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Star Review

STAR REVIEW

The complex role of SIRT6 in carcinogenesis

Batia Lerrer†, Asaf A. Gertler† and Haim Y. Cohen*

The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel

*To whom correspondence should be addressed. Tel: +972 3 531 8383; Fax: +972 3 738 4058; Email: Haim.Cohen@biu.ac.il

†These authors have contributed equally to this work.

Abstract

SIRT6, a member of the mammalian sirtuins family, functions as a mono-ADP-ribosyl transferase and NAD⁺-dependent deacylase of both acetyl groups and long-chain fatty acyl groups. SIRT6 regulates diverse cellular functions such as transcription, genome stability, telomere integrity, DNA repair, inflammation and metabolic related diseases such as diabetes, obesity and cancer. In this review, we will discuss the implication of SIRT6 in the biology of cancer and the relevance to organism homeostasis and lifespan.

Introduction

Sirtuins are a conserved family of proteins with deacylase and/or mono-ADP (adenosine di phosphate)-ribosyltransferase activities that require the cellular metabolite NAD⁺ (nicotinamide adenine dinucleotide) to perform their functions (1). The founding member silent information regulator Sir2 was discovered in *Saccharomyces cerevisiae* to be a key regulator of transcriptional silencing (2). Overexpression (OE) of Sir2 extended lifespan in yeast whereas deletion of this gene reduced lifespan (3,4). There are seven Sir2 homologues in mammals (SIRT1–SIRT7) which differ in their cellular compartment localizations, catalytic activities and cellular functions. SIRT6 and SIRT7 are found in the nucleus while, SIRT3, SIRT4 and SIRT5 are found in the mitochondria. SIRT2 is cytosolic and SIRT1 has been found in both the cytosol and the nucleus (5,6).

Sirtuins have been implicated in multiple cellular processes including transcription, metabolism, fat mobilization, DNA repair, stress responses, apoptosis, tumorigenesis and aging. As deacetylases, sirtuins transfer the acetyl group from the lysine side chain of a protein to the cofactor NAD⁺, generating deacetylated substrate, OAADPr (2′-O-acetyl-ADP-ribose) and NAM (nicotinamide); the latter acts as an endogenous inhibitor to sirtuins (7–9).

Among the seven mammalian sirtuins, SIRT6 is a critical regulator of diverse processes, including DNA repair, gene expression, telomere maintenance, metabolism and aging. SIRT6 acts through both deacylation of acetyl groups and long-chain fatty acyl groups such as myristoyl, and as a mono-ADP-ribosyl transferase (10,11). SIRT6-deficient mice are small and at 2–3 weeks of age develop a severe premature aging phenotype that includes profound lymphopenia, loss of subcutaneous fat, lordokyphosis, metabolic defects and eventual death at about 4 weeks old (12).

In contrast, male mice overexpressing exogenous SIRT6 (MOSES) mice have a significantly longer lifespan than wild-type (WT) mice (13). Although no differences were found between WT and SIRT6-transgenic females, young transgenic males (6 months old) had lower serum insulin-like growth factor 1 (IGF1) levels than WT male littermates. The IGF1 signaling pathway is a key factor in the regulation of lifespan (14). Therefore, the extension in lifespan observed only in males overexpressing SIRT6 can be potentially explained by the reduction in IGF1 signaling. In addition to the effect on lifespan, MOSES mice are protected against the physiological damages caused by diet-induced obesity, including reduced accumulation of visceral fat, improved blood lipid profile, glucose tolerance and insulin secretion (15). Thus, SIRT6 is a regulator that links energy homeostasis to lifespan.

SIRT1 possesses several similarities to SIRT6. Aside from cellular localization and homology (6,16), nutrient deprivation induces the expression of both proteins (17,18). When either is knocked out, mice will die postnatally, stressing their importance for survival (12,19). They also play a major role in the regulation of multiple metabolic processes such as glucose and lipid metabolism (16,20). Kim *et al.* (21) showed that SIRT1

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Abbreviations

BER	base excision repair
DSB	double-strand break
HR	homologous recombination
MEF	mouse embryonic fibroblast
MOSES	mice overexpressing exogenous SIRT6
OE	overexpression
WT	wild type

deacetylates FOXO3a (forkhead box O3a), which subsequently enhances the formation of a SIRT1-FOXO3a-NRF1 (SFN) protein complex on the promoter of SIRT6 and positively regulates SIRT6 expression. Interestingly, the interaction was markedly enhanced in the absence of glucose. Therefore, regulation of SIRT6 under specific conditions is partially via SIRT1.

In addition to the regulatory effects of SIRT1 and SIRT6 on metabolism, SIRT3 also acts as a metabolic regulator. SIRT3, a major mitochondrial deacetylase has been reported to regulate major aspects of mitochondrial biology including: reactive oxygen species detoxification, ATP (adenosine triphosphate) generation, mitochondrial dynamics, nutrient oxidation and the mitochondrial UPR (unfolded protein response) (22). When compared with WT littermates, HFD (high-fat diet) fed SIRT3 KO mice show accelerated obesity, insulin resistance, hyperlipidemia and steatohepatitis (23). Furthermore, SIRT3 was found to act as a tumor suppressor by decreasing reactive oxygen species and maintaining genomic stability (24). Loss of SIRT3 stabilizes HIF1α (hypoxia-inducible factor 1α) and shifts cellular metabolism toward increased glycolysis (25). Taken together, sirtuins' effect on metabolism seem to be, in part, overlapping and redundant.

SIRT6 can bind chromatin and deacetylate H3K9 (histone H3 lysine 9) (26) and H3K56 (27,28). Histone deacetylation is associated with heterochromatin formation and decreased chromatin accessibility (29). A recent study from our lab found that the histone deacetylase activity of SIRT6 is nucleosome dependent. SIRT6 associates with the nucleosome and deacetylates at high efficiency only when histones H3 and H4 are packaged as nucleosomes (30). Other substrates which are deacetylated by SIRT6 are the double strand break resection protein CtIP [C-terminal binding protein (CtBP) interacting protein] and the histone acetyltransferase GCN5 (31,32).

Aging is one of the major risk factors of cancer. Therefore, the regulation of longevity by SIRT6 strongly suggests SIRT6 as a key factor in tumorigenesis. Indeed, a series of studies from recent years identified SIRT6 as a key regulator of cancer and cancer related pathways. In this review, we will discuss its role in regulating cellular pathways relevant to cancer including: metabolism, apoptosis or proliferation, inflammation and genome stability.

Metabolism

Glucose homeostasis

Glycolysis

SIRT6 is a key regulator of metabolism. In addition to the above-mentioned metabolic phenotypes observed in SIRT6-deficient and MOSES mice (12,13), Deng and his colleagues (21) also demonstrated that liver-specific deletion of SIRT6 in mice causes increased glycolysis. The severe hypoglycemia of SIRT6$^{-/-}$ mice was later explained by Zhong *et al.* who demonstrated that SIRT6 regulates glucose homoeostasis via suppressing the expression of multiple glycolytic genes. This allows efficient ATP production through mitochondrial oxidative phosphorylation instead of glycolysis. Indeed, loss of SIRT6 in cells increases glycolysis and diminishes mitochondrial respiration (33). One of the main positive regulators of this switch is the transcription factor HIF1α which is a key mediator in cellular adaptation to nutrient and oxygen stress (34). Thus, the model suggested by Zhong *et al.* was that under normal conditions, SIRT6 inhibits transcription of glycolytic genes, including Glut1 (glucose transporters 1), Pfk1 (phosphofructose kinase 1), Pdk1 (pyruvate dehydrogenase kinase 1) and LDHa (lactate dehydrogenase) which is involved in fermentation. These results demonstrate SIRT6 functions as a co-repressor of HIF1α transcriptional activity by deacetylating H3K9 at HIF1α target gene promoters. Indeed, in the absence of HIF1α, SIRT6 binding to glycolytic genes promoters was impaired. This process maintains a proper flux of glucose to the mitochondrial tricarboxylic acid cycle for efficient ATP production. When SIRT6 is inactivated, HIF1α is activated, acetylation of glycolytic genes promoters increases concurrent with increased expression of multiple metabolic genes, and ultimately results in an increase in glycolysis and a decrease in mitochondrial respiration. A rescue of this metabolic shift was observed in SIRT6-deficient cells when HIF1α was downregulated (33).

This metabolic reprogramming is also observed in cancer cells even in the presence of oxygen. In contrast to normal cells, cancer cells generate the energy needed for their cellular processes by increasing aerobic glycolysis, a phenomenon termed the Warburg effect (35). Based on these results, in 2012, Sebastian *et al.* demonstrated direct evidence for the function of SIRT6 in tumorigenesis. Injection of WT mouse embryonic fibroblasts (MEFs) into immunodeficient mice does not generate tumors. However, SIRT6 KO MEFs formed tumors. Re-expression of SIRT6 in KO MEFs completely abolished tumor formation. These results demonstrate a fundamental role for SIRT6 as a tumor suppressor. Indeed, the authors were able to show that SIRT6 is selectively downregulated in several human cancers. It was also shown that SIRT6 acts as a tumor suppressor by inhibiting the switch towards anaerobic glycolysis, depending on its function as repressor of HIF1α-dependent genes. The researchers knocked down a key glycolytic enzyme using shRNA, Pdk1, in order to suppress glycolysis in SIRT6 KO MEFs. In these cells there was inhibition in anchorage-independent cell growth in soft agar and also a severe reduction in tumor formation *in vivo*.

Because in most cancer cells, increased glycolysis *per se* is not sufficient to provide a growth advantage, the authors also showed that SIRT6 functions as a novel regulator of ribosome metabolism by corepressing avian myelocytomatosis viral oncogene homolog (MYC) transcriptional activity (36,37). MYC, a master regulator of cell proliferation, regulates ribosome biogenesis and protein synthesis by controlling the transcription and assembly of ribosome components (38). In SIRT6-deficient cells, MYC controls tumor growth specifically by regulating the ribosomes. Indeed, SIRT6-deficient tumor cells exhibit high levels of ribosomal protein gene expression (36).

The interaction between SIRT6 and the transcription factor RUNX2 (runt-related transcription factor 2) was reported recently by Choe *et al.* RUNX2 mediates breast cancer metastasis and regulates the expression of genes associated with tumor growth, migration and invasion. In MCF7 cells overexpressing RUNX2, the levels of proglycolytic genes PDK1, LDHA, and HK2 (hexokinase-2) were significantly elevated. RUNX2 increased glucose uptake and utilization by reducing the levels of SIRT6 at both the transcriptional and post-translational levels (39). As mentioned above, SIRT6 increased mitochondrial oxygen

consumption through oxidative phosphorylation (36). However, RUNX2 repressed the expression of SIRT6, and as a result reduced oxygen consumption (39).

The role of SIRT6 in controlling glycolysis was confirmed by Wu *et al*., who checked the function of SIRT6 in muscle invasive urothelial carcinoma of the bladder. It was found that SIRT6 OE inhibits the proliferation of bladder cancer cells and that SIRT6 suppresses glycolysis in urothelial carcinoma of the bladder. A dramatic decline of SIRT6 expression when bladder cancer progressed from T2 to T4 was also observed (40). In bladder and prostate cancer cell lines it was found that E2F1, the founding member of E2F family transcription factors, binds directly to SIRT6 promoter and suppresses SIRT6 transcription thereby promotes cancer cell glycolysis. Deacetylation of E2F1 by HDAC1, promotes the dissociation of E2F1 from the SIRT6 promoter region (41). These findings confirmed the role of SIRT6 as a tumor suppressor by its role in inhibiting glycolysis.

Gluconeogenesis

The influence of SIRT6 on gluconeogenesis was also described. In order to support the rapid proliferation and the requirement for high ATP/ADP and ATP/AMP ratios, cancer cells use large amounts of glucose. Gluconeogenesis generates glucose from non-carbohydrate precursors and is important for tumor cells growth (42). SIRT6 has been reported to downregulate gluconeogenesis in hepatocytes by enhancing GCN5-mediated acetylation and inhibition of PGC-1α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha). Deacetylation of K549 in GCN5 promotes phosphorylation of ser307 (serine in position 307) and thr735 (threonine). In this modified state, GCN5 is activated, resulting in hyperacetylation and a decrease in amount and activity of its substrate, PGC-1α. In addition, a decrease in gluconeogenic gene expression is observed (32). Other studies also demonstrated a role of SIRT6 and the tumor suppressor p53 in the regulation of glucose levels through suppression of gluconeogenesis. p53 transcriptionally activates expression of SIRT6, which specifically interacts with FOXO1 to promote its deacetylation. Deacetylation of FOXO1 is necessary for its nuclear exclusion, resulting in downregulation of FOXO1-activated genes G6PC (glucose-6-phosphatase) and PCK1 (phosphoenolpyruvate carboxy kinase), both of which are rate-limiting enzymes of gluconeogenesis. Thus, p53 is involved in a gluconeogenesis inhibition pathway by enhancing SIRT6 expression and subsequent FOXO1 nuclear exclusion (42). Interestingly, a previous study by Kanfi *et al*. showed that mice deficient in p53 have significantly decreased SIRT6 levels (17). Thus, it would be of great interest to explore under which conditions p53 regulates SIRT6 in cancer cells.

Fat homeostasis

SIRT6 was shown to be a key regulator of fat homeostasis and obesity (15) which are associated with increased risk of several cancer types (43,44). In comparison to WT littermates, HFD fed MOSES mice do not accumulate triglycerides and have significantly lower levels of LDL (low-density lipoprotein) cholesterol (15). Moreover, Elhanati *et al*. found that the mechanism underlying the improved cholesterol phenotype in MOSES mice under normal diet is due to a SIRT6-dependent repression of SREBP1 (sterol regulatory element binding proteins) and SREBP2 in the liver. Interestingly, these proteins are transcription factors that serve as master regulators of lipogenesis and adipogenesis (45–47). This regulation by SREBP1 and SREBP2 is achieved by controlling PPARγ (peroxisome proliferator-activated receptor γ) (48), C\EBP (CCAAT-enhancer-binding protein) (49) and other important adipogenic gene expression. Indeed, Kanfi *et al*. showed that SIRT6 repressed the expression of PPARγ dependent genes in adipose tissues of HFD fed MOSES mice (15). It is worth mentioning that SIRT6 regulates NFκB's (nuclear factor kappa-light-chain-enhancer of activated B cells) transcriptional activity through its RELA (p65) subunit, which can hetrodimerize with C\EBP to form a transcription factor heterodimer, suggesting direct regulation of SIRT6 on C\EBP (50). In addition, activation of PPARγ by rosiglitazone increases SIRT6 expression and ameliorates hepatic steatosis in rats (51). Furthermore, hepatic-specific SIRT6 KO in mice results in fatty liver formation and enhanced triglyceride synthesis (21).

SIRT6 negative regulation of lipid metabolism suggests another layer of SIRT6 regulation of metabolic processes. These phenotypes together with personalized treatment can help fight cancer growth/regrowth. This possibility was demonstrated by blocking lipid synthesis in several cancer types, allowing them to overcome tumor regrowth after angiogenic treatment withdrawal (52). Despite this cumulative data, the challenge remains to explore whether this role of SIRT6 in fat homeostasis influences different stages of cancer formations, and whether there is a connection between them.

Genomic stability and DNA repair

In mammals, regulation of genomic stability is linked to both aging and tumor suppression (53). Preservation of DNA integrity is critical to cellular and organismal function. Therefore, multiple mechanisms have evolved to protect and repair damaged DNA. While SIRT6 and p53 interact to regulate different aspects of the cell—which will be discussed later—it is not clear whether and how they interact to mediate and control DNA repair and genome stability. Single-stranded DNA lesions are repaired via nucleotide excision repair (NER) or base excision repair (BER), depending on the type of lesion. Double-strand breaks (DSBs) are repaired by nonhomologous end-joining or homologous recombination (HR) (54).

SIRT6 has a key role in regulating several DNA repair pathways and maintenance of genomic stability in cells. Originally, it was demonstrated that SIRT6 acts as a suppressor of genomic instability by regulating BER. SIRT6 deficiency leads to sensitivity to a spectrum of DNA damaging agents such as H_2O_2, methyl methanesulfonate and ionizing radiation that are consistent with BER defects (12). In addition, deletion of SIRT6 in cells results in genomic instability, including chromosomal breaks and fusions (55). The chromosome fusion observed in SIRT6 deficient cells might be also explained by an article published by Michishita *et al*. They showed that SIRT6 associates specifically with telomeres and that SIRT6 deletion leads to telomere dysfunction with end-to-end chromosomal fusions and premature cellular senescence. SIRT6 deacetylates H3K9 at telomere chromatin in S phase and is required for the stable association of Werner ATP-dependent helicase, which has a role in genome stability during DNA replication and telomere metabolism, and is the mutated factor in Werner syndrome (27).

In addition to its role in BER, SIRT6 was also shown to play a major role in DSB repair. Jackson and his colleagues showed that SIRT6 has a role in promoting DNA end resection, a crucial step in DSB repair by HR. SIRT6 promotes resection by deacetylating the DSB resection protein CtIP (31). Another study showed that in response to DSBs, SIRT6 forms a macromolecular complex with the DSB repair factor, DNA-PK (DNA-dependent protein kinase). SIRT6 is required for mobilization and stabilization of the DNA-PK catalytic subunit (DNA-PKcs) on chromatin, adjacent

to the DSB site. SIRT6 associates dynamically with chromatin and is necessary for an acute decrease in global cellular acetylation levels on H3K9 in response to DNA damages (56). In addition to these findings, Gorbunova and her colleagues showed that in mammalian cells subjected to oxidative stress, SIRT6 is recruited to DNA DSBs loci and stimulates DSB repair through both nonhomologous end-joining and HR. SIRT6 enhances DSB repair under oxidative stress by mono-ADP-ribosylating PARP1 (poly-ADP-ribose polymerase 1), activating PARP1's poly-ADP-ribosylase activity (57). Last, a recent study by Toiber *et al*. found SIRT6 to be one of the most rapidly recruited factors to DNA DSB damage sites. SIRT6 directly recruits the ATP-dependent chromatin remodeler SNF2H to DNA break sites, allowing the opening of chromatin and also the recruitment of downstream DNA repair factors such as p53-binding protein 1 (53BP1), replication protein A (RPA) and BRCA (breast cancer 1, early onset) (58). As mentioned above, SIRT6 was shown to induce apoptosis in cancer cells via the ATM kinase pathway in a p53/p73-dependent manner using its mono-ADP-ribosyltransferase activity (59).

Interestingly, Gorbunova and her colleagues demonstrate a new role for SIRT6 in a different aspect of genome stability. SIRT6 is also a suppressor of L1 [Long interspersed nuclear elements (LINE1)] retrotransposon activity. L1 activity has been implicated in a variety of age-related disorders including neurodegeneration and cancer. SIRT6 regulates the packaging of L1 elements into transcriptionally repressive heterochromatin by mono-ADP ribosylating the nuclear corepressor protein, KAP1 (KRAB-associated protein 1). During aging, SIRT6 is depleted from L1 loci, relieving this repression. L1 increased activation can then contribute to the pathology of age-related diseases (60).

Taken together, it is clear that SIRT6 influences and regulates genome stability, DNA repair and aging through several unique pathways and that this regulation is also critical for carcinogenesis.

Apoptosis and cell proliferation

SIRT6 OE induces massive apoptosis in a variety of cancer cell lines, but not in normal, non-transformed cells. This phenomenon is mediated by the mono-ADP ribosyltransferase activity and not by the deacetylase activity of SIRT6. SIRT6 promotes apoptosis through either p53 or p73 signaling and requires ATM (ataxia telangiectasia mutated) kinase to initiate this response (59).

Survivin is a prosurvival protein that blocks apoptosis by inhibiting AIF (apoptosis inducing factor) dependent apoptotic pathways (61) and possibly caspase3\7\9 mediated apoptosis (62–65). It is ubiquitously expressed during development and absent in most normal tissues (61,66,67). Survivin expression is re-activated in most cancers (68) and is associated with tumor aggression and decreased patient survival (69). Recently, Min *et al*. used mouse models specific for liver cancer initiation to demonstrate that the survival of initiated cancer cells is controlled by a c-Jun, c-Fos, SIRT6 and survivin cascade. C-Jun and c-Fos are important regulators of tumor development and c-Fos is regulated by c-Jun. During liver tumor initiation, c-Jun suppresses cell death through induction of survivin expression. This expression of survivin is controlled by SIRT6, which modulates histone deacetylation and NFκB binding at the survivin promoter. SIRT6 expression is regulated by c-Fos and enhances cell death by repressing survivin. The inhibition of c-Fos by c-Jun in early tumor stages will block SIRT6 expression. SIRT6 repression of survivin was also demonstrated in endometrial cancer cells with lower expression of SIRT6 (70). Therefore, increasing the levels of SIRT6 or targeting the antiapoptotic activity of survivin at the initiation stage can impair cancer development and may provide a novel preventive strategy in the initiation of tumor development (71).

Another article providing additional evidence for the role of SIRT6 as a tumor suppressor was recently published by Zhang *et al*. They showed that SIRT6 expression is significantly reduced in human ovarian cancer tissues compared to normal tissues. SIRT6 OE inhibited the proliferation of ovarian cancer cells, while downregulation of SIRT6 enhanced their growth. In addition, SIRT6 OE reduced the expression of Notch 3, both at the mRNA and protein levels (72). However, Notch 3 OE blocked this anti-proliferative effect of SIRT6 in ovarian cancer cells. Notch 3 signaling pathway is involved in tumor progression of ovarian carcinoma and higher Notch 3 expression is associated with poor prognosis (73). Taken together, these results suggested that the anti-proliferative effect of SIRT6 on ovarian cancer cells is through downregulation of Notch 3.

In human glioma cells, SIRT6 acts as tumor suppressor. SIRT6 protein and mRNA levels were markedly downregulated when compared to those in normal brain tissues. SIRT6 OE repressed glioma cell growth while SIRT6 KD (knockdown) facilitated growth. In these samples, SIRT6 expression was negatively correlated with the expression of PCBP2 (Poly(rC)-binding protein 2) (74). PCBP2 is a member of the PCBP family that regulates tumor development. PCBP2 plays an important role in posttranscriptional and translational regulation by interacting with single-stranded poly(rC) motifs in target mRNAs (75). SIRT6 inhibits PCBP2 expression by binding to PCBP2 promoter region and deacetylates H3K9, thereby acting as tumor suppressor (74).

Further evidences for the role of SIRT6 in liver cancer were recently described. SIRT6 was downregulated in HCC (hepatocellular carcinoma) cells (76). SIRT6 OE induced apoptosis in human HepG2 (hepatocarcinoma cells) whereas KD of SIRT6 promoted growth of these cells, demonstrating that SIRT6 can act as a tumor suppressor (77). A similar effect was also found in other cancer types. The mRNA and protein levels of SIRT6 are decreased in NSCLC (non-small cell lung cancer) specimen. SIRT6 OE in human NSCLC cell lines inhibited their proliferation, whereas SIRT6 KD promoted it (78,79). SIRT6 negatively regulates Twist1 expression (78). Twist1, a member of the family of basic helix-loop-helix transcription factors, has been found to be a key factor in the promotion of metastasis of cancer cells and is also known to induce EMT (epithelial–mesenchymal transition) (80). Thus, SIRT6 inhibits NSCLC cells proliferation by suppressing Twist1 expression (78).

Taken together, in liver and lung cancer, SIRT6 plays an anti-cancer role by promoting apoptosis. Recently, Azuma *et al*. reported that in primary cancer tissues from patients with NSCLC, high cytoplasmic versus low nuclear expression of SIRT6 is associated with more aggressive cancer and with poor prognosis. In addition, SIRT6 KD in A549 cells showed improvement in paclitaxel sensitivity (81). Taken together, in liver and lung cancer, SIRT6 plays an anticancer role by promoting apoptosis.

Interestingly, in contrast to these findings, Kim *et al*. described a mechanism involving cAMP (cyclic AMP) signaling dependent ubiquitin degradation of SIRT6 in lung cancer cells. SIRT6 degradation is mediated via the PKA (protein kinase A)-dependent inhibition of the Raf-MEK-ERK pathways. Reduced SIRT6 expression augments γ-ray-induced apoptosis of NSCLC cells, suggesting that SIRT6 might have potentially oncogenic roles in the tumorigenesis and progression of lung cancer (82). Ming *et al*., also reported an oncogenic role of SIRT6 in human SCC (skin squamous cell carcinoma). SIRT6 levels were higher

in SCC samples compared to healthy specimens. SIRT6 acts an oncogene in the skin by promoting the expression of COX-2 (cyclooxygenase-2), an enzyme involved in inflammation, proliferation, and survival (83).

Also, in prostate tumors, SIRT6 was overexpressed compared to normal prostate tissue. SIRT6 KD in human prostate cancer cells led to sub-G_1 phase arrest of cell cycle and increased apoptosis (84). Although important, there is not enough evidence to determine whether these effects of SIRT6 on cell proliferation are valid for different non-cancerous cell-lines and tissues. Nevertheless, SIRT6 KO in primary chondrocytes, MEFs and embryonic stem cells impaired their proliferation (12,85).

Taken together, it evident that SIRT6 plays a major role in cell proliferation. It would be of a great interest to further characterize which cancer type requests SIRT6 activation or repression in order to effect tumor growth. Moreover, in order to translate this knowledge to human therapy, one must also define which of these regulated pathways is unique to cancerous cells and if not, how to specifically target SIRT6 in cancer cells.

Inflammation

Chronic inflammation is a risk factor of cancer development. The inflammatory microenvironment in a malignant tumor is now known to be an essential part of cancer development. The malignant microenvironment influences tumorigenesis and cancer progression by supplying growth factors that contribute to cancer cell proliferation, angiogenesis, invasion, metastasis and signals that lead to EMT (86). Previous studies showed that NAD^+ levels influence the capacity of inflammatory cells to secrete cytokines such as tumor necrosis factor α (TNFα), interleukin 6 (IL6), IL1β, interferon γ (IFNγ), IL2 and IL8. These findings led Bauer *et al.* to investigate SIRT6 regulation of proinflammatory cytokines. They found that SIRT6 OE in pancreatic cancer cells increased TNFα and IL8 production at the mRNA and protein levels (87). IL8 is also frequently detected in high concentrations in the plasma of PDAC (pancreatic ductal adenocarcinoma) patients and plays a key role in the pathogenesis of this tumor by promoting local inflammation, increasing angiogenesis and promoting EMT (88).

One of the products of SIRT6 deacetylase activity is OAADPr, which can be hydrolyzed to ADPr. ADPr activates the Ca^{2+} channel TRPM2 (Transient receptor potential cation channel, subfamily M, member 2), causing Ca^{2+} to enter the cells. This induces the expression of IL8 and TNFα. Another cellular function that strongly depends on Ca^{2+} levels promoted by SIRT6 is cell migration. Therefore, the authors suggest inhibiting SIRT6 activity as a therapeutic approach for treating pancreatic cancer (87). However, it has yet to be determined whether mice with reduced SIRT6 activity develop less pancreatic cancer, particularly given the high frequency of SIRT6 mutation observed in human pancreatic tumors.

TNFα is one of the key mediators implicated in inflammation-associated cancers. High doses of local TNFα can cause hemorrhagic necrosis to the tumor via selective destruction of tumor blood vessels. However, TNFα can act as an endogenous tumor promoter when produced in the tumor microenvironment (89). These results implicate a role for SIRT6 in the expression of pro-inflammatory and proangiogenic cytokines, thus contributing to different stages of cancer progression. Indeed, in 2009 Van Gool *et al* were the first to discover that SIRT6 regulates TNFα at the post-transcriptional level, connecting metabolism, inflammation and SIRT6 together (90). In 2013, Jiang *et al* deciphered this post-transcriptional mechanism showing that SIRT6 can remove myristoyl modifications from lysine 19 and 20 of TNFα by a novel enzymatic mechanism of SIRT6: deacylation of long chain fatty acids. This demyristoylation promotes TNFα secretion from the cell (10). Altogether, inhibition of SIRT6 may help combat cancer-induced inflammation, angiogenesis, and metastasis. Interestingly, in one of the fundamental publications of SIRT6, Kawahara *et al.* showed a role for SIRT6 as an anti-inflammatory enzyme by controlling NFκB-dependent gene expression (91). NFκB, which is activated by TNFα, has a role in the initiation and progression of cancer. Its targets are involved in different aspects of tumorigenesis, including cell proliferation, angiogenesis, survival, invasion, metastasis and EMT (92). SIRT6 binds to the NFκB RELA subunit and deacetylates H3K9 at NFκB target gene promoters, leading to repression of NFκB signaling (91).

Taken together, it seems that more research is needed to fully understand the role of SIRT6 in inflammatory pathways and their relevance to cancer formation and progression.

Conclusions and future directions

SIRT6 as a double-edged sword

In this review, we summarized the activities of SIRT6 and their relevance to cancer. SIRT6 has essential roles that impact several cancer and aging-related pathways. These include prevention of genomic instability, maintenance of telomere integrity and regulation of metabolic homeostasis. It is evident that SIRT6 plays a dual role in cancer as both a tumor suppressor and an oncogene (summarized in Table 1). One of the main challenges is to decipher the mechanisms and conditions that control SIRT6 regarding cancer fate. SIRT6 is a chromatin-bound protein and is known to deacetylate H3K9 and H3K56. Interestingly, it was found that acetylation of H3K56 is increased in multiple types of cancer including, liver, breast, thyroid and colon cancer (93). Thus, it might be that the absence of SIRT6 contributes to tumor formation by increased H3K56 acetylation.

The distribution of SIRT6 in human cancer cells was examined by several different groups (36,40,106). Analysis of data from the TCGA (Cancer Genome Atlas database) and the Cancer Cell Line Encyclopedia (CCLE) revealed, that SIRT6 is deleted in 20% of all cancers analyzed and in 35% of ~1000 cancer cell lines collected. Among pancreatic and colorectal cancer cell lines specifically, 63 and 29% were deleted in the SIRT6 locus respectively. Analysis of 55 human colorectal carcinomas showed that SIRT6 expression is down regulated in early stages, and these low levels are maintained during cancer progression (36). These findings support a tumor suppressor role for SIRT6, as potentially its mutation is required for tumor development. Indeed, eight different spontaneous, SIRT6 point mutations discovered in a variety of human cancers inhibited SIRT6's ability to fully repress HIF1α and MYC transcriptional activity, leading to a glycolytic switch and cellular transformation (106).

Interestingly, several of these mutations decrease SIRT6 deacetylase activity, demonstrating the critical role of acetylation status in carcinogenesis (106). These findings and the fact that SIRT6 levels are reduced in human NSCLC (78) support the hypothesis that reduced SIRT6 levels might cause tumor formation and maintenance (36).

However, accumulating data suggests an oncogenic role of SIRT6 in different cancer types (81,101). For example, in human SCC (83,97), head and neck squamous cell carcinoma (HNSCC) (103) and Chronic Lymphocytic Leukemia (CLL) (104,105), SIRT6 is upregulated. In addition, SIRT6 desensitizes breast cancer

Table 1. Summary of SIRT6 role in cancer

Cancer type	Cancer origin		Role of SIRT6	Pathways and regulators	Reference
	Cell line	Tissue[a]			
NSCLC	A549, H1975, H2009, EBC-1, RERF-LC-AI	98	Patients with high cytoplasmic expression and low nuclear expression of SIRT6 exhibited the worst prognosis		(81)
	A549, NCI-H23, 16HBET	36	SIRT6 is downregulated in NSCLC tissues and cell lines. SIRT6 inhibited NSCLC cell proliferation by down-regulation of Twist1	SIRT6 inhibited Twist1 expression in NSCLC cells	(78)
	A549	No	SIRT6 has a tumor suppressor effect	SIRT6 inhibits proliferation, causes G0/G1 phase retardation and induces apoptosis	(79)
	H1299, A549	No	Possible oncogenic roles in the tumorigenesis and progression of lung cancer	cAMP signaling promotes ubiquitin-proteasome-dependent degradation of SIRT6	(82)
Colon carcinoma	HCT 116	No	Tumor suppressor through c-Myc suppression	USP10 interacts with and deubiquitinates SIRT6, protecting it from proteosomal degradation	(94)
HCC	HepG2, HEK293	8	Tumor suppressor through inhibition of ERK1/2 phosphorylation		(77)
	Huh7, Hep3B, PLC/PRF/5, HepG2	70	SIRT6 is a tumor suppressor	hMOF up-regulates SIRT6 via its promoter and inhibits the expression of SIRT6 target genes	(95)
	HepG2, Huh7	No	SIRT6 promotes tumorigenicity	TGF-β1/H2O2/HOCl upregulates SIRT6, which suppresses senescence induction, thus promoting tumorigenicity	(96)
	No	73,[b]	SIRT6 is a tumor initiation suppressor	c-Fos transcriptionally up-regulates SIRT6, which represses Survivin, leading to apoptosis	(71)
	Primary mouse hepatocytes	139	SIRT6 as a potential tumor suppressor	SIRT6 is down-regulated in HCC, leading to liver hypomethylation	(76)
SCC	SCC12, SCC13, SCC O11, O12, O22, O28	7	Upregulated SIRT6 expression in premalignant and malignant keratinocyte lesions	SIRT6 is a miR-34a target. MiR-34a is down-regulated in SCC cells	(97)
	No	6	SIRT6 is upregulated and functions as an oncogene	SIRT6 promoted expression of COX-2 by repressing AMPK signaling thereby, increasing cell proliferation and survival in the skin epidermis	(83)
Breast cancer	No	65	Methylation and hypermethylation of SIRT6 gene and promoter were analyzed—no significant changes found		(98)
	MCF7, Hs578t	150	SIRT6 (tumor suppressor) expression was lower in malignant BC tissues or cell lines that expressed high levels of RUNX2	RUNX2 inhibits mitochondrial respiration through SIRT6 repression via transcriptional and post-translational mechanisms	(39)
	MCF7, transformed MEFs	No	Nuclear SIRT6 expression in breast cancer is associated with poorer survival	SIRT6 mediates paclitaxel and epirubicin resistance. SIRT6 regulates the acetylation status and expression levels of FOXO3a and p53	(99)
	MCF-7, MDA-MB-231, Hs578T, HBL-100, BT474	126,[b]	SIRT6 as a tumor suppressor	Activated AKT1 phosphorylates SIRT6 on Ser338, promoting its degradation in an MDM2-dependent manner. Loss of SIRT6 results in trastuzumab resistance in breast cancer cells overexpressing HER2	(100)

Table 1. *Continued*

Cancer type	Cancer origin: Cell line	Cancer origin: Tissue[a]	Role of SIRT6	Pathways and regulators	Reference
Bladder urothelial carcinoma	5637, RT4, UMUC3	157 and 256	Dramatic decline of SIRT6 expression when bladder cancer progressed from T2 to T4. Over expression of SIRT6 inhibits the proliferation of bladder cancer cells	SIRT6 suppresses glycolysis in bladder cancer cells	(40)
Retinoblastoma	No	18	SIRT6 is expressed in retinoblastoma (no comparison to normal tissue)		(101)
Ovarian cancer	SKOV3, OVCAR3	32	Decreased expression of SIRT6 in ovarian cancer tissues	SIRT6 inhibits ovarian cancer cell proliferation via down-regulation of Notch3 expression	(72)
Endometrial cancer	16 endometrial cancer cell lines	No	SIRT6 demonstrates a tumor suppressor effect. SIRT6 protein expression is lower in endometrial cancer cells	SIRT6 induces apoptosis to endometrial cancer cells by repressing survivin	(70)
Gliomas	T98G, U87MG, A172, U251, CCF-STTG1	31	SIRT6 suppresses glioma growth *in vitro* and *in vivo*	SIRT6 is down-regulated in gliomas and correlates with PCBP2 expression. SIRT6 down-regulates PCBP2 by deacetylating H3K9ac	(74)
Prostate/bladder cancer	PC3, DU145/5637, UMUC3	No	SIRT6 as a tumor suppressor	E2F1 suppresses transcriptionally SIRT6 under hypoxia, leading to enhanced glycolysis	(41)
Pancreas adenocarcinoma	BxPC-3, PDAC	[b]	SIRT6 is proinflammatory and proangiogenesis	SIRT6 regulates cytokine expression and cell migration	(87)
	BxPC-3, Capan-1	No		Quinazolinedione-SIRT6 inhibitors sensitize cancer cells to gemcitabine and to olaparib	(102)
HNSCC	No	34	SIRT6 is upregulated in peripheral blood of HNSCC patients and returns to normal after surgery	SIRT6 is a potential prognostic marker for HNSCC in peripheral blood	(103)
CLL	No	32	Increased levels of SIRT6 in CLL specimens	Other HDACS including SIRT1 were elevated as well	(104)
	No	148	Patients with higher SIRT6 expression had shorter overall survival than patients with lower SIRT6 expression	Findings are correlated to NAMPT expression	(105)
Acute myeloid leukemia	No	79	Patients with higher SIRT6 expression had shorter overall survival than patients with lower SIRT6 expression	Findings are correlated to NAMPT expression	(105)
Diffuse large B-cell lymphoma		396			
Pancreatic ductal adenocarcinomas/colorectal carcinomas	Transformed MEFs	36/55,[b]	SIRT6 acts as a tumor suppressor *in vivo*	SIRT6 controls cancer cell proliferation by corepressing Myc transcriptional activity. SIRT6 expression is down-regulated in human cancers	(36)
Mixed	GP2-293, HeLa,,HT1080, HCC70, HCC1954, MDA-MB-231	No	SIRT6 induces apoptosis in cancer cells	SIRT6 over-expression selectively kills cancer cells in a p53/p73 dependent manner via the ATM pathway. SIRT6's Mono-ADP ribosyltransferase activity is required	(59)
	Immortalized MEFs	12 tumor types	SIRT6 is a tumor suppressor	Different SIRT6 mutants fail to repress glycolysis and cellular transformation. Deacetylase activity is critical for SIRT6 tumor-suppressor (not demyristoylase)	(106)

[a]Number of clinical specimens.
[b]Additional mouse models.

cells to anti-cancer drugs (99) whereas inhibition of SIRT6 sensitizes pancreas adenocarcinoma cells to anti-cancer drugs (102). Possible oncogenic, proangiogenic and cell migratory roles of SIRT6 were also published (82,87,96). Therefore, it seems that SIRT6 role in carcinogenesis may be tissue and context dependent. The reduced SIRT6 levels in many cancer types suggest negative regulation of SIRT6. It would be interesting to investigate the mechanism underlying this negative regulation.

The endogenous regulation of SIRT6

Recent studies revealed molecular regulators of SIRT6 at the protein stability level (Figure 1). For example, the ubiquitin ligase CHIP (carboxyl terminus of Hsp70-interacting protein) stabilizes SIRT6 by ubiquitinating it at lysine 170 thus, preventing SIRT6 canonical ubiquitination by other ubiquitin ligases. Interestingly, CHIP deletion accelerates aging and reduces the life span in mice (107). Also, Stohr *et al.* showed that in the liver of ApoE$^{-/-}$ (apolipoprotein E) mice, ITCH, which is an E3 ubiquitin ligase, ubiquitinates SIRT6. Loss of ITCH in the liver increases the expression of SIRT6 by reducing its ubiquitination, thus protecting the liver from hepatic lipid infiltration (108). In breast cancer cell lines, SIRT6 was phosphorylated by the kinase AKT1 at Ser338. This induced the interaction and ubiquitination of SIRT6 by MDM2 (Mouse double minute 2 homolog), targeting SIRT6 for protease-dependent degradation (100).

As mentioned above, SIRT6 is downregulated in colon cancers and this downregulation is in correlation with poor survival prognosis (36). It was found that the tumor suppressor USP10, (mammalian ubiquitin-specific peptidase 10), deubiquitinates SIRT6 and protects SIRT6 from proteasome-mediated degradation in human colon cancer cells (94). In addition, USP10 prevents p53 degradation by deubiquitinating it (109).

Taken together, it seems that controlling SIRT6 protein levels by preventing its ubiquitination might have a therapeutic potential against tumorigenesis.

Yet, the main challenge is to develop the therapeutic tools to regulate SIRT6 levels. Several conditions and pathways regulating SIRT6 were discovered. For example, SIRT6 levels increase upon nutrient deprivation in cultured cells, in mice after fasting, and in rats fed a DR (dietary-restricted) diet (17). Studies indicated that in humans, long-term DR with adequate intake of nutrients results in several metabolic adaptations that reduce the risk of developing type 2 diabetes, cardiovascular disease and cancer (110). These findings suggest that small molecule mimickers of DR can potentially be used to increase SIRT6 levels and treat cancer.

Another therapeutic approach could be the induction or repression of transcription factors that control SIRT6 expression levels such as c-Jun (via c-Fos) and FOXO3a (21,71). For example, down regulation of RUNX2 which repress SIRT6 expression at both transcriptional and post-translational levels. It was found that endogenous SIRT6 expression levels were lower in malignant breast cancer tissues and cell lines that expressed high levels of RUNX2 (39,95). Another potential target could be hMOF (human Males-absent on the first protein), a histone acetyltransferase that can significantly increase the protein and mRNA levels of SIRT6 in hepatocellular carcinoma by binding to its promoter. When hMOF was overexpressed, SIRT6 downstream genes were downregulated (95). Since promoter hyper methylation is an important gene silencing mechanism thought to be involved in early stages of carcinogenesis (42), Wang *et al.* examined promoter methylation status and mRNA expression levels of SIRT6 and explored the relationship between methylation and mRNA expression in breast cancers. No significant association between promoter methylation status and expression profiles of SIRT6 were observed in invasive breast cancers (98).

However, such an approach of manipulating transcription factors that regulate SIRT6 is not applicable as it requires isolating the effect of these transcription factors on SIRT6. Thus, an alternate approach is to take advantage of the regulation on SIRT6 at the post transcriptional level by microRNA's (miRs). Several miRs were shown to regulate SIRT6. For example, miR33a and miR33b (from the introns of SREBP2 and SREBP1 respectively), repress SIRT6 levels by binding to its 3′ UTR (45), and miR-766 and miR34a decrease the expression of SIRT6 in cells (97,111). Yet, it still remains a challenge to target these miRs and their regulation in tumors.

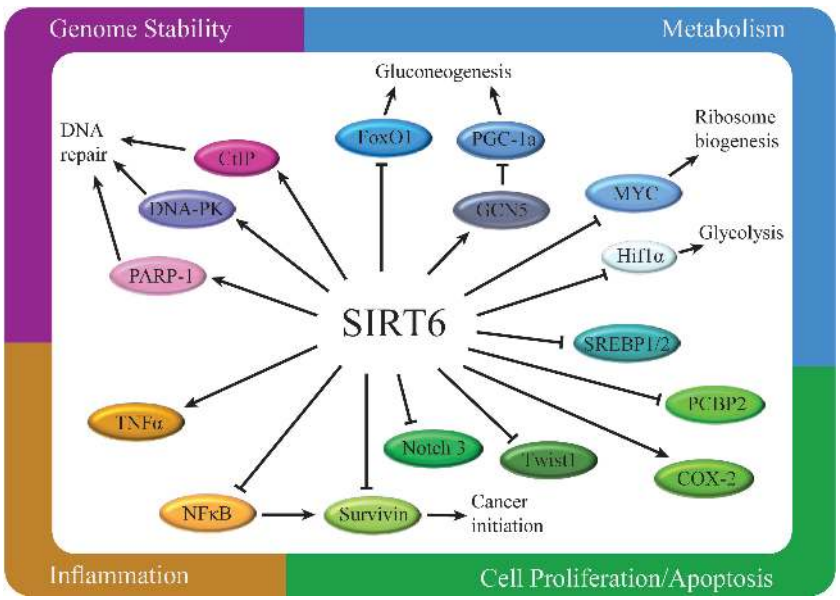

Figure 1. Regulation of multiple cellular functions by SIRT6. The various targets of SIRT6 in cancer related pathways such as metabolism, genome stability, cell proliferation apoptosis and inflammation.

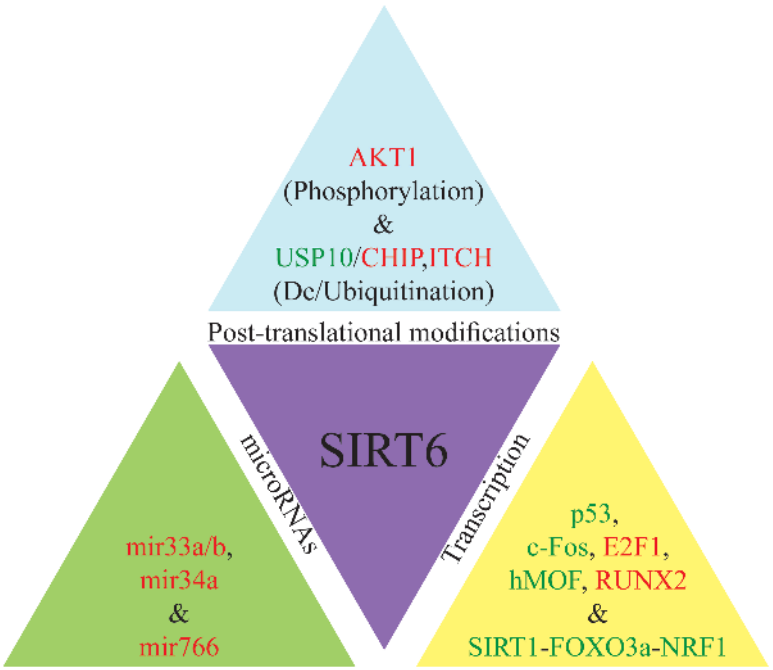

Figure 2. From transcriptional to post-translational regulation of SIRT6. Downregulators or upregulators of SIRT6 are labeled in red or green, respectively.

Finally, there are many endeavors targeting the challenge of finding SIRT6 small molecules therapeutics (112–114). Currently, there are no known selective, specific, effective and direct pharmacological activators of SIRT6 that can be used to treat cancer at any of its stages. Recently, several articles were published demonstrating small molecule inhibitors and some activators of SIRT6 in the form of acyls and acylated peptides. However, despite their potential, these molecules are not SIRT6-specific and exert their effect on other sirtuins as well (115–118).

Altogether, it is evident that SIRT6 is a key regulator of tumorigenesis via multiple pathways (Figure 2). Future exciting studies will provide novel insights for additional targets of SIRT6 and their relevance to cancer. Therefore, we predict that in the near future SIRT6 will become a primary target for the pharmaceutical industry for cancer therapy. Our primary challenge would be to develop novel tools to achieve this goal.

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References

1. Gertler, A.A. et al. (2013) SIRT6, a protein with many faces. Biogerontology, 14, 629–639.
2. Landry, J. et al. (2000) The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. Proc. Natl. Acad. Sci. USA, 97, 5807–5811.
3. Kaeberlein, M. et al. (1999) The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. Genes Dev., 13, 2570–2580.
4. Imai, S. et al. (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature, 403, 795–800.
5. Houtkooper, R.H. et al. (2012) Sirtuins as regulators of metabolism and healthspan. Nat. Rev. Mol. Cell Biol., 13, 225–238.
6. Michishita, E. et al. (2005) Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. Mol. Biol. Cell, 16, 4623–4635.
7. Naiman, S. et al. (2012) The contentious history of sirtuin debates. Rambam Maimonides Med. J., 3, e0022.
8. Rajendran, R. et al. (2011) Sirtuins: molecular traffic lights in the crossroad of oxidative stress, chromatin remodeling, and transcription. J. Biomed. Biotechnol., 2011, 368276.
9. Haigis, M.C. et al. (2010) Mammalian sirtuins: biological insights and disease relevance. Annu. Rev. Pathol., 5, 253–295.
10. Jiang, H. et al. (2013) SIRT6 regulates TNF-α secretion through hydrolysis of long-chain fatty acyl lysine. Nature, 496, 110–113.
11. Pan, P.W. et al. (2011) Structure and biochemical functions of SIRT6. J. Biol. Chem., 286, 14575–14587.
12. Mostoslavsky, R. et al. (2006) Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell, 124, 315–329.
13. Kanfi, Y. et al. (2012) The sirtuin SIRT6 regulates lifespan in male mice. Nature, 483, 218–221.
14. van Heemst, D. (2010) Insulin, IGF-1 and longevity. Aging Dis., 1, 147–157.
15. Kanfi, Y. et al. (2010) SIRT6 protects against pathological damage caused by diet-induced obesity. Aging Cell, 9, 162–173.
16. Boutant, M. et al. (2014) SIRT1 metabolic actions: Integrating recent advances from mouse models. Mol. Metab., 3, 5–18.
17. Kanfi, Y. et al. (2008) Regulation of SIRT6 protein levels by nutrient availability. FEBS Lett., 582, 543–548.
18. Kanfi, Y. et al. (2008) Regulation of SIRT1 protein levels by nutrient availability. FEBS Lett., 582, 2417–2423.
19. Cheng, H.L. et al. (2003) Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice. Proc. Natl. Acad. Sci. USA, 100, 10794–10799.
20. Etchegaray, J.P. et al. (2013) The histone deacetylase SIRT6: at the crossroads between epigenetics, metabolism and disease. Curr. Top. Med. Chem., 13, 2991–3000.

21. Kim, H.S. et al. (2010) Hepatic-specific disruption of SIRT6 in mice results in fatty liver formation due to enhanced glycolysis and triglyceride synthesis. Cell Metab., 12, 224–236.
22. McDonnell, E. et al. (2015) SIRT3 regulates progression and development of diseases of aging. Trends Endocrinol. Metab., 26, 486–492.
23. Hirschey, M.D. et al. (2011) SIRT3 deficiency and mitochondrial protein hyperacetylation accelerate the development of the metabolic syndrome. Mol. Cell, 44, 177–190.
24. Kim, H.S. et al. (2010) SIRT3 is a mitochondria-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress. Cancer Cell, 17, 41–52.
25. Finley, L.W. et al. (2011) SIRT3 opposes reprogramming of cancer cell metabolism through HIF1α destabilization. Cancer Cell, 19, 416–428.
26. Michishita, E. et al. (2008) SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. Nature, 452, 492–496.
27. Michishita, E. et al. (2009) Cell cycle-dependent deacetylation of telomeric histone H3 lysine K56 by human SIRT6. Cell Cycle, 8, 2664–2666.
28. Yang, B. et al. (2009) The sirtuin SIRT6 deacetylates H3 K56Ac *in vivo* to promote genomic stability. Cell Cycle, 8, 2662–2663.
29. Görisch, S.M. et al. (2005) Histone acetylation increases chromatin accessibility. J. Cell Sci., 118(Pt 24), 5825–5834.
30. Gil, R. et al. (2013) SIRT6 exhibits nucleosome-dependent deacetylase activity. Nucleic Acids Res., 41, 8537–8545.
31. Kaidi, A. et al. (2010) Human SIRT6 promotes DNA end resection through CtIP deacetylation. Science, 329, 1348–1353.
32. Dominy, J.E. Jr et al. (2012) The deacetylase Sirt6 activates the acetyltransferase GCN5 and suppresses hepatic gluconeogenesis. Mol. Cell, 48, 900–913.
33. Zhong, L. et al. (2010) The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1alpha. Cell, 140, 280–293.
34. Seagroves, T.N. et al. (2001) Transcription factor HIF-1 is a necessary mediator of the pasteur effect in mammalian cells. Mol. Cell. Biol., 21, 3436–3444.
35. Vander Heiden, M.G. et al. (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science, 324, 1029–1033.
36. Sebastián, C. et al. (2012) The histone deacetylase SIRT6 is a tumor suppressor that controls cancer metabolism. Cell, 151, 1185–1199.
37. Lerrer, B. et al. (2013) The guardian: metabolic and tumour-suppressive effects of SIRT6. EMBO J., 32, 7–8.
38. van Riggelen, J. et al. (2010) MYC as a regulator of ribosome biogenesis and protein synthesis. Nat. Rev. Cancer, 10, 301–309.
39. Choe, M. et al. (2015) The RUNX2 Transcription Factor Negatively Regulates SIRT6 Expression to Alter Glucose Metabolism in Breast Cancer Cells. J. Cell. Biochem., 116, 2210–2226.
40. Wu, M. et al. (2014) Expression and function of SIRT6 in muscle invasive urothelial carcinoma of the bladder. Int. J. Clin. Exp. Pathol., 7, 6504–6513.
41. Wu, M. et al. (2015) E2F1 enhances glycolysis through suppressing Sirt6 transcription in cancer cells. Oncotarget, 6, 11252–11263.
42. Zhang, P. et al. (2014) Tumor suppressor p53 cooperates with SIRT6 to regulate gluconeogenesis by promoting FoxO1 nuclear exclusion. Proc. Natl. Acad. Sci. USA, 111, 10684–10689.
43. Wolin, K.Y. et al. (2010) Obesity and cancer. Oncologist, 15, 556–565.
44. Polednak, A.P. (2008) Estimating the number of U.S. incident cancers attributable to obesity and the impact on temporal trends in incidence rates for obesity-related cancers. Cancer Detect. Prev., 32, 190–199.
45. Elhanati, S. et al. (2013) Multiple regulatory layers of SREBP1/2 by SIRT6. Cell Rep., 4, 905–912.
46. Horton, J.D. (2002) Sterol regulatory element-binding proteins: transcriptional activators of lipid synthesis. Biochem. Soc. Trans., 30(Pt 6), 1091–1095.
47. Horton, J.D. et al. (1999) Sterol regulatory element-binding proteins: activators of cholesterol and fatty acid biosynthesis. Curr. Opin. Lipidol., 10, 143–150.
48. Fajas, L. et al. (1999) Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. Mol. Cell. Biol., 19, 5495–5503.
49. Le Lay, S. et al. (2002) Insulin and sterol-regulatory element-binding protein-1c (SREBP-1C) regulation of gene expression in 3T3-L1 adipocytes. Identification of CCAAT/enhancer-binding protein beta as an SREBP-1C target. J. Biol. Chem., 277, 35625–35634.
50. Ray, A. et al. (1995) Concerted participation of NF-kappa B and C/EBP heteromer in lipopolysaccharide induction of serum amyloid A gene expression in liver. J. Biol. Chem., 270, 7365–7374.
51. Yang, S.J. et al. (2011) Activation of peroxisome proliferator-activated receptor gamma by rosiglitazone increases sirt6 expression and ameliorates hepatic steatosis in rats. PLoS One, 6, e17057.
52. Sounni, N.E. et al. (2014) Blocking lipid synthesis overcomes tumor regrowth and metastasis after antiangiogenic therapy withdrawal. Cell Metab., 20, 280–294.
53. Lombard, D.B. et al. (2005) DNA repair, genome stability, and aging. Cell, 120, 497–512.
54. Bassing, C.H. et al. (2004) The cellular response to general and programmed DNA double strand breaks. DNA Repair (Amst)., 3, 781–796.
55. Lombard, D.B. (2009) Sirtuins at the breaking point: SIRT6 in DNA repair. Aging, 1, 12–16.
56. McCord, R.A. et al. (2009) SIRT6 stabilizes DNA-dependent protein kinase at chromatin for DNA double-strand break repair. Aging, 1, 109–121.
57. Mao, Z. et al. (2011) SIRT6 promotes DNA repair under stress by activating PARP1. Science, 332, 1443–1446.
58. Toiber, D. et al. (2013) SIRT6 recruits SNF2H to DNA break sites, preventing genomic instability through chromatin remodeling. Mol. Cell, 51, 454–468.
59. Van Meter, M. et al. (2011) SIRT6 overexpression induces massive apoptosis in cancer cells but not in normal cells. Cell Cycle, 10, 3153–3158.
60. Van Meter, M. et al. (2014) SIRT6 represses LINE1 retrotransposons by ribosylating KAP1 but this repression fails with stress and age. Nat. Commun., 5, 5011.
61. Liu, T. et al. (2004) Rapid induction of mitochondrial events and caspase-independent apoptosis in Survivin-targeted melanoma cells. Oncogene, 23, 39–48.
62. O'Connor, D.S. et al. (2000) Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin. Proc. Natl. Acad. Sci. USA, 97, 13103–13107.
63. Shin, S. et al. (2001) An anti-apoptotic protein human survivin is a direct inhibitor of caspase-3 and -7. Biochemistry, 40, 1117–1123.
64. Banks, D.P. et al. (2000) Survivin does not inhibit caspase-3 activity. Blood, 96, 4002–4003.
65. Mesri, M. et al. (2001) Cancer gene therapy using a survivin mutant adenovirus. J. Clin. Invest., 108, 981–990.
66. Adida, C. et al. (1998) Developmentally regulated expression of the novel cancer anti-apoptosis gene survivin in human and mouse differentiation. Am. J. Pathol., 152, 43–49.
67. Ambrosini, G. et al. (1997) A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat. Med., 3, 917–921.
68. Tamm, I. et al. (1998) IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res., 58, 5315–5320.
69. Altieri, D.C. et al. (1999) Survivin apoptosis: an interloper between cell death and cell proliferation in cancer. Lab. Invest., 79, 1327–1333.
70. Fukuda, T. et al. (2015) Putative tumor suppression function of SIRT6 in endometrial cancer. FEBS Lett., 589, 2274–2281.
71. Min, L. et al. (2012) Liver cancer initiation is controlled by AP-1 through SIRT6-dependent inhibition of survivin. Nat. Cell Biol., 14, 1203–1211.
72. Zhang, J. et al. (2015) The histone deacetylase SIRT6 inhibits ovarian cancer cell proliferation via down-regulation of Notch 3 expression. Eur. Rev. Med. Pharmacol. Sci., 19, 818–824.
73. Jung, S.G. et al. (2010) Prognostic significance of Notch 3 gene expression in ovarian serous carcinoma. Cancer Sci., 101, 1977–1983.
74. Chen, X. et al. (2014) The histone deacetylase SIRT6 suppresses the expression of the RNA-binding protein PCBP2 in glioma. Biochem. Biophys. Res. Commun., 446, 364–369.
75. Makeyev, A.V. et al. (2002) The poly(C)-binding proteins: a multiplicity of functions and a search for mechanisms. RNA, 8, 265–278.
76. Marquardt, J.U. et al. (2013) Sirtuin-6-dependent genetic and epigenetic alterations are associated with poor clinical outcome in hepatocellular carcinoma patients. Hepatology, 58, 1054–1064.
77. Zhang, Z.G. et al. (2014) Sirt6 suppresses hepatocellular carcinoma cell growth via inhibiting the extracellular signal-regulated kinase signaling pathway. Mol. Med. Rep., 9, 882–888.

78. Han, Z. et al. (2014) Sirtuin SIRT6 suppresses cell proliferation through inhibition of Twist1 expression in non-small cell lung cancer. Int. J. Clin. Exp. Pathol., 7, 4774–4781.
79. Cai, Y. et al. (2014) Radiosensitization effect of overexpression of adenovirus-mediated SIRT6 on A549 non-small cell lung cancer cells. Asian Pac. J. Cancer Prev., 15, 7297–7301.
80. Wushou, A. et al. (2014) Twist-1 up-regulation in carcinoma correlates to poor survival. Int. J. Mol. Sci., 15, 21621–21630.
81. Azuma, Y. et al. (2015) SIRT6 expression is associated with poor prognosis and chemosensitivity in patients with non-small cell lung cancer. J. Surg. Oncol., 112, 231–237.
82. Kim, E.J. et al. (2015) Cyclic AMP signaling reduces sirtuin 6 expression in non-small cell lung cancer cells by promoting ubiquitin-proteasomal degradation via inhibition of the Raf-MEK-ERK (Raf/mitogen-activated extracellular signal-regulated kinase/extracellular signal-regulated kinase) pathway. J. Biol. Chem., 290, 9604–9613.
83. Ming, M. et al. (2014) SIRT6 promotes COX-2 expression and acts as an oncogene in skin cancer. Cancer Res., 74, 5925–5933.
84. Liu, Y. et al. (2013) Inhibition of SIRT6 in prostate cancer reduces cell viability and increases sensitivity to chemotherapeutics. Protein Cell, 4, 702–710.
85. Piao, J. et al. (2013) Sirt6 regulates postnatal growth plate differentiation and proliferation via Ihh signaling. Sci. Rep., 3, 3022.
86. Hagerling, C. et al. (2014) Balancing the innate immune system in tumor development. Trends Cell Biol, 25, 214–220.
87. Bauer, I. et al. (2012) The NAD+-dependent histone deacetylase SIRT6 promotes cytokine production and migration in pancreatic cancer cells by regulating Ca2+ responses. J. Biol. Chem., 287, 40924–40937.
88. Fernando, R.I. et al. (2011) IL-8 signaling plays a critical role in the epithelial-mesenchymal transition of human carcinoma cells. Cancer Res., 71, 5296–5306.
89. Balkwill, F. (2006) TNF-alpha in promotion and progression of cancer. Cancer Metastasis Rev., 25, 409–416.
90. Van Gool, F. et al. (2009) Intracellular NAD levels regulate tumor necrosis factor protein synthesis in a sirtuin-dependent manner. Nat. Med., 15, 206–210.
91. Kawahara, T.L. et al. (2009) SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene expression and organismal life span. Cell, 136, 62–74.
92. Bassères, D.S. et al. (2006) Nuclear factor-kappaB and inhibitor of kappaB kinase pathways in oncogenic initiation and progression. Oncogene, 25, 6817–6830.
93. Das, C. et al. (2009) CBP/p300-mediated acetylation of histone H3 on lysine 56. Nature, 459, 113–117.
94. Lin, Z. et al. (2013) USP10 antagonizes c-Myc transcriptional activation through SIRT6 stabilization to suppress tumor formation. Cell Rep., 5, 1639–1649.
95. Zhang, J. et al. (2014) The histone acetyltransferase hMOF suppresses hepatocellular carcinoma growth. Biochem. Biophys. Res. Commun., 452, 575–580.
96. Feng, X.X. et al. (2015) Sirtuin 6 promotes transforming growth factor-β1/H2O2/HOCl-mediated enhancement of hepatocellular carcinoma cell tumorigenicity by suppressing cellular senescence. Cancer Sci., 106, 559–566.
97. Lefort, K. et al. (2013) A miR-34a-SIRT6 axis in the squamous cell differentiation network. EMBO J., 32, 2248–2263.
98. Wang, D. et al. (2014) The promoter methylation status and mRNA expression levels of CTCF and SIRT6 in sporadic breast cancer. DNA Cell Biol., 33, 581–590.
99. Khongkow, M. et al. (2013) SIRT6 modulates paclitaxel and epirubicin resistance and survival in breast cancer. Carcinogenesis, 34, 1476–1486.
100. Thirumurthi, U. et al. (2014) MDM2-mediated degradation of SIRT6 phosphorylated by AKT1 promotes tumorigenesis and trastuzumab resistance in breast cancer. Sci. Signal., 7, ra71.
101. Orellana, M.E. et al. (2015) Expression of SIRT2 and SIRT6 in retinoblastoma. Ophthalmic Res., 53, 100–108.
102. Sociali, G. et al. (2015) Quinazolinedione SIRT6 inhibitors sensitize cancer cells to chemotherapeutics. Eur. J. Med. Chem., 102, 530–539.
103. Lu, C.T. et al. (2014) The potential of SIRT6 and SIRT7 as circulating markers for head and neck squamous cell carcinoma. Anticancer Res., 34, 7137–7143.
104. Wang, J.C. et al. (2011) Histone deacetylase in chronic lymphocytic leukemia. Oncology, 81, 325–329.
105. Cagnetta, A. et al. (2015) APO866 Increases Antitumor Activity of Cyclosporin-A by Inducing Mitochondrial and Endoplasmic Reticulum Stress in Leukemia Cells. Clin. Cancer Res., 21, 3934–3945.
106. Kugel, S. et al. (2015) Identification of and molecular basis for SIRT6 loss-of-function point mutations in cancer. Cell Rep, 13, 479–488.
107. Ronnebaum, S.M. et al. (2013) The ubiquitin ligase CHIP prevents SirT6 degradation through noncanonical ubiquitination. Mol. Cell. Biol., 33, 4461–4472.
108. Stöhr, R. et al. (2015) ITCH modulates SIRT6 and SREBP2 to influence lipid metabolism and atherosclerosis in ApoE null mice. Sci. Rep., 5, 9023.
109. Yuan, J. et al. (2010) USP10 regulates p53 localization and stability by deubiquitinating p53. Cell, 140, 384–396.
110. Rizza, W. et al. (2014) What are the roles of calorie restriction and diet quality in promoting healthy longevity? Ageing Res. Rev., 13, 38–45.
111. Sharma, A. et al. (2013) The role of SIRT6 protein in aging and reprogramming of human induced pluripotent stem cells. J. Biol. Chem., 288, 18439–18447.
112. Lin, H. (2013) Modulators for sirt6 and assays for screening same. Google Patents. US20130345155, CN103403553 A, WO2012088268A2, WO2012088268A3.
113. Sauve, A. et al. (2013) Activation and activators of sirt6. Google Patents. US20130029930 A1, WO2011081945A2, WO2011081945A3.
114. Bruzzone, S. et al. (2013) Rejuvenating sirtuins: the rise of a new family of cancer drug targets. Curr. Pharm. Des., 19, 614–623.
115. Feldman, J.L. et al. (2013) Activation of the protein deacetylase SIRT6 by long-chain fatty acids and widespread deacylation by mammalian sirtuins. J. Biol. Chem., 288, 31350–31356.
116. Kokkonen, P. et al. (2012) Peptides and Pseudopeptides as SIRT6 Deacetylation Inhibitors. ACS Med. Chem. Lett., 3, 969–974.
117. Parenti, M.D. et al. (2014) Discovery of novel and selective SIRT6 inhibitors. J. Med. Chem., 57, 4796–4804.
118. Sinclair, D.A. et al. (2014) Small-molecule allosteric activators of sirtuins. Annu. Rev. Pharmacol. Toxicol., 54, 363–380.