



Function of Sirtuins in Cancer Stem Cells

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Abstract

Cancer stem cells (CSCs) are self-renewing cancer cells in tumors that are suggested to be responsible for tumor initiation, progression and relapse. The molecular mechanisms of CSCs biology could provide some novel managements for cancer treatment. Sirtuins, as a family of class III deacetylases, are recently found to play essential roles in the maintenance and differentiation of various cancer stem cells, including glioma stem cells (GSCs), colorectal cancer stem cells (CCSCs), breast cancer stem cells (BCSCs), pancreatic cancer stem cells (PCSCs), chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) stem cells. Herein, we reviewed the advanced studies that linking sirtuins, especially SIRT1 and SIRT2, to cancer stem cells and will also discuss how these enzymes could be used in biological pharmaceuticals of tumors.

Keywords

Sirtuins, Cancer stem cells, Epigenetic regulation, Pharmaceuticals

Abbreviations

ABCB5: ATP-binding Cassette B5, ADP: Adenosine Diphosphate, ALDH1: Aldehyde Dehydrogenase 1, ALDH1A1: Aldehyde Dehydrogenase 1A1, ALL: Acute Lymphoblastic Leukemia, AML: Acute Myeloid Leukemia, BAX: Bcl2-Associated X Protein, BCR-ABL: Breakpoint Cluster Region-C-Abl Oncogene 1, BCSC: Breast Cancer Stem Cell, CCSC: Colorectal Cancer Stem Cell, CD24: Cluster of Differentiation 24, CD44: Cluster of Differentiation 44, CD90: Cluster of Differentiation 90, CD133: Cluster of Differentiation 133, CD200: Cluster of Differentiation 200, CDK6: cyclin-dependent kinase 6, CTE: C-Terminal Extension, CLL: Chronic Lymphocytic Leukemia, CML: Chronic Myeloid Leukemia, CNSC: Cancerous Neural Stem Cell, CPEB1: Cytoplasmic Polyadenylation Element Binding Protein 1, CSC: Cancer Stem Cell, EpCAM: Epithelial Cell Adhesion Molecule, DNA: Deoxyribonucleic Acid, ESA: Epithelial Specific Antigen, FLT3-ITD: Fms-Like Tyrosine Kinase 3 Internal Tandem Duplication, GFAP: Glial Fibrillary Acidic Protein, GSC: Glioma Stem Cell, HAT: Histone Acetyltransferase, HCAM, Homing Cell Adhesion Molecule, HDAC: Histone Deacetylase, HIF-1 α : Hypoxia Inducible Factor 1 α , HSA, Heat Stable Antigen, HSC: Hematopoietic Stem Cell, LSC: Leukemia Stem Cells, MPT: Mitochondrial Permeability Transition, NAD: Nicotinamide Adenine Dinucleotide, NF- κ B: Nuclear Factor- κ B, NICD: Notch Intracellular Domain, NTE: N-Terminal Extension, OX2G: OX-2 Membrane Glycoprotein, PCSC: Pancreatic Cancer Stem Cell, PROM1: Prominin-1, ROS: Reactive Oxygen Species, Sox2: Sex Determining Region Y-box 2, SP: Side Population, TERT: Telomerase Reverse Transcriptase, THY1: Thy-1 Cell Surface Antigen, TKI: Tyrosine Kinase Inhibitor, USP22: Ubiquitin Specific Peptidase 22.

Short Review

In common tumor theories, tumor is an abnormal somatic cell proliferation caused by accumulation of random mutations in essential genes that control cell growth. However, the mutation theory have been challenged by various contradictions [1], such as oncogene activation appearance in normal cells, a slower growth rate of some tumor cells than that of normal cells, normal tissue development by injecting malignant teratoma cells into normal blastaea, silent metastatic cancers, tumor occurrence via transplanting normal tissue into the spleen, and outpouring relapse of cancers [2]. More importantly, minor subpopulations of cancer cells have aberrant self-renewal capacities to differentiate into several lineages of cancer cells. These self-renewing cancer cells are called cancer stem cells (CSCs), which are supposedly responsible for tumorigenesis, tumor spread, tumor resistance and tumor relapse [3].

As so far, CSCs can be identified by some cell surface markers such as cluster of differentiation 24 (CD24, or heat stable antigen CD24, HSA), cluster of differentiation 44 (CD44, also referred to as homing cell adhesion molecule, HCAM), cluster of differentiation 90 (CD90, also known as THY1), cluster of differentiation 133 (CD133, also known as prominin-1, PROM1), cluster of differentiation 200 (CD200, also called OX-2 membrane glycoprotein, OX2G), epithelial specific antigen (ESA, also called epithelial cell adhesion molecule, EpCAM), ATP-binding cassette B5 (ABCB5), nestin and so on [4]. The CSCs hypothesis provides a reasonable cellular mechanism to explain the therapeutic tolerances and duter behaviors exhibited in tumors, and CSCs has been suggested to be a significant barrier for efficient cancer therapy.

Recently, accumulative evidences showed that epigenetic mechanisms were key regulators during the process of differentiation from stem cells to specialized cells. In the development of tumors, aberrant epigenetic changes may help cancer cells to up or downregulate cancer stem cell markers in order to give rise a heterogeneous population of tumor cells [5]. Therefore, epigenetic regulation in cancer stem cells may provide new therapy for cancer treatment. Among them, histone acetylation is an important gene transcriptional regulation, which conducted by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDACs are grouped into 4 classes including class I, II, III and IV [6].

Class III HDACs, also called sirtuins, are a family of proteins composed of 7 members including SIRT1-7 [7]. As shown in figure 1, they are nicotinamide adenine dinucleotide (NAD⁺) dependent

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	N-terminal domain	Core	C-terminal domain	Size	Location
SIRT1	244	498	746aa	62.0 kDa	Euchromatin, cytoplasm
SIRT2	65	340	388aa	41.5 kDa	Cytoplasm
SIRT3	126	382	399aa	43.6 kDa	Mitochondria, cytoplasm
SIRT4	45	314aa	35.2 kDa	Mitochondria	
SIRT5	41	309	310aa	33.9 kDa	Mitochondria
SIRT6	35	274	355aa	39.1 kDa	Heterochromatin
SIRT7	90	331	400aa	44.8 kDa	Nucleolus

Figure 1: Schematic overview of *Homo. Sapiens* sirtuins family members SIRT1–7, NAD-binding pocket (green), zinc-binding domain (red), and their size and intracellular locations.

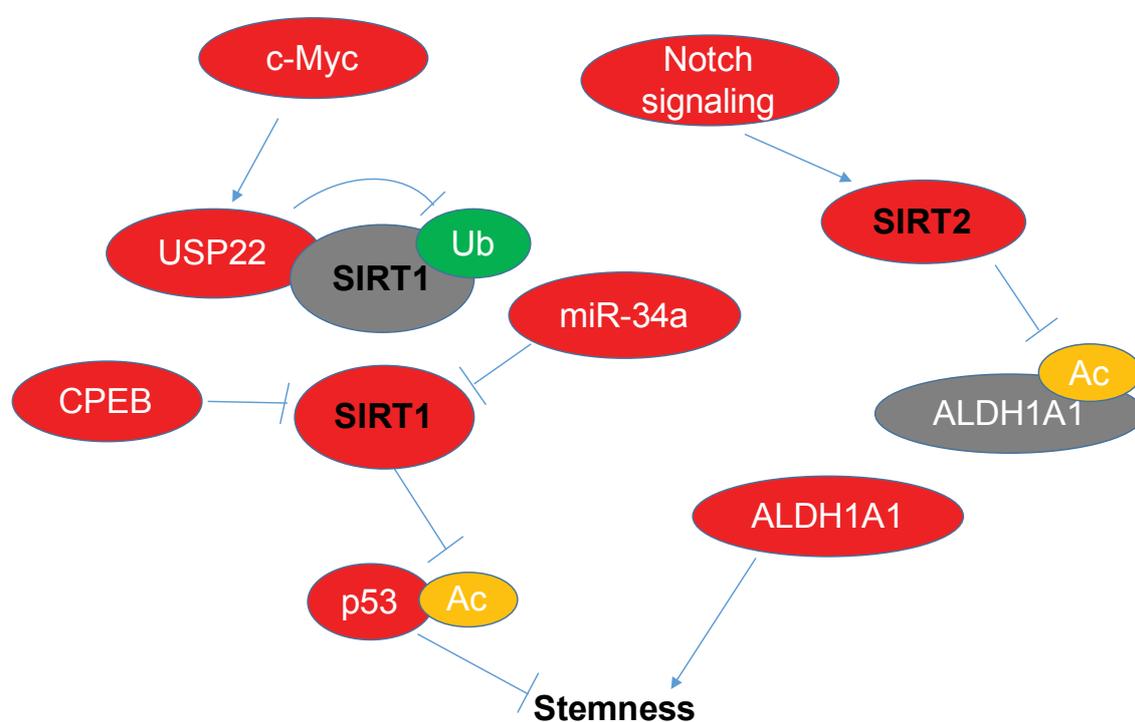


Figure 2: Schematic overview of molecular mechanisms of SIRT1/2 in CSCs, Ub: ubiquitination (green), Ac: acetylation (yellow), activated molecule (red), inactivated molecule (grey)

deacetylases and/or mono-[Adenosine diphosphate (ADP)-ribosyl] transferases that contain an N-terminal extension (NTE), a C-terminal extension (CTE), an NAD binding domain and a zinc-binding domain [8-10]. And they have different localizations: SIRT1/6/7 are localized in nucleus; SIRT1/2/3 are localized in cytoplasm; while SIRT3/4/5 are localized in mitochondria [10,11]. Sirtuins participated in multiple biological functions including chromatin structure and gene expression regulation, metabolic homeostasis, programmed cell death, aging, telomere activity, DNA repair, and cell differentiation [12]. Recently, more and more evidences showed that sirtuins, especially SIRT1 and SIRT2, played essential roles in the maintenance and differentiation of various cancer stem cells.

As the most important member in sirtuins, SIRT1 regulates multiple cellular processes including DNA repair, aging, metabolism, cell cycle, and survival [13] and plays an important role in maintaining self-renewal and differentiation of embryonic stem cells and hematopoietic stem cells (HSCs), especially under conditions of outside stress [14]. SIRT1 is a controversial histone deacetylase in tumorigenesis as it deacetylates both oncogenes such as β -catenin,

survivin, and nuclear factor- κ B (NF- κ B) and tumor suppressors such as p53 and fork-head class O (FOXO) transcription factor [15]. But recently studies showed that SIRT1 was highly expressed in cancer stem cells, including glioma stem cells (GSCs) [16], colorectal cancer stem cells (CCSCs) [17], breast cancer stem cells (BCSCs) [18], pancreatic cancer stem cells (PCSCs) [19], chronic myeloid leukemia (CML) [20] and acute myeloid leukemia (AML) stem cells [21] and acted as an important promoters in the maintenance and self-renew properties of these CSCs.

High expression of SIRT1 was found in CD133-positive glioma cells, which played an important role in tumor recovery and radiotherapy resistance. Knockdown of SIRT1 expression in these cells enhanced radiosensitivity and radiation-induced apoptosis *in vitro* and the mean survival rate of radiotherapy-treated mice bearing glioblastoma-CD133-positive tumors was significantly improved by SIRT1 knockdown as well [22]. Further study showed that SIRT1 increased expression in GSCs (CD133, sex determining region Y-box 2 (Sox2), and nestin positive) and cancerous neural stem cells (CNSCs) and played an important role in escaping p53-dependent

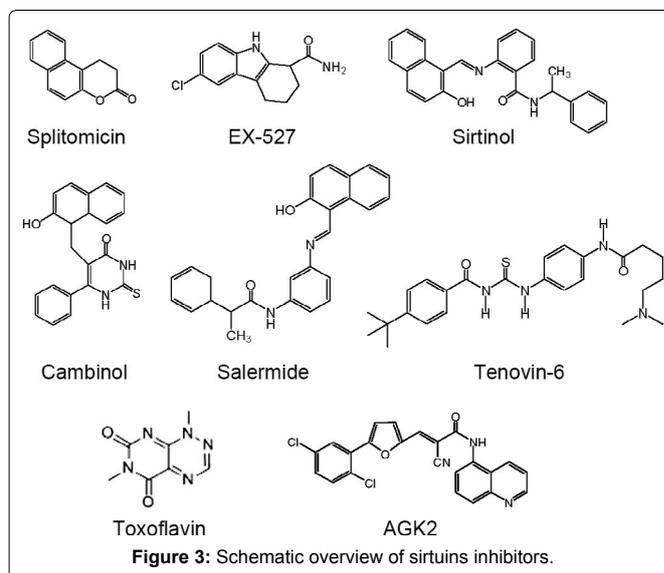
tumor surveillance, thus induced oncogenic transformation and retained the neural cancer stemness of the cells [16]. In addition, SIRT1 expression was decreased in cells of glial type (glial fibrillary acidic protein (GFAP)-positive) after differentiation from GSCs [16]. Besides, another study also showed that translation of SIRT1 in nestin and notch intracellular domain (NICD)-positive GSCs was suppressed by cytoplasmic polyadenylation element binding protein 1 (CPEB1), a key modulator that induced differentiation at the post-transcriptional level [23]. These evidences indicated that SIRT1 played an essential role in GSCs and could be a therapeutic target of GSCs.

SIRT1 is overexpressed in human leukemia stem cells (LSC) and its inhibition increased apoptosis in LSC and suppressed proliferation of CML stem cells or primitive progenitor cells. Besides, inhibition SIRT1 in CML stem cells facilitated to eliminate CSCs by Imatinib, a breakpoint cluster region-c-Abl oncogene 1 (BCR-ABL) tyrosine kinase inhibitor (TKI), in part via p53 activation [20]. In SIRT1 homozygous knockout BALB/c mice models, CD150-negative side population (SP) CML stem cells was also reduced and the maintenance of these cells was compromised. It seemed that SIRT1 knockout reduced cyclin-dependent kinase 6 (CDK6) expression and activated p53 through deacetylation instead of increasing its expression [24]. Except for CML stem cells, in primary human fms-like tyrosine kinase 3 internal tandem duplicated (FLT3-ITD) AML stem cells, SIRT1 was also selectively expressed in a high level and was enhanced by proto-oncogene c-MYC-induced high expression of ubiquitin specific peptidase 22 (USP22), a deubiquitinase (Figure 2). Besides, SIRT1 inhibition combined with FLT3 inhibitors promoted reduction of FLT3-ITD AML stem cells [21]. These evidences indicated that inhibition of SIRT1 network combined with TKIs or FLT3 inhibitors could be an attractive approach for BCR-ABL CML and FLT3-ITD AML treatment.

In addition, low level of miR-34a and high level of its target SIRT1 were found in CD44 positive and CD24-negative BCSCs, and further study showed that miR-34a inhibited proliferative potential of BCSCs and downregulated the expression of CSC markers aldehyde dehydrogenase 1 (ALDH1), polycomb ring finger proto-oncogene Bmi1 and homeobox transcription factor Nanog partially by downregulating SIRT1 (Figure 2) [18]. Such mechanism might also occur in PCSCs [19]. In addition, many colorectal cancer specimens had strong SIRT1 expression, which had an obvious correlation with poor prognosis of colorectal cancer patients. Besides, SIRT1 was highly expressed in colorectal CSC-like cells and its expression had a colocalization with CD133, a current common used marker of CCSCs. Further study showed that SIRT1 knockdown reduced proportion of CD133-positive cells, dampened colony and sphere formation *in vitro*, and restrained tumorigenicity *in vivo*. Importantly, the expressions of Oct4, Cripto, Nanog, telomerase reverse transcriptase (TERT) and Lin28, which are stemness-associated genes, were decreased by SIRT1 deficiency [17]. These evidence indicated that SIRT1 played a crucial role in keeping the characteristics of stemness in colorectal cancer cells and SIRT1 may act as a potential prognostic factor of tumor recovery risk for cancer patients, and will contribute to providing a new therapeutic method for cancer treatment.

SIRT2 was another deacetylase that is increasingly identified as a essential regulator of cell cycle, cellular necrosis and cytoskeleton reorganization [25]. It's recently reported to play an important role in carcinogenesis [26] and early lineage commitment of embryonic stem cell [27]. Recent research showed that SIRT2 could be induced by Notch signaling, thus leading to aldehyde dehydrogenase 1A1 (ALDH1A1) deacetylation and enzymatic activation to promote BCSCs (Figure 2) [28]. SIRT2 was also highly expressed in CSCs and its activity is required for CSCs proliferation arrest induced by resveratrol, a potent SIRT1 inhibitor [29]. However, in skin tumors but not normal skin, SIRT2 deletion up-regulates the stem cell marker CD34 [30]. No doubt, much more exploration should be conducted to make sure the exactly role of SIRT2 in CSCs.

Other members of sirtuins also have been demonstrated to play an important role in tumorigenesis and cancer development [31]. For



example, SIRT3 limits reactive oxygen species (ROS) levels in cancer cell, thus induces the degradation of hypoxia inducible factor 1 α (HIF-1 α) and high level of SIRT3 may suppress intracellular acidification, inhibit Bcl2-associated X protein (BAX) activation and subsequent mitochondrial permeability transition (MPT) induction [32]. SIRT4 inhibits mitochondrial glutamine metabolism and responses to deoxyribonucleic acid (DNA) damage in cancer cells [33]. SIRT6 regulates glucose metabolism in tumors and acts as a tumor suppressor [34]. SIRT7 deacetylates H3K18 and maintains oncogenic transformation [35]. In addition, these sirtuins, especially SIRT6 [36,37], SIRT7 [38] and SIRT3 [39], also have been demonstrated to play important roles in the maintenance and differentiation of normal stem cells. They might also act as key regulators in CSCs, but their roles are remained to be further explored.

CSCs are a small subset of cells that are responsible for initiation, development, and recurrence of tumors. Therefore, it is important to understand the molecular mechanism of CSCs for translational applications using CSCs in the treatment of patients with cancer. Sirtuins are an important family of HDACs and have been shown to tightly relate to CSCs and play essential roles in the maintenance and self-renew properties of CSCs. These evidences indicated that sirtuins must be important pharmaceutical targets for cancer therapy.

During past several years, some sirtuins inhibitors were identified and developed to anti-cancer drug candidates, including splitomicin analogs, the indole derivative EX-527 (selisistat), sirtinol, cambinol, salermide, tenovins, toxoflavin, and AGK2 [40] (Figure 3). Several splitomicin derivatives were shown to inhibit SIRT2 and exerted antiproliferative properties in MCF7 breast cancer cells [41]. Another small molecular compound, EX-527 is a cell-permeable, specific inhibitor of SIRT1 and shown to increase acetylation at Lys382 of p53 and activate it [42]. Above all, a potent human SIRT1/2 inhibitor, sirtinol [43] was shown to induce senescence-like growth arrest in breast cancer cells, lung cancer cells [44], and enhance chemosensitivity to cisplatin and camptothecin in prostate cancer cells [45]. Besides, Cambinol and salermide are also SIRT1/2 inhibitors and showed anticancer properties [46,47]. Recently, some studies also explored salermide derivatives as sirtuins inhibitors and tested its efficient in cancer stem cells [48,49]. Tenovin-6 was identified as SIRT1/2/3 inhibitor [50] and antitumor was showed in acute lymphoblastic leukemia (ALL) [51], chronic lymphocytic leukemia (CLL) [52], gastric cancer [53] and colon cancer [54]. Besides, a SIRT1 specific inhibitor toxoflavin was also screened out and showed good anticancer effect [55]. Another SIRT2 potent and selective inhibitor AGK2 was also shown to induce apoptosis in glioma [56]. These studies indicated that sirtuins inhibitor may be efficient drug candidates for cancer therapy, but their effects on CSCs are remained to be explored.

In conclusion, sirtuins play essential roles in CSCs regulation and could be candidate molecular target in cancer treatment. However, the exactly function and mechanism of sirtuins have not been fully elucidated, much more work should be carried out.

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