetylates several transcription factors involved in the regulation of cell cycle progression and apoptosis consistent with a role in the fundamental processes underlying cancer (FIGURE 2).

SIRT1 deacetylates the tumor suppressor p53 to inhibit its transcriptional activity, resulting in reduced apoptosis in response to various genotoxic stimuli (50, 75). On the other hand, in cultured primary cells, SIRT1 is required for the expression of the tumor suppressor p19^{ARF}, which promotes p53 stability (16). MEFs (mouse embryonic fibroblasts) lacking SIRT1 have an increased resistance to senescence induced by chronic oxidative stress, a phenomenon associated with decreased levels of the tumor suppressor p19^{ARF} and thus p53 levels (16). Conversely, in the same cellular system, SIRT1 is not required for oncogene-induced senescence, despite the fact that the latter is also known to involve p19^{ARF}. Of note, however, Langley et al. reported that SIRT1 localizes to PML bodies to attenuate p53-dependent cellular senescence induced by PML-IV or activated Ras overexpression (45).

SIRT1 associates with the tumor suppressor HIC1, which has been shown to act synergistically with p53 in tumorigenesis (13). Both SIRT1 and HIC1 can bind the SIRT1 promoter to repress gene transcription and thus allow p53 acetylation and activation (13). HIC1 cells exhibit elevated levels of SIRT1, hypoacetylated p53, and enhanced resistance to DNA damage-induced apoptosis, which can be reversed by expression of dominant-negative SIRT1. Interestingly, HIC1 expression is progressively reduced during aging due to promoter methylation, which would lead to higher SIRT1 levels, reduced p53 activity, and prolonged life span. Concomitantly, such a model would also predict a higher propensity to form tumors due to higher SIRT1 activity consistent with the inverse relationship between longevity and tumor suppression (11).

SIRT1 has been shown to regulate the transcription factor NF-κB as well as several forkhead family transcription factors, including FOXO1, FOXO3a, and FOXO4 (10, 19, 56, 72, 76, 78). SIRT1 deacetylates and inactivates NF-κB, leading to enhanced cell death in response to the inflammatory cytokine TNF-α (78). NF-κB is required for the transcription of growth factors and cytokines involved in inflammation, which has been linked to diseases such as Type 2 diabetes and cancer (39). Other NF-κB target genes include antiapoptotic factors such as cIAP (cellular inhibitor of apoptosis) and selective members of the Bcl2 family and the antioxidant MnSOD (manganese superoxide dismutase), which protect cells from TNF-α-induced apoptosis (38).

MnSOD is also a target of forkhead transcription factors, and SIRT1 enhances its expression in a FOXO-dependent manner (28). Forkhead factors regulate...
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The work summarized above represents a strong body of evidence supporting a role for SIRT1 in cancer development, in particular as a promoter of cancer cell survival, bearing the characteristics of a potential oncogene (33). Yet it would be of great importance to determine whether this involvement in oncogenesis reflects a more fundamental, causative role of altered SIRT1 activity for tumor progression by determining the incidence of aberrant SIRT1 expression in tumors.

SIRT1 Functions in Metabolic Regulation

Organismal energy homeostasis is achieved by the coordinate action of central and peripheral signals that control appetite and dictate efficient nutrient distribution among tissues to sustain body functions (24). Disturbances in such circuits lead to abnormal weight control and associated pathological side effects, and recent data suggest that they may also underlie key aspects of tumorigenesis (61, 62).

SIRT1 in homeostatic organ functions: regulating glucose metabolism

Ageing is associated with several pathological conditions resulting in aberrant organismal metabolic functions (49). Prevalent among them is Type 2 diabetes, which shows almost an exponential incidence rate increase after the age of 20–30 years (54). Type 2 diabetes is associated with decreased insulin secretion and the development of insulin resistance, which in turn is a major risk factor for cardiovascular disease as well as a series of other medical conditions collectively known as the metabolic syndrome (20).

In response to elevated glucose levels, e.g., following a meal, insulin is secreted by the β-cells found in the pancreatic islets of Langerhans to promote glucose uptake and catabolism in peripheral tissues. The recent discovery that Ku70 interaction with the pro-apoptotic protein Bax is regulated by acetylation can provide an alternative route by which SIRT1 promotes cancer cell survival (17). SIRT1-mediated deacetylation of Ku70 preserves its association with Bax, which is inhibited by CBP-driven Ku70 acetylation, thus preventing the translocation of Bax to mitochondria to initiate apoptosis (17).

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Furthermore, SIRT1 appears to have a protective role against glucose-induced cytotoxicity in pancreatic β-cells, an underlying cause of β-cell degeneration seen in diabetic patients with chronically high plasma glucose levels (42). The cytotoxic effects of increased glucose concentrations are attributed to elevated mitochondrial oxidation rates, which lead to increased ROS production. Under these conditions, SIRT1 was shown to be required in sustaining FOXO1-mediated transcription of MafA and NeuroD, two transcription factors required for the expression of the Insulin 2 gene to prevent apoptosis (42).

Another site of SIRT1 function in glucose metabolism is the liver, where SIRT1 interacts with the transcriptional co-activator PGC-1α in a ternary complex with hepatocyte nuclear factor 4 (HNF-4) to induce gluconeogenesis following fasting (67) (FIGURE 3). In promoting glucose production, the hepatic function of SIRT1 appears to be the opposite of that in the pancreas, where it promotes insulin secretion, thus leading to glucose utilization by peripheral tissues. In addition to β-cells, high SIRT1 expression was also observed in pancreatic α-cells (58), which play a central role in organismal responses to fasting by stimulating, among others, hepatic gluconeogenesis. Whether these apparently opposing functions reflect a broader role for SIRT1 in balancing body glucose homeostasis by coordinating multiple organ responses to a common stimulus is a notion worth investigating.

Overall, the above evidence suggests a multiple impact of SIRT1 function on body glucose homeostasis through its role in insulin secretion and contribution to survival in the context of pancreatic β-cells and gluconeogenesis in the liver. Thus the activity of SIRT1 and possibly other sirtuins (77) may also be a relevant therapeutic target for diabetes, where aberrant glucose homeostasis and β-cell dysfunction are key manifestations of the disease.

**SIRT1 function in lipid metabolism**

Another tissue of interest in metabolic regulation is the adipose tissue. The adipose tissue is the major site of triglyceride storage, an important energy source when glucose availability is limited. During fasting and starvation, adipose triglyceride (TG) stores are mobilized to give rise to free fatty acids (FFA), which can be utilized by other tissues for energy production (24). Furthermore, the metabolic activities of adipose tissue may have an impact on organismal longevity since adipose-specific ablation of IGF receptor results in an approximately 18% increase in life span in mice (5).

SIRT1 was shown to suppress adipocyte differentiation by inhibiting the adipogenic factor PPARγ (peroxisome proliferator-activated receptor-γ) through the transcriptional co-repressor NCoR (60). Furthermore, it was suggested that SIRT1 is required for TG mobilization since SIRT1+/− animals exhibited low levels of blood FFAs following fasting or β-adrenergic stimulation. Interestingly, the bacterial sirtuin ortholog CobB deacetylates and activates the enzyme acetyl-CoA synthase (ACS), which catalyses the synthesis of acetyl-CoA, a key molecule in mitochondrial oxidation and lipid synthesis (69, 70). Recently, Hallows et al. demonstrated that SIRT1 also activates the mammalian acetyl-CoA synthase 1 (AceCS-1) via deacetylation, raising the possibility that a phylogenetically conserved molecular mechanism mediated by SIRT1 and possibly other sirtuins modulates lipid metabolism by regulating intracellular acetyl-CoA levels (32).

Dyslipidemia is a common feature of the metabolic syndrome-associated disorders, including cardiovascular disease and atherosclerosis (24). Thus it would be of interest to investigate whether modulation of SIRT1 activity can be considered as a possible target for treating these conditions or symptoms thereof.

**Neuroprotective and Cardioprotective Roles of SIRT1**

Immunohistochemical studies have identified the heart and central nervous system as sites of high murine SIRT1 expression during embryogenesis and in adult animals (68). Furthermore, mice with genetic ablation of the SIRT1 locus exhibit multiple developmental defects, some of which are consistent with this localization (14). Experimental evidence suggests that SIRT1 has a protective role against neuronal and cardiac damage.

**FIGURE 3. Function of SIRT1 in tissues relevant to organismal energy homeostasis**

Despite evidence for SIRT1 functions in skeletal muscle and the brain, little is known about its role in regulating metabolic functions of these tissues. Similarly, the role of SIRT1 in pancreatic α-cells, where it is highly expressed, is unknown. TGs, triglycerides; NEFAs, nonesterified fatty acids; also referred to as free fatty acids (FFAs); NCoR, nuclear co-repressor; AcCoA, acetyl-CoA; AceCS-1, acetyl-CoA synthase-1; Ucp2, uncoupling protein 2; PPARα, peroxisome proliferator-activated receptor-α; PGC-1α, PPARγ coactivator-1α.
In the Wallerian degeneration slow (\textit{wld}) mice, increased nuclear NAD$^+$ underlies the protection exhibited in the neurons of these mice against neurodegenerative agents (2). Importantly, the neuroprotective effects of NAD$^+$ require SIRT1 (2). Moreover, sirtinol and resveratrol, two compounds that inhibit and activate SIRT1, respectively, affect this process in a manner consistent with the proposed involvement of SIRT1 (2). Similar effects of sirtinol and resveratrol were also observed in an independent in vitro model of cerebral ischaemia (65).

These observations also extend to the heart. In isolated neonatal rat cardiomyocytes, sirtuin inhibition by either nicotinamide or sirtinol induced cell death in a p53-dependent manner (1). SIRT1 overexpression also caused an increase in cardiomyocyte size and protected cells from apoptosis following serum starvation (1). Importantly, SIRT1 levels were dramatically increased in a dog model of heart failure, possibly a result of a failed attempt to prevent cell death (18).

Risk of neurodegenerative and cardiovascular pathological conditions such as Alzheimer’s and ischaemic heart disease, respectively, increase dramatically with age and together account for the vast majority of death rates (31, 41). A causative role for the devastating outcomes of ischemic conditions is also connected to how promptly they are treated so as to minimize tissue damage. This, in combination with lifestyle factors such as diet, which is proposed to affect sirtuin function, renders sirtuins an important potential target for preventative treatments in the context of these diseases.

**SIRT1 and Muscle Mass Maintenance**

SIRT1 deacetylates and thus negatively modulates the transcription factor MyoD, which is one of the key executors of the muscle differentiation programme (27). SIRT1 activity in turn is dictated by the progressively decreasing levels of NAD$^+$ during muscle differentiation, thus alleviating SIRT1-mediated MyoD suppression and allowing differentiation (27).

SIRT1 was also found acting in conjunction with HDAC4 to regulate the activity of another transcription factor with roles in muscle differentiation, myocyte enhancing factor 2 (MEF2) (79). HDAC4 recruits a small ubiquitin-like modifier (SUMO) E3-ligase, which attaches SUMO to lysine residues on MEF2 that are previously deacetylated by SIRT1. This leads to inhibition of MEF2-mediated transcription (79). It is unclear, however, whether this function of SIRT1 also leads to inhibition of muscle differentiation and whether it is sensitive to intracellular NAD/NADH levels.

Multiple factors contribute to muscle mass reduction during ageing and diseases such as cancer or muscular dystrophy. These include oxidative and inflammatory damage due to neutrophil and macrophage recruitment as well as muscle wasting due to increased protein catabolism (52, 57).

Balanced protein turnover is paramount for the maintenance of proper muscle mass (52). Increased protein catabolism associated with cachexia is attributed to the action of specific E3 ubiquitin ligases that target proteins for proteasome-mediated proteolysis (29). Two such proteins have been identified, MuRF1 (for muscle RING finger 1) and MAFbx/atrogen-1 (for muscle atrophy F-box), which are transcriptionally controlled by the NF-$\kappa$B and FOXO pathways, respectively (29). NF-$\kappa$B activation in response to the inflammatory cytokine TNF-$\alpha$ leads to suppression of MyoD gene expression, whereas MyoD is also proposed to be a substrate of the MAFbx E3 ligase (29). Furthermore, MyoD activity is required for the postproliferative differentiation of precursor muscle cells following injury (57).

Since SIRT1 was found to modulate both NF-$\kappa$B and FOXO transcription factors in heterologous systems (see above), in conjunction with its role in regulation of MyoD and MEF2, a broader role of SIRT1 in the control of muscle mass maintenance during injury and ageing is conceivable.

**Reproduction**

There is an inverse correlation between age and fecundity in that, as animals age, their ability to produce viable offspring decreases (59). Experimental evidence in model organisms suggests that a hormonal cue from the reproductive system, most likely IGF, regulates life span in both the nematode and the mouse (40).

SIRT1 is highly expressed in the developing spermatocytes, and deletion of the \textit{SIRT1} gene leads to severe sperm abnormalities and sterility (51). In this context, SIRT1 would appear to be important to the reproductive capacity of the animal, thus defying its role in longevity based on the above. Interestingly, reduction of IGF signaling starting at the time of hatching in \textit{C. elegans} extends life span and delays reproduction, whereas IGF signaling reduction in the adult increases life span to the same extend without affecting reproduction (40). Thus it is possible that SIRT1 holds a role in the adult reproductive system that extends beyond embryonic development,
possibly by controlling IGF signaling through its documented roles in regulating forkhead factor activity.

Conclusion

Ageing-related changes in body physiology involve multiple target tissues with varying consequences on organismal fitness, both at the macroscopic as well as the molecular level (41). Since a clear increasing trend in world population age is evident (http://www.who.int/ageing/en/), the possibility to alleviate ageing-related phenotypes with the aim to ameliorate life quality is highly desirable.

Members of the sirtuin protein family have been implicated in the regulation of organismal longevity from yeast to mice (30). In this article, we have reviewed current evidence implicating SIRT1, the best-studied mammalian sirtuin, in molecular processes linked to physiological tissue changes associated with ageing. Recent work supports the involvement of other sirtuins in some of these phenotypes (55, 77). Of note, mice lacking SIRT6 exhibit several characteristics of premature ageing, which, at the cellular level, can be attributed to defects in base-excision repair (BER), a mechanism employed by cells to prevent chromosomal instability (55).

A focused effort to develop HDAC inhibitors has been long underway following evidence that many cancers, and in particular leukemias, exhibit aberrant chromatin acetylation patterns (53). Similarly, early development of pharmacological agents that modulate sirtuin activity (3) provides a promising outlook for our ability to intervene in the progression of degenerative processes associated with ageing (FIGURE 4). Furthermore, the functional synergism observed between SIRT1 and other sirtuins and HDACs (32, 79) can provide a novel framework of investigation into combinatorial therapies with a broader targeting of protein deacetylases.

The multiple sites and the heterogeneous nature of proposed SIRT1 functions mean that bioavailability of SIRT1 modulators to target tissues will be a crucial factor for the success of such compounds. In this regard, dissecting the tissue-specific roles of SIRT1 in whole organisms and the use of animal disease models to investigate the specificity and mode of action of SIRT1-targeting drugs would provide an essential tool. Finally, since little is known about the intracellular pathways that regulate SIRT1 activity, identifying such signalling inputs will be of substantial interest. This knowledge would provide additional routes for the study and modulation of SIRT1 in the context of adaptive cellular responses to nutrient availability, which are important homeostatic mechanisms often disturbed in human disease.

References


