Sirtuin 1 (SIRT1): The Misunderstood HDAC

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The sirtuin family of NAD-dependent histone deacetylases (HDACs) consists of seven mammalian proteins, SIRT1–7. Many of the sirtuin isoforms also deacetylate nonhistone substrates, such as p53 (SIRT1) and α-tubulin (SIRT2). The sirtuin literature focuses on pharmacological activators of SIRT1 (e.g., resveratrol, SRT1720), proposed as therapeutics for diabetes, neurodegeneration, inflammation, and others. However, many of the SIRT1 activator results may have been due to artifacts in the assay methodology (i.e., use of fluorescently tagged substrates). A biological role for SIRT1 in cancer has been given less scrutiny but is no less equivocal. Although proposed initially as an oncogene, we present herein compelling data suggesting that SIRT1 is indeed a context-specific tumor suppressor. For oncology, SIRT1 inhibitors (dual SIRT1/2) are indicated as potential therapeutics. A number of sirtuin inhibitors have been developed but with mixed results in cellular systems and animal models. It is unclear whether this has been due to poorly understood model systems, signalling redundancy, and/or inadequately potent and selective tool compounds. This review provides an overview of recent developments in the field of SIRT1 function. While focusing on oncology, it aims to shed light on new concepts of expanding the selectivity spectrum, including other sirtuins such as SIRT2. (*Journal of Biomolecular Screening* 2011:1153-1169)

Key words: epigenetics, metabolic diseases, cancer and cancer drugs, immune system diseases, CNS and PNS diseases

INTRODUCTION

Members of the silent information regulator family (SIRT or sirtuins) are evolutionarily conserved NAD-dependent protein deacetylases and adenosine diphosphate (ADP)–ribosylases. There are seven known isoforms (SIRT1–7) that differ in their subcellular localization, substrate specificities, and functions (**Table 1**).[1–3] An initial report indicated that SIRT1, 6, and 7 are predominantly nuclear, SIRT2 is cytoplasmic, and SIRT3–5 are localized in the mitochondria.[4] However, it has subsequently been shown that movement of SIRT1 and SIRT2 between cytoplasmic and nuclear compartments may occur,[5,6] perhaps indicative of substrate localization. Sirtuins catalyze the deacetylation of lysine residues on histones and various proteins, resulting in a deacetylated product, nicotinamide, and O-acetyl-ADP-ribose.[7] For at least SIRT1, kinetic analysis indicates that lysine deacetylation activity follows a sequential mechanism, whereby the acetylated substrate and NAD(+) must both bind prior to initiating catalysis.[8] It should be pointed out that although all seven sirtuins are classified as deacetylases (largely based on structural homology), the catalytic activity of SIRT4–7 has been associated primarily with ADP-ribosylation. Indeed, little or no lysine deacetylase activity has been found with purified SIRT4 or SIRT6 enzymes.[9,10] It is possible that the biological functions of these isoforms are more closely related to ADP-ribosylation than with lysine acetylation or recognize a specific lysine-containing motif in a protein substrate yet to be determined. Additional less well-characterized posttranslational modifications have been associated with sirtuin activity, such as depropionylation,[11] but their biological impact has yet to be demonstrated.

Sirtuins have garnered a great deal of interest in the short time since their discovery. As such, a treatise on the entire sirtuin family could fill volumes. The founding member of this class of deacetylases, SIRT1 (homolog of yeast silent information regulator, Sir2), is the most widely studied of the sirtuins. Early reports in yeast described Sir2/SIRT1 as a promoter of genomic stability and enhancer of viability/lifetime.[12,13] SIRT1 has consequently been associated with aging processes, neural plasticity, and memory, as well as a variety of human disease conditions such as metabolic syndrome, inflammation, neurodegeneration, and cancer.[3,14] Because of these pleiotropic functions, SIRT1 has gained considerable interest by the pharmaceutical industry as a prospective drug target. However, it is unclear whether an activator inhibitor approach will produce the desired therapeutic outcome in the absence of (or minimal) unwanted side effects. A thorough understanding of sirtuin biology is needed to establish physiologically relevant enzymatic and cellular assays to screen for these pharmacological modulators. The complexities of SIRT1 biology and screening assays have revealed the potential for artifacts and/or misinterpretation. This review is devoted to summarizing the

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Table 1. The Sirtuin Family of Protein Deacetylases/ADP-Ribosyltransferases: Known Crystal Structures, Subcellular Localization, Substrates, and Phenotypes from Knockout/Deficient Mice

SIRT Isoform	*Crystal Structure*	*Localization*	*Substrates*	*Knockout Mouse Phenotype*
SIRT1	Human (none), yeast (Hst2),[169-171] *Escherichia coli* (cobB),[172] *Thermotoga maritima* (Sir2-Tm),[173-175] *Archaeoglobus fulgidus* (Sir2-Af1/2)[176-178]	Nucleus (can shuttle to cytoplasm)	H3K9, H4K16,[15,16] p53,[24,25] p73,[26] FOX01, FOX03a, FOX04,[27-30] PTEN,[31] NICD,[32] MEF2,[33] HIF-1α, HIF-2α,[34-36] TAF(I)68,[37] SREBP-1c,[38] β-catenin,[39] RelA/p65,[40,41] PGC1α,[42] BMAL1, Per2,[43,44] Ku70,[45] XPA,[46] SMAD7,[47] cortactin,[48] IRS-2,[49,50] APE1,[52] PCAF,[53] TIP60,[54] p300,[20,28,55,56] SUV39H1,[57] AceCS1,[58,59] PPARγ,[60] ERα,[61] AR,[62] LXR[63]	Reduced life span, tumor suppressor, or oncogene depending on mouse genetic background, genomic instability, developmental defects, autoimmune syndrome[17,23,39,100-102,117,119,179]
SIRT2	Human[180]	Cytoplasm (can shuttle to nucleus)	H4K16,[181] H3K56,[20] α-tubulin[182]	None reported
SIRT3	Human[183]	Mitochondria (can shuttle to nucleus)	H4K16,[184] H3K56,[20] Ku70,[185] IDH2,[186,187] HMGCS2,[188] GDH,[187] AceCS,[58,189] SdhA,[190] SOD2,[191,192] LCAD[193]	Normal, increased reactive oxygen species (ROS) levels, decreased adenosine triphosphate (ATP) levels[194,195]
SIRT4	None reported	Mitochondria	Glutamate dehydrogenase (GDH) (ADP-ribosylation)[196]	Normal, GDH activation[197]
SIRT5	Human[198]	Mitochondria	Cytochrome C,[187] CPS1[199]	Urea cycle dysfunction, hyperammonemia following fasting[199]
SIRT6	Human[10]	Nucleus	H3K9,[200,201] H3K56[202]	Lethal around 4 weeks, lymphopenia, decreased subcutaneous fat, bone loss, decreased IGF1, metabolic defects[203]
SIRT7	None reported	Nucleus (nucleolus)	RNA Pol I(?),[204] p53[159]	Reduced life span, kyphosis, decreased subcutaneous fat, degenerative cardiac hypertrophy[159]

No deacetylation activity has been found with SIRT4 thus far. It is unclear whether RNA polymerase I is a direct target of SIRT7.[204] Although deacetylation activity has been reported for SIRT6, other reports suggest it is primarily an ADP ribosyltransferase.[10]

biochemical/biological activities of SIRT1, status of chemical modulators (activators, inhibitors), assay considerations, and suggestions for paths forward in sirtuin drug discovery.

SIRT1 substrates—histones

Yeast and mammalian Sir2/SIRT1 will deacetylate a variety of histones at specific lysine residues: H1 (K26), H3 (K9, K14), and H4 (K16) in vitro.[15,16] Of these mark changes observed biochemically, elevation in H3K9 and H4K16 acetylation is seen in SIRT$^{-/-}$ MEF cells.[17] Loss of H4K16 acetylation and H4K20 trimethylation are commonly observed in human cancers.[18] In yeast, there is an age-associated increase in H4K16 acetylation and concomitant decrease in Sir2 (SIRT1) protein, causing reduced transcriptional silencing.[19] These early observations were key drivers of investigations into the role of SIRT1 in cancer and aging. Recently, SIRT1 was found to suppress p300-mediated H3K56 acetylation[20,21] at the Bclaf1 promoter, thereby reducing T cell activation.[21] Sirt1 knockout mice develop an autoimmune syndrome, with increased T cell activation and disruption of CD4+ T cell tolerance.[22,23] Given this evidence, SIRT1, perhaps via H3K56 deacetylation, may play a role in innate and adaptive immune function.

SIRT1 substrates—nonhistones

SIRT1 can deacetylate a number of nonhistone substrates, suggesting a broader role than just epigenetic silencing. One of

the first substrates of SIRT1 identified was p53 (p53K382), a well-known tumor suppressor, which resulted in repression of p53-dependent apoptosis in response to DNA damage and oxidative stress.[24,25] SIRT1 deacetylates quite a number of other nonhistone proteins, including other tumor suppressors, transcription factors, signaling proteins, enzymes, and nuclear hormone receptors: p73 (putative tumor suppressor involved in cell cycle and apoptosis)[26]; FOX01, FOX03a, and FOX04 (Forkhead transcription factors)[27–30]; PTEN (tumor suppressor regulating PI3K/AKT pathway)[31]; NICD (Notch1 intracellular domain)[32]; myocyte enhancer factor, MEF2 (transcription factor)[33]; hypoxia-inducible factors HIF-1α, -2α[34–36]; TAF(I)68 (Pol I transcription)[37]; SREBP-1c (transcription factor modulating hepatic lipogenesis)[38]; β-catenin (component of WNT signaling pathway)[39]; RelA/p65 (NFκB signaling; immune function)[40,41]; PGC1α (nuclear coactivator involved in control of gluconeogenic genes)[42]; BMAL1 and Per2 (circadian rhythm/clock proteins)[43,44]; Ku70 (DNA repair protein)[45]; XPA (nucleotide excision repair)[46]; SMAD7 (TGFβ-induced signaling protein)[47]; cortactin (promotes cell migration)[48]; IRS-2 (neuroprotection)[49,50]; Tau (neurodegeneration)[51]; APE1 (apurinic/apyrimidinic endonuclease-1, base-excision repair pathway)[52]; PCAF[53]; TIP60[54]; p300 histone acetyltransferases (inflammation, DNA damage response)[20,28,55,56]; SUV39H1 (histone methyltransferase)[57]; AceCS1 (acetyl CoA synthetase)[58,59]; and PPARγ,[60] ERα,[61] AR,[62] and LXR[63] nuclear hormone receptors.

Given the multitude of potential substrates and the lack of a defined consensus sequence,[64] it is possible that SIRT1 substrate selectivity may be defined by cellular localization in the presence of SIRT1 chaperones/partners in a given complex. SIRT1 has been reported to shuttle between the cytoplasm and the nucleus upon various stimuli and has "compartment-dependent" functions.[6,65,66]

SIRT1 AND METABOLIC FUNCTIONS

SIRT1 can be considered a master regulator of cellular metabolic pathways.[67] SIRT1 plays a direct role in transcriptional silencing and, via direct interaction with specific corepressors, exerts profound effects on metabolic pathways such as lipogenesis and gluconeogenesis. Data suggest that SIRT1 is involved in the central regulation of food intake[68] and regulation of the circadian clock.[69] Its major function in metabolism may be to act as a "nutrient sensor." SIRT1 has been shown to affect lipogenesis through the regulation of mitochondrial acetyl-CoA synthetase[58] and PPARγ.[70] Interestingly enough, PPARγ directly interacts with SIRT1 and inhibits SIRT1 activity, forming a highly regulated negative feedback loop.[60] SIRT1 has also been implicated in control of metabolic glucose. The deacetylation of LXR by SIRT1 suggests a role in cholesterol and lipid homeostasis.[63] SIRT1 promotes gluconeogenic gene induction and fatty acid oxidation at least in part via PGC1α.[71,72] SIRT1 overexpression in the liver attenuates insulin resistance.[73] Similarly, SIRT1 has been downregulated in insulin-resistant C2C12 cells and muscle tissues, and SIRT1 knockdown has produced insulin resistance.[74] This effect was thought to be mediated by PTP1B silencing at the chromatin level. Insulin secretion may also be regulated by SIRT1 repression of UCP2 in pancreatic cells.[75] Subsequently, there have been a multitude of reports linking SIRT1 activation to enhanced fatty acid oxidation, gluconeogenesis, insulin secretion, insulin sensitivity, and decreased lipogenesis (reviewed in Haigis and Guarente[76] and Liang et al.[77]). Therefore, it is not surprising to find great excitement surrounding the development of SIRT1 activators for metabolic diseases.

SIRT1 AND NEURODEGENERATION

SIRT1 has been postulated to serve a neuroprotective function, suggesting that SIRT1 activators may be effective therapeutics for treating neurodegeneration diseases such as Alzheimer disease. Alzheimer disease is characterized by deposition of β-amyloid (or amyloid β, Aβ) plaques and Tau protein–containing tangles. It is believed that these proteins may be causative in the neuronal and functional loss, which eventually results in death. Tau protein is deacetylated by SIRT1, albeit with a modest effect in vitro.[51] Tau acetylation prevents degradation of phosphorylated tau (p-tau), so deacetylation (by SIRT1) may be neuroprotective. There is compelling evidence linking SIRT1 to Aβ-peptide production. For example, in mixed cortical cultures, SIRT1 overexpression inhibits Aβ-induced NFκB activation and cytotoxicity, presumably via the deacetylation of RelA/p65 (K310).[40,78] Neuronal cells from SIRT1 transgenic mice display higher α-secretase activity and attenuated Aβ peptide (1–40 and 1–42) production.[79] Introducing a SIRT1 transgene to a mouse model of Alzheimer disease (APPswe/PSEN1dE9) similarly causes an activation of α-secretase and subsequent reduction in Alzheimer disease–related Aβ peptides.[80] Conversely, reduction of SIRT1 via expression of a dominant negative SIRT1 construct augments the accumulation of Aβ peptides by neuronal cells in vitro.[81] It is conceivable that SIRT1 activators may attenuate Aβ peptide and Tau production and thereby have utility in Alzheimer disease therapy. Indeed, SIRT1 lentiviral insertion in the hippocampus was neuroprotective in an inducible p25 transgenic mouse model of Alzheimer disease.[82]

As mentioned, the activity of SIRT1 may be regulated by its nuclear/cytoplasmic localization. Upon exposure to neuronal cell precursor differentiation conditions, SIRT1 quickly translocates from the cytoplasm to the nucleus and later moves back to the cytoplasm.[65] Overexpression of SIRT1 in these cells promoted differentiation, but this was not seen with a cytoplasmically restricted SIRT1 mutant.[65] Similarly, cytoplasmic SIRT1 (but not nuclear) increased NGF-induced neurite

outgrowth of PC12 cells.[66] Both studies suggest that SIRT1 localization was critical to activity.

SIRT1 AND THE IMMUNE SYSTEM

SIRT1 is a potent negative regulator of NFκB transcription, at least in part due to direct RelA/p65 deacetylation.[40] Data from both overexpression and knockout/down models are consistent with an anti-inflammatory role for SIRT1. In mouse macrophages overexpressing SIRT1, NFκB activity is decreased relative to WT, whereas this effect is not seen with knock-in of a catalytically inactive SIRT1 [H355A] mutant.[83] Mice with myeloid-specific SIRT1 knockout display hyperacetylated NFκB, resulting in elevated transcriptional activation of proinflammatory macrophage target genes.[41] Smokers and patients with chronic obstructive pulmonary disease (COPD) have decreased lung SIRT1 levels (especially in alveolar macrophages, airway epithelium, and alveolar epithelium).[84] Cigarette smoke reduces SIRT1 levels in a human monocyte-macrophage cell line (MonoMac6), leading to acetylation of the RelA/p65 subunit of NFκB and elevation in levels of interleukin (IL)–8. It was postulated that reduction of SIRT1 and resulting NFκB hyperactivation could possibly be the basis of the chronic inflammatory response, such as in COPD.[84] In primary mouse peritoneal macrophages, SIRT1 suppresses AP-1 transcriptional activity and COX-2 expression.[85] It was also found that RAW264.7 macrophage-like cells transfected with SIRT1 demonstrated improved phagocytic function. In the neonatal cardiomyocyte, SIRT1 may depress the inflammatory response, as adenoviral overexpression of SIRT1 in these cells blocked phenylephrine-stimulated MCP1 mRNA (monocyte chemoattractant protein–1) and acetylation of RelA/p65 and PGC1α.[86] SIRT1 may modulate NFκB as a pivotal point in the regulation of endotoxin tolerance, shepherding the transition from an inflammatory to an adaptive state. Following LPS stimulation of THP-1 monocytic cells or infusion in vivo, SIRT1 protein transiently decreases (8–12 h) and later returns to[87] or exceeds baseline.[88] SIRT1 binds to the promoters of tumor necrosis factor α (TNFα) and IL-1β in the THP1 cell, deacetylates RelA/p65 (presumably when in complex at the promoter), and represses transcription of these proinflammatory cytokines.[88] It is believed that, over time, expression of NAMPT (nicotinamide phosphoribosyltransferase, a key enzyme in NAD biosynthesis) and thus NAD+ is increased, stimulating RelB expression and recruitment to sites of transcription. This "feed-forward" loop resulting in sustained epigenetic silencing may be the mechanism that produces endotoxin tolerance.[88]

As mentioned previously, SIRT1-null mice display autoimmune-like symptoms, such as the accumulation of immune complexes in liver and kidney, as well as the presence of antinuclear antibodies.[23] It is thought that HIV-1 may act to interfere with SIRT1 function and decrease immune surveillance. HIV-1 Tat protein binds to the deacetylase domain of SIRT1 and inhibits SIRT1-mediated deacetylation of RelA/p65.[55,89] Consequently, NFκB transactivation is greatly potentiated, causing T cell hyperactivation, increased viral replication, and eventually the depletion of CD4+ T cells.

Targeted deletion of SIRT1 in specific T cell populations reveals a contrasting view to global knockout (i.e., where data suggest that SIRT1 dampens the inflammatory response and suppresses immune response). However, when SIRT1 was deleted specifically in CD4+ T cells and Foxp3+ Treg cells, activation, proliferation, and cytokine production were unaltered relative to wild type.[90] Also seen was an increase in Foxp3 expression, a protein associated with an immunosuppressive phenotype, and enhancement of allograft survival. As these results were recapitulated by SIRT1 inhibitors, EX527 and splitomicin,[90] one could certainly make a case for the use of SIRT1 inhibitors in preventing allograft rejection.

DICHOTOMIC FUNCTIONS OF SIRT1 IN CANCER

Studies of SIRT1 function in cancer are littered with seemingly contradictory data, with some suggesting SIRT1 is a tumor suppressor and others indicating that SIRT1 may be tumorigenic. Expression data are equivocal, as SIRT1 is overexpressed in certain tumors (prostate, hepatocellular carcinoma, breast ovarian epithelial, gastric carcinoma, colorectal, melanoma)[91–98] while reduced in several others (bladder, colon carcinoma, glioblastoma, ovary, and prostate).[17,99] In vivo, the presence or absence of other mutations in key tumor survival pathways may serve to determine the fate of SIRT1 function. Another thought to consider is that SIRT1 may normally protect the cell from oncogenic transformation, but upon or after transformation, SIRT1 signaling is co-opted to promote malignant growth (i.e., those pathways that serve to protect normal cells may prevent the death of cancer cells). The various lines of evidence are presented herein with the suggestion that SIRT1 function is context dependent.

SIRT1 as a tumor suppressor

SIRT1 knockout and transgenic mouse studies led to the speculation that under certain circumstances, SIRT1 may act as a tumor suppressor.[100] In the presence of $APC^{min/+}$ mutation, SIRT1 overexpression reduced polyp formation in mice, a potential precursor to colorectal cancer.[39] However, when using $APC^{min/+}$, $SIRT^{-/-}$ mice, the presence of intestinal polyps was unchanged relative to $APC^{min/+}$, $SIRT^{+/+}$ counterparts (i.e., the absence of SIRT1 did not yield a greater number of polyps).[101] Additional studies support the concept of SIRT1 as a tumor suppressor. For example, $SIRT1^{+/-}$, $p53^{+/-}$ mice develop tumors

in multiple organs.[17] In another model, homozygous deletion of SIRT1 in mice led to the development of prostatic intraepithelial neoplasia, which was thought to be mechanistically linked with decreased autophagy.[102] In contrast, skin papilloma development was unchanged by SIRT status (SIRT1$^{-/-}$ or SIRT1$^{+/-}$ mice) in a model of chemically induced skin carcinogenesis.[101]

Nucleotide excision repair (NER) is conducted by the careful orchestration of many proteins, including APE1, NBS1, XPA, and XPC, to remove and, where possible, fix damaged DNA. Apurinic/apyrimidinic endonuclease-1, APE1, is essential for the repair of single-strand DNA lesions. Nijmegen breakage syndrome protein, NBS1, is part of the larger MRE11-RAD50-NBS1 nuclease complex involved in the detection and repair of damaged DNA. XPA and XPC (xeroderma pigmentosum proteins A or C) are also responsible for sensing the DNA breaks and recruiting repair proteins to the site of damage. It was recently reported that SIRT1 could deacetylate and activate each of these proteins (APE1, NBS1, XPA, XPC) directly, enhancing NER and cell survival.[46,52,103,104] SIRT1 knockdown causes cells to become more sensitive to killing by a variety of genotoxic stresses—for example, UV, H2O2, and MMS (abasic DNA damaging agent)—potentially due to hyperacetylation of these NER factors.

SIRT1$^{-/-}$ MEFs and keratinocytes with SIRT1 knockdown show dramatically reduced XPC levels, increased AKT Ser473 phosphorylation, and a diminished ability to excise base lesions (cyclobutane pyrimidine dimers) caused by UV irradiation.[103] The effect on AKT phosphorylation is consistent with previous data showing that SIRT1 deacetylates PTEN, counteracting acetylation-induced repression of its phosphatase activity, thereby activating AKT.[31] Given the well-known oncogenic role of AKT, these data would strongly suggest a tumor-suppressing role for SIRT1 under these conditions. Downstream of AKT, SIRT1 has been shown to interact with TSC2, a member of the mammalian target of rapamycin complex.[105] SIRT1 negatively regulates mTOR signaling, implying that targeting SIRT1 would enhance mTOR activity, which is contraindicated in cancer therapy. To complicate the situation further, SIRT1 is phosphorylated (Ser47) by mTOR, resulting in the inhibition of SIRT1 catalytic activity.[106] More recently, it was shown that the AKT/mTOR pathway is activated in BRCA1-mutated cancers mediating tumorigenesis.[107] BRCA1 mutant-dependent breast cancers were found to have decreased SIRT1 and increased survivin expression compared to controls.[108] Overexpression of SIRT1 hindered BRCA1-dependent cancer formation in vitro.

Survivin and other parts of the WNT signaling pathway have been shown to foster the proliferative state of stem cells.[109] SIRT1 was reported to deacetylate β-catenin, leading to inhibition of proliferation of colon cancer cells.[39] The non-oncogenic cytosolic form of β-catenin was found to be enhanced by SIRT1, coinciding with co-localization of nuclear SIRT1 and the oncogenic form of β-catenin in a cohort of 81 human colon tumors.[39] However, in another study, SIRT1 shRNA caused a reduction in Dishevelled proteins DVl-2 and DVl-3 in T47D and HEK-293 cells, suggesting multiple regulation points in the WNT signaling by SIRT1.[110] Stem cell function highly depends on the transcription factor environment. For example, c-Myc is part of a transcription factor network that can reverse the differentiated phenotype back to pluripotent and proliferative stem cells (reviewed in Papp and Plath[111]). In the same way described for the p53-HIC1-SIRT1 axis and its function on the SIRT1 promoter, the oncogenic transcription factor c-Myc constitutes a negative feedback loop with SIRT1 leading to downregulation of c-Myc activity, another potential antitumor effect.[112] In light of the putative stem cell function of c-myc, SIRT1-mediated c-myc downregulation in cancer stem cells may lead to differentiation, a trait highly aligned with a potent tumor suppressor function.

SIRT1 as tumor promoter/oncogene

There is evidence that SIRT1 may play an oncogenic role by inactivation of tumor suppressors and/or activation of oncogenic proteins. HIC1 (hypermethylated in cancer 1) is a tumor suppressor that can be either epigenetically silenced or deleted in various cancers.[113,114] HIC1 constitutes a circular regulatory feedback loop with p53 and SIRT1, such that inactivation/absence of HIC1 derepresses SIRT1, triggering SIRT1 activation and p53 inactivation via deacetylation.[115,116] Hic1$^{+/-}$, Apc$^{+/\Delta 716}$ double-heterozygous mice have increased numbers of intestinal polyps, leading to hyperplasia.[117] If indeed the HIC1-SIRT1-p53 loop were in effect, this would provide indirect evidence for SIRT1 as a tumor promoter (i.e., in the context of HIC1 and APC). SIRT1 may also promote oncogenesis via the stabilization of N-Myc. N-Myc induces SIRT1 transcription in neuroblastoma, which then enhances N-Myc stability in a positive feedback loop.[118] This is thought to be accomplished by SIRT1-induced repression of MKP3 phosphatase, leading to increased ERK phosphorylation (i.e., decreased de-phosphorylation) and then N-Myc Ser62 phosphorylation, which protects N-Myc from degradation.[118] These observations are seemingly contradictory to the tumor suppressor activities of SIRT1 mentioned previously. One could speculate that specific genetic disruptions (i.e., such as seen with Hic1) effect a molecular switch, turning SIRT1 from a tumor suppressor to an oncogene. For N-Myc, the specific mutation effecting this switch has yet to be elucidated.

Some of the most intriguing animal data for SIRT1 as an oncogene come from the recent 2011 American Association for Cancer Research (AACR) meeting, where Serrano[119] reported that SIRT1 transgenic crossed with PTEN-null mice produced aggressive thyroid and prostate carcinomas. These thyroid tumors were enriched in cMyc and cMyc targets (but not p53,

p65, or β-catenin) and displayed elevated p-AKT levels. More studies will be required to understand the interaction of the PI3k/mTOR pathway, SIRT1, and Myc. However, this is the first evidence clearly demonstrating an oncogenic role for SIRT1 in vivo.

An alternative oncogenic hypothesis is that cancer cells develop addictive mechanisms by which SIRT1 overexpression is coupled with aberrant silencing of tumor suppressors.[120] Addition of transforming growth factor β (TGFβ), known to stimulate epithelial-to-mesenchymal transition (EMT), to immortalized human mammary epithelial cells (hTERT-HMEs) produces a profound elevation in SIRT1 and N-cadherin, as well as a substantial reduction in E-cadherin via direct epigenetic silencing.[121] E-cadherin levels were restored by SIRT1 depletion in vitro. Anchorage-independent growth and migration of hTERT-HME cells are inhibited by either SIRT1 shRNA or miR-200a. SIRT1 and miR-200a display an inverse relationship, as SIRT1 is overexpressed whereas miR-200a is depressed in breast cancer biopsies.[121] These authors suggest that miR-200a functions as a tumor suppressor in breast cancer and that reduction in miR-200a removes the repression of SIRT1, producing SIRT1 overexpression, thus supporting induction of EMT. Other ways SIRT1 could influence tumor suppressor function is via the interaction with other oncogenes such as Ski. Ski interacts with SIRT1, increasing the binding of p53 to SIRT1 and stabilizing the p53-SIRT1 interaction. Deacetylation of p53 (by SIRT1) is enhanced, which suppresses p53-dependent transcriptional activation.[122] As Ski facilitates the p53-SIRT1 interaction, the authors speculated that Ski may convey SIRT1 substrate selectivity. Given the multiplicity of activities attributed to SIRT1 and subcellular localization, it seems quite plausible that adapter proteins might exist to associate SIRT1 with specific substrates and effector functions. This role for SIRT1, however, is only relevant in those tumors that have intact/competent p53.

SIRT1 silencing is reported to cause apoptosis of many tumor lines (colorectal, breast, cervical) but not noncancerous lines (epithelial origin: ARPE-19, HTB-125; and normal fibroblasts [NDF]) via a p53-, Bcl-2- and Bax- independent mechanism.[123] In the HCT116 colorectal tumor line, co-silencing of FoxO4 and SIRT1, but not FoxO3, rescues the cells from apoptosis[123] (i.e., suggesting FOXO4 is required for the proapoptotic effect in the absence of SIRT1). It is quite possible then that the differential sensitivity to SIRT1 siRNA (cancer vs. normal) has a genetic basis, but the enabling molecular target(s) are as yet undetermined. In pancreatic cancer, SIRT1 levels are inversely correlated with patient survival. SIRT1 knockdown in pancreatic tumor lines induces G1 growth arrest and apoptosis (MiaPaCa, PANC1).[124] SIRT1 interference also decreases viability and clonogenic survival of human melanoma tumor lines (A375, Hs294T, G361)[97] and inhibits proliferation of breast (MCF7) and NSCLC (H1299, p53-deficient) cells.[125,126] It should be noted that knockdown of both SIRT1 and SIRT2 was required to induce cell cycle arrest and kill MCF-7 cells in vitro,[125] suggesting that dual SIRT1/2 inhibitors might be required for maximal efficacy in breast cancer.

SIRT1 has been linked to mechanisms of DNA repair, putatively serving to protect normal cells against potential carcinogenic agents and/or environmental stresses. Another possibility is that during malignant growth, SIRT1 provides an antiapoptotic stimulus to maintain the aberrant growth and lifetime of the transformed cells. O'Hagan et al.[127] have postulated that chronic exposure to inflammation and/or carcinogens incurring DNA damage could initiate epigenetic silencing of tumor suppressor genes. Using their exogenous gene promoter model (containing the CpG island segment of E-cadherin), these investigators found that both SIRT1 and EZH2 were transiently recruited to the DNA break site, as well as DNMT1 and DNMT3B. DNA methylation and engagement of DNMT3B were dependent on SIRT1.[127] Albeit infrequent in occurrence, gene silencing and DNA methylation were observed in the CpG island region. These authors provide a compelling argument for SIRT1 involvement in aberrant CpG island DNA methylation and gene silencing contributing to the initiation and/or maintenance of cancer. This may explain why SIRT1 and EZH2 proteins are more abundant in cancerous (*Nkx3.1*$^{-/-}$; *Pten*$^{-/-}$ mouse model) versus normal prostate tissue[128] and perhaps also the association of SIRT1 overexpression with CIMP-high (CpG island methylator phenotype) and microsatellite instability in colorectal cancer.[96]

Upon DNA damage, the cell triggers a number of key proteins that begin the process of DNA repair and maintain genomic integrity, including Ku70, TIP60, and E2F1. Ku70, a protein that binds to the ends of DNA double-strand breaks and initiates nonhomologous end-joining, is deacetylated by SIRT1.[45] In a model system of radiation-induced DNA damage (luciferase reporter gene), SIRT1 expression enhanced annealing of DNA breaks in vitro; this was not seen using dominant-negative, catalytically inactive SIRT1 cells.[45] As such, SIRT1 could be acting to enhance DNA repair and cell survival by deacetylation of Ku70. TIP60, TAT-interacting protein 60 kD, is a histone acetyltransferase required for double-stranded DNA break (DSDB) repair following radiation.[129,130] TIP60 and the MRE11-RAD50-NBS1 (MRN) complex act cooperatively to activate ATM kinase following DNA damage. MRN is thought to direct ATM and TIP60 to the site of DSDBs, where TIP60 and ATM become activated.[129] H2AX is then phosphorylated by ATM (Ser139) and acetylated (Lys5) by TIP60,[54,131] causing cell cycle arrest and apoptosis. TIP60 also acetylates p53 (K120), enhancing the binding of p53 to proapoptotic genes.[54] SIRT1 acts as a negative regulator of TIP60, directly deacetylating TIP60, inhibiting TIP60 activation, and promoting TIP60 degradation.[54,131] As such, one could envision SIRT1 acting to dampen the apoptotic response and promote tumor survival.

A common recurring theme in SIRT1 and cancer is that of promoting cellular resistance toward damaging conditions. SIRT1 has been associated with drug resistance[120] or oxidative stress.[25,27,29] The interaction between E2F1 (i.e., a "proapoptotic" factor induced by DNA damage) and SIRT1 perhaps illustrates the conditional nature of SIRT1 activity. SIRT1 is elevated following etoposide-induced DNA damage, and this is thought to be mediated by E2F1 (stabilized by ATM phosphorylation).[132] E2F1 is also deacetylated by SIRT1 and may even recruit SIRT1 to its own promoter. This suggests that a feedback loop between SIRT1 and E2F1 may exist to finely control the DNA damage response. In U2OS cells co-transfected with SIRT1 and E2F, SIRT1 was found to inhibit the apoptotic activity of E2F1.[132] This effect was also seen with HCT116 $p53^{-/-}$ cells indicating a p53-independent mechanism. In H1299 non–small cell lung carcinoma, SIRT1 knockdown or addition of a nonselective SIRT inhibitor, nicotinamide, sensitized cells to etoposide-induced apoptosis.[132]

SIRT1 may also function to cultivate a microenvironment conducive to tumor growth and metastasis. SIRT1 is highly expressed in the nuclei of vascular endothelium.[133] RNA silencing of SIRT1, but not SIRT-2, -3, or -5, abolishes endothelial cell migration and sprout formation in vitro. The deacetylase activity of SIRT1 is required for these activities as a dominant negative mutant [H363Y] mimics the effects of SIRT1 siRNA.[133] As one might expect from the in vitro observations, knockout of SIRT1 in mice (endothelial cell specific: *Tie2Cre^{tg}; SIRT1$^{flox/-}$*) or zebrafish (antisense) produced a blunted angiogenic phenotype. Subsequent data from this laboratory revealed that SIRT1, via deacetylation of NCID, acts as a negative regulator of Notch signaling in endothelial cells.[32] Notch is ubiquinated and degraded under deacetylation conditions, so SIRT1 could conceivably control the levels of Notch. As Notch coordinates various aspects of blood vessel growth, the authors speculated that SIRT1 may act as "a rheostat" to finely tune Notch responses in endothelial cells.[32] Cortactin is an F-actin binding protein and Src substrate that, in its acetylated form, attenuates cell migration. Cortactin has been shown to potentiate metastasis in breast and esophageal cancer models.[134,135] SIRT1 deacetylates cortactin and promotes cell migration in vitro,[48] suggesting a role for SIRT1 in metastasis.

PHARMACOLOGICAL MODULATION OF SIRT1

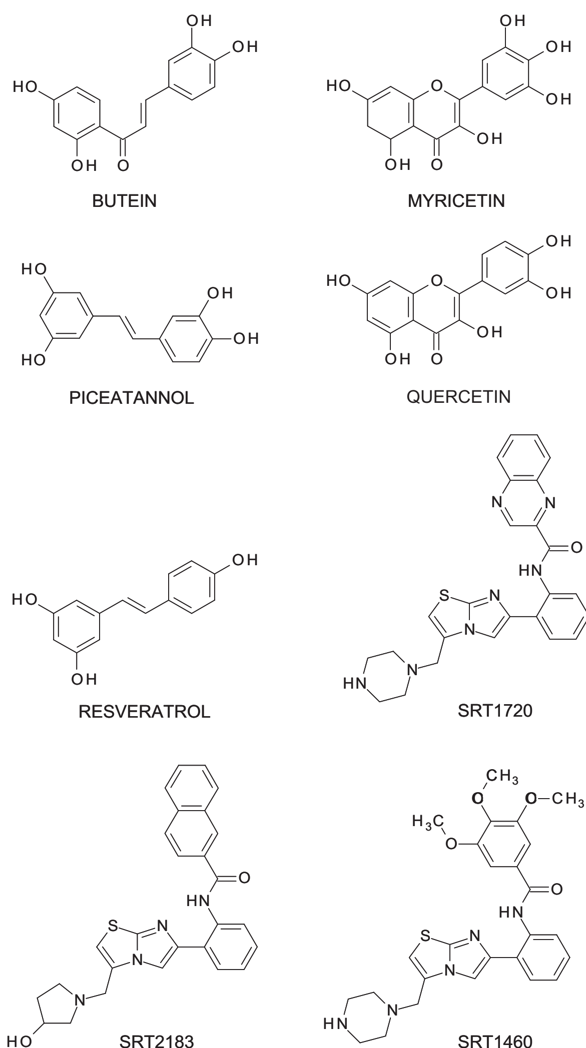
Enzymatic assays

There are now many assay formats to screen SIRT1 enzyme for both activators and inhibitors. These include fluorescence,[136–139] time-resolved fluorescence, chemiluminescence,[140] microfluidic mobility shift,[140] fluorescence polarization,[141] mass spectrometry,[141,142] capillary electrophoresis,[143] high-performance liquid chromatography (HPLC),[7,144] and radioactive[145–147] formats. These are described in more detail within this issue.[142]

SIRT1 activators

SIRT activators are being pursued in hopes of providing benefit to patients with neurodegenerative, inflammatory/autoimmune, and metabolic disease (perhaps also cancer in certain tumor types). However, the most widely published SIRT1 activators have caused a great deal of controversy. It is questioned whether many of these compounds are de facto SIRT1 activators or assay artifacts. This debate has arisen from a combination of issues associated with enzymatic assay format and compound selectivity. One of the earliest assays used for SIRT1 screening, commercialized by BioMol (Plymouth Meeting, PA) as Fluor de Lys,[148] contained a fluorescent-labeled peptide (p53-AMC) for a substrate. The first SIRT1 activators identified with this assay were polyphenolic plant-derived compounds: resveratrol, butein, quercetin, piceatannol, and myrcetin (**Table 2**).[148,149] Subsequently, much more potent and efficacious SIRT1 activators were reported as potential therapeutics for the treatment of diabetes (e.g., SRT1720, SRT2183, and SRT1460),[141] using a fluorescently labeled substrate in a fluorescence polarization assay. It was realized quickly that SIRT1 biochemical activation by resveratrol could only be demonstrated when the substrate was fluorescently tagged.[150–152] Using a variety of nonfluorometric assay formats, it was found that all of these four compounds (resveratrol, SRT1720, SRT2183, and SRT1460) did not activate SIRT1 when using native peptide or protein substrates.[152,153] Furthermore, SRT1720 and SRT2183 decreased acetylated p53 levels in SIRT1-deficient (siRNA) and null ($SIRT^{-/-}$ MEFs) cells, again suggesting SIRT1 was not the direct target. One unconfirmed report claimed that SRT1720 and SRT2183, but not resveratrol, were actually p300 HAT inhibitors, accounting for their cellular effect on p53 acetylation in the absence of enzymatic modulation.[153] The value of resveratrol, SRT1720, SRT2183, and SRT1460 as SIRT1 activators becomes even more dubious when taking into account that they inhibit quite a number of nonepigenetic targets (e.g., G-protein-coupled receptors [GPCRs], enzymes, and transporters).[152] The selectivity of piceatannol for SIRT1 is also questionable as it inhibits tyrosine kinases such as Lck[154] and mitochondrial ATP synthase (as do other polyphenols).[155] It is evident that more highly selective SIRT1 activators, which show correlative activity in both enzymatic and cellular SIRT1-dependent assays, are still needed to understand SIRT1 biology and therapeutic potential.

Table 2. Small-Molecule Activators of SIRT1



	Fold Rate Increase	
	@100 μM [refs. 148,149]	*$EC_{1.5}$ (%Max)* [ref. 142]*
Butein	8.53	
Myrcetin	3.19	
Piceatannol	7.90	
Quercetin	4.59, 2.15	
Resveratrol	13.4, 4.66	46.2 μM (201%)
SRT1720		0.16 μM (781%)
SRT2183		0.36 μM (296%)
SRT1460		2.90 μM (447%)

*Concentration of compound required to increase enzyme activity by 50%.

SIRT1 inhibitors

A selective (vs. other HDACs) SIRT1 inhibitor, EX527 (aka SEN-196), was initially reported with an $IC_{50} \approx 100$ nM in enzymatic assays.[156] EX527 (1 μM) was later shown to increase p53[K382] acetylation in the presence of etoposide (but not in the absence) and had no effect on the expression of p53 target genes, cell viability, or proliferation in various tumor lines.[157] This result was quite unexpected as both SIRT1 knockdown and overexpression of catalytically inactive SIRT1 mutants induce proliferation, apoptosis, and/or DNA damage repair repression in cancer cell lines.[24,45,97,123,125,126] One possible explanation is that the antitumor effects observed with knockdown are independent of catalytic activity and that the point mutation inactivating the enzyme also disrupts the protein–protein interactions that are indeed responsible for SIRT1 activity. p53 is a protein harboring multiple acetylation sites,[158] and it is worthwhile noting that the same lysine residue in p53 (K382) is also targeted by SIRT7, another member of the SIRT family.[159] This may reflect a functional redundancy, although the role and activity of SIRT7 are poorly established.

A new selectivity concept for sirtuin inhibitors in oncology has emerged, based on the observation that multi-isoform SIRT inhibitors such as cambinol,[160] sirtinol,[126] salermide,[161] JGB1741,[162] suramin analogs,[163] and the tenovins[164] are effective in inducing cancer cell death (**Table 3**). These observations were recently strengthened by simultaneous RNAi-mediated gene silencing of both SIRT1 and SIRT2, which potently induced apoptosis in MCF-7 breast cancer cells, whereas the individual knockdowns were ineffective.[125] In addition, both dual SIRT1/2 inhibitors sirtinol and salermide, but not EX527, induced p53[K382] acetylation and stabilization in MCF7 cells. Pharmacological inhibition of SIRT1 and SIRT2 is also efficacious in xenograft models, as exemplified by tenovin-6 (ARN-8 melanoma)[164] and cambinol (Daudi Burkitt lymphoma).[160] This lends credence to the belief that dual SIRT1/2 inhibition may provide therapeutic benefit in cancer. There are an increasing number of publications reporting the discovery of novel dual SIRT1/2 inhibitors[165–167] as well as inhibitors of SIRT1–3.[168] It remains to be seen whether dual- or broad-spectrum sirtuin inhibitors may prove to be efficacious and safe in the clinic.

CONCLUSION

It is evident that a great deal has been learned about the biology of sirtuins since their original discovery in yeast. Although classified as type III histone deacetylases, the sirtuins are both deacetylases and ADP-ribosyltransferases, with substrates that include nonhistone proteins. There is evidence that several sirtuins translocate between subcellular

Table 3. Small-Molecule Inhibitors of SIRT1

NICOTINAMIDE EX527 SPLITOMYCIN

SALERMIDE SIRTINOL CAMBINOL

JGB-1741 TENOVIN-6

	SIRT1 IC_{50} (μM)	*SIRT2 IC_{50} (μM)*
NADH	17 000[205] (Sir2)	11 000[205] (human)
Nicotinamide	95.1,[125] 130[205] (HST2)	1.16,[125] 32.3[206]
EX527	0.16,[207] 0.38,[125] 0.098[156]	48.5,[207] 32.6,[125] 19.6[156]
Splitomycin	≈100,[162] *60,[146] *74[208]	
Salermide	76.2[125]	45[125]
Sirtinol	131,[209] 37.6[125]	57.7,[209] 103.4[125]
Cambinol	40.7,[167] 57.9,[207] 56[160]	47.9,[167] 40.7,[207] 59[160]
JGB1741	15[162]	>100[162]
Tenovin-6	21[164]	10[164]

References listed as superscripts above the IC_{50} value.

*Yeast Sir2 enzyme used; all others human SIRT enzyme.

compartments depending on stimuli and form various complexes that can be highly regulated by both positive and negative feedback circuits. SIRT1 is to date the best studied of the sirtuins. SIRT1 is involved in the regulation of metabolic, neuronal, immune, and tumor function (**Figs. 1, 2**). However, despite the multitude of data, both molecular and pharmacological, the outcome of selective SIRT1 modulation in vivo is still not completely clear. Data from various formats explored for both SIRT1 enzymatic and cell-based assays suggest great caution should be exercised in assay construction and interpretation of the data. In the case of SIRT1 activator assays, the presence of a fluorophore-tagged substrate in certain assays has led to misleading results. It is difficult to reconcile the lack of biological activity for the sole potent and selective SIRT1 inhibitor, EX527, with the opposing results of SIRT1 catalytically inactive mutants. Moreover, other published inhibitors exert promiscuity with equivocal evidence for inhibition of SIRT1-dependent deacetylase activity as the sole mechanism behind the phenotypic response observed in cancer cells. Perhaps other SIRT1-selective and/or multi-isoform selective compounds will shed light on this situation. Advances in structural biology for the sirtuin family will be quite impactful in guiding further development of isoform-selective and/or broad-spectrum SIRT inhibitors. Co-crystallization with a chemical probe compound (i.e., not just apo-protein) may better aid in understanding the potential Binding modes of activators/inhibitors and facilitate medicinal chemistry strategies. It should be noted that although the structures for SIRT1 from several lower species have been solved (**Table 1**), a crystal structure for human SIRT1 is yet to be reported. Known sirtuin isoform crystal structures are outlined and referenced in **Table 1**.

In drug discovery, SIRT1 cell-based assays are potentially complicated by the multitude of substrates—that is, which substrate(s) is driving the biology/phenotype in question in that specific cell type? Do they differ between cell types and stimuli? Is there redundancy between sirtuin isoforms? Comparative data with specific activators/inhibitors and/or molecular tools (overexpression, knockdown, catalytically inactive mutants) will be helpful in this regard. Having the appropriate animal models will also be critical to understanding SIRT1 biology. For oncology, using xenografts in SIRT1-deficient mice with different genetic backgrounds may produce different results (e.g., those with inactive HIC1 or PTEN; see **Table 1**). Animal model selection may affect patient tailoring strategies in oncology if indeed a preferred genetic background lends itself to sirtuin modulation.

In summary, the existing data suggest that SIRT1 activators may be useful for the treatment of diabetes/metabolic disease, inflammation, and/or neurodegeneration. However, for cancer, the burden of evidence indicates that broad-spectrum SIRT1/2 inhibitors may provide therapeutic benefit in select tumor types. These opposing approaches bring up many questions, such as whether SIRT1 activators will promote cancer (no evidence thus far) or whether SIRT1 (and SIRT2) inhibitors will exacerbate metabolic disease, inflammation, and/or neurodegeneration (data equivocal at best). More potent and selective compounds will need to be tested in vivo to unequivocally answer these questions. The preponderance of data is indeed compelling enough to justify drug discovery efforts for both SIRT1 activators and SIRT1/2 inhibitors.

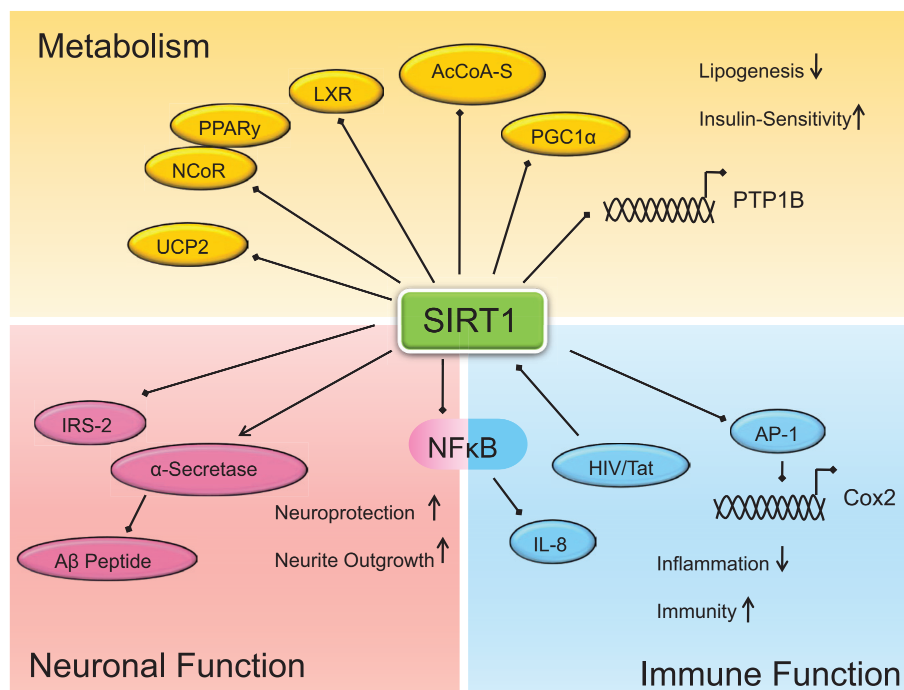


FIG. 1. Diverse roles of SIRT1 in biological pathways. SIRT1 is a central regulator with impact on metabolic, neuronal, and immunological functions. SIRT1 enhances insulin sensitivity, and aberrant function is associated with the etiology of type 2 diabetes mellitus. In addition, SIRT1 has been shown to be neuroprotective and anti-inflammatory, both via deacetylation of NFκB. Arrows with diamond-shaped ends show inhibitory action via deacetylation.

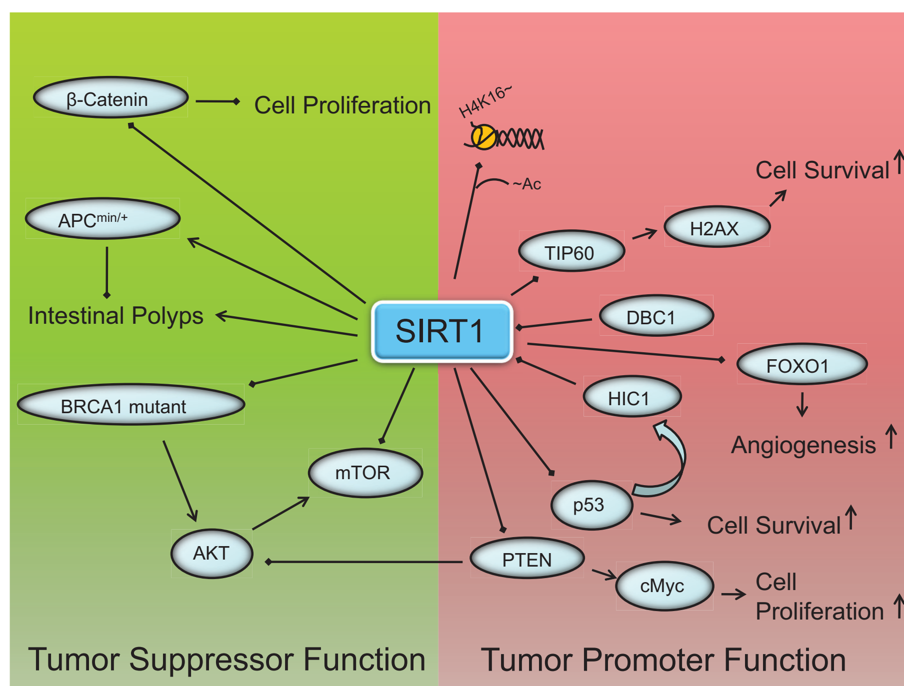


FIG. 2. SIRT1's dual roles in cancer. SIRT1 functions as a context-dependent tumor suppressor and oncogene. SIRT1 has direct repressive activity on a number of cancer-relevant pathways involving mTOR, mutated BRCA1, and APC. However, in the context of mutations (e.g., negative regulators such as HIC1 and DBC1), SIRT1 activity is enhanced, tipping the balance toward increased cancer cell survival, angiogenesis, and cell cycle progression. Arrows with diamond shaped ends show an inhibitory activity.

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