The Critical Role of the Class III Histone Deacetylase SIRT1 in Cancer

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Abstract

Gene expression and deacetylase activity of the class III histone deacetylase SIRT1 are up-regulated in cancer cells due to oncogene overexpression or loss of function of tumor suppressor genes. SIRT1 induces histone deacetylation and methylation, promoter CpG island methylation, transcriptional repression, and deacetylation of tumor suppressor proteins. SIRT1 may play a critical role in tumor initiation, progression, and drug resistance by blocking senescence and apoptosis, and promoting cell growth and angiogenesis. SIRT1 inhibitors have shown promising anticancer effects in animal models of cancer. Further screening for more potent SIRT1 inhibitors may lead to compounds suitable for clinical trials in patients. [Cancer Res 2009;69(5):1702–5]

Introduction

The class III histone deacetylases (HDAC), also known as the sirtuins (SIRT1-7), are the silent information regulator 2 (Sir2) family of proteins. Distinct from other HDACs, the sirtuins are not modulated by the inhibitors of Class I, II, and IV HDACs, such as SAHA. SIRT1, the founding member of the class III HDACs, shares a catalytic domain of ~275 amino acids with other sirtuins, and uses NAD^+ to mediate the deacetylation of histone and nonhistone proteins. Although well-documented to extend the life span of yeast, worms, flies, and mammals, SIRT1 has more recently been implicated in the initiation and progression of various malignancies.

Modulation of SIRT1 Gene Expression and Deacetylase Activity

Modulation of SIRT1 mRNA expression by oncogenes and tumor suppressor genes. Overexpression of SIRT1 in cancer cells occurs partly at the level of transcription. Two p53 binding sites in the SIRT1 gene promoter normally repress SIRT1 gene transcription (Fig. 1*A*). The tumor suppressor hypermethylated in cancer 1 (HIC1) forms a transcription repression complex with SIRT1, and this complex directly binds the SIRT1 promoter and represses its transcription (1). Inactivation of HIC1 due to promoter hypermethylation in cancer cells results in up-regulated SIRT1 gene expression. Importantly, HIC1−/− mice are tumor prone and show a SIRT1-dependent block in apoptosis induction (1). The oncogenic RNA binding protein HuR associates with the 3′ untranslated region of SIRT1 mRNA, stabilizing the SIRT1 mRNA, and increasing SIRT1 expression level (2). In contrast, the cell cycle checkpoint kinase 2 (Chk2), a tumor suppressor, phosphorylates HuR and reduces SIRT1 expression (2). Moreover, under cellular stress or DNA damage, the cell cycle and apoptosis regulator E2F1 directly induces SIRT1 transcription by binding to the SIRT1 promoter. However, SIRT1 can bind and deacetylate E2F1, and inhibit E2F1-induced transcription of target genes including SIRT1 itself (3).

Modulation of SIRT1 deacetylase activity by protein-protein interaction and sumoylation. Through protein-protein interactions, the enzymatic activity of SIRT1 can be modulated by proteins including active regulator of SIRT1 (AROS), the tumor suppressor deleted in breast cancer 1 (DBC1), and the proapoptotic nuclear desumoylase SENP1 (Fig. 1*A*). By direct binding to the NH_2 terminus of SIRT1 protein, AROS enhances the deacetylase activity of SIRT1, thereby increasing SIRT1-mediated p53 deacetylation, both *in vitro* and *in vivo*, and inhibiting p53-mediated transcriptional activity (4). DBC1, which is homozygously deleted in some breast cancer patients, acts as a native inhibitor of SIRT1 by direct binding to the catalytic domain of SIRT1 protein (5). Depletion of DBC1 stimulates SIRT1-mediated p53 deacetylation and inhibits p53-mediated apoptosis; and these effects can be reversed in cancer cells by concomitant knockdown of SIRT1, demonstrating that DBC1 promotes p53-mediated apoptosis via SIRT1 inhibition (5). Moreover, desumoylation by SENP1 reduces the deacetylase activity of SIRT1. Under cellular stress, SENP1 directly binds to the COOH terminus of SIRT1 protein, desumoylates and inactivates SIRT1, resulting in p53 acetylation and activation of apoptosis (6).

The Biological Function of SIRT1 in Cancer

SIRT1 regulates histone deacetylation and methylation. Recruitment of SIRT1 to its target gene promoter results in deacetylation of histone proteins at H1 lysine 26 (H1-K26Ac), H4 lysine 16 (H4-K16Ac), and H3 lysine 9 (H3-K9Ac; Fig. 1*B*; ref. 7). SIRT1 has no effect on H3-K4 methylation, a mark associated with open chromatin, but induces the production of H4 monomethyl K20 (H4-mono-MeK20) and H3 tri-methyl K9 (H3-tri-MeK9), two marks associated with repressed chromatin, and the production of H3 dimethyl K79 (H3-di-MeK79), a "boundary" separating active and inactive chromatin domains (7). SIRT1 not only directly recruits and deacetylates suppressor of variegation 3 to 9 homologue 1 (SUV39H1), resulting in the trimethylation of H3K9 (8), but can also form a protein complex with SUV39H1 and nucleomethylin. The protein complex binds histone H3 Lys9 (H3K9) in the rDNA locus, dimethylates (H3-di-MeK9) and deacetylates H3K9 (H3K9Ac), and represses rRNA transcription, thus establishing silent chromatin in the rDNA locus, and protecting cells from energy deprivation and apoptosis (9). Moreover, SIRT1 physically interacts with the histone acetyltransferase p300 and p300/CBP-associated factor to induce histone hypoacetylation.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi:10.1158/0008-5472.CAN-08-3365

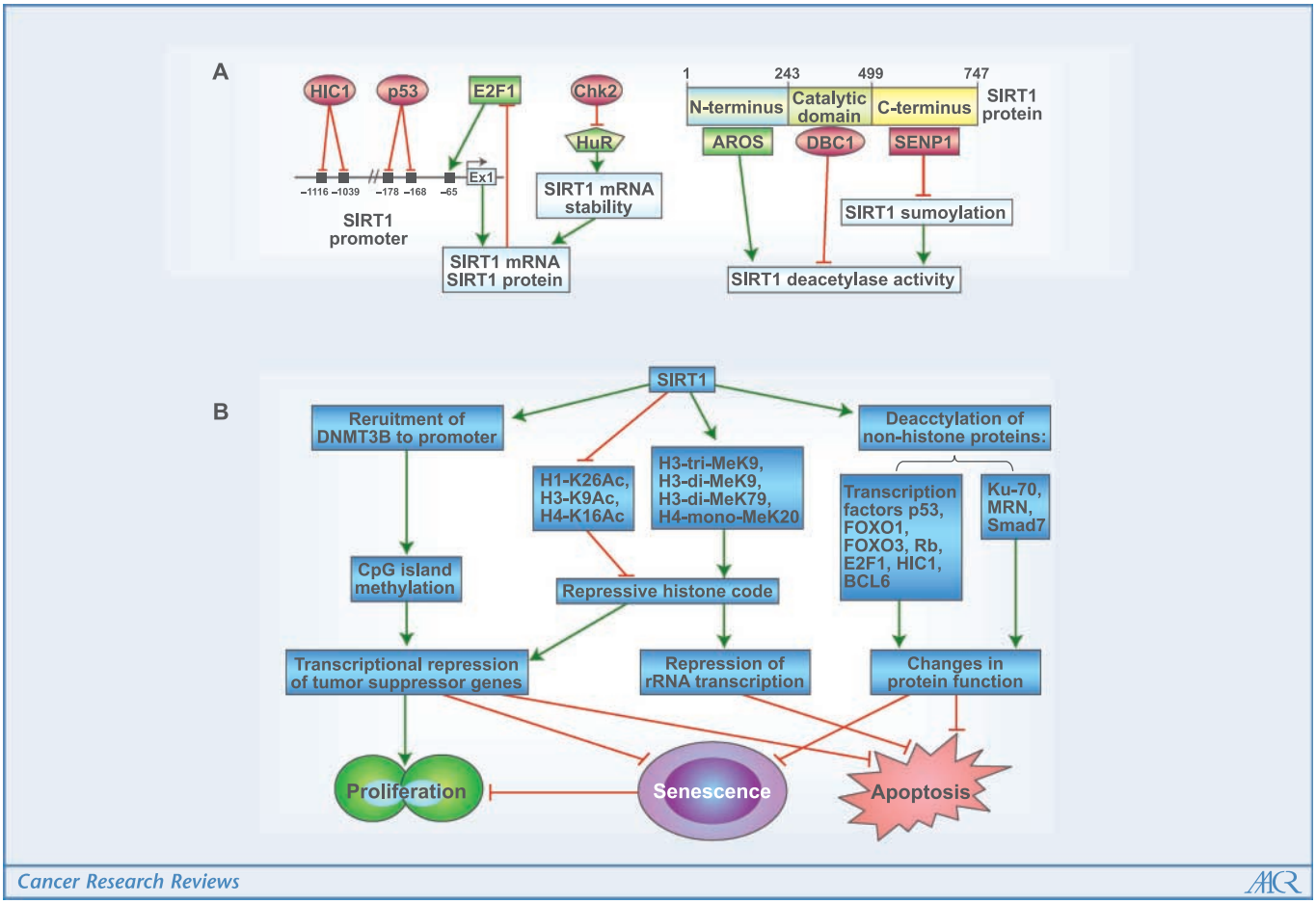

Figure 1. Modulation of SIRT1 gene expression and deacetylase activity (*A*), and the diverse biological functions of SIRT1 in cancer (*B*). *A,* SIRT1 gene expression is modulated at both transcriptional and posttranscriptional levels, and its deacetylase activity is modulated through protein-protein interaction and sumoylation at its three protein domains. *Pink ovals,* tumor suppressor genes; *bright green pentagons,* oncogenes. Proteins that cannot be classified as typical tumor suppressors or oncogenes are labeled with bright green squares for their stimulation of SIRT1 expression or activity, or with pink squares for their inhibition of SIRT1 expression. *Negative numbers,* transcription factor-binding sites at SIRT1 promoter relative to the transcription start site (labeled with an arrow at the beginning of exon 1). *Red* ⊥, inhibition; *green arrows,* enhancement. *B,* SIRT1 blocks cancer cell apoptosis and senescence and promotes cell proliferation by modulating histone deacetylation, histone methylation, and promoter CpG island methylation, repressing tumor suppressor gene transcription and deacetylating nonhistone proteins.

SIRT1 induces heritable CpG island methylation at the gene promoter. In cancer cells, SIRT1 localizes to promoter elements of aberrantly silenced tumor suppressor genes in which 5 CpG islands are densely hypermethylated, and inhibition of SIRT1 leads to the re-expression of these genes (10). The tumor suppressor genes repressed by SIRT1 include E-cadherin, which is often repressed during epithelial tumorigenesis, progression, and metastasis, secreted frizzled-related proteins, mismatch repair gene MLH1, the transcription factors GATA-4 and GATA-5, p27, and cellular retinoid binding protein 1 (10). Importantly, in cells transfected with exogenous E-cadherin promoter constructs, SIRT1 is recruited to the promoter after the induction of DNA double-strand break, and the recruitment of SIRT1 is required for the further recruitment of DNA (cytosine-5)-methyltransferase 3β (DNMT3B) and the subsequent heritable CpG island methylation of the exogenous promoter (Fig. 1*B*; ref. 11).

SIRT1 deacetylates nonhistone proteins in cancer cells. The nonhistone deacetylation targets of SIRT1 can be divided into three groups: (*a*) transcription factors including p53, the proapoptotic tumor suppressor FOXO proteins, E2F1, HIC1, BCL6, TAF_I68, retinoblastoma protein (Rb), PGC1α, peroxisome proliferator-activated receptor γ, p73, the androgen receptor, nuclear factor κB, myocyte enhancing factor 2, MyoD, HDAC4, and others; (*b*) DNA repair proteins (chromatin-related enzymes) including Ku-70 and MRE11-RAD50-NBS1 (MRN); and (*c*) signaling factors such as endothelial nitric oxide synthase and Smad7 (Fig. 1*B*). By directly binding and deacetylating p53, FOXO1, FOXO3, E2F1, BCL6, and TAF_I68, SIRT1 suppresses the transcriptional activity of p53, FOXO1, FOXO3, E2F1, and RNA polymerase I but, in contrast, enhances the function of BCL6 (3, 12, 13), which is involved in the pathogenesis of diffuse large-cell lymphoma and Burkitt's lymphoma.

SIRT1 blocks senescence, cell differentiation, and stress-induced apoptosis, and promotes cell growth, angiogenesis, and vasodilation. It has been well-documented that SIRT1 overexpression can block stress-induced apoptosis by modulation of histone deacetylation, histone methylation and promoter methylation, repression of tumor repressor gene transcription, and deacetylation of nonhistone proteins such as p53 (13), FOXO, E2F1 (3), Rb, BCL6 (12), Ku-70, and Smad7 (Fig. 1*B*). Deacetylation of Ku-70 blocks BAX conformational change and translocation into the mitochondria, and consequent mitochondrial apoptosis. However, it should be noted that SIRT1 is not always antiapoptotic

due to its ability to inhibit the *trans*-activation potential of nuclear factor κB and to directly deacetylate β-catenin.

SIRT1 can promote cancer cell growth by blocking cellular senescence and differentiation (Fig. 1*B*). The SIRT1 inhibitor, sirtinol, can promote cell senescence in breast and lung cancer cells. Overexpression of SIRT1 may block senescence by direct deacetylation of p53, FOXO, E2F1, and/or RB, or by enhancing the activation of S6K1 signaling, which decreases the expression of p16. Moreover, SIRT1 represses the expression of differentiation-associated genes and retards cell differentiation, and SIRT1 expression in tumor cells correlates with a reduced histologic level of tissue differentiation (14, 15).

SIRT1 is also a critical modulator of angiogenesis, vasodilation, and blood supply. As a consequence of FOXO1 deacetylation, SIRT1 blocks endothelial cell senescence, promotes endothelial cell growth, vascular sprouting, branching morphogenesis, and blood vessel formation in mice. Moreover, by directly binding to and deacetylating the endothelial nitric oxide synthase, SIRT1 stimulates its activity and increases the expression of endothelial nitric oxide, leading to vasodilation, and increased blood supply.

Overexpression of SIRT1 during Experimental Carcinogenesis and in Human Tumor Tissues

Overexpression of SIRT1 during experimental carcinogenesis. SIRT1 overexpression is an early event during the immortalization of normal human urothelial cells by hTERT, the catalytic subunit of telomerase. In mouse epithelial JB6 cells, SIRT1 gene expression is up-regulated by the carcinogenic aluminum oxide, and the up-regulation of SIRT1 is essential for the activation of activator protein-1, a well-established event associated with tumor promotion. SIRT1 is, therefore, suggested to play an important role in carcinogenesis induced by aluminum oxide nanoparticles (16). Additionally, SIRT1 is recruited to the promoter region of DNA double-strand break, and the recruitment of SIRT1 is required for the subsequent heritable CpG island methylation of the promoter (11), which is frequently associated with tumor initiation.

Overexpression of SIRT1 in human tumor tissues. SIRT1 is consistently up-regulated in malignant cells or tissues from patients with leukemia, glioblastoma, prostate, colorectal, or skin cancer (Supplementary Table S1; refs. 14, 15). SIRT1 is the only HDAC that is significantly overexpressed in leukemia lymphoblasts as compared with normal lymphoblasts. In glioblastoma cells purified from patients, SIRT1 expression is 4.9-fold higher in poorly differentiated, stem cell marker CD133-positive tumor cells than those more differentiated CD133-negative tumor cells (15). SIRT1 is also significantly elevated in poorly differentiated adenocarcinomas, compared with normal counterparts, in three transgenic mouse models of prostate cancer and in human prostate tumor tissues (14). In colorectal cancer tissues, SIRT1 is consistently up-regulated in cancer cells, compared with adjacent normal epithelial cells, and up-regulation of SIRT1 has been suggested to be a useful marker for the diagnosis of colorectal cancer. Moreover, overexpression of SIRT1 is frequently observed in tumor tissues from skin cancer patients.

Overexpression of SIRT1 in chemo-resistant tumor cells and tissues. Ectopic overexpression of SIRT1 induces P-glycoprotein expression and renders cancer cells resistant to the chemotherapy drug doxorubicin (17). SIRT1 is overexpressed in chemo-resistant leukemia, neuroblastoma, osteosarcoma, ovarian, and breast cancer cells, compared with their drug-sensitive counterparts. Biopsies from cancer patients treated with chemotherapeutic agents also express a higher level of SIRT1, compared with untreated samples (17).

Small Molecule SIRT1 Inhibitors as Anticancer Agents

A number of nonspecific and specific inhibitors of SIRT1 have been discovered by testing natural products, by biochemical- or cell-based screening of small molecule libraries using a forward chemical genetics approach, or by chemical synthesis (reviewed in ref. 18). These include the physiologic inhibitor nicotinamide, the 2-hydroxynaphthaldehyde derivative sirtinol, the coumarin derivative splitomicin, the splitomicin derivative HR73 and cambinol (12), the tenovins (19), and the indole derivative EX527. The structure and the biochemical character of some of the representative inhibitors are summarized in Supplementary Table S2. Consistent with structural diversity, the inhibitors exert biological functions through different mechanisms, such as SIRT1 protein precipitation for sirtinol, competing with the histone peptide substrate but not NAD^+ for cambinol (12), and mimicking the effect of nicotinamide for tenovins (19). Importantly, a number of chemically synthesized epigenetic multiple ligands have been found to simultaneously inhibit nonhistone and histone methyltransferases, SIRT1 and SIRT2, and induce dramatic differentiation and apoptosis in cancer cells (20).

The only SIRT1 inhibitors that have been tested in animal models of cancer are cambinol and the tenovins. Cambinol selectively inhibits SIRT1 and SIRT2, causes BCL6 and p53 hyperacetylation and apoptosis in Burkitt lymphoma cells, and enhances chemotherapy-induced apoptosis in various cancer cells. In nude mice xenografted with Burkitt lymphoma cells, monotherapy with cambinol suppresses tumor growth (12). Tenovin 1 and tenovin 6 selectively inhibit SIRT1 and SIRT2, acetylate p53 and histone proteins, repress tumor cell growth, and induce apoptosis *in vitro*. When injected into nude mice xenografted with human melanoma cells as a monotherapy, tenovin 6 effectively represses tumor growth (19).

Implications and Future Directions

SIRT1 expression and deacetylase activity are repressed in normal nonmalignant cells by tumor suppressor proteins such as p53, Chk2, HIC1, and DBC1. The overexpression of oncogenes or the loss of function of tumor suppressor genes in precancer and cancer cells leads to SIRT1 overexpression or enzymatic activation, resulting in aberrant histone deacetylation and methylation, promoter CpG island methylation, transcriptional repression, deacetylation, and inactivation of nonhistone tumor suppressor proteins such as p53, FOXO proteins, Rb, E2F1, and Ku-70. Because these biological changes block senescence, cell differentiation, and stress-induced apoptosis, and promote cell growth, angiogenesis, blood supply, and resistance to chemotherapy, SIRT1 may play a critical role in tumor initiation, progression, and drug resistance. Further studies of the modulation of SIRT1 gene expression and deacetylase activity by tumor suppressor proteins and oncoproteins will shed light on the role of SIRT1 in tumor initiation and the role of SIRT1 inhibitors in cancer prevention. Further screening for more potent SIRT1 inhibitors and *in vivo* testing of their anticancer efficacy in animal models of cancer will eventually lead to compounds suitable for clinical trials in patients with precancer

conditions, and in cancer patients as monotherapy or in combination with other anticancer agents.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 9/7/2008; revised 11/24/2008; accepted 11/25/2008; published OnlineFirst 02/24/2009.

Grant support: Cancer Institute New South Wales (T. Liu, G. Marshall), Cure Cancer Australia (T. Liu, G. Marshall), and National Health and Medical Research Council Australia (T. Liu, G. Marshall).

Children's Cancer Institute Australia for Medical Research is affiliated with University of New South Wales and Sydney Children's Hospital.

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