SIRT1 activators as novel therapy for cancer

Marwah Saad Joudah*, Basma Talib Al-Sudani, Inam Sameh Arif ***
College of Pharmacy, Branch of Pharmacology and Toxicology, Mustansiriyah University, Iraq.
**** College of Pharmacy, Branch of Clinical Laboratory Sciences, Mustansiriyah University, Iraq.***

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

Article Info:

Received 22 Apr 2019
Accepted 20 June 2019
Published 1 Aug 2019

Corresponding Author email:
basma_alsudani@uomustansiriyah.edu.iq
orcid: https://orcid.org/0000-0001-6253-3599

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 that –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 ١ كعلاج جديد للسرطان

***انعام سامح عارف ، **بسمة طالب السوداني ،*مروة سعد جودة**
*** كلية الصيدلة، فرع الادوية والسموم، الجامعة المستنصرية، العراق***
**** كلية الصيدلة، فرع العلوم المختبرية، الجامعة المستنصرية، العراق***

الخلاصة:

سرتوين (٧-١) هو انزيم يعتمد على النيكوتين ادنين داي نيوكليوتايد ليكون فعالا مما يجعله في الفئة الثالثة من الانزيمات المثبطة. يتمثل نشاط السرتوين (٧-١) في عملية التمثيل الغذائي، بقاء الخلية او وفاتها وكذلك اصلاح الحمض النووي وقمع الجينات والاستجابات الالتهابية وعملية الشيخوخة والوقاية العصبية بالاضافة الى المساعدة في علاج السرطان. أولت بعض الجزيئات التي يمكن ان يكون لها تأثيرعلى نشاط السرتوين (٧-١) اهتماما كبيرا مؤخرا، نظرا لحقيقة مدى فائدة هذا الانزيم. في هذا المقال نحاول القاء الضوء على المركبات المنشطة للسرتوين١ واستخدامها في العلاج من خلال تنشيط السرتوين ١، من اكثر هذه المركبات هو الريسفيراترول، وهو مركب طبيعي- بفضل امكاناته التنشيطيه للسرتوين١ يمكن ان يساعد في علاج السمنة، ومنع تكوين المرض، وكذلك انخفاض في وظائف القلب وفقدان الخلايا العصبية المرتبطة

بالشيخوخة؛ ومع ذلك، فان الريسفيراترول يتسم بضعف التوافر البايلوجي، ولهذا السبب تم تطوير المركبات والجزيئات المعدلة هيكليا. تم استخدام جزيئات اخرى مختلفة عن الريسفيراترول مثل سرتوين١٧٢٠، سرتوين٢١٠٤ وسرتوين٢٣٧٩ بالاضافة الى غيرها، وقد اظهرت امكانية تنشيط اكبر للسرتوين١ من ريسفيراترول.

الكلمات المفتاحية: سرتوين١ - انزيم مثبط – سرطان

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 deacelyated 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.

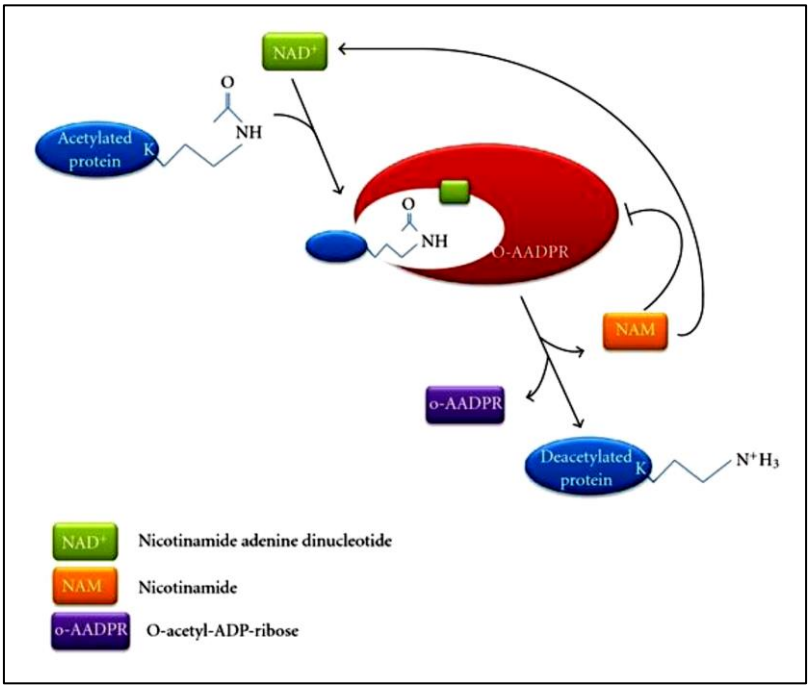

Figure (1): Protein deacetylation by Sirtuins [17].

Researchers have discovered seven sirtuins in humans i.e. SIRT1-7 [18]; [19]: [20]. SIRT1, 6 and 7are 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].

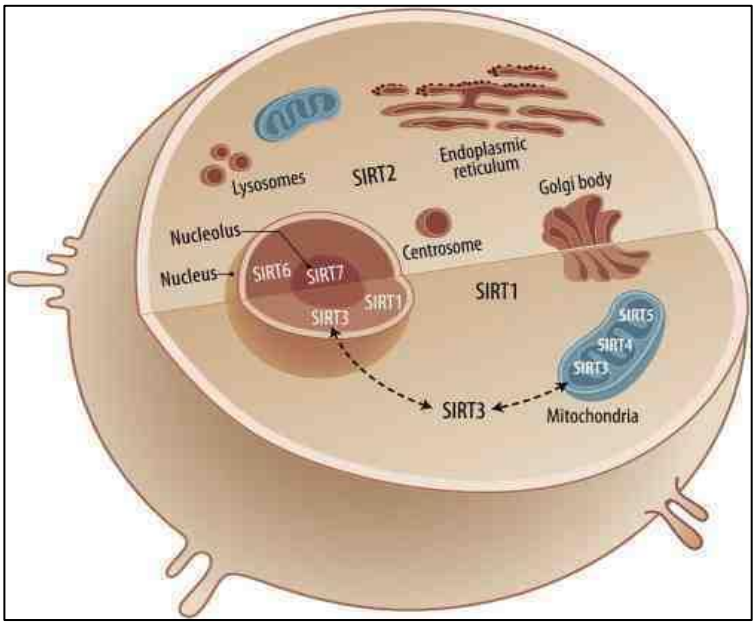

Figure (2): All 7 proteins of the Sirutins family of histones have deacetylase activity except for SIRT4 [25].

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 deacetylate 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 ß-cell function, improve sensitivity for Insulin and increase the mass of ß 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].

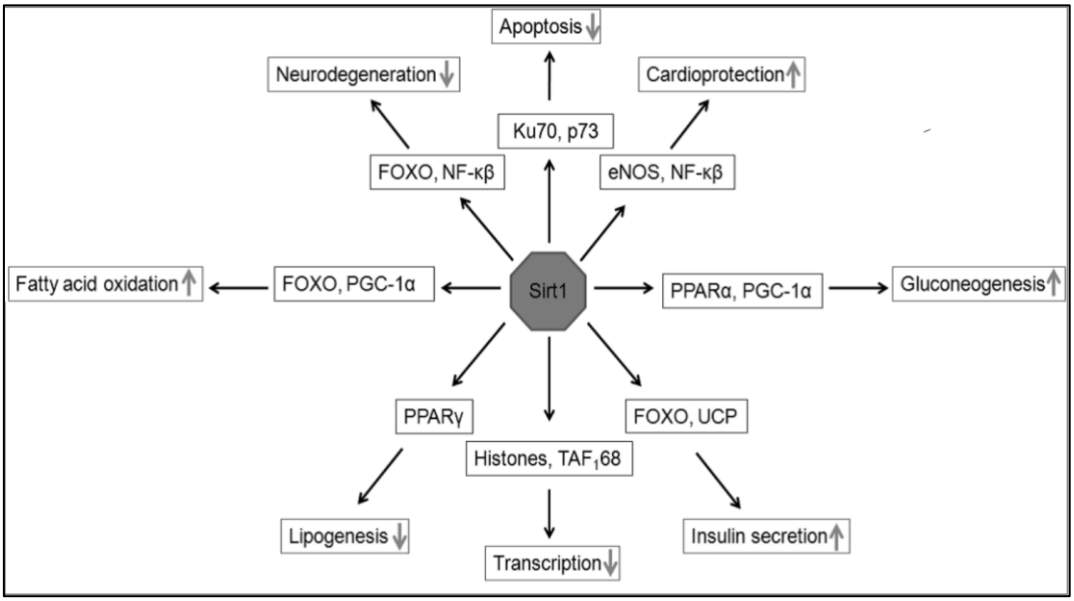

Figure (3): SIRT1 affects various cellular events. SIRT1 can deacetylate several proteins in the cell and thereby influence various cellular processes ranging from cell proliferation, metabolism to diseases [29].

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 keratosis, 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].

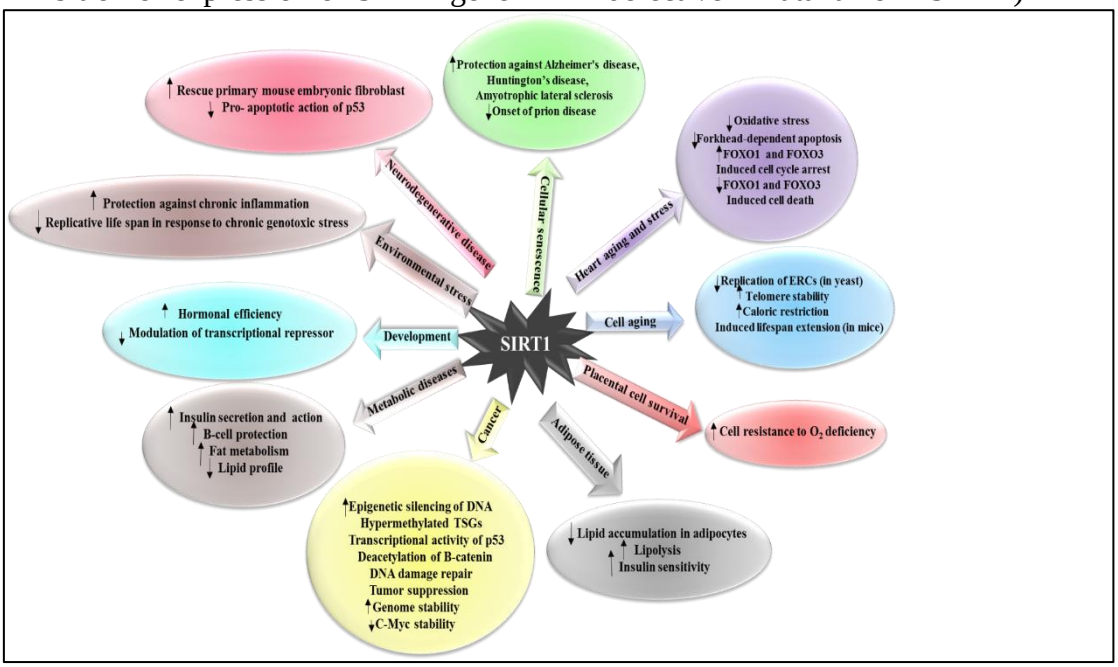

Figure (4): Activation and inhibition of many cellular processes by SIRT1 [42].

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 de-condensation 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 SIIRT1 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 imidazo-thiazoles. Third generations STACs are basically benzimidazoles and include STAC-9, STAC-5 and STAC-10 [71]; [72].

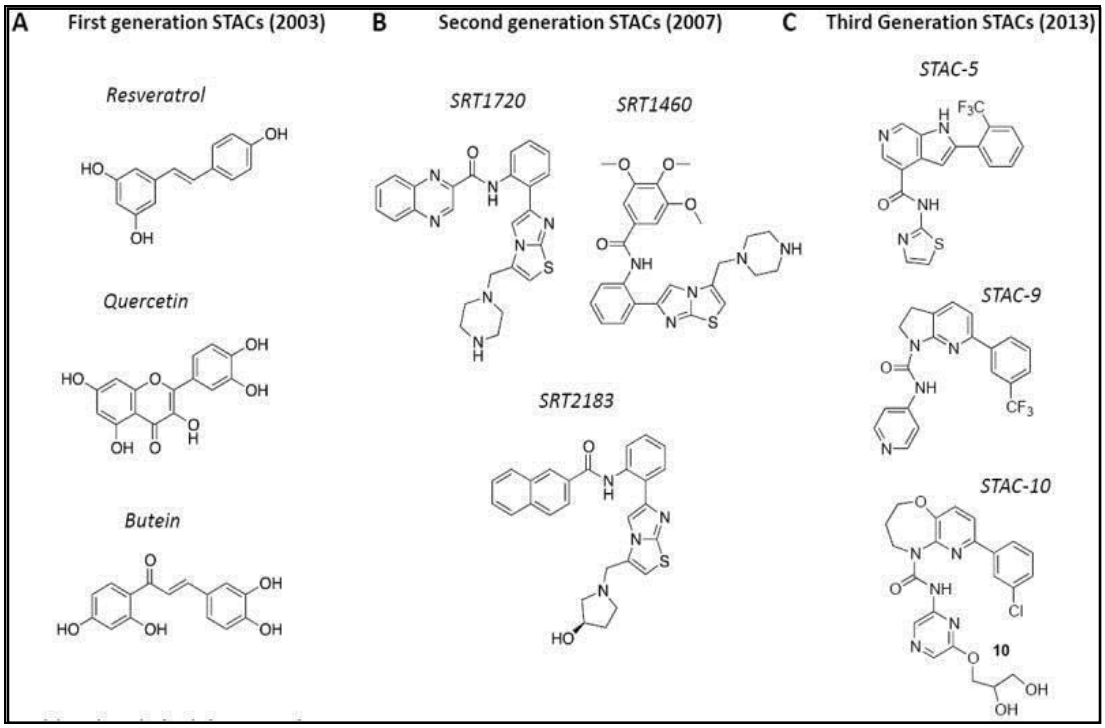

Figure (5): Sirtuin activating compounds (STACs) aiming at extending life and healthspan [71].

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) Benzimidazoles 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 (K_M) 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.

References:

1- Rine, J., Strathern, J.N., Hicks, J.B., Herskowitz, I. A suppressor of mating-type locus mutations in Saccharomyces cerevisiae: evidence for and identification of cryptic mating-type loci, (1979). Genetics 93(4), 877–901.
2- Haigis, M.C., and Sinclair, D.A. Mammalian sirtuins: biological insights and Disease relevance. (2010). Annu Rev Pathol 5, 253-295.
3- Ota, H., Eto, M., Ogawa, S., Iijima, K., Akishita, M., Ouchi, Y. Sirt1/eNOS axis as a potential target against vascular senescence, dysfunction and atherosclerosis. Journal of Atherosclerosis and Thrombosis, (2010). 17(5), 431–435.
4- Avogaro, A., De Kreutzenberg, S.V., Fadini, G.P. Insulin signaling and life span. Pflugers Archive European Journal of Physiology, (2010). 459(2), 301–314.
5- Schumacker, P.T. A tumor suppressor SIRTainty. Cancer Cell, (2010). 17(1), 5–6.
6- Esteves, A.R., Lu, J., Rodova, M., et al., Mitochondrial respiration and respiration- associated proteins in cell lines created through Parkinson's subject mitochondrial transfer. Journal of Neurochemistry, (2010). 113(3), 674–682.
7- Albani, D., Polito, L., Forloni, G., Sirtuins as novel targets for Alzheimer's disease and other neurodegenerative disorders: experimental and genetic evidence. Journal of Alzheimer's Disease, (2010). 19(1), 11–26.
8- Dali-Youcef, N., Lagouge, M., Froelich, S., Koehl, C., Schoonjans, K., Auwerx, J., Sirtuins: the ’magnificent seven’, function, metabolism and longevity. Annals of Medicine, (2007). 39(5),335–345.
9- Qiu, X., Brown, K.V., Moran, Y., Chen, D., Sirtuin regulation in calorie restriction. Biochimica et Biophysica Acta., (2010). 1804(8), 1576–1583.
10- Hamilton, B., Dong, Y., Shindo, M., et al., A systematic RNAi screen for longevity genes in C. elegans. Genes and Development, (2005). 19(13), 1544–1555.
11- Hansen, M., Taubert, S., Crawford, D., Libina, N., Lee, S.J., Kenyon, C., Lifespan extensio by conditions that inhibit translation in Caenorhabditis elegans. Aging Cell, (2007). 6(1), 95–110.
12- Kaeberlein, M., McVey, M., Guarente, L., The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes and Development., (1999). 13(19), 2570–2580.
13- Mueller, I., Zimmermann, M., Becker, D., Floemer, M., Calendar life span versus budding life span of Saccharomyces cerevisiae. Mechanisms of Ageing and Development, (1980). 12(1), 47–52.
14- Rogina, B., Helfand, S.L., Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proceedings of the National Academy of Sciences of the United States of America, (2004). 101(45), 15998–16003.
15- Sinclair, D.A., Guarente, L., Extra chromosomal rDNA circles—a cause of aging in yeast. Cell, (1997). 91(7), 1033–1042.
16- Ghosh, S., George, S., Roy, U., Ramachandran, D., Kolthur-Seetharam, U., NAD: a master regulator of transcription. Biochimica et Biophysica Acta., (2010). 1799(10-12), 681– 693.
17- Sauve, A.A., Wolberger, C., Schramm, V.L., Boeke, J.D., The biochemistry of sirtuins. Annual Review of Biochemistry, (2006). 75, 435–465.
18- Frye, R.A., Phylogenetic classification of prokaryotic and eukaryotic Sir2-

like proteins. Biochemical and Biophysical Research Communications,(2000). 273(2):793–798.
19- Michishita, E., Park, J.Y., Burneskis, J.M., Barrett, J.C., Horikawa, I., Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. Molecular Biology of the Cell, (2005). 16(10), 4623–4635.
20- Michan, S., and Sinclair, D., Sirtuins in mammals: insights into their biological function. Biochem, (2007). J 404, 1-13.
21- Huang, J.Y., Hirschey, M.D., Shimazu, T., Ho, L., Verdin, E., Mitochondrial sirtuins. Biochimica et Biophysica Acta, (2010). 1804(8), 1645–1651.
22- North, B.J., Verdin, E., Interphase nucleo-cytoplasmic shuttling and localization of SIRT2 during mitosis. PLoS One., (2007). 2(8), 784.
23- Tanno, M., Sakamoto, J., Miura, T., Shimamoto, K., Horio, Y., Nucleocytoplasmic shuttling of the NAD+-dependent histone deacetylase SIRT1. Journal of Biological Chemistry, (2007). 282(9), 6823–6832.
24- Canto´, C. and Auwerx, J., Targeting Sirtuin 1 to Improve Metabolism: All YouNeed Is NAD? Pharmacol Rev., (2012). 64, 166–187.
25- David, J., A Reversible Cause of Aging.Tsunster Innovations.Dec 27(2013).
26- Frye, R.A., "Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity". Biochem. Biophys. Res. Commun., (1999). 260 (1), 273–9.
27- Donmez, G., and Outeiro, T. F. SIRT1 and SIRT2: emerging targets in neurodegeneration. EMBO Mol. Med. (2013). 5, 344–352.
28- Autiero, I., Costantini, S., Colonna, G., Human Sirt-1: molecular modeling and structure-function relationships of an unordered protein. PLoS One, (2009). 4, 7350.
29- Lakshminarasimhan, M., Biochemical and structural characterization of Sirtuins from mammals and Thermotoga maritime. Thesis, (2012).
30- Gilbert RE, et al. SIRT1 activation ameliorates hyperglycaemia by inducing a torpor-like state in an obese mouse model of type 2 diabetes. Diabetologia. 2015; 58:819–827. doi: 10.1007/s00125-014-3485-4.
31- Luu L, et al. The loss of Sirt1 in mouse pancreatic beta cells impairs insulin secretion by disrupting glucose sensing. Diabetologia. 2013; 56:2010–2020. doi: 10.1007/s00125-013-2946-5.
32- Libert, S., Pointer, K., Bell, Eric L., Das, A., Cohen, Dena E., Asara, John M., Kapur, K.,Bergmann, S., Preisig, M., Otowa, T., et al., SIRT1 Activates MAO-A in the Brain to Mediate Anxiety and Exploratory Drive . Cell, (2011). 147, 1459–1472.
33- Stunkel, W., Peh, B.K., Tan, Y.C., Nayagam, V.M., Wang, X., Salto-Tellez, M., Ni, B., Entzeroth, M., Wood, J., Function of the SIRT1 protein deacetylase in cancer. Biotechnol J., (2007). 2, 1360–1368.
34- Bradbury, C.A., Khanim, F.L., Hayden, R., Bunce, C.M., White, D.A., Drayson, M.T. et al., Histone deacetylases in acute myeloid leukaemia show a distinctive pattern of expression that changes selectively in response to deacetylase inhibitors. Leukemia, (2005). 19: 1751–1759.
35- Huffman, D.M., Grizzle, W.E., Bamman, M.M., Kim, J.S., Eltoum, I.A., Elgavish, A., Nagy, T.R., SIRT1 is significantly elevated in mouse and human prostate cancer. Cancer Res., (2007). 67, 6612–6618.
36- Deng, C.X., SIRT1, Is It a Tumor Promoter or Tumor Suppressor? Int J Biol Sci., (2009). 5, 147–152.

37- Lim, C.S., SIRT1: Tumor promoter or tumor suppressor? Med Hypotheses., (2006). 67, 341–344.
38- Vaziri, H., Dessain, S.K., Ng Eaton, E., Imai, S.I., Frye, R.A., Pandita, T.K., Guarente, L., Weinberg, R.A., SIR2 (SIRT1) functions as an NAD-dependent p53 deacetylase. Cell, (2001). 107, 149–159.
39- Luo, J., Nikolaev, A.Y., Imai, S., Chen, D., Su, F., Shiloh, A., Guarente, L., Gu, W., Negative control of p53 by Sir2alpha promotes cell survival under stress. Cell, (2001). 107, 137– 148.
40- Zhao, W., Kruse, J.P., Tang, Y., Jung, S.Y., Qin, J., Gu, W., Negative regulation of the deacetylase SIRT1 by DBC1. Nature, (2008). 451, 587–590.
41- Kim, J.E., Chen, J., Lou, Z., DBC1 is a negative regulator of SIRT1. Nature, (2008). 451, 583–586.
42- Rahman, S. and Islam, R., Mammalian Sirt1insights on its biological functions. Cell Communication and Signaling, (2011). 9 (1), 11.
43- Pruitt, K., Zinn, R.L., Ohm1, J.E., McGarvey, K.M., Kang1, S.L., Watkins, D.N., Herman, J.G., Baylin, S.B., Inhibition of SIRT1 reactivates silenced cancer genes without loss of promoter DNA hypermethylation. PLoS Genet, (2006). 2, 40.
44- Potente, M., Ghaeni, L., Baldessari, D., Mostoslavsky, R., Rossig, L., Dequiedt, F., Haendeler, J., Mione, M., Dejana, E., Alt, F.W., Zeiher, A.M., Dimmeler, S., SIRT1 controls endothelial angiogenic functions during vascular growth. Genes Dev., (2007). 21, 2644–2658.
45- Kruszewski, M., and Szumiel, I. Sirtuins (histone deacetylases III) in the cellular response to DNA damage--facts and hypotheses. DNA Repair (Amst) (2005). 4, 1306-1313.
46- Gerhart-Hines, Z., Rodgers, J.T., Bare, O., Lerin, C., Kim, S.H., Mostoslavsky, R., Alt, F.W., Wu, Z., Puigserver, P., Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. EMBO J., (2007). 26, 1913–1923.
47- Wang, R.H., Sengupta, K., Li, C., Kim, H.S., Cao, L., Xiao, C., Kim, S., Xu, X., Zheng, Y., Chilton, B., Jia, R., Zheng, Z.M., Appella, E., Wang, X.W., Ried, T., Deng, C.X., Impaired DNA damage response, genome instability, and tumorigenesis in SIRT1 mutant mice. Cancer Cell., (2008). 14, 312–323.
48- Firestein, R., Blander, G., Michan, S., Oberdoerffer, P., Ogino, S., Campbell, J., Bhimavarapu, A., Luikenhuis, S., de Cabo, R., Fuchs, C., Hahn, W.C., Guarente, L.P., Sinclair, D.A., (2008). The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth. PLoS ONE, (2008). 3,2020.
49- Yuan, J., Minter-Dykhouse, K., Lou, Z.A., c-Myc-SIRT1 feedback loop regulates cell growth and transformation. J Cell Biol., (2009). 185,203–211.
50- Haigis MC, Guarente LP. Mammalian sirtuins--emerging roles in physiology, aging, and calorie restriction. Genes Dev. 2006; 20:2913–2921. [PubMed: 17079682]
51- Bordone L, Guarente L. Calorie restriction, SIRT1 and metabolism: understanding longevity. Nat Rev Mol Cell Biol. 2005; 6:298–305. [PubMed: 15768047]
52- Blander G, Guarente L. The Sir2 family of protein deacetylases. Annu Rev Biochem. 2004; 73:417–435. [PubMed: 15189148]
53- Tissenbaum HA, Guarente L. Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. Nature. 2001; 410:227–230. [PubMed: 11242085]
54- Burnett, C., Valentini, S., Cabreiro, F., Goss, M., Somogyv_ari, M., et al., Absence of effects of Sir2 overexpression on lifespan in C.

elegans and Drosophila, Nature, (2011). 477, 482–485.
55- Viswanathan M, Guarente L. Regulation of Caenorhabditis elegans lifespan by sir-2.1 transgenes. Nature. 2011; 477:E1–2. [PubMed: 21938026]
56- Banerjee KK, et al. dSir2 in the adult fat body, but not in muscles, regulates life span in a dietdependent manner. Cell Rep. 2012; 2:1485–1491. [PubMed: 23246004]
57- Kanfi Y, et al. The sirtuin SIRT6 regulates lifespan in male mice. Nature. 2012; 483:218–221. [PubMed: 22367546]
58- Satoh A, et al. Sirt1 Extends Life Span and Delays Aging in Mice through the Regulation of Nk2 Homeobox 1 in the DMH and LH. Cell Metab. 2013; 18:416–430. [PubMed: 24011076]
59- Anderson RM, et al. Nicotinamide and PNC1 govern lifespan extension by calorie restriction in Saccharomyces cerevisiae. Nature. 2003; 423:181–185. [PubMed: 12736687]
60- Lin SJ, et al. Calorie restriction extends Saccharomyces cerevisiae lifespan by increasing respiration. Nature. 2002; 418:344–348. [PubMed: 12124627]
61- Greer EL, Brunet A. Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in C. elegans. Aging Cell. 2009; 8:113–127. [PubMed: 19239417]
62- Wang Y, Tissenbaum HA. Overlapping and distinct functions for a Caenorhabditis elegans SIR2 and DAF-16/FOXO. Mech Ageing Dev. 2006; 127:48–56. [PubMed: 16280150]
63- Raynes R, et al. Heat shock and caloric restriction have a synergistic effect on the heat shock response in a sir2.1-dependent manner in Caenorhabditis elegans. J Biol Chem. 2012; 287:29045– 29053. [PubMed: 22778258]
64- Cohen, H. Y., Miller, C., Bitterman, K. J., Wall, N. R., Hekking, B., et al., Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase, Science (2004). 305, 390–392.
65- Morris BJ. Seven sirtuins for seven deadly diseases of aging. Free Radic Biol Med. 2013; 56:133– 171. [PubMed: 23104101]
66- Bordone L, et al. SIRT1 transgenic mice show phenotypes resembling calorie restriction. AgingCell. 2007; 6:759–767. [PubMed: 17877786]
67- Chen D, et al. Tissue-specific regulation of SIRT1 by calorie restriction. Genes Dev. 2008; 22:1753–1757. [PubMed: 18550784]
68- Harper CE, Patel BB, Wang J, Arabshahi A, Eltoum IA, Lamartiniere CA. Resveratrol suppresses prostate cancer progression in transgenic mice. Carcinogenesis 2007; 28:1946–53.
69- Harikumar KB, Kunnumakkara AB, Sethi G, Diagaradjane P, Anand P, Pandey MK, et al. Resveratrol, a multitargeted agent, can enhance antitumor activity of gemcitabine in vitro and in orthotopic mouse model of human pancreatic cancer. Int J Cancer 2010; 127:257–68.
70- Gong J, Wang H, Lou W. Associations of sirtuins with clinicopathological parameters and prognosis in non–small cell lung cancer. Cancer Manag Res. 2018; 10: 3341–3356
71- Hubbard BP, Sinclair DA. Small molecule SIRT1 activators for the treatment of aging and age-related diseases. Trends Pharmacol Sci 2014;35: 146–54.
72- Carafa V, Altucci L, and Nebbioso A. Dual Tumor Suppressor and Tumor Promoter Action of Sirtuins in Determining Malignant Phenotype. Front Pharmacol. 2019; 10: 38.
73- Howitz, K. T., Bitterman, K. J., Cohen, H. Y., Lamming, D. W., Lavu, S., Wood, J. G., Zipkin, R. E., Chung,

P., Kisielewski, A., Zhang, L. L., Scherer, B., and Sinclair, D. A. (2003) *Nature* 425**,** 191–196.

74- Milne, J.C., Lambert, P.D., Schenk, S., Carney, D.P., Smith, J.J., Gagne, D.J., Jin, L., Boss, O., Perni, R.B., Vu, C.B., et al. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. (2007) Nature 450, 712–716.

75- Dai H, et al. SIRT1 activation by small molecules: kinetic and biophysical evidence for direct interaction of enzyme and activator. J Biol Chem. 2010; 285:32695–32703. [PubMed: 20702418]

76- Hubbard BP, et al. Evidence for a common mechanism of SIRT1 regulation by allosteric activators. Science. 2013; 339:1216–1219. [PubMed: 23471411]

77- Bishayee A. Cancer prevention and treatment with resveratrol: from rodent studies to clinical trials. Cancer prevention research. 2009 Apr 28:1940-6207.

78- Wolter F, Clausnitzer A, Akoglu B, Stein J. Piceatannol, a natural analog of resveratrol, inhibits progression through the S phase of the cell cycle in colorectal cancer cell lines. The Journal of nutrition. 2002 Feb 1;132(2):298-302.

79- Jeong JH, An JY, Kwon YT, Rhee JG, Lee YJ. Effects of low dose quercetin: Cancer cell-specific inhibition of cell cycle progression. Journal of cellular biochemistry. 2009 Jan 1;106(1):73-82.

80- Alcaín FJ, Villalba JM. Sirtuin activators. Expert opinion on therapeutic patents. 2009 Apr 1;19(4):403-14.

81- Sinclair DA, Guarente L. Small-molecule allosteric activators of sirtuins. Ann Rev Pharmacol Toxicol 2014; 54:363–80.

82- Akhtar W, Khan MF, Verma G, Shaquiquzzaman M, Rizvi MA, Mehdi SH, Akhter M, Alam MM. Therapeutic evolution of benzimidazole derivatives in the last quinquennial period. European journal of medicinal chemistry. 2017 Jan 27; 126:705-53.

83- Chauhan D, Bandi M, Singh AV, Ray A, Raje N, Richardson P, et al. Preclinical evaluation of a novel SIRT1 modulator SRT1720 in multiple myeloma cells. Br J Haematol 2011; 155:588–98.

84- Lahusen TJ, Deng CX. SRT1720 induces lysosomal-dependent cell death of breast cancer cells. Mol Cancer Therap 2015; 14:183–92.