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SIRTUINS -THE REGULATORS OF LIFESPAN

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INTRODUCTION

Past decade researches on science of aging, has identified important genes and pathways that control the aging process (Guarente, 2011). Included among these is a family of NAD^+–dependent protein deacetylases called sirtuins. Sir2 (silent information regulator 2) protein of yeast *Sacchromyces cervisiae* and its conserved orthologues in other prokaryotes and eukaryotes are collectively known as sirtuins (Li and Kazgan, 2011). Initially SIRT2 was shown to extend lifespan in budding yeast. Since then, many sirtuins have been shown to regulate longevity in other organisms including mammals. Sirtuins exhibit deacetylase or ADP-ribosyltransferase activity and they belong to the class III protein deacetylase family of enzymes which require NAD^+ as a co-factor for their enzymatic activity (Nakagawa and Guarente, 2011). Requirement of NAD^+ for their enzymatic activity connects sirtuins to cell metabolism and age related diseases. High levels of NAD^+ stimulate the sirtuins activity and high levels of NAM inhibit SIRT2 activity (Li and Kazgan, 2011).

History of Sirtuins

Sir2 (silent information regulator 2) of yeast *Saccharomyces cerviciae* is the founding member of the sirtuin family (Finkel, *et al*., 2009). David Sinclair along with Leonard Guarente made the beginning in sirtuin research in 1997. Their famous experiment at Massachusetts Institute of Technology, established that the resveratrol (a polyphenol compound in grape skins and wine) causes lifespan extension in yeast and the resveratrol induced lifespan extension was mediated by SIRT2 activity. This finding lead to the world wide attention to consider the SIRT2 as the ultimate "longevity gene". David Sinclair and Leonard Guarente went on to propose the extra-chromosomal rDNA circle (ERCs) theory of yeast ageing. According to this theory, rDNA repeats of yeast genome undergo homologous recombination during cell division to form toxic ERC and with successive divisions mother cell over-runs with ERCs. As the ERCs titrate the essential cellular elements, increase in the number of ERCs causes ageing and ultimate death of the yeast. But the presence of SIR2 deacetylases the histone proteins that shroud the DNA and thus suppresses the homologous recombination of rDNA and prevents the ERC formation extending the lifespan of yeast (Haigis and Sinclair, 2010).

Mammalian Sirtuins

Seven sirtuins are identified in mammals and are named numerically as SIRT1 to SIRT7. They contain highly conserved "core domain" in common but they differ in their N and C terminal domains which make them to exhibit highly divergent biological functions. (Haigis and Guarente, 2010). Mammalian sirtuins are found in different compartments of the cells. SIR1, SIR6 and SIR7 are localized in nucleus. SIR 3, SIR4 and SIR5 are found in mitochondria. SIRT2 is present in cytoplasm (Verdin *et al*., 2010). Subcellular localization of sirtuins is attributed to localization signals at their amino terminal. Sirtuins present in nucleus contain nuclear localization signal and the nuclear export signals. Exposure to nuclear localization signal versus nuclear export signals determines the localization of these sirtuins in the nucleus. Similarly the sirtuins like SIRT3, SIRT4 and SIRT5 possess N-terminal mitochondrial sequences which direct them to be localized in the mitochondria (Tanno, *et al*., 2007). Among the mammalian sirtuins SIR2, SIR3, SIR5 and SIR7 exhibit deacetylase activity, SIRT4 have only ADP-ribosyltransferase activity but, SIRT1 and SIRT6 posses both

deacetylation and relatively weak ADP-ribosyltransferase activity (Michishita, *et al*,. 2008).

Sirtuins mediate deacetylation reaction NADH enhances the SIRT2 activity which mediates the CR induced lifespan extension in yeast. Presence of SIRT2 suppresses the homologous recombination of rDNA

Sirtuins as Enzymes

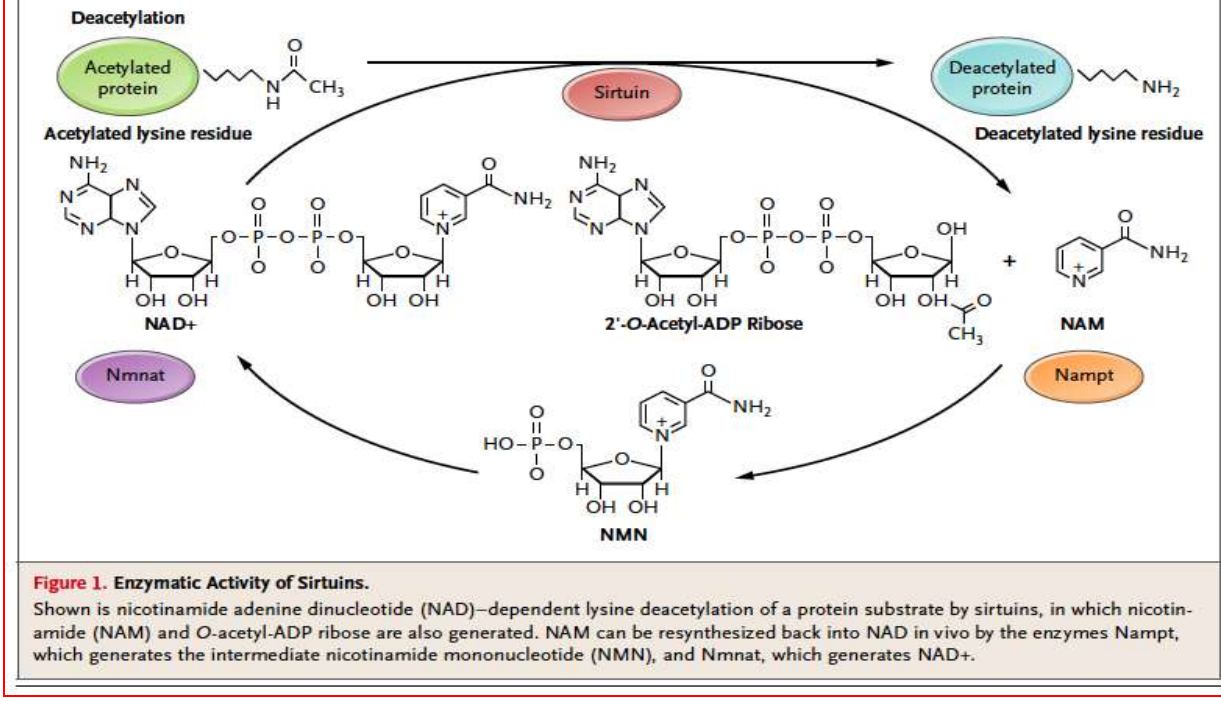

Figure 1. Enzymatic Activity of Sirtuins.
Shown is nicotinamide adenine dinucleotide (NAD)–dependent lysine deacetylation of a protein substrate by sirtuins, in which nicotinamide (NAM) and *O*-acetyl-ADP ribose are also generated. NAM can be resynthesized back into NAD in vivo by the enzymes Nampt, which generates the intermediate nicotinamide mononucleotide (NMN), and Nmnat, which generates NAD+.

(Guarente, 2011)

where in, they first cleave NAD^+ to produce nicotinamide (NAM) and then the acetyl group of substrate is transferred to ADP-ribose moiety of NAD^+ resulting in generation of O-acetyl-ADP ribose and deacetylated substrate. NAM generated during this reaction act as an endogenous inhibitor of sirtuins and it gets converted into nicotinamide mononucleotide (NMN) by the NAD^+ salvage pathway enzyme nicotinamide phosphoribosyl-transferase (Nampt). Subsequently, nicotinamide mononucleotide adenyltransferase (Nmnat) regenerates NAD^+ from NMN. Rate of NAD^+ biosynthesis regulate the activity of sirtuins (Houtkooper *et al*., 2010)

Sirtuins and Calorie Restriction

Calorie restriction (CR), a 20-40% reduction in calories consumed below *ad libitum* intake has been shown to extend the lifespan of numerous organisms from yeast to higher mammals by up to 50% (Eomb *et al*., 2010). During calorie restriction (CR) the increased levels of NAD^+ or reduced concentration of through deacetylation of histone proteins that shroud DNA and thus preventing the formation of extra chromosomal rDNA circles which are the cause of the yeast aging and death (Sinclair and Guarente, 1997).

Mammalian SIRT1, the homologue of SIRT2 mediates calorie restriction induced life extension in mammals. CR induced SIRT1 activity in mammals downregulate pro-ageing pathways like growth hormone signaling and oxidative stress induced signaling. Simultaneously CR induced sirtuin activity upregulates activity of anti-ageing pathways like stress resistance pathways and DNA repair pathways (Li and Kazgan, 2011). In mammals CR triggers metabolic changes which are implicated in longevity which include physiological changes that improve glucose homeostasis. In humans and rodents the CR reduces the insulin and glucose levels and improves insulin sensitivity (Guarente and Kenyon, 2000).

Sirtuins and DNA Repair

Eukaryotic cell faces more than 1 million DNA lesions / day and these lesions need to be repaired in order to avert chromosomal instability, mutations and cell death /dysfunction. Despite the presence of multiple DNA repair pathways like homologous repair (HR), non-homologous end joining (NHEJ), base excision repair (BER), nucleotide repair (NER) and mismatch repair (MMR), unrepaired DNA damages increasingly accumulate with advancement of the age, resulting in manifestations of ageing. Reactive oxygen species (ROS) are the major factors that cause DNA damages (Lombard *et al*., 2008). SIRT 6 is implicated in DNA repair mechanisms. It deacetylases many factors which take part in DNA repair process and facilitates both BER and double strand breaks (DSBs) repair mechanisms (Martinez-Pastor and Mostoslavsky, 2012). SIRT6 activates poly-ADP ribose polymerase 1 (PARP1) by mono-ADP-ribosylation and thus facilitates PARP1 mediated DNA damage repair by BER and DSB repair systems. SIR6 mediated deacetylation of DNA polymerase (*pol*β) also initiates DNA repair mechanism by BER. SIR6 also deacetylates the factors like Werner helicase, Nijmegen breakage syndrome 1 (NBS1) factor and DNA-dependent Protein Kinase (DNA-PK) to promote double-strand break repair mechanisms (Mostolavsky *et al*., 2006).

Sirtuins and Oxidative Stress

Reactive oxygen species (ROS) are the group of compounds derived from incomplete oxidation of molecular oxygen during mitochondrial respiration. Most important ROS include superoxide anion (O_2^{*-}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^*) (Murphy, 2009). Enzymatic mechanisms involving antioxidant enzymes activity (CAT, GPx, and SOD) operating inside the cells seem to be insufficient to neutralize ROS insults resulting in oxidative damage of the cellular macromolecules, a state called "oxidative stress" (Merksamer e*t al*., 2013).

Sirtuins Against ROS

Mammalian nuclear SIRT1 and mitochondrial SIRT3 are implicated in protection against ROS. SIR1 mediated activation of PGC 1-α (Peroxisomes proliferator-activated gamma receptor co-activator 1 alpha) promote the expression of oxidative stress genes including glutathione peroxidase, catalase and manganese superoxide dismutase. SIRT1 mediated inactivation of nuclear factor kappa B (NF-κB) suppresses inducible nitric oxide synthase (iNOS) activity and reduce cellular ROS load (Marksamer *et al*., 2013). SIRT1 meditaed activation of FOXO family of transcription factors upregulate antioxidant genes to alleviates the RSO mediated oxidative stress (Motta *et al*., 2004). SIRT1 induced activation of Heat Shock Factor Protein 1 (HSF1) enhances the production of various heat shock proteins (Westerheide *et al*., 2009). SIRT3 mediated deacetylation enhance the catalytic activity of SOD2 to reduce cellular ROS. SIRT3 promote the conversion of oxidized to reduced glutathione by activating isocitrate dehydrogenase2 (IDH2) thus offering the protection against the ROS (Zhang *et al*., 2011).

Sirtuins and Metabolism

Mitochondria are the crucial intracellular organelle involved in energy production, metabolism and intracellular signaling. Mitochondrial number and activity changes in response to variety physiological conditions such as nutrients exercise and change in the temperature / oxygen levels as well as during ageing (Wenjuan *et al*., 2012). SIRT3, SIRT4 and SIRT5 are mainly, if not exclusively (Scher *et al*., 2007), localized to mitochondria (Onyango *et al*., 2002). Sirtuins the molecular sensors of cellular energy balance and regulate metabolic responses to change in nutritional availability in multiple tissues (Haigis and Sinclair, 2010)

Sirtuins in Lipid Metabolism

SIRT3 activates long chain acyl-CoA dehydrogenase (LCAD) to promote β-oxidation of FAs in to acetyl-CoA (Hirschey, 2011). SIRT3 also activate 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) to promote ketone body production and their utilization as an alternative source of energy (Shimazu, 2010). SIRT3 activates acetyl-CoA

synthetase 2 (ACS2), the enzyme that convert acetate into acetyl-CoA - in extra-hepatic tissues. Overall, SIRT3 facilitates lipolysis and mobilization of fatty acids in liver and in turn promote peripheral use of lipid-derived acetate and ketone bodies as alternate source of energy during fasting (Wenjuan *et al*., 2012).

Sirtuins in Carbohydrate Metabolism

Glucose homeostasis is maintained by the liver, in addition to pancreas in changing nutrient conditions. During CR/fasting hepatocytes induce gluconeogenesis to supply other tissues with glucose. SIRT1 activate FOXO1 transcription factor to promote the FOXO1-dependent transcription of hepatic gluconeogenic genes (Frescas *et al*., 2005). SIRT1 deacetylates the transcriptional co-activator PGC-1α in liver and drives the expression of gluconeogenic genes and repression of glycolytic genes during fasting (Rodgers *et al*., 2005). SIRT3 also regulates carbohydrate metabolism in cancer cells. Preference of cancer cells for glucose utilization as a source of energy is referred to "**Warburg effect**" and this effect can be alleviated by the upregulation of SIRT3. Hypoxia-inducible factor 1α (HIF 1α), when stabilized by ROS, induce expression glycolytic genes. Hyperacetylated peptidyl-prolyl isomerase cyclophilin D (PPID) helps maintain active state of hexokinase II (HXK2), facilitating the glycolytic process and facilitate the survival and growth of cancer cells. Presence of SIRT3 activates SOD2 that reduce the cellular ROS resulting unstabilized HIF1α. Net result is downregulation of glycolytic genes and cancer cell death for want of energy. SIRT3 also deacetylates to inactivate PPID in turn inhibiting hexokinase II. Thus downregulation of glycolysis and energy deprived cancer cells dies (Wenjuan *et al*., 2012).

Sirtuins and Old-age Diseases

Sirtuin are promising potential therapeutic agents in variety of human diseases including diabetes and neurodegenerative diseases. Modulation of sirtuin activity has been shown to impact the course of several aggregate forming neurodegenerative disorders including Alzheimer's disease, Parkinson's disease and Huntington's disease (Herskovits and Guarente, 2013).

Use of SIRT1 modulators has been described as an attractive potential therapeutic solution for treating multiple diseases states including diabetes, aging, and neurodegenerative, metabolic or cardiovascular diseases. Small molecules that act as modulator of sirtuins have been developed, and are currently under clinical trial (Athie-Cuervo, 2011).

Sirtuins in Diabetes

Beta-cell mitochondria serve as sensors of cellular energy levels and thus link the glucose exposure to insulin release. Efficient coupling of electron transport with ATP production causes the glucose induced release of insulin from β-cells of pancreas. Hyperglycemia/ obesity/ fatty acid excess induced superoxide radicals cause up-regulation of a protein called uncoupling protein 2 (UCP-2). Proton leak through the UCP2 results in uncoupling of electron transport and ATP production leading to reduced ATP : ADP ratio and loss of glucose stimulated insulin release (Bordone *et al*., 2006).

Sirtuins Enhance β-Cell Response

Direct effect: SIRT1downregulates expression of UCP-2 protein and promote efficient coupling of electron transport with ATP production in absence of UCP2 and resulting enhanced glucose stimulated insulin release from β-cell (Li and Kazgan, 2011).

Indirect effect: SIRT1 and SIRT3 induced upregulation of antioxidant genes (SOD2, GPx1, catalase & SOD) reduces the cellular superoxide radicals load resulting in downregulation of UCP-2 and enhanced production of ATP, in turn increasing cellular response to circulating glucose (Nakagawa and Guarente, 2011).

Sirtuins and Cardiovascular Diseases (CVD)

Cardiovascular disease is the leading cause of death in the world cholesterol biosynthesis, immune system and vascular endothelial cell function interactions determine the progress of the disease. Higher LDL cholesterol, vascular injury,

increased inflammation and deposits of cholesterol below vascular endothelium are considered as the risk factors (Haigis and Sinclair, 2010). SIRT1 activity gives the protection against the hypertrophy in cardiac and smooth muscle cells. SIRT1 inhibits angiotensin receptor1 expression to reduce ROS levels and protect smooth muscles against the oxidative insults. SIRT1 also activates endothelial nitric oxide synthase (eNOS) to enhance nitric oxide production. Nitric oxide induced blood vessel relaxation lowers the blood pressure and reduce the number of atherosclerotic plaques (Nisoli *et al*., 2005).

SIR1 activate Liver X receptor (LXR) to upregulates the ATP binging cassette transporter A1 (ABCA), a transporter protein that causes efflux of cholesterol from peripheral tissues i e., reverse Cholesterol Transport (RCT). The overall effect is slowdown atherosclerotic plaque formation (Guarente, 2011). SIR1 also activates nuclear bile acid receptor called farnesoid X receptor (FXR) by deacetylation. Activated FXR reduces the lipogenesis via inhibition of sterol-regulatory element- binding protein 1C (SREBP1C) and fatty acid synthase (FAS). SIRT1 stimulated FXR also increased expression of PPARα promoting fatty acid β-oxidation and increased expression of the VLDL receptor that promotes TG clearance (Wlaker *et al*., 2010).

Sirtuins and Neurodegenerative Diseases

Mitochondria play a central role in aging as mitochondrial dysfunction increases with age and produces harmful levels of reactive oxygen species which leads to cellular oxidative stress (free-radical theory of aging). Oxidative stress is highly damaging to cellular macromolecules and is also a major cause of neurodegenerative disorders. Sirtuins are potential therapeutic targets in a variety of human diseases including diabetes and neurodegenerative disease. Modulation of sirtuin activity has been shown to impact the course of several aggregate-forming neurodegenerative disorders including Alzheimer's disease and Parkinson's disease. Sirtuins can influence the progression of neurodegenerative disorders by modulating transcription factor activity and directly by deacetylating proteotoxic species (Herskovits and Guarente, 2013**).**

Alzheimer's Disease (AD)

Neurodegenerative diseases are sobering obstacle to the healthy aging. AD an important neurodegenerative condition that affects one third of the people who wins the longevity lottery (Haass and Selkoe, 2007). Amyloid-β peptide (APP) plaques and neurofibrillary tangles (τ -tangles) in critical brain areas are pathological hallmarks of Alzheimer's disease. Amyloid precursor protein cleavage by the β-secretase and γ-secretase complexes leads to the formation of amyloid-β (Aβ) peptides that can aggregate and form amyloid plaques. Acetylated Tau protein is involved in neurofibrillary tangle (tau tangles) formation (Herskovits and Guarente, 2013**).**

Sirtuins in Alzheimer's Disease (AD)

SIRT1 mediated deacetylation of retinoid acid receptors β **(RARβ) enhances the** expression of metallopeptidase 10 (ADAM10) that code for α-secretase activity, thus promoting a non-amyloidogenic pathway for processing APP. SIRT1 mediated deacetylation of tau protein cause ubiquitination to form ubiquitinated tau which is not involved in neurofibrillary tangles formation thus reducing the tau tangles (Zhou *et al.*, 2003).

Parkinson's disease (PD)

Ageing is the single most significant factor that influences the clinical presentation and course and progression of PD. A movement disorder causing tremor, rigidity and postural instability. Also causes cognitive and behavioral changes including sleep impairments, olfactory deficits and neuropsychiatric disorders. Significant loss of dopaminergic neurons in substantia nigra and accumulation of intracytoplasmic Lewy bodies, (inclusions of contain α- synuclein & ubiquitin) are the leading causes of PD. Activation of SIRT1 by genetic means or resveratrol treatment has been found to be protective in mouse models of PD (Blesa *et al*., 2012).

Sirtuin in Parkinson's disease

SIRT1 and SIRT2 are implicated in PD and are known to exert opposite effect on PD models of neurodegeneration. SIRT1 exert

neuroprotective effect and SIRT2 activity is detrimental to the neuronal health in PD. Multiple studies have shown that SIRT1 induced activation of heat shock factor 1 (HSF1) promote the transcription of molecular chaperones including heat shock protein 70 that regulate homeostasis of cellular proteins (Westerheide *et al*., 2009). SIRT2 induced Foxo3a deacetylation, leads to increased levels of the pro-apoptotic factor Bim and neuronal death (Raynes *et al*., 2012). SIRT2 activity also increase the number of Lewy bodies in turn leading to neuronal death (Garske *et al*., 2007). More recently SIRT1 was found to modulate memory formation and synaptic plasticity. Here, SIRT1 promote CREB (cAMP responsive element binding) expression by repressing micro RNA 134 (miR134) and induces brain derived neurotrophic factor (BNDF), which has crucial role to play in normal cognition (Gao *et al*., 2010).

CONCLUSION

Evidences suggest that sirtuins mediate beneficial effects of calorie restriction in mammals. Mammalian sirtuins are involved in many central pathways governing the physiology and are required to maintain a delicate balance between metabolism and ageing. As the SIRT1 plays significant role in metabolic diseases like diabetes and neurodegenerative diseases, control of SIRT1 activity through calorie restriction or through small molecules could be a valuable treatment strategy for treating multiple diseases states including diabetes, aging, neurodegenerative and cardiovascular diseases. Indeed two different SIRT1-activating compounds are now in a diverse set of phase 1 or phase 2 human trials. Apart from SIRT1, other sirtuins may have therapeutic potential, in this regard the role of SIRT3 is of special interest, as it is involved in suppressing one of the important cause of ageing, reactive oxygen species in mitochondria. Sirtuins are unique class of proteins that link the protein acetylation to metabolism and exert profound effect on mammalian physiology and diseases of aging.

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