SIRT1 activators as novel therapy for cancer

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DOI: https://doi.org/10.32947/ajps.19.03.0410

Abstract:
Sirutins 1-7 (SIRT1-7) is an enzyme that depends on NAD+ to be activated, making it a member of the 3rd class of Deacetylase enzymes. SIRT1-7’s activity is involved with metabolism, cell survival and/or death as well as DNA repair, gene repression, inflammatory responses, the aging process, neuroprotection in addition to possibly helping with the treatment of cancer.

Molecules that could have a modifying effect on SIRT1-7’s activity has caught a great attention recently, owing to the fact of how beneficial this enzyme could be. In this review, we attempt to shed a light on these activator compounds and their use in Sirutin activation therapy, particularly SIRT1, for it is the most researched type. One of these compounds is Resveratrol, a natural compound – due to its SIRT 1 activation potential – could help in the treatment of obesity, prevention of tumor formation as well as decrease in heart function and neuronal loss related to aging; however, Resveratrol has poor bioavailability, which is why structurally reformulated compounds and molecules have been developed. Other molecules that are different from Resveratrol such as SRT1720, SRT2104 and SRT2379 in addition to others, have been used and shown greater activation potential for SIRT1 than Resveratrol.

Key words: SIRT1 - Deacetylase - Cancer

منشطات السرتوين 1 كعلاج جديد للسرطان

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الخلاصة:
سرتوين (SIRT1-7) هو الأنزيم يتمتع على النيكوتينوين ادينين دي نيوكلوتايد ليكون فعالاً مما يجعله في الفئة الثلاثة من الالتهابات المثبتة. يتمثل نشاط السرتوين (SIRT1-7) في عملية التثبيط الغذائي، بقاء الخلية، وظائفها وكذلك أصلح الحمض النووي وقمع الجينات والاستجابات الإنتهاطية وعملية الشيخوخة والوقاية العصبية بالإضافة إلى المساعدة في علاج السرطان. أولت بعض الجزيئات التي يمكن أن تكون لها تأثير على نشاط السرتوين (SIRT1-7) اهتماماً كبيرة مؤخراً، نظراً لحقيقة مدى فائدة هذا الانتزيم. في هذا المقال نحاول إلقاء الضوء على المركبات المشابهة للسرتوين واستخدامها في العلاج من خلال تثبيط السرتوين 1، من أكثر هذه المركبات هو الريسيراتروال، وهو مركب طبيعي - يفضل إمكاناته التشخيصية للسرتوين 1 يمكن أن يساعد في علاج السمنة، ومنع تكوين المرض، وكذلك انتفاخ في وظائف القلب وفقدان الخلايا العصبية المرتبطة.
Introduction

Sirtuins are group of proteins found in almost all living organisms from yeast to mammals. All are derivatives of the yeast protein known as silent information regulator 2 (Sir2) [1] and their primarily target acetylated lysines of various peptides and proteins, including histones. For that purpose, all sirtuins possess a catalytic domain and an extremely conserved core domain with NAD⁺-binding site [2]. Besides similarities in sequences, sirtuins of different organisms have similar functions even though the roles played by sirtuins in mammals are far more complicated than those performed in yeasts. Since sirtuins are mainly involved in ageing and cellular stress, they have been linked to disorders related to ageing such as arthrosclerosis [3], type II diabetes [4], cancer [5], Parkinson’s disease [6] and Alzheimer’s disease [7]. Sirtuins are involved in energy generation via NAD⁺-dependent deacetylation and o-ADP ribosylation. These reactions take place in response to alterations in the Nicotinamide adenine dinucleotide/Nicotinamide adenine dinucleotide phosphate (NAD⁺/NADPH) ratio of the cell. It seems they have role in prolonging life and promoting health in different organisms like yeasts, flies and nematodes [8]. This is also reflected by the fact that certain pharmacological agents, stress or caloric restriction can activate these proteins [9]. They are essentially involved in widening lifespan through caloric restriction in simple organisms [10]; [11]; [12]; [13]; [14]; [15].

Nevertheless, growing number of evidences support association between sirtuins and genomic instability, cancer processes and disorders of ageing. Most important of these evidences is considered to be the correlation between activity of sirtuin and cellular metabolic state [16]. Compared with typical deacetylases, sirtuins serve to be target-specific type III protein lysine deacetylases that support the connection between transcription regulation and cell metabolism. In short, an exclusive NAD⁺-dependent enzymatic reaction takes place during deacetylation. This enzymatic reaction initiates when an amide is cleaved from NAD⁺ resulting in Nicotinamide (NAM) production and formation of a covalent ADP-ribose-peptide-imidate intermediate (ADPR). As shown in (Figure 1) [17], the intermediate is converted to O-acetyl-ADP-ribose accompanied with release of deacetylated protein from the complex. Since activity of sirtuin depends on NAD⁺, it is understandable that a number of evidences supporting the link between NAD⁺, NAD⁺ generating pathways and activities of sirtuins are increasing.
Researchers have discovered seven sirtuins in humans i.e. SIRT1-7 [18]: [19]: [20]. SIRT1, 6 and 7 are predominantly located in nucleus, while SIRT3, 4 and 5 are chiefly found in mitochondria [21]. Also, SIRT1 and 2 are found in cytoplasm [22]: [23] as illustrated in (Figure 2). Two Nuclear Localization signals are located within SIRT1, and two nuclear exportation signals as well [23]. SIRT1 could be present in the nuclear or cytoplasmic compartments, which is determined by the balanced functionality of these signals. This also explains the difference in location of SIRT1 within different cell types and tissues. [24].

![Figure 1: Protein deacylation by Sirtuins](image1)

**Figure (1):** Protein deacylation by Sirtuins [17].

![Figure 2: All 7 proteins of the Sirutins family of histones have deacytlyase activity except for SIRT4](image2)

**Figure (2):** All 7 proteins of the Sirutins family of histones have deacytlyase activity except for SIRT4 [25].
**Sirtuin1**

The SIRT1 gene in humans encodes a protein called NAD-dependent deacetylase sirtuin-1 or simply sirtuin1 protein [26]. Other than histones, SIRT1 acts as a catalyst in the deacetylation process of a large number of non-histone substrates found in the nucleus and cytoplasm. It also plays a role in other different cell functions, such as stabilizing the genome, inflammation suppression, protection from neurodegeneration and improving plasticity of the synapses [27]. 747 amino acids combine to form human SIRT1 which can be split into four chief portions. Amino acids 1-182 constitute the N-terminal domain, amino acids 183-243 constitute the allosteric site, amino acids 244-498 constitute the catalytic core and amino acids 499-747 constitute the C-terminal domain [28]. The amino and carboxyl terminal domains have non-α, non-β structure and these disorganised regions of the protein work as flexible linkers with its particular substrate proteins. It has been determined through anchor analysis that 14 disorganised binding regions for specific substrates are present in SIRT1. Moreover, amino and carboxyl terminals are essentially involved in action of this protein.

**Role of SIRT1 in cellular functions and disease states**

In terms of functionality, human SIRT1 is the mammalian Sirtuin which is most extensively characterised. At the beginning, SIRT1 was thought to be involved in chromatin silencing and extension of lifespan mediated by calorie control; however, its role in different phenomena like metabolism, apoptosis, cell senescence, fat mobilisation, mitochondrial biogenesis, neuronal diseases and diabetes [29] (Figure 3) has recently been confirmed. Discovery of deacetylable activity of SIRT1 and its first non-histone substrate p53 had spurred researchers to identify different SIRT1 substrates ranging from enzymes that participate in metabolism to transcription factors [29] (Figure 3). SIRT1 plays its role in different processes like lipogenesis, insulin secretion, gluconeogenesis and fatty acid oxidation via deacetylation of transcription factors like PPARγ, PGC-1α, p53, NF-κβ and FOXO family. Previous studies have tackled the role of SIRT1 in diabetes, focusing more have on type 2 diabetes, where SIRT1 activation proved to enhance β-cell function, improve sensitivity for Insulin and increase the mass of β cell to offset hyperglycaemia [30]; [31]. The deacetylation carried out by SIRT1 can lead to different consequences depending on the kind of cell or tissue and other exterior factors like carcinogen or calorie constraint. For instance, when SIRT1 deacetylate PGC-1α, it results in upregulation of PGC-1α and induction of mitochondrial biogenesis in hepatocytes. Simultaneously, it can also cause a change in energy generation in skeletal muscles by amplifying oxidation of fatty acids and via glucose conservation [2]. A number of studies are aimed at exploration of SIRT1’s involvement in aging-related diseases like neurological diseases, diabetes and cancer. Its involvement in depression and anxiety has recently been deciphered [32]. Literature is rich in different arguments related to the involvement of SIRT1 in cancer. Some claim that it is an oncogene while other are of the view that it behaves as a tumour suppressor and to a great extent this is connected with deacetylation of p53 at Lys382 residue thereby repressing its transactivation capability [2].

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Figure (3): SIRT1 affects various cellular events. SIRT1 can deacetylate several proteins in the cell and thereby influence various cellular processes ranging from cell proliferation, metabolism to diseases [29].

Sirtuin1 and cancer
A significant increase in levels of SIRT1 has been recorded in patients of primary colon cancer [33], acute myeloid leukemia [34] and human prostate cancer [35]. Similarly, SIRT1 was found to be highly expressed in all forms of non-melanoma skin cancers like Bowen’s disease, actinic keratoses, and Squamous and basal cell carcinomas [36]. Considering these reports indicating increased expression of SIRT1 in cancers, a hypothesis that SIRT1 acts as a tumour promoter was developed [37]. Primary evidence supporting this hypothesis was provided by the studies demonstrating physical interaction between SIRT1 and p53. It was found that deacetylation of the p53 mediated by the SIRT1, particularly at Lys382 present on carboxyl end results in attenuation of activities executed by p53 [38]; [39].

Recently, two reports have shown that high degree of p53 acetylation resulting in upregulation of activities mediated by p53 occurs when SIRT1 activity is inhibited by the DBC1 (deleted in breast cancer-1). The DBC1 was previously cloned from a sequence (8p21) homozygously deleted in breast cancer. It makes a stable complex with SIRT1 thereby inhibiting its activity. In agreement with this, deacetylation of the p53 was promoted by knocking down DBC1 using RNA interference (RNAi). As a consequence, apoptosis mediated by the p53 was inhibited. These consequences were reverted in cells by RNAi-mediated lowering of endogenous SIRT1 simultaneously [40]; [41].

As shown in figure 5, SIRT1 also plays role in epigenetic silencing of the DNA-hyper methylated tumour suppressor genes (TSGs) in tumour cells. Re-expression of the tumour suppressor genes (TSGs) can be caused by SIRT1 inhibition which can be done in different ways i.e. using short RNAi, increased synthesis of a dominant negative protein or using a pharmacologic agent [42]. As a consequence, the cell signalling networks involved in causing cancer which are activated by reduced expression of the TSGs in several different tumours are blocked. In addition to these, E-cadherin gene gets re-expressed as a result of SIRT1 inhibition in colon and breast cancer cell lines. Protein coded by this gene forms complex with β-catenin and WNT signalling pathway which is constitutively active can be suppressed by
reactivation of this gene [43]. It has also been reported that SIRT1 behaves as a critical modulator of endothelial angiogenic activities. In vitro development of a vascular-like network is prevented by the inhibition of expression of SIRT1 gene [42]. Moreover, increased expression of wild-type SIRT1 caused amplified migratory and sprout forming activity of endothelial cells [44]. This was not the case with the SIRT1 H363Y (deacetylase-defective mutant of SIRT1) [38]; [39].

**Figure (4): Activation and inhibition of many cellular processes by SIRT1** [42].

**Sirtuin1 in DNA damage repair**

According to a hypothesis, the DNA damage repair is influenced by the SIRT1 as it alters the chromatin structure where there is damage. Transient SIRT1 inactivation at the site of damage can be caused by activation of the PARP which in turn occurs due to elevation in the NAD at the damage site. This leads to decondensation of the deacetylated inactivation thereby providing access to enzymes for the DNA damage repair [45]. Acetylation of the p53 induced by the DNA damage results in activation of p53 and either apoptosis or growth blockage. When the DNA is damaged, SIRT1 specifically binds with the Lys382 residue of the p53 protein present on the carboxyl end leading to its deacetylation. Alteration in this residue has shown to be linked with the activation of p53 as transcriptional factor. In human cells, transcriptional function of p53 is lowered by production of SIRT1. On the other hand, p53-mediated radio sensitivity and apoptosis is amplified by production of a catalytically inactive SIRT1. It implies that Sirt1 regulated function of p53 protein [38]. Androgen receptor (AR) signalling induced by dihydrotestosterone (DHT) needs activity of the NAD-dependent SIRT1 which deacetylates the lysine residue of the AR. Interactions between carboxyl and amino termini of the AR induced by coactivator are inhibited by SIRT1. It has also been reported that SIRT1 inhibits DHT-induced cell proliferation in prostate cancer. It indicates that there is a functional association between SIRT1 and AR this is a significant finding in the field of prostate cancer [46]. Conversely, analysis of public database by some other investigators revealed that expression of SIRT1 was decreased in several cancers like ovarian cancer, prostate cancer, bladder cancer and glioblastoma [47]. It has been shown by Firestein et al., that increased expression of
SIRT1 in the APCmin/+ mice results in a reduction in development of colon cancer. Accordingly, this decrease depends on the capability of the SIRT1 to cause deacetylation of β-catenin and promotion of cytoplasmic restriction of oncogenic forms of β-catenin which is restricted in nucleus [48]. It has recently been found that SIRT1 is crucial for the DNA damage repair and hence for maintenance of genetic integrity as shown in (Figure 4).

Role of SIRT1 in tumour suppression has also been reported by Yuan et al. According to them tumour suppression involves c-Myc-SIRT1 feedback loop that works for regulation of cellular transformation and c-Myc activity. Binding of c-Myc with the promoter of SIRT1 results in expression of SIRT1. But SIRT1 causes deacetylation of c-Myc thereby reducing its stability as shown in (Figure 4). Hence, transformational activity of c-Myc is at risk when SIRT1 is present [49]. In the p53+/- model, when expression of SIRT1 was increased in response to irradiation, frequency of fatal thymic lymphomas was low and average survival was high [2] indicating once again that SIRT1 behaves in a tumor suppressing manner. Function of SIRT1 as a tumour suppressor is evident from the abovementioned studies. Hence, risk of developing cancer can be decreased thereby allowing extension of lifespan through augmenting SIRT1 function to improve metabolic conditions [36].

Role played by SIRT1 in development of cancer remains questionable. Depending on the cellular location or targets, it can behave as tumour promoter as well as a tumour suppressor.

**Sirtuin 1 activation:**
Increasing SIRT1 activity pharmacologically has shown great effects in delaying age-related diseases as well as slowing the onset of aging. The silent information regulator (SIR) genes can in fact decrease the chances of cancer, diabetes and cardiovascular disease, and therefore can increase the longevity and arbitrate the beneficial effects of Calorie restriction (CR) [50]: [51]. The first evidence of the link between sirtuins and aging was discovered in budding yeast, where overexpression of the Sir2 gene led to suppression of rDNA circle formation-which is a known cause of aging in yeast-and therefore increased longevity of the species [52]. Increased lifespan of worms and flies was also the result of overexpression of Sir2 homologs [53], [14]. These results were challenged [54] but recently reaffirmed [55]: [56]. In mice, the effect of 2 Sirutins in lifespan extension was studied. Overexpression of SIRT6 overexpression increased the lifespan of male mice when attempted in the whole body [57], while overexpression of SIRT1 showed effect when overexpressed only in the brain [58]. Other studies showed that CR-mediated lifespan extension requires Sir2 in *S. cerevisiae* [59]: [60] and in flies [14]. In worms, while still controversial [61], multiple groups showed that the presence of SIRT1 is a partial requirement for the CR-mediated longevity effects, depending on the diet [62]: [63]. In mammals, SIRT1 proteins levels are induced by both fasting and by CR in numerous tissues [64], and deletion of SIRT1 prevents some of the behavioral changes and health benefits of CR [65]. Furthermore, genetically modified mice with whole body SIRT1 overexpression display phenotypes resembling CR [66], and those with SIRT1 overexpressed in their brains have longer lives [65]. An important discovery was the tissue specificity of the effect of SIRT1 in the CR response, where elimination of SIRT1 in the liver is expandable for this effect [67]. Compatible with its character in the CR response, upregulation of SIRT1 could have a reversing effect on a variety of age-related diseases in animal models, such as Alzheimer’s, Type II diabetes and even cancer [65]. A number of studies have shown that SIRT1 plays a protective role in the reduction of tumor numbers as well
as their growth, particularly in prostate and colon cancer.

**SIRT1 activator compound and cancer treatment:**

Evidence for clinical benefits of SIRT1-activating compounds (STAC) in cancer treatment have been recently revealed. [68]; [69]; [70]. These compounds are divided into three generations. Polyphenols such as resveratrol, quercetin and butein are included in first generation. Second generation STACs include SRT1720 and SRT1460, these are basically imidazothiazoles. Third generations STACs are basically benimidazoles and include STAC-9, STAC-5 and STAC-10 [71]; [72].

**Figure (5):** Sirtuin activating compounds (STACs) aiming at extending life and healthspan [71].

In the recent years, there has been a rapidly advancing target of finding molecules that slow aging. Small molecule SIRT1 activators include (A) Resveratrol (and similar phenols): an example of 1st generation molecules [73], (B) Imidazothiazoles: an example of 2nd generation molecules [74], and (C) Benimidazoles and urea-based scaffolds: examples of 3rd generation molecules [75]; [76].

Resveratrol is a natural polyphenol (3,4’,5-trihydroxy-trans-stilbene) and is obtained from grapes, peanuts, different berries and other natural plant sources. Studies have demonstrated its effect on initiation, promotion and progression, all three stages of cancer. This is achieved by modulation of signal transduction pathways which control cancer development. Extensive *in vitro* studies and clinical studies on rodents are available to describe its effects on wide spectrum of human tumours. Some natural metabolites of resveratrol have also been involved in tumour suppression and being studied further [77]. Piceatannol (trans-3,4,3′,5′-tetrahydroxystilbene or 3-hydroxyresveratrol) is a natural analog or metabolite of resveratrol. Its effects on controlling ovarian, colorectal and other cancer have been studied over the years and it exhibited promising results. The compound works by significantly reducing the proliferation rate of cancer cells. [78].

Quercetin, is another dietary polyphenol and is more potent than the resveratrol. It is found in leafy green vegetables, broccoli, green tea and black tea. It shows
anti-tumor, anti-oxidant and anti-inflammatory activities. It directly inhibits the proliferation of tumour cells thereby inhibiting its growth. [79]: [80]. Butein (3,4,2',4'-tetrahydroxychalone) is a plant-based polyphenol structurally belonging to the class of chalcones. Numerous studies involving the butein and its effect on the cancer cells showed that it inhibited the growth of cancer cells in bladder, breast, cervical cancer and more [81]. STAC1 or SRT1460 and STAC-2 have similar mechanism of action of activation of SIRT1 i.e. by lowering the peptide (KM) and producing other pharmacological actions but unlike resveratrol they show increased effects in inhibiting the tumour growth. However, these compounds are controversial and needs to be researched further. [73] STAC-5, STAC-9 and STAC-10 belong to the third generation of sirtuin activators and are chemically relate to the class of compounds called benzimidazoles. Various studies on its SAR revealed that they show potent bioactivities in the prevention and treatment of cancer. They also show antimicrobial and antiviral properties. [82]

SRT1720 having a structure N-[2-[3-(1-Piperazinylmethyl) imidazole [2,1-b] thiazol-6-yl] phenyl]-2-quinoxaline-carboxamide hydrochloride, is a controversial SRT1 activator compound. A study performed in animal model showed that it exhibits anti-tumour activity. It prevents the growth of multiple myeloma cells and induces apoptosis followed by the activation of caspases, production of ROS and other biochemical pathways. [83]

Another study performed on the effects of SRT1720 on the breast cancer cells concluded that it inhibited the growth of cancer cells by the process of necrosis. Its effects were more profound in tumours containing increased SIRT1. However, furthers studies needs to be performed to eliminate the doubt that it promotes tumour in few cases. [84].

Conclusions
Sirtuins belong to the class of deacetylases and are known to be responsible for the expression of genes in response to that of different metabolic status. Studies have demonstrated that the modulation of SIRT1 results in inhibition of tumours. Above that numerous studies have confirmed the wide range of health benefits provided by the regulation of SIRT1. These include metabolism, apoptosis, cell senescence, fat mobilization, mitochondrial biogenesis, neuronal diseases and diabetes.
SIRT1 performs functions such as DNA damage repair and tumour suppression. SIRT1 repairs the damages DNA by the virtue of its property that it can alter the structure of chromatin. Although the role of SIRT1 as tumour suppressor when activated needs to be studied further but the data available currently indicates that SIRT1 activation via STACs can open gateways to multiple novel therapeutics for the treatment of cancer. Mostly these STACs follow the apoptotic pathway for the control of tumour, however, they might also control tumour via the necrosis.
Resveratrol, butein and other STAC agents of first generation activated SIRT1 and inhibit the growth and development of tumour in various animal and clinical models. However, a large number of variants of resveratrol are currently under study. Piceatannol is a natural analog of resveratrol and it reduces the proliferation rate of cancer cells in multiple cancers such as ovarian, colon and rectal. Second generation STACs such as SRT1720, SRT1460 are also found to be associated with the inhibition of tumour growth, however, they are shown to be involved in promoting the metastasis too. The fact that the new analogs show anti-cancer effects increased up too many folds signifies that further research on SIRT1 activators can allow us to develop drugs and compounds that can effectively control, inhibit and prevent cancer growth.
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