International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 8
August 2017

In Silico Protein – Protein Interaction Studies Of Sirtuins As Anti-Inflammatory And Anti-Cancer Agents

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ABSTRACT

Sirtuins are NAD+ dependent deacetylase found nearly in all species, have comparable activities in human physiology and several diseases.Sirtuins (SIRT) has diverse roles in various biological processes, including caloric restrictionchanges in glucose metabolism, tumorigenesis and life span.Sirtuin modulates the inflammatorytranscription factor and various cancers pathways that leads to major role in cancer. From several decades, scientist's areknown that activation of sirtuins extends life span and inhibits tumorigenesis. The in silico experimental reports shown that all types of Sirtuins are showing protein interactions that are related to anti-inflammatory and anticancer proteins.

Keywords: Sirtuins, Anti-Ageing

INTRODUCTION

Inflammation is a fundamental defensive response that sometimes goes askew and becomes a major cofactor in the pathogenesis of many chronic human diseases, including cancer. Inflammatory responses play critical roles at different stages of tumor growth, including initiation, promotion, malignant conversion, invasion, and metastasis. Inflammation also affects immune surveillance and responses to therapy [1]. Links between cancer and inflammation were first made in the nineteenthcentury, based on observations that tumors often arose at sites of chronic inflammation and that inflammatory cells were present in biopsied samples from tumors [(2,3)]. Normal physiologic processes are accompanied by changes in levels and activity of sirtuins. For example, circadian rhythm is controlled by NAD+ generation and cyclical activation and deactivation of SIRT1 and SIRT6. The circadian clock can influence chronic or acute inflammation. Chronic inflammation may be a causative factor in a variety of cancers. In general, the longer theinflammation persists, the higher the risk of cancer[2]. Hence, acute inflammation, such as occurs in response to a momentary infection, is not regarded as a risk factor for the growth of neoplasia, although many of the same molecular mediators are generated in both acute and chronic inflammation. In general, inflammatory leukocytes such as neutrophils, monocytes, macrophages, and eosinophils provide the soluble factors that are thought to mediate the development of inflammation-associated cancer, although other cells, including the cancer cells themselves, also participate[4].

The physiological relationship among inflammation and cancer has been observed from many years. In 1863, Virchow hypothesized that the basis of cancer was at sites of chronic inflammation, in part grounded on his hypothesis that some classes of irritants, together with the tissue injury and subsequent inflammation they cause, enhance cell proliferation. Today, the causal relationship between inflammation, innate immunity and cancer is more widely accepted; however, many of the molecular and cellular mechanisms mediating this relationship remain unsolved. Moreover, tumor cells may appropriate key mechanisms by which inflammation interfaces with cancers, to further their colonization of the host[5,17].

A considerable body of evidence supports the conclusion that chronic inflammation can impact an individual to cancer, as demonstrated by the association between chronic inflammatory bowel diseases and the increased risk of colon carcinoma[(2,3)].

Sirtuins are members of a family of evolutionarily conserved enzymes with nicotinamide adenine dinucleotide $(NAD)^+$-dependent deacetylase or mono-[ADP-ribosyl] transferase activity and they can be found in nearly all species[6].The circumstance that sirtuinsrequire NAD for their enzymatic activity connects

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 8
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metabolism to aging and aging related diseases[7]. Sirtuins belong to class III histone deacetylase family of enzymes. Sirtuins are a large family of protein-modifying enzymes highly conserved throughout bacteria, archaea, and eukaryotes. The founding member of the sirtuin family, silent information regulator 2 (Sir2), was first identified through a genetic screen in Saccharomyces cerevisiae as necessary for silencing of the mating-type information locus, HM1. Mammalian sirtuins have seven isoforms (SIRT1–7), which possess principally histone deacetylase (SIRT1, SIRT2, SIRT3, SIRT5 and SIRT7) or monoribosyltransferase activity (SIRT4 and SIRT6)[(6,7)]. Each sirtuin is characterized by an approximately 275 amino acid conserved catalytic core region and by unique additional N-terminal and C-terminal sequences of variable length. Later work showed Sir2 and its homologs to function primarily as nicotinamide adenine dinucleotide (NAD+)-dependent deacetylases, with specific family members reported to possess mono-ADP ribosyl transferase, demalonylase, or desuccinylase activity[21].

Table 1: Sirtuins diversity and regulatory effects on various cells:

Sirtuin	Location	Enzyme Activity	Disease area	Therapeutic strategy/ Functions	Targets	High Expression	References
SIRT1	Nuclear/ cytoplasmic	Deacetylase	Metabolic, neurological, cardiovascular, renal, cancer, mitochondrial	Activation Cell survival, Regulation, Inflammation	p53, FOXO1, FOXO4, COUP-TF, CTIP2, NF-kB-p65, NCOR, histone H1, histone H4, KU70, p300, BCL11A, Tat, PGC1a, MEF2, eNOS, ACS1, E2F1, AR, p73, SMAD7, NBS1, RB, TLE1, IRS2, LXR, PPAR-γ AROS, SUV39H1, WRN, DBC1, TORC2	Brain, skeletal muscle, heart, kidney and uterus	[(9,12,15,17)]
SIRT2	Nuclear/ cytoplasmic	Deacetylase	Neurological, metabolic, cancer	Inhibition/ activation? Cell cycle regulation	Alpha-Tubulin, HOXA10, FOXO, histone H4, 14-3-3 protein, PGC1α	Brain	[(9,12,15)]
SIRT3	Mitochondrial	Deacetylase	Metabolic, mitochondrial	Activation	ACS2	Brain, heart, liver, kidney and brown adipose tissue	[(9,12,15)]
SIRT4	Mitochondrial	ADP-ribosyl-transferase	Metabolic, mitochondrial	Inhibition?	GDH, IDE, ANT2, ANT3, Glutamate dehydrogenase	Pancreatic b-cells, brain, liver, kidney and heart	[(6,9,7,12,15)]
SIRT5	Mitochondrial	Deacetylase/ Demalonylase /Desuccinylase	Neurological	Unknown	Carbamoyl phosphate synthase 1, Cytochrome c, Urea cycle	Brain, testis, heart, muscle and lymphoblast	[(9,12,15)]
SIRT6	Nuclear	Deacetylase/ADP-ribosyl-transferase	Cancer	Activation	Histone H3K9, CPS1 H3K46, TNF-alpha	Brain, muscle, heart, ovary and bone cells (absent in bone marrow)	[(9,12,15,16,43)]
SIRT7	Nuclear	Deacetylase	Cardiovascular	Activation Regulation of rRNA transcription, cell cycle regulation	RNA polymerase I, p53, H3K18, Histone	Peripheral blood cells, CD33+ myeloid bone marrow precursor cells	[(9,12,13,15)]

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As specified in the table, discovery of cellular substrates as well as overexpression and knockout models deliver validation and therapeutic strategy to target sirtuins in various diseases of ageing. Development of small-molecule modulators of sirtuin activity would validate the genetic lead obtained in animal models. ACS, acetyl-CoA synthetase; ANT, ADP/ATP carrier protein; AR, androgen receptor; AROS, active regulator of SIRT1; BCL11A, B-cell CLL/lymphoma 11A (zinc finger protein); COUP-TF, chicken ovalbumin upstream promoter-transcription factor (also known as NR2F1); CTIP2, COUP-TF interacting protein 2 (also known as BCL11B); DBC1, deleted in breast cancer 1; E2F1, E2F transcription factor 1; eNOS, endothelial nitric oxide synthase; GDH, glutamate dehydrogenase; IDE, insulin-degrading enzyme; IRS2, insulin receptor substrate 2; LXR, liver X receptor; MEF2, myocyte-specific enhancer factor 2; NBS1, Nijmegen breakage syndrome 1; NCOR, nuclear receptor co-repressor; NF-kB, nuclear factor-kB; PGC1a, peroxisome proliferator-activated receptor-g co-activator 1a; RB, retinoblastoma protein; SUV39H1, suppressor of variegation 3-9 homologue 1; TLE1, transducin-like enhancer of split 1; TORC2, transducer of regulated cAMP response element binding protein 2; WRN, Werner syndrome protein.

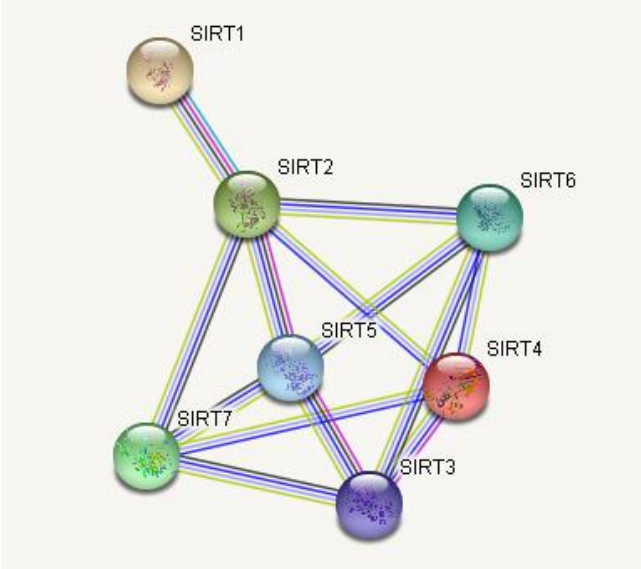

Fig1: Protein Interaction Process of all types of sirtuins from String v 10.5

The sirtuin/Sir2 (Silent information regulator 2) family of NAD+-dependent deacetylases and mono-ADP-ribosyltransferase plays a part in several cellular processes including gene silencing, cell cycle regulation and life span extension in yeast and animals. However, information about their occurrence and role in the plant genomes is scarce. Recently, two presumed sirtuin genes encoding a SIRT4-like and a SIRT7-like protein respectively were identified in the grapevine genome. Starting from the putative coding sequences present in the database, we have been able to obtain two long fragments of the true coding sequences for each sirtuin gene. Moreover, we did not observe mono-ADP-ribosyltransferase activity in either of the proteins. Finally, we investigated the expression of both sirtuin genes under conditions of stress, such as in the presence methyl jasmonate and UV-C irradiation. Preliminary assays showed that neither methyl jasmonate nor UV-C rays influence the expression of these grapevine Sirtuin genes[11].

SIRTUINS ASSOCIATION IN ANTI-INFLAMMATION

Following general concepts are relevant to the role of sirtuins in inflammation:

1. The requirement for NAD+ as cofactor supports sirtuin function in redox and bioenergy sensor[(1,2)].

2. While sirtuin-dependent deacetylation activities dominate our present understanding of the functional roles of sirtuins in inflammation, other attributes such as ADP ribosylation (SIRT4) and removal of succinyl, malonyl, and glutamyl groups from lysine residues (SIRT5) may be important in inflammation[(18,19)].
3. Acetyl CoA levels and its support of histone-acetylation and other proteins are linked to nutritional status of cell. Fasted or survival state of cell utilizes protein deacetylation with SIRT[19].
4. Inflammatory effects of SIRT1 on target cells can be a double-edged sword, since low levels accentuate early acute inflammation-related autotoxicity by increasing NFκB RelA/p65 activity, and prolonged increases in SIRT1 throughout late inflammation are associated with immunosuppression and increased mortality[20].
5. Adoptive transfer of inflammatory cells or overexpression ofinflammatory cytokines promotes the development of tumors.

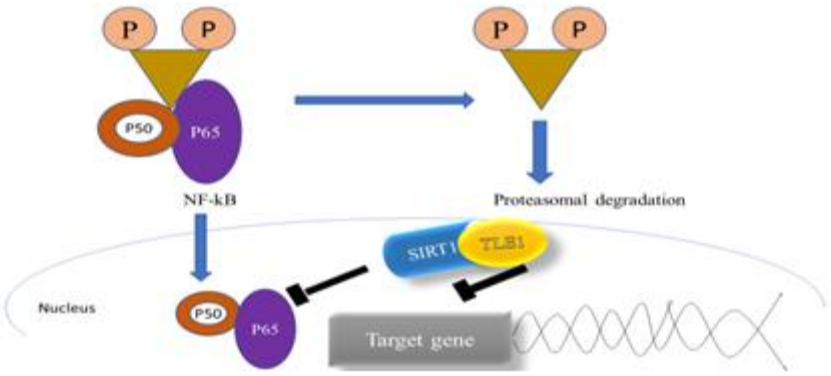

Fig2: SIRT1 represses the transcriptional activity of nuclear factor-kB (NF-kB) through deacetylation of the p65 subunit, and through interaction with transducin-like enhancer of split 1 (TLE 1).

Sirtuins biology in cancer

SIRT1 can activate stress defense and DNA repair mechanisms, and therefore aids in the preservation of genomic integrity. SIRT1 also functions in the regulation of metabolism and maintaining the integrity of the genome, and has thus been described as a potential tumor suppressor. Another significant role for SIRT1 in cancer is its suppression of the apoptosis inhibitor survivin in breast cancer susceptibility gene 1 (BRCA1)-associated breast cancers. Notably, both breast cancer and hepatic cell carcinoma revelation reduced SIRT1 levels compared with normal tissues[9]. SIRT1 similarly may have suppressive activity in tumor cell growth by suppressing NF-κB, a transcription factor that plays a central role in the regulation of innate and adaptive immune responses and carcinogenesis[7]. The dysregulation of NF-κB can lead to the onset of tumorigenesis and tumor malignancy. SIRT1 mRNA and protein levels were significantly lower in cell lines derived from mammary tumors lacking BRCA1 compared with cell lines derived from tumors without BRCA1 mutation. Furthermore, SIRT1 activity is required for suppressing survivin transcription. BRCA1 binds to the SIRT1 promoter and rises SIRT1 expression, which in turn inhibits survivin by changing the epigenetic modification of histone H3. Thus, decrease of survivin via SIRT1 activity may play an important role in BRCA1-associated mammary tumor formation[(7,9)].

SIRT2 is a tumor suppressor gene that has an essential role in maintaining the integrity of mitosis by positively regulating the activity of anaphase-promoting complex/cyclosome. Its dysfunction leads to genetic instability and tumorigenesis. During mitosis, SIRT2 is relegalized from the cytoplasm to the nucleus and serves as a histone deacetylase with a preference for histone H4 lysine. Overexpression of SIRT2 can

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 8
August 2017

pointedly prolong the mitotic phase and delay mitotic exit. Thus, it has been proposed that SIRT2 might function as a mitotic checkpoint protein in G2-M to prevent the initiation of chromosomal instability, particularly in response to microtubule inhibitor-mediated mitotic stress. Numerous studies have shown that tumors that express high levels of SIRT2 are not approachable to chemotherapy, specifically microtubule poisons. Human SIRT2 is most predominantly expressed in the brain[10].SIRT7 binds specific promoters and deacetylates H3K18Ac, causing repression of transcription. SIRT1 is also responsible for site-specific deacetylation at H3K18Ac in cancer cells. SIRT7 plays a critical function in maintaining properties of cancer cells, including escape from cell contact inhibition and anchorage-independent growth[13].Newly, hypoacetylation of histone H3 acetyl lysine 18 (H3K18Ac) has been reported to be a general marker of tumor prognosis and oncoviral transformation. H3K18Ac has also been linked to tumorigenesis, as well as poor prognosis and aggressive tumor phenotypes. Furthermore, human cancer cell xenografts that absence SIRT7 exhibit markedly compact oncogenicity in mice[9].

Expression of numerous sirtuins is altered in various types of cancers. For example, SIRT1, 4, 5, and 7 have been described as upregulated in certain cancers, while reduced SIRT1 levels have been reported in breast cancer and hepatic cell carcinoma[(2,9,23)]. SIRT2 is downregulated ingastrointestinal carcinomas, as well as in melanomas, in which a mutation in its catalytic domain has been shown to eliminate its enzymatic activity. SIRT6 is also downregulated in pancreatic cancer and colon adenocarcinoma[16]. The case of SIRT3 is more complex in the meantime it has been found to be upregulated or downregulated in diverse types of breast cancer[(8,10)]. SIRT7 knockdown in human cells induces cell cycle arrest and apoptosis. Some sirtuins, such as SIRT2 and SIRT6, seem to function as tumor suppressors, but others, such as SIRT1, are apparently bifunctional, operating as both tumor suppressors and oncogenic factors, depending on cellular context and study conditions. Recent findings have suggested that these contradictory activities of sirtuins might be a "double-edged sword"; however, the mechanisms underlying these functions remain unknown[8].

Table 2: Regulation of cancers by various Sirtuins

Sirtuins classes	Types of sirtuins	Cancer	Regulation	References
I	a) SIRT1 b) SIRT2 c) SIRT3	Brest cancer, hepatic cell carcinoma Various types of Brest cancer Oral cancer	Up regulation Down regulation Down regulation	Yeung et al [7] Pais et al [10] Turki et al [22]
II	SIRT4	Brest cancer, hepatic cell carcinoma	Up regulation	Tao et al[18]
III	SIRT5	Brest cancer, hepatic cell carcinoma	Up regulation	Jintang et al [21]
IV	a) SIRT6 b) SIRT7	Pancreatic cancer, colon adenocarcinoma Brest cancer, hepatic cell carcinoma	Down regulation Up regulation	Lee et al[16] Vakhrusheva et al [13]

TYPES OF SITUINS

SIRT1

The utmost extensive study, however, has been directed toward the functions of SIRT1, the founding member of this Sirtuin family and the mammalian ortholog of yeast Sir2[31]. Yeast Sir2 that was initially characterized as an NAD^+ dependent histone deacetylase (so called Class III HDAC that is structurally and biochemically diverse from Class I, II and IV HDACs). Activation of SIRT1 may lead to anti-inflammatory activity through

International Journal of Engineering Technology Science and Research
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www.ijetsr.com
ISSN 2394 – 3386
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attenuation of NF-kB activity, the master regulator of innate immune responses [(7,9, 43)], known as a major metabolic regulator, epigenetically reprograms inflammation by altering histones and transcription factors such as NFκB and AP1. Growing evidence wires that inflammation sequentially links immune, metabolic, and mitochondrial bioenergy networks; sirtuins are essential regulators of these networks[10]. SIRT1 deacetylates histone H4 lysine 16 (H4K16) as well as histone H3 lysine 9 and 14 (H3K9 and H3K14, respectively).Additionally, SIRT1 deacetylates histone H1 lysine 26 (H1K26) and is involved in the deposition of histone variants. These modifications of histone tails are closely related to gene silencing and heterochromatin formation that may underlie certain biological processes. Notably, global genomic hypoacetylation at H4K16 is a hallmark of human cancer cells, both cell lines and clinical samples.SIRT1 modifies histones through deacetylation of K9 in histone H3 (H3K9) and K16 in histone H4 (H4K16) and deacetylates many nonhistone proteins that are involved in cell growth, apoptosis, neuronal protection, adaptation to caloric restriction, organ metabolism and function, cellular senescence, and tumorigenesis[31]. The biological roles of SIRT1, however, are mostly revealed through its deacetylation of a growing number of non-histone substrates that are involved in a wide variety of cellular functions, particularly in metabolic, oxidative/genotoxic and oncogenic stress responses.

SIRT1 was also found to play an important role in modulating the development and progression of inflammation through deacetylating histones and critical transcription factor such as nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1), thus leading to transcriptional repression of various inflammation-related genes[(7,30)].

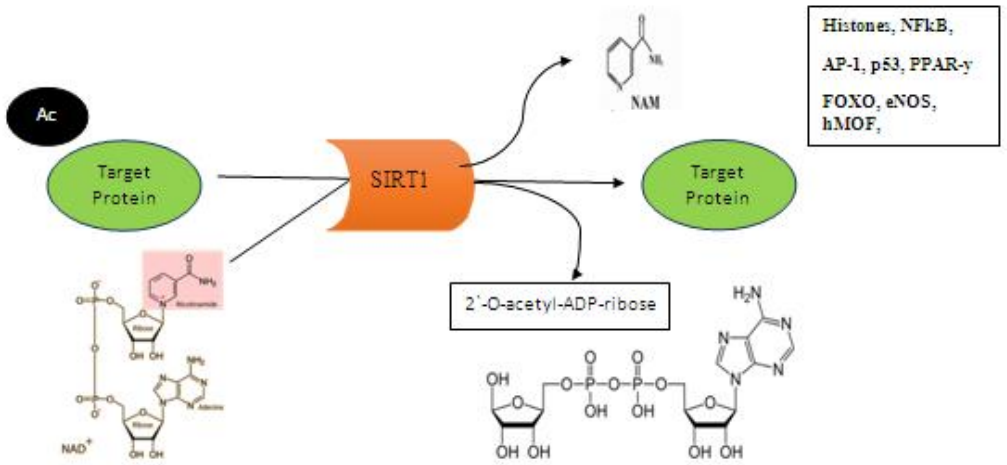

Fig 3: Deacetylation of various target proteins

The deacetylation reaction removes an acetyl group from the lysine sidechains of a protein substrate while cleaving NAD+ in the process to generate the deacetylated protein 2′-*O*-acetyl ADP-ribose and nicotinamide.

Mechanisms of SIRT1 on cancer

Tumor-suppressing roles of SIRT1

SIRT1 is the most studied Sirtuin. Several genetic studies provide evidence that SIRT1 suppresses tumor formation [8]. One of the most notable targets of SIRT1 is p53, which plays a critical role in cell-cycle checkpoint regulation, apoptosis, and tumor suppression. It has been shown that overexpression of SIRT1 deacetylates p53, leading to the suppression of p53 activity [(31,32)]. Studies on SIRT1 transgenic mice showed that conditional SIRT1 overexpression suppresses the incidence of intestinal tumor, spontaneous carcinomas and sarcoma, as well as carcinogen-induced liver cancer. The tumor-suppressing role of SIRT1 may come from its ability to improve genomic stability by regulating chromatin and DNA repair. *Sirt1*$^{-/-}$ mouse embryos

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
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display altered histone modification accompanied with impaired DNA damage response and reduced DNA damage repair [(15,31)]. In line with the decreased genome stability, *Sirt1*$^{+/-}$*p53*$^{+/-}$ mice develop tumors in various tissues.

Table 3: Various roles of SIRT1 in Anti-inflammation, Anti-cancer and related regulatory substrates

Role of SIRT1	SUBSTRATES	Regulation	REFERENCES
Transcriptional factors	P53, FOXO1, FOXO3a, NF-kB, c-MYC, N-MYC, E2F1, and HIF-1 α/HIF-2 α	Cell cycle progression and promoting survival under various conditions	Roth et al [15], Philipp et al [47]
DNA repair	Ku70, RAD51, NBS1, APE1, XP/C, and WRN	Improving DNA damage repair	Philipp et al [47]
Nuclear receptor	LXR, FXR, ERα, AR, PPARγ, PGC1α	Metabolic regulator	Roth et al [15]
circadian clock related factor	CLOCK and PER2	Metabolism regulation	Philipp et al [47]
Histone modifying enzymes	SUV39H1, p300, TIP60 and PCAF	Gene expression regulation	Yeung et al [7]
Cell signaling molecules	STATE3, β-catenin and SMAD7	Cell death and differentiation	Roth et al [15]
Anti-Inflammation	NF-kB, and AP-1	Reprograms inflammation	Yinon et al [30]
Tumor-suppression	RelA/p65 subunit of NF-kB at Lysine 319, HIF-1**α**	Inhibition of tumor formation through inhibition of tumor promoting transcription factors	Yeung et al [7]
Tumor-promotion	SIRT1 deacetylates p53 at lysine 382., Ku70	negatively regulates p53 transactivation activity	Yeung et al [7]

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www.ijetsr.com
ISSN 2394 – 3386
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The tumor-suppressing role of SIRT1 may come from its ability to deacetylate and inactivate certain tumor-promoting transcription factors, such as NF-κB and HIF-1α. SIRT1 deacetylates RelA/p65 subunit of NF-κB at lysine 310 and inhibits its transcription activity, thereby augmenting TNF-α-induced apoptosis. Overexpression of SIRT1 suppresses the growth and angiogenesis of fibrosarcoma HT1080 tumors in a mouse xenograft model by deacetylating and inactivating HIF-1α.The tumor-suppressing role of SIRT1 could also be due to its ability to suppress the transcription tumor promoting genes by deacetylation of histones. BRCA1 binds to the SIRT1 promoter and increases SIRT1 expression, which in turn inhibits Survivin by deacetylating H3K9. Therefore, ablation or mutation of BRCA1 results in increased Survivin level and promotes tumor growth by suppressing SIRT1 expression.

Tumor-promoting role of SIRT1

Current genetic studies on mice provided awareness into the oncogenic activity of SIRT1 *in vivo*. SIRT1 overexpression leads to increased thyroid and prostate tumorigenesis in *Pten*$^{+/-}$ mice[15]. Based on mRNA analysis, C-MYC levels are increased when SIRT1 is overexpressed. In human papillary thyroid carcinomas, SIRT1 is overexpressed and SIRT1 level positively correlates with C-MYC level[8]. It has been shown in another study that *Sirt1* knockout suppresses BCR-ABL transformation of mouse bone marrow cells and the development of a CML-like disease in a mouse model. A recent study indicated that enterocyte-specific inactivation of Sirt1 decreases tumor number and size in the APC+/min mouse model of intestinal tumorigenesis[48]. In addition, studies in many cancer cell lines showed that inhibiting SIRT1 or decreasing SIRT1 can inhibit cancer cell proliferation.

The role of SIRT1 in DNA repair and genome stability can also account for SIRT1's tumor-promoting the role. A recent study revealed SIRT1 help to acquire mutations for drug resistance in CML cells. SIRT1 alters both homologous recombination (HR) and error-prone, non-homologous end joining (NHEJ) DNA repair pathways by regulating the key components in these pathways, KU70 and NBS1 (Nijmegen breakage syndrome 1). SIRT1 enhances error-prone DNA damage repair, resulting in acquisition of genetic mutation for CML drug resistance. As noted in previous section, role of SIRT1 in DNA repair and genome stability has also been used to explain the tumor-suppressing role of SIRT1[14]. It is proposed that SIRT1 might differentially regulate genome stability in normal cells versus cancer cells. In normal cells, SIRT1 promotes genomic stability by activating DNA repair with high fidelity, thereby suppressing tumor progression. In cancer cells, SIRT1 activates DNA repair with low fidelity to protect cells from deleterious impact of DNA damage. However, the error-prone DNA damage repair allows mutation acquisition in cancer cells and evolution toward high-grade malignancy.

The oncogenic role of SIRT1 is further supported by its role in inhibiting cell death mediated by tumor suppressors. SIRT1 has been shown to promote cell survival by deacetylating and inhibiting the function of p53[49]. SIRT1 deacetylates p53 at lysine 382 and negatively regulates its transactivation activity. Overexpression of SIRT1 strongly attenuates p53-dependent apoptosis upon DNA damage and oxidative stress. Forkhead box O (FOXO) transcription factors are an important family of tumor suppressors that modulate the expression of genes involved in cell cycle control, apoptosis and DNA repair. SIRT1 regulates various cellular processes by deacetylating FOXO family members. For example, SIRT1 deacetylates FOXO1 and inhibits FOXO1-induced apoptosis in prostate cancer cells. Furthermore, SIRT1 mediated deacetylation of FOXO3a facilitates its ubiquitination and degradation. Interestingly, FOXO transcription factors are downstream of PTEN. PTEN acetylation level is also increased upon *Sirt1* knockout and SIRT1 can directly deacetylate PTEN. However, it has been shown that deacetylation of FOXO proteins by SIRT1 can also activate their transcriptional activities. Daitoku *et al.* showed that SIRT1 potentiates transcription mediated by FOXO1 by deacetylation in mice. In line with this, FOXO1 activation *via* SIRT1 is closely involved in Tamoxifen-resistance in human breast cancer MCF-7 cells. SIRT1 inhibits FOXO3-induced cell death but increases FOXO3-mediated cell cycle arrest and resistance to oxidative stress. These findings suggest that the regulation of FOXO by SIRT1 may be complex[(8,9)].In addition, twoother tumor suppressors, deleted in bladder cancer 1 (DBC1) and hypermethylated in cancer 1 (HIC1) have been found to affect SIRT1. DBC1 binds to the N-terminus of SIRT1 and inhibits its enzymatic activity[33].

International Journal of Engineering Technology Science and Research
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ISSN 2394 – 3386
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Protein-Protein Interaction studies for SIRT1

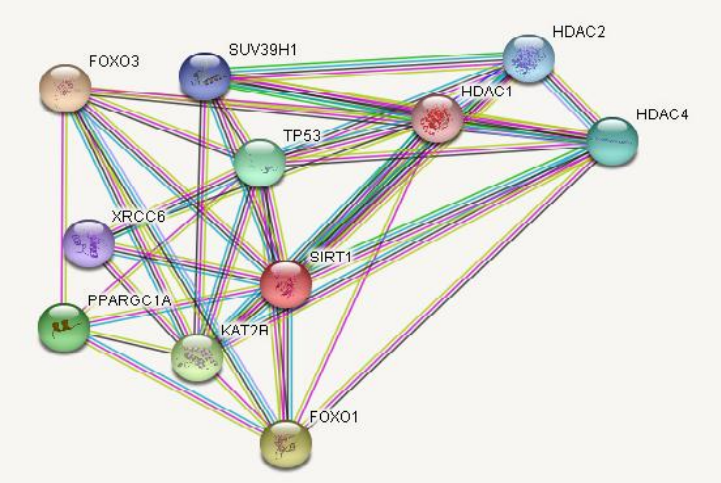

Fig 4: Protein Interaction of SIRT1 with other proteins

SIRT1 has shown interactions with proteins like FOXO3 (Forkhead box O3 - Participates in post-transcriptional regulation of MYC), XRCC6 (X-ray repair complementing defective repair in Chinese hamster cells 6- role in chromosome translocation), PPARGC 1A (Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; Transcriptional coactivator for steroid receptors and nuclear receptors), KAT2B (K(lysine) acetyltransferase 2B; Functions as a histone acetyltransferase (HAT) to promote transcriptional activation), FOXO1 (Forkhead box O1; Transcription factor that is the main target of insulin signaling and regulates metabolic homeostasis in response to oxidative stress), HDAC4 (Histone deacetylase 4; Responsible for the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3 and H4)), HDAC2 (Histone deacetylase 2), HDAC1 (Histone deacetylase 1), TP53 (Tumor protein p53; Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type) and SUV39H1 (Suppressor of variegation 3-9 homolog 1).

SIRT2

Sirtuin 2 (SIRT2) is an NAD+-dependent protein deacetylase whose targets include histone H4 lysine 16, p53, and α-tubulin.SIRT2 is the closest homolog to Hst2 from S. cerevisiae, which is a cytoplasmic H4 lysine 16 (H4 K16) histone deacetylase that disrupts telomeric silencing and increases rDNA silencing upon overexpression [69].SIRT2 is predominantly localized in the cytoplasm but can transiently translocate to the nucleus during G2/M transition and deacetylate histone H4K16, thereby modulating chromatin condensation during metaphase.SIRT2 deacetylates ϵ-N-acetyllysine residues on a variety of protein substrates [70]. Deacetylation of p53 regulates its effect on apoptosis. It has been shown that SIRT2 plays a role in the mitotic checkpoint to arrest cells if DNA damage is detected[31]. Various other substrates have been recently reported, suggesting that SIRT2 relates to multiple cellular processes. SIRT2 knockout mouse studies have pointed to a tumor suppressor role of SIRT2. *Sirt2* knockout mice look grossly normal, but starting from 10 months of age, they tend to have more tumors than the wild type. This effect is attributed to the role of SIRT2 in regulating the cell cycle by deacetylating APC/C. The hypothesis is that SIRT2 is important for the cell cycle and thus without SIRT2, abnormal cell division occurs and thus tumor arises[15].

However, the increased spontaneous tumor formation in $Sirt2^{-/-}$ mice may be strain-dependent and is not seen in another study. $Sirt2^{-/-}$ cells have increased DNA damage and aberrant cell cycle progression compared with wild type cells. Although no increased spontaneous tumorigenesis was observed in the knockout mice up to one year of age, increase tumorigenesis was observed in an induced skin tumor model. A recent report also suggested that SIRT2 can deacetylate and promote the degradation of ATP-citrate lyase, which is important for lipid biosynthesis and thus tumor growth. Inhibition of SIRT2 promotes ATP-citrate lyase stability and thus may promote tumor growth[(8,13,15)].

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ISSN 2394 – 3386
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SIRT2 inhibition has been shown to increase the levels of tumor suppressor genes, such as p53 and p21. Increased p53 level is achieved through deacetylation of p53, but the mechanism for increased p21 level is not clear. SIRT2 inhibition or knockdown can interfere with cancer cell metabolism, e.g. the Warburg effect. SIRT2 can deacetylate and activate lactate dehydrogenase A (LDH-A). LDH-A is over-expressed in many cancer cells and is responsible for the increased production of lactate in cancer cells.

Thus, inhibiting SIRT2 can potentially inhibit lactate production in cancer cells and disrupt cancer cell metabolism. Additionally, SIRT2 may exert tumor-promoting function by epigenetically silencing tumor suppressors, such arrestin domain-containing 3 (ARRDC3) in basal-like breast cancer cells, by controlling histone acetylation [(10,15)].

Table 4: Role of SIRT2 in tumorigenesis and tumor suppression

Role of SIRT2	Substrate	Regulation	References
Deacetylates ϵ-*N*-acetyllysine transcription factors	p53	Increase the levels of tumor suppressor genes, effect on apoptosis	Zhao et al [70]
Deacetylates ϵ-*N*-acetyllysine transcription factors	p65, FOXO1, FOXO3a	effect on apoptosis,	Zhao et al [70] Jin et al [65]
Deacetylates lysine 40 of α-tubulin During G_2/M phase	α-tubulin	disrupts telomeric silencing	Gesine, et al [67]
Acetylation of p53 by p300	P53, p300	cellular stress stimulates the DNA-binding capacity of p53 and enhances its biological function *in vivo*	Robert et al [66]
Histone deacetylation	H4 K16	disrupts telomeric silencing and increases rDNA silencing upon overexpression	Séverine et al. [69]

However, in gliomas and gastric carcinomas,SIRT2 levels are downregulated. Aneuploidy, a hallmark of gliomas, is marked by a defective mitotic spindle checkpoint that causes chromosomal instability in such tumors. SIRT2 may thus function at a mitotic checkpoint to ensure chromosomal stability, with downregulation of SIRT2 potentially leading to chromosomal instability and consequently tumorigenesis. Chemical inhibition of SIRT1 with selective and potent inhibitors has not been shown to prevent proliferation of multiple cancer cell lines[(39,45)].

Furthermore, another study reported increased SIRT2 expression in 6 of 11 human pancreatic adenocarcinomas, and SIRT2 was found to be up-regulated in human breast cancer and hepatocellular carcinoma. Deacetylation of p53 by SIRT1 and SIRT2 therefore counteracts p53-dependent cell cycle arrest, and SIRT1/SIRT2 inhibition is therefore expected to enhance p53-mediated apoptosis [67]. Next to its role as an anticancer target, SIRT2 also holds promise as a target for the treatment of neurodegenerative disorders in that SIRT2 inhibition in primary neuronal and invertebrate models of Parkinson and Huntington diseases rescues neurotoxicity induced by α-synuclein and huntingtin proteins, respectively.

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IJETSR
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ISSN 2394 – 3386
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Protein-Protein Interaction studies for SIRT2

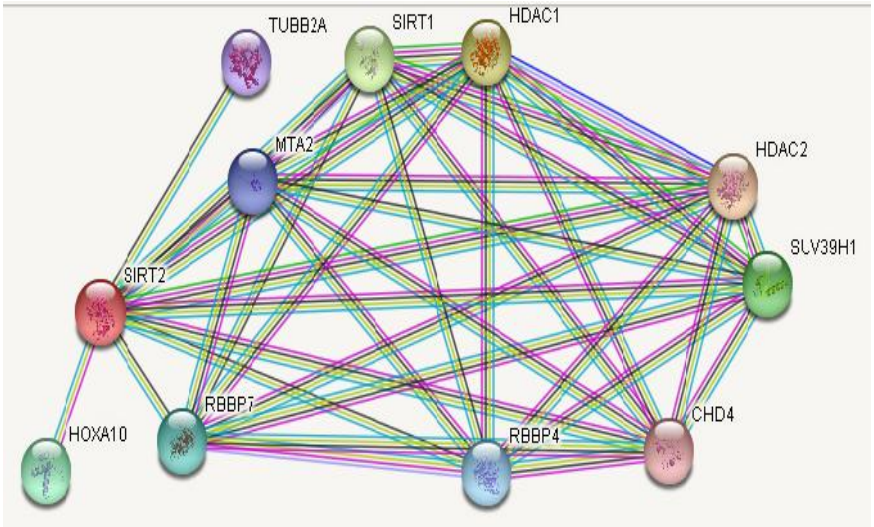

Fig 5: Protein Interaction of SIRT2 with other proteins

SIRT2 has shown interactions with proteins like TUBB2A (Tubulin, beta 2A class IIa; Tubulin is the major constituent of microtubules), HOXA10 (Homeobox A10; Sequence-specific transcription factor which is part of a developmental regulatory system), MTA2 (Metastasis associated 1 family, member 2; May be involved in the regulation of gene expression as repressor and activator), RBBP4 (Retinoblastoma binding protein 4; Core histone-binding subunit that may target chromatin assembly factors), CHD4 (Chromodomain helicase DNA binding protein 4; Component of the histone deacetylase NuRD), SUV39H1 (Suppressor of variegation 3-9 homolog 1), HDAC 1 and 2 (Histone deacetylase) and SIRT1 (Sirtuin 1; NAD-dependent protein deacetylase that links transcriptional regulation directly to intracellular energetics and participates in the coordination of several separated cellular functions)

SIRT3

SIRT3 is one of the major mitochondrial NAD^+-dependent deacetylase, has a key role in cancer and has been shown to regulate the activity of many mitochondrial proteins.SIRT3 has been found to regulate many aspects of mitochondrial function, such as metabolism, ATP generation and modulation of the response to oxidative stress. The function of SIRT3 may be different depending on the cell-type and tumor-type; thereby, SIRT3 may act as an oncogene or a tumor suppressor.The first substrate identified for SIRT3, acetyl-CoA synthetase 2 (AceCS2) and glutamate dehydrogenase (GDH) have shown that SIRT3 may have a regulatory role in mitochondrial metabolism as AceCS2 localizes to the mitochondria, and is activated by deacetylation. SIRT3-dependent deacetylation also increases the enzymatic activity of GDH, which is involved in the oxidation of amino acids.

SIRT3 is central to the maintenance of appropriate mitochondrial function by limiting oxidative stress, and reducing reactive oxygen species (ROS) production with a decrease in mitochondrial membrane potential.The expression of two antioxidant proteins including mitochondrial superoxide dismutase 2 (SOD2) and catalase is increased by overexpression of SIRT3. FoxO3a, a transcriptional factor that upregulates SOD2 and catalase, can be deacetylated by SIRT3, providing a mechanism by which SIRT3 decreases ROS levels. Given the facts that SIRT3 localizes to mitochondria and that mitochondrial ROS are important in cancer, SIRT3 may have a key role in carcinogenesis. By deacetylating proteins involved in multiple mitochondrial processes, SIRT3 can coordinate global shifts in mitochondrial activity, thereby indicating some implications for tumor proliferation. [60] Loss of SIRT3 triggers oxidative damage, ROS-mediated signaling and metabolic reprogramming that can work together to lead to carcinogenesis. On one hand, SIRT3 is pro-survival and prevents cell death in response to stress and starvation. [60] In addition, it can protect from cell death in response to hypoxia or the apoptotic inducer staurosporine. On the other hand, SIRT3 is pro-apoptotic under

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ISSN 2394 – 3386
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certain pathway conditions [61].SIRT3 has been identified as a tumor suppressor, which directly links SIRT3 to metabolic processes. SIRT3 seems to be involved in carcinogenesis; thereby, SIRT3 inhibitors/activators might have some potential therapeutic benefits.

SIRT3 contains a conserved enzymatic core with two domains, including a large Rossmann fold domain that binds NAD^+ and a small domain formed by two insertions of the large domain binding to a zinc atom. The acetylated peptide substrate binds to the cleft between the two domains.A few key enzymes involved in energy production in the mitochondria have been identified as SIRT3 substrates. Deacetylation of AceCS2 at lysine 642 by SIRT3 activates AceCS2 activity, providing increased acetyl-CoA to feed into the tricarboxylic acid cycle (TCA).The cofactor-binding pocket can be divided into three regions: the adenine ribose moiety of NAD^+, the nicotinamide ribose moiety and the catalytic center located deep inside the pocket. Structural evidence suggests that the binding of acetyl-lysine positions the nicotinamide group of $NADs^+$ within a highly-conserved pocket where the carboxamide of NAD^+ interacts with a conserved aspartate residue.

It seems that the major function of SIRT3 is to promote mitochondrial metabolism and suppress the production of reactive oxygen species (ROS), as Sirt3−/− mice or cells have decreased ATP production and increased ROS levels. Strong evidence exists to support a tumor-suppressor role of SIRT3. Increased ROS levels in Sirt3−/− MEF cells are associated with increased mitochondrial DNA damage.SIRT3 enhances acetyl-CoA production by deacetylating acetyl-CoA synthetase 2[31]. *Sirt3* knockout itself does not transform MEF cells, but readily transform MEF cells when another oncogene, *Ras* or *Myc*, is overexpressed. In contrast, $Sirt3^{+/+}$ MEF cells cannot be transformed by the overexpression of *Ras* or *Myc*. Increased ROS level seems to be important for the tumor-permissive phenotype. Consistent with the tumor-permissive phenotype, $Sirt3^{-/-}$ mice develope mammary tumors over 24 months while $Sirt3^{+/+}$mice do not. $Sirt3^{-/-}$ MEF cells have increased ROS levels and glycolysis, like the Warburg effect in cancer cells[8]. Correspondingly, $Sirt3^{-/-}$ MEF cells proliferate faster than $Sirt3^{+/+}$ cells. This effect is caused by increased ROS, decreased proline hydroxylase activity (the enzyme that hydroxylates and destabilizes HIF-1α), and thus increased HIF-1α level in the absence of SIRT3. These results are further confirmed by another study. SIRT3 is heterozygously or homozygously deleted in about 20% of all human cancers and about 40% of breast and ovarian cancers[15].

In human cancer cell lines, overexpression of SIRT3 reverses the Warburg effect and decreases cell proliferation. It has also been reported that SIRT3 promotes oxidative phosphorylation (the opposite of Warburg effect) at least partially through deacetylation of cyclophilin D and the accompanied dissociation of hexokinase II from mitochondria. Another possible molecular mechanism for the tumor suppression role of SIRT3 is suggested by studies in cardiac hypertrophy. SIRT3 is shown to suppress cardiac hypertrophy by activating FOXO3a, which increases MnSOD and decreases ROS. ROS can activate RAS, which further activate MAPK and AKT pathways to promote cell growth and proliferation. SIRT3 can deacetylate and destabilize F-box protein S-phase kinase associated protein 2 (Skp2), a protein that supports tumorigenesis by promoting the ubiquitination and degradation ofseveral tumor suppressors[(22, 31, 48)].

Table 5: Role of SIRT3 in cell apoptosis and cell proliferation

Role of SIRT3	Substrate	Activity	References
Deacetylates and destabilize	F-box protein skp2	Supports tumorigenesis	Chen et al [53]
triggers oxidative damage ROS-mediated signaling metabolic reprogramming	ROS	lead to carcinogenesis	Finely et al [51]
Overexpression of SIRT3 in mitochondria	SOD2 and Catalase	Antioxidant-production	Sundaresan et al [52]
Suppress cardiac hypertrophy by activating	FOXO3a	Promote cell growth and proliferation	Chen et al [53]
SIRT3 represses the expression of nuclear-encoded mitochondrial and some stress-related genes	ZFAT and WAPAL	Anti-apoptotic and oncogenic activity	Hening Lin et al [62]

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SIRT3 has also been reported to be present in the nucleus. Nuclear SIRT3 represses the expression of nuclear-encoded mitochondrial and some stress-related genes, including *Zfat* and *Wapal*. Both ZFAT and WAPAL have anti-apoptotic and oncogenic functions. By suppressing their expression, SIRT3 may suppress tumor formation. In contrast, SIRT3 also physically binds to and deacetylates KU70, protecting cells from stress-mediated cell death in cardiomyocytes. These studies indicate the previously unappreciated and sometimes contradicting roles of SIRT3 in the nucleus.

Fig6: SIRT3 and the hallmarks of cancer

SIRT3 can link to six hallmarks of cancer, such as resisting cell death, genomic instability and mutation, sustaining proliferative signaling, dysregulation of cellular energetics, tumor-promoting inflammation and invading growth suppressors

SIRT3 appears coupled with the tumor suppressor p53 during the initiation of its expression in the mitochondria, usually by abrogating p53 activity to enact growth arrest and senescence. Subsequently, p53 is regarded as a new target for SIRT3 deacetylation in bladder cancer as SIRT3 can rescue p53-induced growth arrest in human bladder tumor-derived EJ-p53 cells[54].Cumulative mitochondrial damage leads to a fall in relative NAD levels and a concomitant fall in SIRT3 activity, which is also associated with growth arrest, senescence and apoptosis. In addition, SIRT3 alters sensitivity of breast cancer cells to tamoxifen (Tam), a commonly used anti-estrogen agent. In sensitive MCF-7 cells, SIRT3 can be significantly increased following exposure to Tam, and transfection of MCF-7 cells with a SIRT3 expression plasmid may decrease cellular sensitivity to Tam and block the Tam-induced apoptosis. Thus, SIRT3 is considered as a potential target for overcoming Tam resistance in the treatment of breast cancer[55].SIRT3 induces survival and protects several cell types from cellular damage by maintaining mitochondrial integrity and function or by enhancing their resistance to stress-mediated cell death. In addition, the overexpression of SIRT3 in cancer may result in increase of survival signals and suppression of apoptotic signals, thus enhancing carcinogenesis[53].

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
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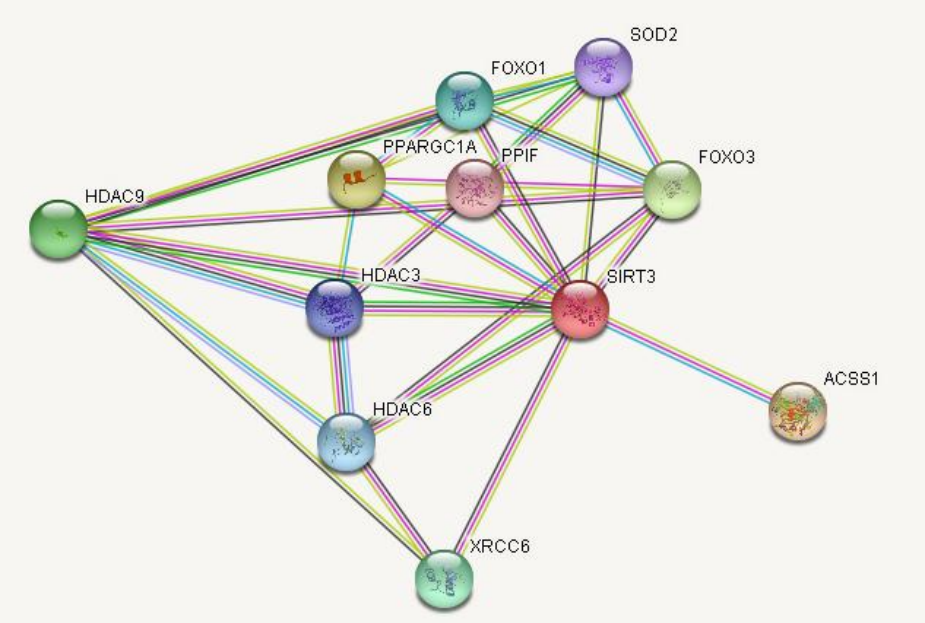

Fig 7: Protein Interaction of SIRT3 with other proteins

SIRT3 has shown interactions with proteins like HDAC3, 6 and 9 (Histone deacetylase), XRCC6 (X-ray repair complementing defective repair in Chinese hamster cells 6), ACSS1 (acyl-CoA synthetase short-chain family member 1; Important for maintaining normal body temperature during fasting and for energy homeostasis), FOXO3 and FOXO1 (Forkhead box), SOD2 (Superoxide dismutase 2, mitochondrial; Destroys superoxide anion radicals which are normally produced within the cells and which are toxic to biological systems), PPARGC 1A (Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha) and PPIF (Peptidylprolyl isomerase F; PPIases accelerate the folding of proteins).

SIRT4

SIRT4 represses glutamate dehydrogenase to suppress insulin signaling through its ADP-ribosylase activity[31]. SIRT4 is one of the least understood sirtuins. SIRT4 inhibits glutamate dehydrogenase (GDH), thereby inhibiting amino acid induced insulin secretion in pancreatic beta cells [36]. The inhibitory effect on GDH seems to contribute to the tumor suppressor role of SIRT4. SIRT4 is down regulated in many cancers and mammalian target of rapamycin complex 1 (mTORC1) upregulates glutamine metabolism and cell proliferation by suppressing SIRT4 [35]. By inhibiting glutamine metabolism, SIRT4 also contributes to DNA damage and repair. Transformed *Sirt4*$^{-/-}$ MEF cells form larger tumors than transformed *Sirt4*$^{+/+}$ MEF cells in allograft tumor formation assay. *Sirt4*$^{-/-}$ mice also developed more lung tumors than *Sirt4*$^{+/+}$ mice at 18–26 months of age.

Table 6: Various roles of SIRT4 in mammalian cell and mitochondria

Role of SIRT4	Substrates	Activity	References
Inhibits tumor cells	GDH	Tumor suppression role	Marcia C., et al [36]
SIRT4-mediated regulation of DLAT lipoyl levels and PDH activity in cells and in vivo	PDH	Lipoamidase activity	Rommel A., et al. [57]

SIRT4 as a cellular lipoamidase that regulates the pyruvate dehydrogenase complex (PDH). Prominently, SIRT4 catalytic efficiency for lipoyl- and biotinyl-lysine modifications is superior to its deacetylation activity. PDH, which converts pyruvate to acetyl-CoA, has been known to be primarily regulated by phosphorylation

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of its E1 component. Rommel A., et al. [57] determined that SIRT4 enzymatically hydrolyzes the lipoamide cofactors from the E2 component dihydrolipoyllysine acetyltransferase (DLAT), inactive the PDH activity. Moreover, SIRT4-mediated regulation of DLAT lipoyl levels and PDH activity in cells and in vivo, in mouse liver.

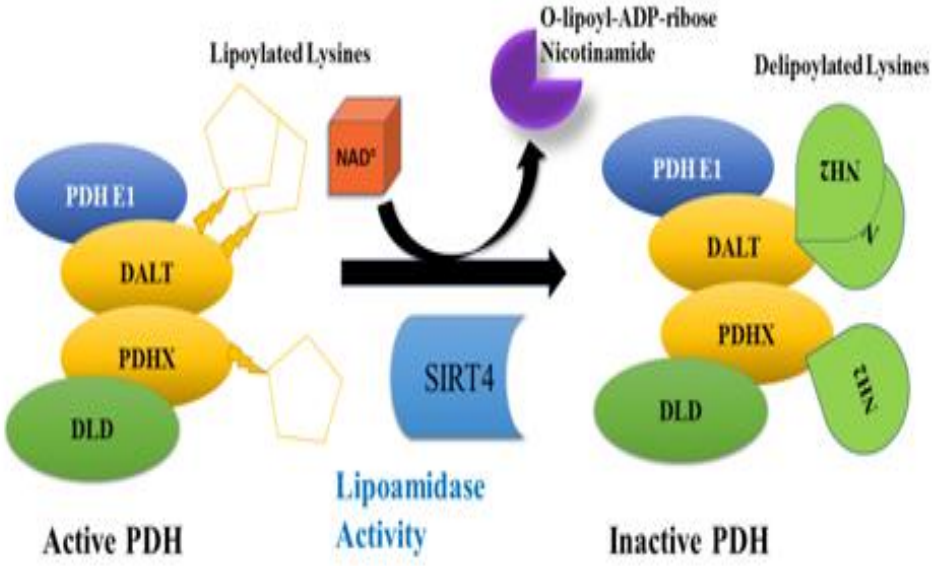

Fig8: SIRT4-mediated regulation of DLAT-lipoyl levels and PDH activity in cells and in vivo, in mouse liver.

Furthermore, metabolic flux switching via glutamine stimulation induces SIRT4 lipoamidase activity to inhibit PDH, highlighting SIRT4 as a guardian of cellular metabolism[57].Certainly, SIRT4 show that glutamine stimulation induces endogenous SIRT4 lipoamidase activity, triggering a reduction in both DLAT lipoyl levels and PDH activity. As the PDH controls pyruvate decarboxylation, fueling multiple downstream pathways, our findings highlight SIRT4 as a critical regulator of cellular metabolism[57].

Protein-Protein Interaction studies for SIRT4

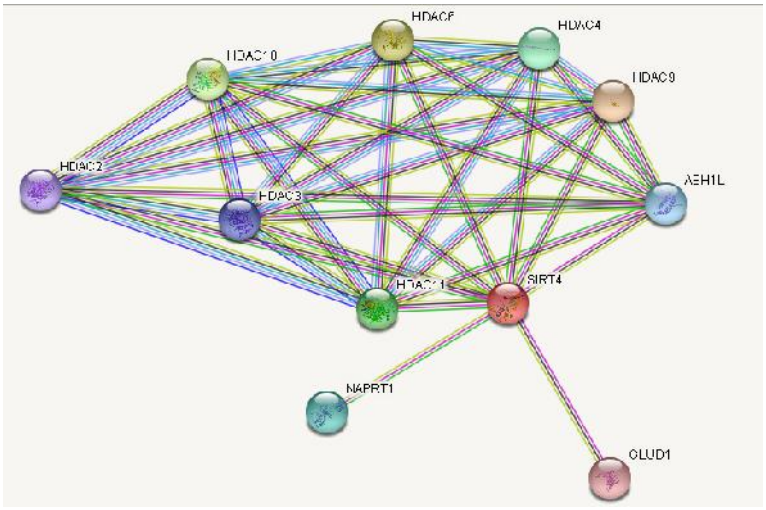

Fig 9: Protein Interaction of SIRT4 with other proteins

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SIRT4 has shown interactions with proteins like HDAC 2,3,4,6,9,10 and 11 (Histone deacetylase), ASH1L (Ash1 (absent, small, or homeotic)-like (Drosophila); Histone methyltransferase specifically methylating 'Lys- 36' of histone H3 (H3K36me)), NAPRT1 (Nicotinate phosphoribosyltransferase domain containing 1; Catalyzes the conversion of nicotinic acid (NA) to NA mononucleotide (NaMN). Essential for NA to increase cellular NAD levels and prevent oxidative stress of the cells) and GLUD1 (Glutamate dehydrogenase 1; Mitochondrial glutamate dehydrogenase that converts L- glutamate into alpha-ketoglutarate. Plays a key role in glutamine anaplerosis by producing alpha-ketoglutarate, an important intermediate in the tricarboxylic acid cycle)

SIRT5

SIRT5 is another mitochondrial sirtuin that is not well understood and has no clear association with cancer. *Sirt5*$^{-/-}$ mice do not exhibit severe phenotype. SIRT5 has weak deacetylase activity and has been demonstrated to have more efficient desuccinylase and demalonylase activity. A recent proteomic study further identified hundreds of proteins with increased succinylation level when SIRT5 is knocked out, suggesting that SIRT5 regulates many metabolic pathways[(21,37)].SIRT5, is a mitochondrial class III NAD+-dependent deacetylase SIRT5 is overexpressed in human NSCLC and high expression of SIRT5 predicts deprived survival. SIRT5 knockdown represses lung cancer cell growth and transformation in vitro and in vivo[38].SIRT5 mRNA level is positively correlated with the expression of Nrf2 in lung cancer tissues and SIRT5 knockdown reduces the expression of Nrf2 and its downstream drug-resistance genes.

SIRT5 regulates ammonia production by controlling glutamine metabolism.Nakagawa *et al*. first reported the role of Sirt5 in regulating the urea cycle through the deacetylation of carbamoyl phosphate synthetase 1 (CPS1), which plays a critical role in the initial order of the urea cycle for ammonia detoxification. Loss of Sirt5 in mice causes enhanced ammonia levels in blood under fasting, calorie restriction, or high protein diet compared to that in the wild type. However, the physiological significance of protein deacylation by Sirt5 is still unknown. In addition, Sirt5 knockout mice have not shown any metabolic phenotypes except for that of the urea cycle. There are no reports yet implicating Sirt5 in tumorigenesis[63].

Protein-Protein Interaction studies for SIRT5

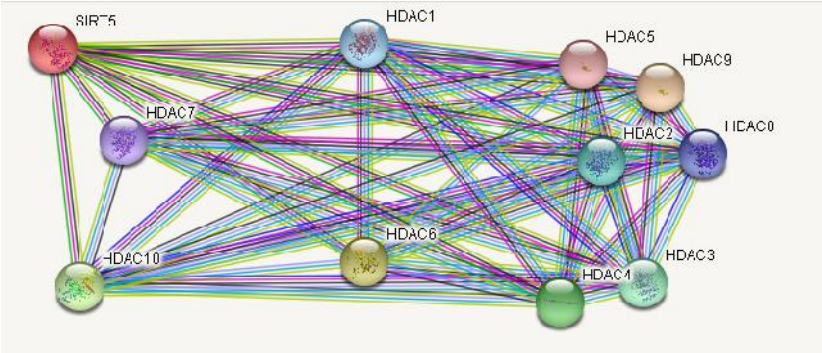

Fig 10: Protein Interaction of SIRT5 with other proteins

SIRT5 has shown interactions with proteins like HDAC 1 to 10 (Histone deacetylase- plays an important role in transcriptional regulation, cell cycle progression and developmental events)

SIRT6

SIRT6 is a nuclear protein that has both ADP-ribosyltransferase and deacetylase activity. SIRT6, an enzyme highly expressed in skeletal muscles, brain, heart, liver, and thymus, affects transcriptional regulation in a tissue-specific manner. A mono-ADP-ribosyltransferase, which can transfer an ADP-ribose moiety from NAD to itself and histones. SIRT6 has both ADP-ribosylase and deacetylase activity and plays a role in base excision repair[31]. The enzyme has a two-domain structure that consists of a large Rossmann fold and a smaller and structurally extra varied sequence containing a Zn2+-binding motif. The C-terminus is required for proper nuclear localization, while the N-terminus is important for chromatin association and for intrinsic catalytic activity[29]. More recently, it has been discovered that SIRT-6 can deacetylate histone H3 lysine-9

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(H3K9) and H3K56. SIRT-6 has been shown to maintain genomic stability and telomere integrity, and to prevent age-related disorders and premature aging. It deacetylation substrates include histone H3K9 and H3K56. By deacetylating H3 associated as HIF-1α and MYC [(39,40)].This mechanism has been used to explain many of the phenotype of Sirt6 knockout mice. However, given that the in vitro deacetylase activity is weak, other activity of SIRT6 has been reported[14]. SIRT6 suppresses the transcription of the target genes of these transcription factors.

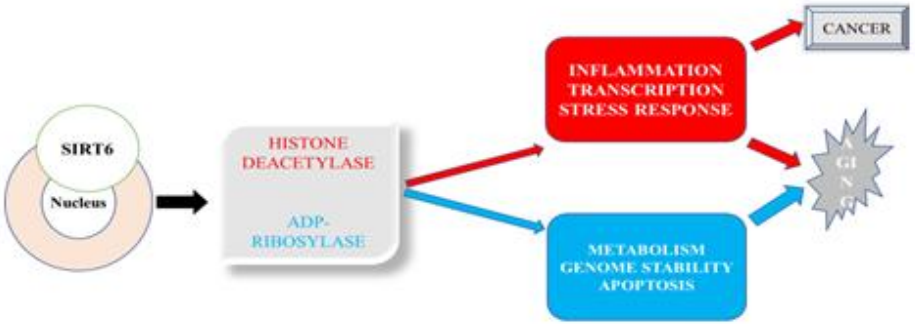

Fig11: Depicts the dual catalytic role of SIRT6 in nucleus: SIRT6, has both Histone deacetylase as well as ADP-ribosylase activity.

Table 7: Anti-inflammatory role of SIRT6

Role of SIRT6	Substrate	Activity	References
Deacetylase activity	H3K9, H3K56, HIF-1α and MYC	deacetylase activity and plays a role in base excision repair	Eriko, et al. [40]
ADP-ribosyltransferase activity	ADP-ribose and NAD	ADP-ribose moiety from NAD to itself and histones	Rui-Hong, et al [31]
Directly deacetylate p65 of NF-κB	NF-kB, IκB	anti-inflammatory	Tiara LA, et al [59]

Indeed, transgenic overexpression of SIRT-6 increases lifespan. Metabolically, the level of SIRT-6 increases in response to the restriction of nutrient availability, and this contributes to lifespan extension. In addition, its critical role in lifespan regulation, SIRT-6 has also been implicated in modulating NF-κB–facilitated inflammatory responses. SIRT-6 deacetylates H3K9 on the promoters of NF-κB target genes to decrease promoter occupancy by the p65 subunit of NF-κB, while SIRT-1 and SIRT-2 directly deacetylate p65 and inhibit its transcriptional activity. Since the regulation of NF-κB activity through revocable acetylation is an effective way to control inflammatory reactions[(16, 58)].

NF-κB is a heterodimeric complex of p50 (encoded by *NFKB1*) and p65 (RELA). In the inactive state, NF-κB complexes are sequestered in the cytoplasm by Iκ-B inhibitory proteins. A variety of signals like oxidative stress and DNA damage stimulate the phosphorylation and subsequent degradation of IκB, which leads to the nuclear translocation of NF-κB. NF-κB controls the activity of genes involved in apoptosis, cell senescence, inflammation, and immunity, and its activity increase with age in many mammalian tissues and stem cells. Indeed, genetic hyperactivation of NF-κB results in diseases in murine models including muscle wasting and obesity-induced insulin resistance.

Thus, both SIRT1 and SIRT6 down-regulate the NF-κB signaling pathway. NF-κB is a heterodimeric complex of p50 (encoded by *NFKB1*) and p65 (RELA). In the inactive state, NF-κB complexes are

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sequestered in the cytoplasm by Iκ-B inhibitory proteins. A variety of signals like oxidative stress and DNA damage stimulate the phosphorylation and subsequent degradation of IκB, which leads to the nuclear translocation of NF-κB. NF-κB controls the activity of genes involved in apoptosis, cell senescence, inflammation, and immunity, and its activity increase with age in many mammalian tissues and stem cells[58]. However, another study by Van Gool et al. indicated that intracellular NAD levels promoted TNFα protein synthesis in SIRT6 dependent manner[59].

SIRT6 is a poor deacetylase *in vitro*, but binds and prefers to hydrolyze long-chain acylated peptides, SIRT6, hypothesize that binding of certain free fatty acids (FFAs) could stimulate deacetylation activity. Definitely, SIRT6 demonstrate that several biologically relevant FFAs (including myristic, oleic, and linoleic acids) at physiological concentrations induce up to a 35-fold increase in catalytic efficiency of SIRT6 but not SIRT1. The activation mechanism is consistent with fatty acid inducing a conformation that binds acetylated H3 with greater affinity. Binding of long-chain FFA and myristoylated H3 peptide is mutually exclusive.

NF-κB has been implicated as a candidate activator of aging-related transcriptional changes in multiple human and mouse tissues. Genetic blockade of NF-κB in the skin of chronologically aged mice reversed the global gene expression program and tissue characteristics to those of young mice. Further, NF-κB blockade increased the proliferative capacity of the skin and reversed several markers of cellular senescence to levels observed in young animals. Therefore, NF-κB blockade may alleviate aspects of aging that are because of hyper-activity of NF-κB. Activation of SIRT1 and/or SIRT6 might be one approach to creating such a blockade.SIRT6 also plays a role in inflammatory pathways, exerting anti-inflammatory effects at the transcriptional level. However, other studies suggest a pro-inflammatory effect on intracellular signaling. Although SIRT6 functions in metabolism, inflammation, and genome maintenance, its molecular functions remain enigmatic.

Protein-Protein Interaction studies for SIRT6

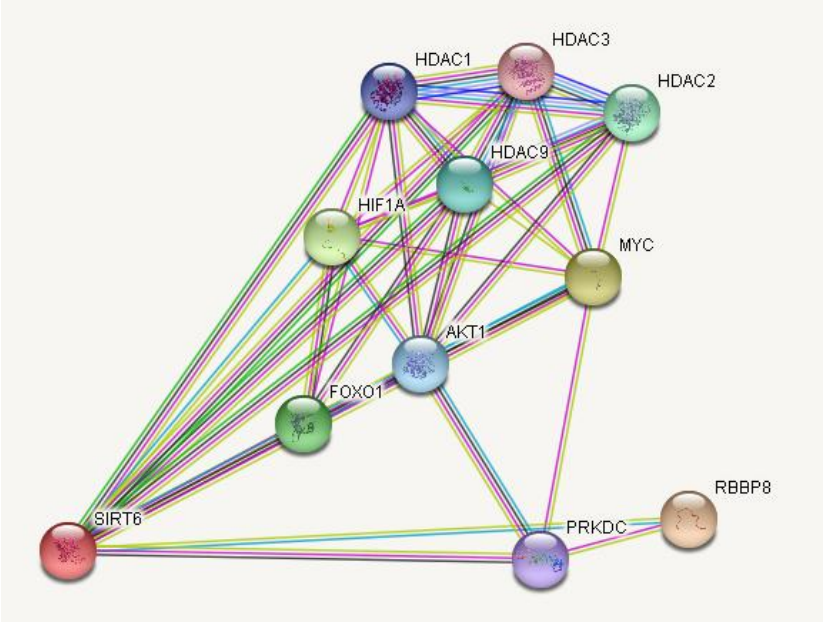

Fig 12: Protein Interaction of SIRT6 with other proteins

SIRT6 has shown interactions with proteins like HDAC1, 2, 3, 9 (Histone deacetylase), HIF1A (Hypoxia inducible factor 1- a master transcriptional regulator of the adaptive response to hypoxia), FOXO1 (Forkhead box O1), AKT1 (V-akt murine thymoma viral oncogene homolog 1- egulate many processes including metabolism, proliferation, cell survival, growth and angiogenesis), MYC (V-myc myelocytomatosis viral oncogene homolog), PRKDC (Protein kinase, DNA-activated, catalytic polypeptide; Serine/threonine-protein kinase that acts as a molecular sensor for DNA damage) and RBBP8 (Retinoblastoma binding protein 8).

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SIRT7

SIRT7 is a nuclear sirtuin and is enriched in nucleoli. Existing evidence suggests that it regulates ribosome biogenesis by controlling rRNAs, tRNAs, and ribosomal protein synthesis.SIRT7 research show that SIRT7 activates RNA polymerase I transcription[(28,41)]. SIRT7 has been demonstrated to be a H3K18-specfic deacetylase. It can be recruited by specific transcription factors, such as ELK4 and MYC, to specific genes (such as genes encoding ribosomal proteins) and repress the expression of these genes by deacetylating H3K18. It has been demonstrated that knockdown of SIRT7 inhibits the colony formation of HT1080 (a fibrosarcoma cell line) and U2OS (an osteosarcoma cell line) on soft agar, and decreases U251 (a glioma cell line) tumor size in mouse xenograft models. SIRT7 knockdown also inhibits adenoviral E1A-induced cellular transformation. SIRT7 plays a grave function in maintaining properties of cancer cells, including escape from cell contact inhibition and anchorage-independent growth. Thus, SIRT7 is a highly selective H3K18Ac deacetylase and has a key role in chromatin regulation, cellular transformation, and tumor formation[42].

Table 8: Diverse role of SIRT 7 in different cells

Role of SIRT7	Substrate	Activity	References
Deacetylates	H3K18Ac (highly selective)	chromatin regulation cellular transformation tumor formation	Matthew et al [42]
Ribosomal protein synthesis	rRNAs, tRNAs	regulates ribosome biogenesis	Ethan, et al [28]
SIRT7 activates	RNA polymerase I	Promotes transcription	Ethan, et al [28]
Oncogenic function	Mice model study suggests	SIRT7 Can function as oncogene	Zwaans et al[64]
SIRT7 knockdown	NME1 and ribosomal protein genes *via* H3K18 acetylation	Increased expression of certain genes NME1, ribosome biogenesis and transcriptional regulation	Ethan, et al [28]

The effects of SIRT7 knockdown might be mediated by the increased expression of certain genes, such as NME1 and ribosomal protein genes *via* H3K18 acetylation[28]. SIRT7 has been revealed to be important for ribosome biogenesis and transcriptional regulation. SIRT7 knockout mice parade complications associated with fatty liver and increased aging in hematopoietic stem cells.

However, the molecular basis for its biological function remains unclear, in part due to the lack of efficient enzymatic activity in vitro[43].The loss of Sirt7 resulted in the inhibition of cell proliferation and the induction of apoptosis, although targets of Sirt7 remain unknown. The deletion of Sirt7 in mice led to a reduction of life span by the development of heart hypertrophy and inflammatory cardiomyopathy[63].According to previous studies of Zwaans et al[64], it has been postulated that Sirt7 can function as an oncogene. Barber et al. showed that while the gain of function of Sirt7 triggers the oncogenic capacity of cancer cells such as anchorage-independent growth and the loss of contact inhibition, the loss of Sirt7 function significantly reduced the tumorigenic potential of cancer cells, suggesting Sirt7 has a tumor-promotive function. [64]

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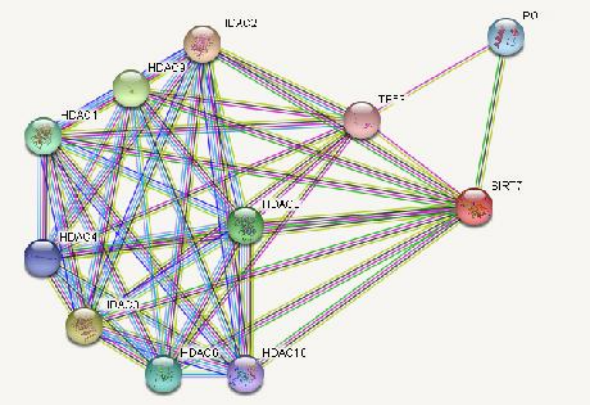

Fig 13: Protein Interaction of SIRT7 with other proteins

SIRT7 has shown interactions with proteins like HDAC 1-4, 6, 8-10 (Histone deacetylase), TP53 **(**Tumor protein p53 **)** and POLI **(**Polymerase (DNA directed) iota; Error-prone DNA polymerase specifically involved in DNA repair).

CONCLUSIONS

Cancer associated with inflammation could be regulated by Sirtuins activity/expression leading to an increased healthy lifespan. Owing to this profile, SIRT1 most profoundly shows the anti-cancer activity. SIRT1, activators have potential applications for multiple therapeutic areas including type 2 diabetes, inflammation, neurodegeneration, cancer and heart disease. Activation of SIRT1 and SIRT3 has been suggested for the treatment of metabolic diseases, whereas SIRT1, SIRT2, SIRT3 andSIRT4 inhibition may be useful in certain cancers and neurodegeneration. SIRT4 suppression tumors mediated by glutamate dehydrogenase(GDH) and show the SIRT4-mediated regulation of DLAT lipoyl levels and PDH activity in cells and in vivo. Even though the data is limited, roles for the other sirtuins, SIRT5 has not been reported any activity as oncogene or tumorigenesis, but this has other desuccinylase and demalonylase activity. SIRT6, mediates the anti-inflammation by NF-Kb inhibition, ADP-ribosyl activity and serves role in DNA-base excision repair. SIRT7 has been demonstrated to be a H3K18-specfic deacetylase activity. Modulation of various Sirtuin activity could lead to a new platform opportunity for drug discovery. For example, current treatments for various types of inflammatory disorders like rheumatoid arthritis treatment. Data so far obtained with SIRT1 activators show improved glucose homeostasis without any of the above side effects. However, SIRT1 is a novel target and comes with the challenge of discovering molecules that are activators rather than inhibitors and would be developed as first-in-class therapeutics. The side-effect profile, if any, is still to be determined.

ACKNOWLEDGEMENT

The authors like to thank Bilaspur University, Bilaspur (CG) for providing necessary facilities and financial support for bringing out the work.

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ABBREVIATIONS

:	Sirtuin
NAD+:	Nicotinamide dinucleotide
SIR 2:	Silent information regulator 2
ATP:	Adenosine triphosphate
ADP:	Adenosine dinucleotide
AMP:	Adenosine monophosphate
Acetyl CoA:	Acetyl coenzyme A
PPAR-γ:	Peroxisome proliferator-activated receptor gamma
PGC-1α:	PPAR-γ coactivator 1 alpha
HIF-1α:	Hypoxia inducing factor alpha
NADPH:	Nicotinamide adenine dinucleotide phosphate
UCP:	Uncoupling protein
NFκB:	Nuclear factor κ B
ROS:	Reactive oxygen species
AMPK:	Adenosine monophosphate-activated protein kinase

mTOR:	Mammalian target of rapamycin.
ATG	autophagy-related protein
ATL	adult T-cell leukemia-lymphoma
BRCA1	breast cancer susceptibility gene 1
FOXO	forkhead-box
H3K18Ac	H3 acetyl lysine 18
HIC1	hypermethylated in cancer 1
HTLV-1	human T-cell leukemia virus
IC50	50% inhibitory concentration
NAD	nicotinamide adenine dinucleotide
NF-κB	nuclear factor-κB
Rb	retinoblastoma
RIP3	receptor-interacting protein kinase 3
Sir2	Silencing information regulator 2
TGF-β	transforming growth factor-β
TNF-α	tumor necrosis factor-α