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Mitochondrial Sirtuins: Their Role in Different Diseases

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Abstract

This review paper aims to provide a comprehensive understanding of the three mitochondrial sirtuins (SIRT3, SIRT4, and SIRT5). Investigating their structural features, enzymatic activities, target proteins, involvement in diverse illness situations, and the therapeutic modulators created for SIRT3 and SIRT5 are the study's goals. We conducted a thorough literature survey, focusing on works that discussed the biochemical and physiological roles of SIRT3, SIRT4, and SIRT5 in the mitochondria. We looked over relevant research papers, reviews, and databases to learn more about their molecular make-ups, enzyme activity, and substrates. Studies indicating their involvement in metabolic pathways, physiological processes, and disease correlations gained considerable attention. Deacetylase activity in SIRT3 affects the TCA cycle, fatty acid oxidation, and glycolysis; SIRT4 affects the amino acids and TCA cycle; and SIRT5's acyl modifications affect the urea cycle, ROS, and glucose oxidation. Therapeutic alternatives are available for both SIRT3 and SIRT5. The review paper emphasizes the crucial functions of SIRT3, SIRT4, and SIRT5 in mitochondrial metabolism and their implications in a variety of illness situations in its conclusion. With a better understanding of their roles and potential therapeutic regulation, metabolic disorders, and other associated diseases may be treated. Unlocking the full potential of mitochondrial sirtuins as therapeutic targets will require more research in this area.

Keywords: ***Mitochondrial sirtuins, SIRT3, SIRT4, SIRT5, TCA cycle, acyl modifications, ROS***

Introduction

Sirtuins are a class of seven NAD+-dependent deacylases that are highly conserved and are involved in the removal of a large number of acyl modifications from cellular proteins and, as a result, regulate cellular processes by deacetylating intracellular targets such as signaling molecules, transcription factors, and enzymes [(1,2).]These proteins are homologous to proteins expressed by the Silent information regulation 2 (Sir2) gene, which have been shown to slow ageing in the yeast species *Saccharomyces cerevisia*e and they are largely conserved across species from bacteria to humans.[(3)]Ageing is a complex process characterized by a variety of cellular changes that occur over the course of an organism's existence. In yeast, it has been demonstrated that adding an extra SIR2 allele can enhance lifespan by 30%, whilst deleting SIR2 reduces lifespan. In agreement with this finding, the increased longevity that is seen with calorie restriction in yeast requires SIR2 activation [(4)]. Structurally, all Sirtuins have a conserved catalytic core (250 amino acids) with two domains- a large NAD+ binding domain known as the Rossmann fold and another small Zn^{2+} containing domain and have distinct N-terminal as well as C-terminal extensions. A hydrophobic cleft is formed by four loops that connect the large and small domains of the enzyme. Both NAD+ and the acetylated substrate bind to the cleft. Among the four

loops, the largest one is conformationally the most dynamic region of the sirtuin enzymes. This loop is referred to as the co-factor binding loop because it forms a part of the NAD+ cofactor binding site**.** When NAD+ is not bound, the cofactor binding loop is highly disordered or flexible but when NAD+ binds it becomes ordered [(5)].

The pocket within the cleft to which NAD+ binds is divided into three sites. A site – adenosine ribose component of NAD+ binds, B site – the nicotinamide ribose component binds, C site – nicotinamide binds[(6)]. After binding of the two molecules, the nicotinamide group of NAD is released when the carbonyl oxygen of the acetyl group attacks the C1' position of the nicotinamide ribose which leads to the formation of the alkylamidate intermediate (N-ribose). The 2'-OH ribose after deprotonation by the enzyme attacks the amidated at the carbonyl carbon leading to the generation of 1',2'- cyclic intermediate. Consequently, the bicyclic intermediate is hydrolyzed to produce 2'-O- acetyl-ADP-ribose. End products include the deacetylated protein and the 2'-O-acetyl-ADP- ribose moiety **(Fig.1)**[(7)]. All seven Sirtuins vary according to their subcellular localization. They are involved in carrying out different post-translational modifications and differ in their substrate affinity. [(8)]

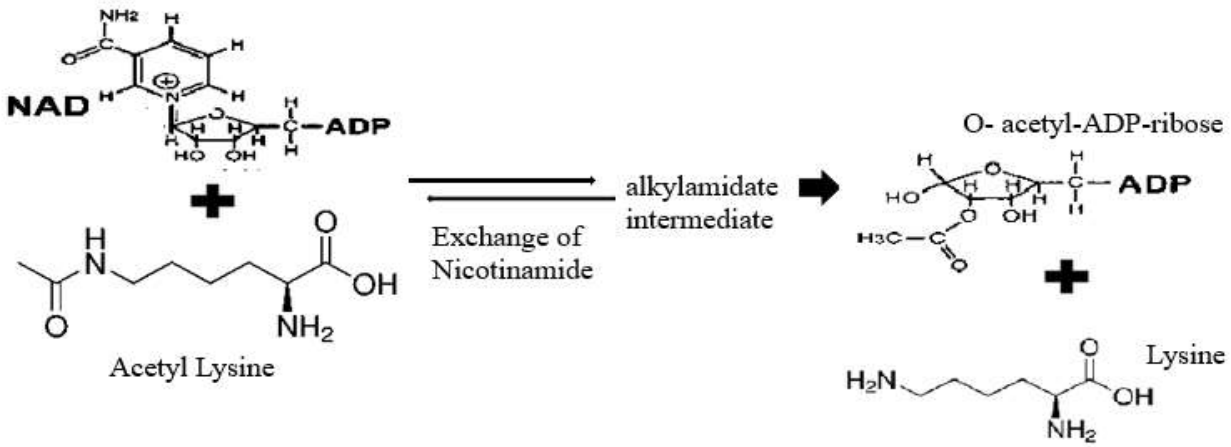

Figure 1: Mechanism of Enzyme-catalysed deacetylation where the 2' carbonyl oxygen attacks N-ribose leading to the formation of O-alkyl amidate which is hydrolyzed into Lysine and OAADPR (Alvalos et. al 2005)

Cytoplasmic Sirtuins

Sirtuin 2 (SIRT 2) is localized in the cytoplasm within the cell and interacts with chromatin in the nucleus [(9,10)]. Sirt2 possesses both deacetylase and demyristoylase activity invitro. SIRT2 is mostly found in the central nervous system and plays important role in regulation of tubulin acetylation, cell cycle and glucose metabolism [(9,11–14)].

Nuclear Sirtuins

Sirtuin 1, Sirtuin 6, Sirtuin 7 are mainly found in the nucleus but all three enzymes have been reported to be localized outside the nucleus [(15)]. Sirtuin 1 can translocate from the nucleus to the cytosol, according to studies on adult mouse heart. It acts as a nucleocytoplasmic shuttling protein. In vivo, the SIRT1 subcellular localization differed from cell to cell as demonstrated by Tanno. et. al. Significance of SIRT 1's cytoplasmic localization is unknown but modifying its nucleocytoplasmic shutting can provide a new therapeutic approach to prevent diseases caused by oxidative stress because SIRT 1 has been found to increase the resistance of a cell to apoptosis. [(16)]. Sirtuin 1 can deacetylate a large number of substrates and therefore it is involved in different physiological processes like control of gene expression, metabolism and ageing. Several transcription factors act as Sirtuin 1 substrates including tumour suppressor protein, p53, members present in the Foxo family, PPARα (peroxisome proliferator-activated receptor-α), NFB (nuclear factor kappa β) and PGC-1 (PPAR coactivator) etc.[(17)]

SIRT6 which is localized within the nucleus possesses both histone deacetylation and ADP- ribosyl transferase activity [(18,19)]. Sirtuin 6 is thought to be essential for cell survival because Sirtuin 6 Knock-out mice died in twenty four days of its birth because of the degenerative process[(20)]. SIRT6 overexpression reduces low-density lipoprotein and triglyceride levels, improves glucose tolerance, and extends the longevity of male mice, according to different studies [(21,22)].

Sirtuin 7 is basically found in the nucleus and nucleolus, although it has also been found in the cytoplasm of

primary fibroblasts. [23,24]. Sirtuin 7's prominent nucleolus localization further suggested a role for Sirtuin 7 (SIRT7) in rDNA transcription. [25].

Mitochondrial Sirtuins

Among the seven Sirtuins, three of them (SIRT3, SIRT4, SIRT5) are found within the mitochondria, an organelle that specializes in energy production, stress responses and signaling [26]. The mitochondrial SIRT3 is the most well-studied. It possesses deacetylase activity towards a large number of metabolic substrates including enzymes of the Electron transport chain (ETC), fatty acid oxidation, amino acid metabolism and the TCA cycle [27].

SIRT4 is a metabolic regulator that inhibits Malonyl CoA decarboxylase, Pyruvatdehydrogenase and pancreatic glutamate Dehydrogenase [28] SIRT5 have a low level of deacylase activity but high levels of desuccinylase, demalonylase, and deglutarylase activity [29]. SIRT5 also plays an important role in pyruvate metabolism via control of oxidative phosphorylation [30].

Structure of Mitochondrial Sirtuins

Human SIRT3 protein contains 399 residues and has a N-terminal mitochondrial targeting sequence. The full-length protein is inert enzymatically, but mitochondrial matrix processing peptidase cleaves 101 residues at the N-terminus, which activates the enzyme [31]. SIRT3 has a two-domain structure, similar to other sirtuins, with a large Rossman fold domain for NAD^+ binding and a smaller domain with a helical bundle and another zinc binding motif formed by two extending loops from the large domain. [31]. In addition to having deacetylation activity, SIRT3 has also been reported to have depalmitoylation and demyristoylation activity [32].

Human SIRT4 crystal structure could not be obtained therefore orthologues of SIRT 4 from *Xenopus tropicalis* and *Danio rerio* were used as model systems in order to study the structure of SIRT4. The sequence similarity within the catalytic core between hSirt4 and xSirt4 was found to be 81% (for zSirt4 and hSirt4 it is 78%). SIRT4 share the same overall domain organization similar to the other Sirtuins. An important structural difference between SIRT4 and all the other sirtuins is in the presence of an extended 12 residue SIRT 4 loop in the Zinc binding domain. The loop lines the active site and is present deep within the catalytic core.

Structure comparison has shown that in the case of SIRT 5, the loop is present as a small surface loop and in other isoforms it is present as a short turn **(Fig.2)**.

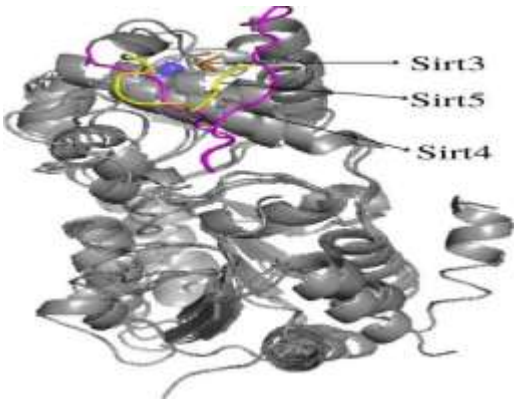

Figure 2: Superimposition of SIRT3, SIRT4, SIRT5 structures showing SIRT4 specific loop extending into the catalytic pocket, present as a surface loop in SIRT5 and short turn in SIRT3.The PDB structures were downloaded from NCBI site and the diagram was made using chimera software.

SIRT4 tends to show weak deacetylation activity but has high delipoylation and debiotinylation activities. They have also been shown to have high activity against 3,3-dimethylsuccinyl (DMS) substrate and 3-hydroxy-3-methylglutaryl (HMG) substrate. ADP-ribosyl transferase activity of SIRT4 has also been found.

SIRT5 has 310 amino acid residues and similar to Sirtuin3 has an N-terminal mitochondrial targeting sequence

(residues 1-36 transit peptide). The monomer of Sirt5 is made up of 14 alpha-helices and 9 beta-strands arranged into two globular domains: a large Rossmann fold domain and a smaller zinc-binding domain. The gap which is formed between the two domains is traversed by many loops. The six parallel Beta strands of the Rossmann fold domain form a central beta-sheet and the nine alpha-helices pack against the sheet. The zinc-binding domain, on the other hand, has a small three-stranded antiparallel beta-sheet and five alpha-helices. Besides having an overall similar structure to other Sirtuins, SIRT 5 has some of its structural variations. SIRT5 possesses two non-hydrophobic residues, Tyr102 and Arg105 located within the substrate-binding pocket. These two residues are involved in recognizing negatively charged acyl-lysine groups (glutaryl, succinyl, malonyl). In addition to this SIRT 5 has a small Ala86 residue (the same place occupied by Phenylalanine in SIRT1, 2 and 3, which allows it to have a larger acyl-binding pocket in comparison to other sirtuins [33].

In addition to having deacetylation activity, SIRT5 also possesses desuccinylation and demalonylation activity. In fact the catalytic efficiency for demalonylation and desuccinylation was found to be much higher compared to deacetylation. The mechanism of desuccinylation and demalonylation catalysed by SIRT5 is similar to that of deacetylation catalysed by class I sirtuins, which involves the production of O-malonyl- or O-succinyl-adenosine 5'- diphosphoribose (O-Ma-ADPR) or O-succinyl-ADPR (O-Su-ADPR).

SIRT3: Targets and functions

SIRT3 has been shown to regulate nearly every aspect of mitochondrial function, including ROS detoxification, energy production, oxidation of nutrients, and the mitochondrial UPR [30,34–39]. These processes are primarily regulated by SIRT3 by the removal of acetyl modifications from mitochondrial proteins involved in the process. SIRT3 has been seen to regulate most of the lysine acetylation within the mitochondria as shown by biochemical methods [40–42]. Sirt3's first reported target was a crucial mitochondrial enzyme called Acetyl CoA synthetase 2. (AceCS2). Acetyl-CoA synthetase 2 which catalyses the production of Acetyl-CoA synthetase was shown to be activated upon deacetylation of lysine residue Lys642 by Sirt3 [43,44]. SIRT3 has been identified to directly alter the activity of a vast variety of target proteins, and hence its role in all key mitochondrial activities, including fatty acid metabolism, electron transport chain, apoptosis, antioxidant defences, and amino acid catabolism, has been suggested [41,45–48]. Sirt3 regulates mitochondrial energy metabolism by controlling the enzymatic activity of major oxidative phosphorylation enzymes via deacetylation [49].

SIRT3 deficiency in the cell enhances glycolysis via two pathways. First, in the absence of SIRT3, Cyclophilin D is heavily acetylated, which activates Hexokinase 2, a crucial enzyme of glycolysis found in the outer membrane of mitochondria. Hexokinase 2 is involved in the first stage of glycolysis, the phosphorylation of glucose to Glucose-6-phosphate [50,51]. The second mechanism involves the transcription factor, HIF1α (modulates expression of glycolytic genes. SIRT3 deletion increases ROS production thus stabilizing HIF1α [52,53].

SIRT3 also regulates beta-oxidation of fatty acids by deacetylation of long-chain acyl CoA dehydrogenase, one of the major enzymes in the process. SIRT3 is also involved in regulating the activity of many other fatty acid oxidation enzymes such as medium-chain specific acyl-CoA dehydrogenase (ACADM) and acyl glycerol kinase (AGK) [54]. In addition to this, Sirt3 also contributes to ketone body synthesis by deacetylation and activation of 3-hydroxy-3-methylglutaryl-CoA synthetase (HMGCS2), which acts as the rate-limiting enzyme in the process [55,56].

One of the key enzymes of Urea Cycle, orinithine transcarbomoylase (OTC), is a substrate for Sirt3. It can upregulate the urea cycle by deacetylating OTC, which suggests that SIRT3 promotes amino acid catabolism and detoxification of ammonia during metabolic stress [57]. In addition, Sirt3 deacetylation activates ETC complex components such as NDUFA9 (complex I) and SDHA (complex II) [58,59]. Besides this, SIRT3 deacetylates IDH2 which is an important enzyme of Tricarboxylic acid cycle. IDH2 causes the oxidation of isocitrate to alpha-ketoglutarate and produces NADPH [60].

Furthermore, SIRT3 appears to have an important role in oxidative stress resistance outside of metabolic pathways. Oxidative stress leads to the accumulation of large amounts of intracellular ROS which in turn causes the destruction of lipids, proteins, nucleic acids and mitochondrial DNA mutations. SIRT3 also helps to reduce the amount of ROS which is produced within the mitochondria by multiple mechanisms. SIRT3 directly regulates superoxide dismutase (SOD2), a key enzyme that acts as superoxide scavenger [61–66].IDH2 which is a TCA cycle enzyme acts as a substrate for SIRT3. Deacetylation of IDH2 by SIRT3 increases the production of NADPH via TCA cycle Second, SIRT3 contributes to the replenishment of the mitochondrial NADPH pool by deacetylating and activating the TCA cycle enzyme IDH2. NADPH in turn influences glutathione reductase which helps the cell to cope with cellular oxidative stress [60].

SIRT3 and Diseases

Cardiovascular Diseases

Cardiac function tends to deteriorate as people age and understanding the underlying mechanisms that cause this dysfunction is critical for the development of future therapeutics. Studies have reported that decreased mitochondrial function in mice leads to a decline in the function of the heart and expedites cardiac hypertrophy. Cardiac hypertrophy involves the enlargement of the myocardium which ultimately leads to cardiomyocyte death, fibrosis, and cardiac pressure or volume overload-induced heart failure [67]. A large number of studies have reported that poor SIRT3 activity is one of the major causes of cardiac hypertrophy and heart failure [68,69]. Nicotinamide mononucleotide adenylyltransferase 3 (NMNAT3) which is the only enzyme of the NAD^+ synthesis pathway acts as a SIRT3 substrate. SIRT3 deacetylates NMNAT3 and activates it. As this enzyme is involved in the synthesis of NAD^+ it directly contributes to the antihypertrophic effects of Sirtuin by supplying NAD^+ within the cardiomyocytes [70,71,72].

SIRT3-deficient mice have abnormal fatty acid oxidation in the mitochondria of the heart [37,73] as well as reduced oxidative phosphorylation complex activity and energy production.[35] As already reported SIRT3 prevent ROS accumulation in the heart. The fact that SIRT3KO mice's cardiomyocytes produce more ROS under normal conditions supports this idea. Cyclophilin D which is thought to be a structural component of mitochondrial permeability transition pore is a SIRT3 target. In SIRT3KO mice Cyclophilin D is hyperacetylated which leads to mitochondrial dysfunction, increased mitochondrial permeability and altered energetics [68]. Normally, fusion is a mitochondrial stress response that promotes mitochondrial function. Another protein optic atrophy 1 (OPA1) which belongs to the family of fusion proteins is a SIRT3 target and loss of SIRT3 causes OPA1 hyperacetylation and impairment in mitochondrial fusion [36].

Moreover, decreased expression of SIRT3 increases the susceptibility of cells derived from heart and adult hearts to ischemic reperfusion injury [74].

Together, all of these studies have proved that SIRT3 regulates many different mitochondrial pathways which are critical to maintain cardiac health. In the absence of SIRT3, most of these pathways are abnormally regulated leading to different heart-related diseases.

Cancer

Various studies have reported that there is a direct correlation between SIRT3 and the progression of Cancer. SIRT3 exhibits both tumour-suppressive and promoting capacity depending on the type of cancer [81]. Under Hypoxic conditions, increased expression of SIRT3 decreases tumorigenesis, by inhibiting glycolysis, ROS production and HIFα stabilization [82]. HIF α, upon stabilization, is able to move into the nucleus and enhance transcription of its target genes involved in cancer [83]. SIRT3 also causes reduction in the level of reactive oxygen species which ultimately leads to HIFα destabilization and degradation [84].

SIRT3 acts as an oncogenic factor in different types of cancer. High SIRT3 expression is linked to a poor prognosis in oesophageal cancer. [85] In addition to this both in case of grade 3 breast cancer [86] and Squamous cell carcinoma of the mouth, overexpression of SIRT3 has been reported [87]. In human melanoma cells, SIRT3 deletion inhibits cell proliferation, colony formation, and migration, as well as causes G1 phase arrest [88]. In addition, SIRT3 can rescue p53-induced growth arrest by abrogating p53 activity in human bladder tumour derived EJ-p53 cells, making p53 a new target for SIRT3 deacetylation in cases of bladder cancer [89].

SIRT3 has also been shown to play a pro-apoptotic role in cancers. For example, SIRT3 expression is very low in breast cancer cells compared to normal breast epithelium [90]. In breast cell lines, SIRT3 has been reported to induce inhibition of glycolysis and reverse metabolic reprogramming [50]. Lower SIRT3 expression is linked to greater aggressiveness and a quicker time to relapse in pancreatic cancer. [91]. In addition to this, metastatic ovarian cancer tissues have significantly downregulated expression of SIRT3 [92]. SIRT3 also deacetylates cyclophilin D, causing hexokinase II to bind to voltage-dependent anion channels and preserve mitochondrial integrity.

SIRT4: Target and functions

SIRT4, like SIRT3 and SIRT5, is found in the mitochondrial matrix and is expressed in numerous human

tissues, with particularly high levels in the heart, liver, kidney, testis, ovary, and prostate. Although it was previously discovered that most Sirtuins serve as lysine deacetylases, later research revealed that SIRT4 has very weak deacetylase activity. All the Sirtuins have a catalytic domain consisting of 275 amino acid residues [93]. The catalytic domain of SIRT4 is unique and closely resembles prokaryotic and other metazoan Sirtuins, according to phylogenetic analysis [3,93]. Additional research has looked at many of SIRT4's enzymatic activity and specific substrates. In addition to having weak deacetylase activity and ADP-ribosyl transferase activity, SIRT4 hydrolyse lipoyl- and biotinyl-lysine modifications more efficiently [94]. Lipoylation is a very rare Post-transcriptional modification because there are only four multicomponent enzymatic complexes having lipolylation as a modification. Four of these include- Pyruvate dehydrogenase complex (PDH), alpha-ketoglutarate dehydrogenase complex (KDH), and branched chain alpha-keto acid dehydrogenase complex (BCKDH) and glycine cleavage system [95]. SIRT4 has been discovered to interact with subunits of all four enzymatic complexes. For example, SIRT4 remove lipoylation from DLAT which is a PDH subunit [83]. It was further discovered that in addition to removing both lipoyl and biotinyl modifications, SIRT4 recognises acyl-lysine modification with a high affinity [111].

In vitro and in vivo, they catalyse the removal of 3-hydroxy-3-methyl glutaryl (HMG), 3- methylglutaryl (MG), and 3-methylglutaconyl from lysine residues [111].

Besides this, SIRT4 has also been found to be an important regulator of lipid metabolism [96].Malonyl CoA decarboxylase (MCD) has been demonstrated to be deacetylated and inactivated by SIRT4. MCD, in turn, stimulates the synthesis of acetyl CoA from malonyl CoA. [97]. PRARα (a ligand-activated transcription factor) has been found to increase transcription of fatty acid metabolism genes. [98]). SIRT4 in turn inhibits PRARα activity thereby supressing hepatic fatty acid oxidation [99]. Both SIRT3 and SIRT4 regulate fatty acid oxidation and they seem to have opposing roles [37,100]. Therefore it remains to be determined how these two mitochondrial enzymes regulate distinct metabolic responses.

SIRT4 and Disease

Heart diseases

The involvement of SIRT4 in the development of heart disease has been revealed. Deficiency of SIRT4 supresses myocardial hypertrophy and fibrosis after Ang II infusion (114). But in case of ischemic heart injury, SIRT4 has ameliorative role (103,104). SIRT4 is highly expressed in numerous organs, including the heart, liver, brain and kidney (105).

A large amount of ROS is generated in the mitochondria by the process of oxidative phosphorylation and the mitochondria are damaged by prolonged oxidative stress [106]. A particularly important enzyme, MnSOD, prevents excessive accumulation of ROS in the mitochondria. MnSOD is involved in catalyzing the production of hydrogen peroxide from superoxide radicals and hydrogen peroxide is thereby converted to H2O by other antioxidant enzymes [106]. MnSOD is a substrate for SIRT3, which is another mitochondrial Sirtuin [102]. SIRT3 causes activation of MnSOD which thereby helps to reduce ROS levels and oxidative stress. The overall activity of MnSOD was seen to be reduced during hypertrophic stress [102]. SIRT4 in turn has been reported to prevent interaction between SIRT3 and MnSOD thereby increasing MnSOD acetylation levels upon Ang II treatment. In mouse liver also, SIRT3 expression is influenced by SIRT4. Therefore, we can conclude that during cardiac hypertrophy SIRT4 competes with MnSOD for binding to SIRT3 and SIRT4 overexpression increases MnSOD acetylation levels [102].

Growing studies have demonstrated the important part played by miRNA in the progression of cardiac hypertrophy and failure of heart [107–109]. It has been recently reported that miR-497 is up-regulated in cardiac hypertrophy and therefore acts as a unique regulator of cardiac hypertrophy [110]. Wimin Xiao et al., 2006 [103] reported for the first time that miRNA 497 is negatively correlated to Sirt4 and target the 3ˈUTR of the protein, there by supressing cardiac hypertrophy.

In case of ischemic heart injury SIRT4 has been reported to have a protective role [106]. Members of the Bcl-2 family play significant role in the cellular apoptosis process [111]. Bax which belongs to this family is mainly found in the cytoplasm or loosely attached to the mitochondria under normal condition [112,113]. Bax translocate to the mitochondria upon apoptotic stimulation. It has been reported that SIRT4 affect Bax translocation and thereby provide protection against hypoxia induced apoptosis. In H9c2 cardiomyoblast cells, this increases the viability of cells and protects against the pathogenesis of ischemic heart injury [109].

Cancer

In several types of cancer for example liver [101,114], endometrial [115,116], lung [101], ovarian, pancreatic, bladder, renal, prostate [101], oesophageal [118], breast [119], lymphoma [119] and leukemia [120], there was a reduction in SIRT4 expression. Previous research found that overexpression of SIRT4 decreased the growth of Myc-driven human Burkitt lymphoma cells and HeLa cells via inhibiting glutamine metabolism [119,121]. Glutamine metabolism tends to play an important role in cancer cell growth by replenishing Tricarboxylic acid cycle intermediate to support increased growth and production of ammonia which neutralizes the acidic metabolites produced during increased glycolysis in cancer cells. As a result, SIRT4 is hypothesised to function as a tumour suppressor by limiting glutamine metabolism via GDH repression.

In addition to playing an important role in glutamine metabolism, SIRT4 is also involved in the regulation of various other key processes in cancer, including progression of the cell cycle, apoptosis, metastasis and growth of tumour. For example in case of DNA damage SIRT4 prevents cell cycle progression by inhibiting glutamine reflux [133]. In lung cancer cells SIRT4 is also known to inhibit mitochondrial fission. This procedure has been shown to promote cancer cell proliferation and prevent apoptosis [113]. SIRT4 inhibits the activity of Mitogen-activated protein kinase and extracellular signal-regulated kinase in lung cells. These protein kinases phosphorylate and activate Drp1 which is involved in promoting mitochondrial fission. As a result of Drp1 inhibition, mitochondrial fission is reduced which in turn decreases cancer cell invasive ability [101].

SIRT5: Target and functions

SIRT5 is the last of the three Sirtuins to be located in the mitochondria. A small fraction of it is also found in the nucleus [30]. Both SIRT4 and SIRT5 exhibit weak lysine deacetylation activity in comparison to SIRT 1,2,3 and 6 [1,3,122]. SIRT5 more efficiently catalyze removal of negatively charged modifications like malonylation, glutarylation and succinylation from lysine residues [123–125]. Additionally, SIRT5 has been newly identified to succinylate histone proteins [126]. SIRT5 crystal structure has revealed that it has a larger substrate binding pocket compared to Sirtuin 2 (possesses high deacetylation activity), therefore succinyl group position better than acetyl group [141]. Backbone hydrogen bonding is the primary mode of interaction between the peptide and the protein.

SIRT5 regulates a number of physiological processes including glucose oxidation, Fatty acid oxidation, ROS management and ammonia detoxification. SIRT5 increases glycolytic flux by promoting demalonylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and additional enzymes involved in glycolysis. As a result SIRT5 Knock-out mice hepatocytes show reduced glycolytic flux [127]. Pyruvate kinase M2 (PKM2) is yet another enzyme involved in glycolysis whose activity is regulated by SIRT5 through desuccinylation. [128]. SIRT5 is also reported to impede the activity of pyruvate dehydrogenase complex (PDC) by desuccinylation. PDC is a crucial enzyme that catalyses the conversion of pyruvate to acetyl CoA, which then enters the TCA cycle. [30,129]). SIRT5 also desuccinylates and activates isocitrate dehydrogense 2 which is an enzyme involved in TCA cycle [130].

SIRT5 and diseases

Neurodegenerative Diseases

In neurodegenerative diseases, mitochondria tends to play a very important role. Different mitochondrial functions such as ATP production, oxidative phosphorylation, maintaining the concentration of free calcium ions etc. are important for survival of neurons. Mitochondria tends to undergo fusion which results in elongation of mitochondria or fission causing their fragmentation in response to different stress conditions in order to maintain their performance. SIRT5 tends to promote mitochondrial elongation under various stressful conditions. Guedouari et al. have reported that during starvation, SIRT5 knock-out increases mitochondrial fission which shows that SIRT5 is involved in protecting the mitochondria from fragmentation under such conditions.

Epilepsy is a neurological condition that affects a lot of people. It is marked by repetitive spontaneous seizures. Kainate (glutamate analog) promotes epilepsy by affecting the receptor of glutamate. A recent study has reported that SIRT5 protects the neurons from epileptic seizures caused by Kainite. Exposure to kainite in mice causes enhanced expression of SIRT5 within the hippocampus whereas deletion of SIRT5 shows degeneration

and loss of neurons. From these studies it can be concluded that SIRT5 protects the neurons against Kainate induced epilepsy.

Cancer

SIRT5 like SIRT3 has both tumour promoter and tumour suppressor activities. SIRT5 is involved in desuccinylation and activation of Superoxide dismutase 1 which reduces the level of reactive oxygen species. In lung tumour cells, SIRT5 acts as a tumour suppressor by activating SOD1. Another important substrate for SIRT5 is glutaminase (catalyses hydrolysis of glutamine to ammonia and glutamate). SIRT5 inhibits glutaminase via desuccinylation. As a result, in human breast cancer cells increased SIRT5 expression reduces the production of ammonia which in turn causes reduction of ammonia-induced mitophagy and autophagy.[121] In breast cancer, SIRT5-mediated desuccinylation stabilises mitochondrial glutaminase (GLS), enhancing proliferation and survival and providing substrates for multiple metabolic pathways and it correlates with a worse outcome for breast cancer.[137]

SIRT3 and 5: Therapeutic targets

Sirtuins have been considered as attractive drug targets because of their wide range of functions in our body. Sirtuins can be targeted for the treatment of metabolic syndrome, cancer, neurological disorders etc. Different therapeutic modulators have been developed targeting both SIRT 3 and SIRT 5. Crystal structures have also been developed in order to understand the mechanism by which the modulators active or inhibit the protein.

As already discussed, the conserved catalytic core consisting of the Rossmann fold and Zinc binding domain has a cleft in between to which both the substrate peptide and NAD+ binds. The acetyl-lysine leads through the tunnel into the active site where it reacts with NAD+ to produce the deacetylated substrate peptide. The majority of pharmacological inhibitors appear to block the peptide or NAD+ binding site, and they typically have medium potency and isoform selectivity. Nicotinamide which is a formed at an intermediate stage has been found to act as a non-competitive inhibitor of Sirtuins. One of the very important modulators of Sirtuins is Resveratrol which is isoform specific.

Resveratrol-like compounds has been found to be an act as activators for SIRT 1 and 3 but acts as an inhibitor for SIRT 5. In order to understand why resveratrol acts as an activator for 5 but inhibitor for SIRT 3, structures of Sirt5/Flour-de-Lys/resveratrol and Sirt3/FlourdeLys-1/Piceatannol (Resveratrol like compound) were solved. In case of Sirt5/Flour-de-Lys/resveratrol, the resveratrol binds between α2-α3 and β8-α13 loops leading to the closure of opening of the active site which traps the bound peptide. This interaction appears to result in a substrate binding mode that is better suited for the next reaction step. There is also an interaction between the coumarin moiety of FdL-1 peptide and the compound which increases the interaction interface.

In case of Sirt3/Flour-de-lys/piceatannol complex, there is an interaction between the piceatannol compound and the coumarin moiety. Superimposition of the two complexes show that in both cases the FdL-1 lysines bind into the hydrophobic tunnel of the active site but shows a different arrangement of the fluorophore pairs. As a result, as compared to the Sirt3/inhibitor complex, the entire FdL1-peptide is displaced in the Sirt5/activator structure. These variations are due to the interaction of piceatannol/resveratrol with the coumarin moiety, which results in non-productive substrate binding in Sirt3 but a productive substrate conformation in Sirt5 [131].

When the hSirt3/Flour-De-lys 1/4'-bromo-resveratrol complex was superimposed on hSirt3 in association with the ACS2 peptide, it was discovered that 4'-bromo-resveratrol occupies part of the NAD+ binding pocket (C-pocket). As a result, this configuration precludes the entrance of the NAD+ nicotinamide moiety into the C-pocket, which is required for catalysis. Piceatannol, another derivate of Resveratrol also inhibits hSirt3 but only weakly. Piceatannol causes non-productive peptide binding by interacting with FdL-1 fluorophore (as is revealed from superimposition of hSirt3/Flour-De-Lys-1/4'-bromo-resveratrol and hSirt3-Flour-De- Lys-1/Piceatannol complex) (**Fig 3A**). Superimposition of hSirt3/4'-bromo resveratrol complexes with FdL-1 and ACS2 peptide, on the other hand, demonstrated that the inhibitor failed to bind to the catalytic pocket when ACS2 peptide was attached. In case of ACS2 peptide it binds to a surface pocket **(Fig 3B)**. Further competition assays demonstrated that hSirt3 suppression is mostly caused by 4'-bromo-resveratrol attaching to the internal site, which competes with the ACS2 peptide having longer C-terminal. The compound also competes for binding to hSirt3 with the nicotinamide moiety of NAD+, indicating that the inner 4'-bromo-resveratrol binding site is necessary for hSirt3 suppression [132].

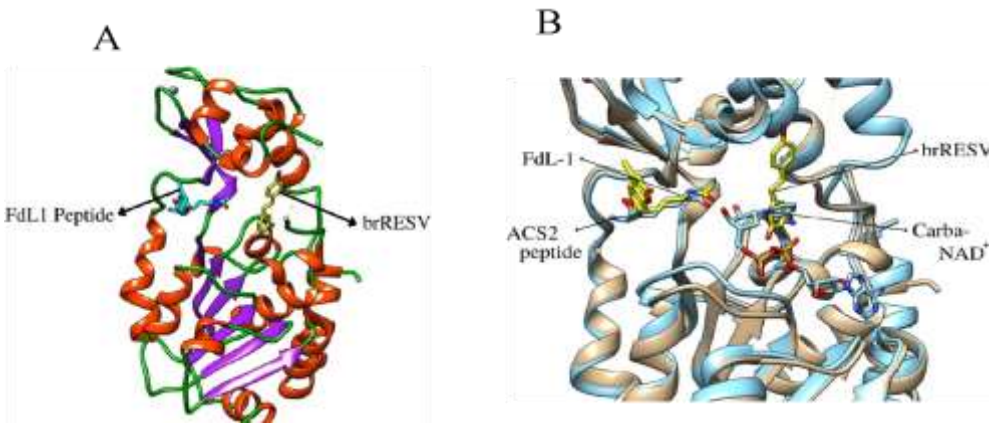

Figure 3: A) Sirt3 bound with 4'-bromo resveratrol and Flour-de-lys peptide (PDB 4C7B) B) Superimposition of Sirt3/FdL- 1/4'bromo-resveratrol and Sirt3-ACS2-carba-NAD+ showing that the resveratrol blocks the C pocket as a result inhibits SIRT 3 activity (PDB 4FVT, visualization tool is chimera)

SIRT5 is involved in regulating cellular metabolism and metabolic energy homeostasis, just like the other two mitochondrial sirtuins making it an attractive drug target. Both peptide and small molecule inhibitors has been identified for SIRT5 through different studies.

Sirtuins' deacetylation mechanism has already been confirmed in several research. The O- alkylamidate intermediate I and the bicyclic intermediate II are the two intermediates that are formed. [7,133]. One of the inhibitors reported for sirtuins with deacetylase activity is Thioacetyl-lysine [31].

SIRT5 is the only Sirtuin member possessing demalonylation and desuccinylion activity, owing to structural differences with other Sirtuins. As a result, a thiosuccinyl-lysine peptide was developed as a SIRT5 selective inhibitor. It was found to be a competitive inhibitor with an IC_{50} value of 5µmol/L[134]. Further research revealed that peptide antagonists with thiosuccinyl-lysine residues at the N- or C-terminus were less effective than those with the residue in the middle. In addition, the lengthier the succinyl peptide appeared to have a stronger inhibitory effect on SIRT5. Another peptide inhibitor identified for SIRT5 is N- carboxyethyl-thiourea-lysine which has been reported to have an IC_{50} value of 5.0 µmol L^{-1} [135]. Other than having peptide inhibitors, many small molecule inhibitors have also been reported for SIRT5. Suramin is a well-known small chemical inhibitor of SIRT5. The crystal structure of SIRT5 bound Suramin and its superimposition with SIRT5-ADP ribose structure has revealed that there are important structural alterations inside the flexible loop that connects the Rossmann fold domain and the Zinc-binding domain. In the SIRT5-ADP ribose structure, this loop appears to be partly disordered, however it is revealed to be ordered and has a distinct confirmation in the SIRT5 bound Suramin structure. As already reported, the pocket to which NAD+ binds in sirtuins is divided into three regions [154]. The adenosine ribose component of NAD+ binds to A site, the nicotinamide ribose component to B site and the C site lies deep inside the pocket to which nicotinamide binds. Suramin inhibits Sirt5 by imitating the contacts between the cofactor's nicotinamide ribose in the B-pocket (His158), nicotinamide in the C-pocket (Phe70), and the peptide in the substrate-binding pocket (Tyr255) [136]. Suramin therefore completely covers the active site of Sirtuin **(Fig. 4).** In addition to Suramin, other small molecule inhibitors of SIRT5 include siritinol, cambinol and nicotinamide which can cause inhibition at the sub-micromolar level.

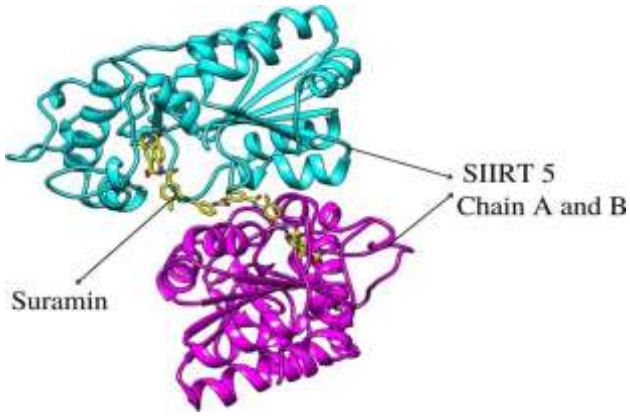

Figure 4: Overall structure of SIRT 5 suramin complex. Two monomers (cyan and magenta) are linked by one molecule of suramin (PDB 2NYR, visualization tool is chimera).

Conclusion

In this review, we talked about the mitochondrial Sirtuins, their overall structural arrangements, enzymatic activities, their role in different disease conditions and SIRT3 and 5 as important therapeutic targets. All the three sirtuins found inside the mitochondria play different roles in regulating various metabolic pathways. Dysregulation of any of the three Sirtuins can lead to mitochondria-related diseases. Sirt3, 4 and 5 has been shown to have different enzymatic activities regulating various substrates. SIRT3 specifically show deacetylation activity whereas SIRT 4 has higher delipoylation, debiotinylation activity. On the other hand SIRT5 has demalonylation, deglutarylation and demalonylation activities. Through their different enzymatic activities, Sirtuins in turn affect the metabolic pathways within the mitochondria. Their role has also been implicated in various diseases like Neurodegearative disease, Heart diseases, cancer etc. Under expression or overexpression of the enzymes ultimately can either protect or lead to development of different diseased-condition.

Many of the therapeutic agents has also been reported for SIRT3 and 5. Resveratrol which is an important modulator was seen to activate SIRT3 but inhibit SIRT 5 through different mechanisms. Besides this 4'-bromo resveratrol acts as an inhibitor for SIRT3 etc. Another inhibitor reported for SIRT3 is the SRT1720 molecule SIRT5 on the other hand is inhibited by suramin which binds to the catalytic site. Other inhibitors of SIRT5 include piceatannol, carbimol etc. For SIRT4, modulators has not yet been reported because the Human SIRT4 structure could not be crystallized. Therefore, by studying the structure of SIRT4 it remains to be seen which molecules can act as activators or inhibitors for SIRT4.

Future Plan

The review paper's extensive explanation provides a solid foundation for ongoing research on mitochondrial sirtuins. Future studies may focus on the precise mechanisms by which SIRT3, SIRT4, and SIRT5 recognize and deacetylate/acetylate particular substrates. By understanding their preferred substrates, we may be better able to understand the regulatory roles they play in various physiological and metabolic processes. For this, it could be required to develop medication delivery methods that specifically target the mitochondria.

More study is required since these sirtuins interact with several cellular pathways and have implications for a number of illnesses. This area of research has the potential to significantly advance medical care for people.

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