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SIRTUIN1: SERENDIPITOUS AND ENIGMATIC MOLECULE

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ABSTRACT

Biological marker or biomarker is an indicator of physiological or pathological changes in an organism. A biomarker is valid only after evaluation and approval by Food and Drug Administration (FDA) committee. Three step evaluation processes for biomarker includes: Validation, Qualification and Utilization. Present review highlights recent trends of biomarker in Diabetes and diabetic nephropathy and an emerging protein a natural boon for life: the Sirtuin. Sirtuin a diverse ancient protein family has its role as a biomarker as well as a metabolite essential for day to day wear and tear of cells in the system of an organism. It is an NAD- dependent class III histone deacetylase and mono ADP- ribosyl transferase enzyme. Sirtuin is a seven member family from sirtuin 1 to 7 (Sirt1- 7) based on their function, composition and localization. Sirt1 is the closest homologue of Sir2 of yeast and with wide range of functions. Hence sirtuin is a molecule of interest for research. Isoforms are localized in nucleus, mitochondria and cytoplasm for respective metabolic functions in the cell

INTRODUCTION

Biology of an organism undergoes physiological as well as pathological regulations which must be monitored to prevent harmful and fatal changes (pathological). To prevent or cure this pathological change we require indicators to monitor and treat called the 'biological marker or biomarkers'. Biomarkers are defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological, pathogenic or pharmacological responses to an intervention", National Institutes of Health Biomarkers Definitions Working Group, 2001 [1]. A genuine biomarker must also be a substitute to clinical endpoint, which is projected by a patient's response to treatment. Biomarkers are approved by Food and Drug Administration (FDA) after thorough validation and qualification [2]. Validation includes assay development, sensitivity, accuracy and reproducibility [3, 4]. Evaluation or qualification is interchangeable terms which are linked with analytical evaluation or qualification of biomarker prior to its clinical application. Qualification of a biomarker bridges validation and clinical endpoints to evaluate reaction between analytical procedures and clinical outcomes of treatment [1].

Steps in biomarker validation

Validation of biomarkers include; analytical part, sample type, sample storage and handling, types of assays, standardized analytical procedures starting from primary immunohistochemistry, Immunoassays to higher omics studies such as genomics, proteomics and metabolomics [5].

Genomics involves molecular biological methods for validation such as, PCR, microarrays etc. Proteomics includes study on protein- protein interaction and ligand binding methods by immunoassays. Metabolomics employs analysis of metabolites by chromatography and mass spectroscopy in biological fluids such as plasma, serum, whole blood, feces, urine, cerebro spinal fluid (CSF), tissue biopsy etc., to finally assess and correlate endpoint with diagnosis [5].

First step in biomarker discovery and/or development is validation. Major points to be considered are:

Matrix selection and sensitivity of measurement/ analysis. If non- invasive or minimal invasive samples are considered then, sensitivity and analytical part is not be compromised. If sample is obtained through an invasive and risky procedure such as surgeries or biopsies, assay preference can be selected based on sample availability [5].

Sample source, sample integrity and reagent integrity: Sample source depends on the population, age and gender. Sample integrity depends on sample handling assessed by freezing- thawing cycles, storage conditions and type of sample. Reagent integrity depends on the type of reagent ex: ELISA plates and reagents or fully automated analyzer reagents or molecular biology buffers etc, stability and storage aspects are also considered [5].

Analytical part to be considered in the laboratory based testing of biomarker is quality control (QC) measures. This has to be

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documented to compare and check performance of previously said aspects to validate biomarker [6]. QC also helps check assay performance by validation samples (VS). In good laboratory practice (GLP) three samples for higher, middle and low ranges are sufficient to validate parameter but while considering biomarker, different concentrations of at least five validation samples with six runs each are to be considered for better comparison and performance of assay and standardization [4,7]. Thus, validation of a biomarker must be completed at pre- clinical stage before drug development phase to avoid lengthy phases of drug development.

Second step in biomarker evaluation as approved by FDA and National Institute of Health (NIH) is qualification. Qualification, as mentioned earlier links analytical method validation and surrogate and/or clinical endpoint [1].

USFDA in the year 2005 described the process of biomarker qualification in three stages [8]:

- ***I stage:*** Exploratory biomarkers, employed in identifying the lead molecule or key parameter by genetic analysis in animals
- ***II stage:*** Probable valid biomarker, to predict outcome considering results of I stage
- ***III stage:*** Known valid biomarker, established and widely accepted for interventional studies or for routine laboratory use

For considering the known valid biomarker from research to the routine investigation, several steps need to be followed.

Institute of medicine (IOM) in 2010 proposed 2step process in qualification which include: [9]

I step: Evaluating strength of link between biomarker with clinical outcome and pathophysiology of disease. This can be evaluated by two types of study designs:

Cohort study for long period, depicting clinical outcome but a slow process

Cross- sectional study simultaneously involves outcome and characteristics of interest in a population. Faster compared to cohort design but lack information regarding interferences developing overtime.

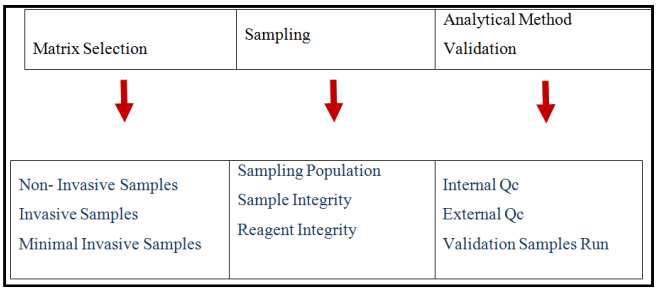

Fig 1 Overview of Biomarker Validation

II step: Confirmatory step, involving interventional studies to assess the outcome. First step is relevant to phase I and phase II in pre- clinical trials and laboratory data. Second step holds good for deriving prognostic value of a biomarker by employing interventional trials for phase III. Thus, second step of qualification outlines are result of interventional effect and clinical outcome of a biomarker. Limitation in intervention may be different clinical outcome in individuals of same population which accounts for their genetic makeup.

Third step and last criteria for biomarker evaluation proposed by Biomarkers definitions working group is termed 'Utilization'. This process shall answer the following important questions to implement biomarker at its best [1].

Whether biomarker coincides with surrogate endpoint or clinical endpoint
How robust and cost effective biomarker is?
Does biomarker follow fit for purpose theory?

"Fit- for purpose theory" holds good for both pharmacokinetics and laboratory analytical validation following American association of pharmaceutical scientists (AAPS) 2003 guidelines and clinical laboratory improvement amendment (CLIA) respectively [10].

After biomarker evaluation under the guidance of biomarkers committee and FDA it is now the turn to take biomarker into the field of diagnostics and pharmaceuticals [11]. To achieve biomarker evaluation and to implement in diagnostics and therapeutics we need to consider Laboratory personnel to check biomarker for accurate and sensitive analytical validation step to improve turnaround time of test

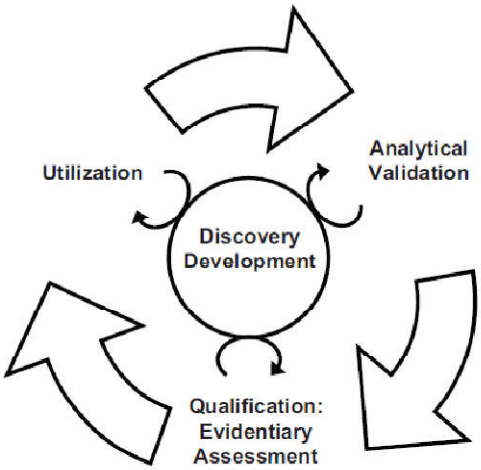

Fig 2 Steps in Biomarker Evaluation

Source: IOM (Institute of Medicine). 2010. Evaluation of biomarkers and surrogate endpoints in chronic disease.
Washington, DC: The National Academies Press.

Clinicians demand for evidence based qualification of biomarker Further, Policy makers concentrate mainly on biomarker utility and cost- effectiveness Biomarker for utility If these criteria are satisfied, then biomarker can be considered positively. First considered biomarker was blood pressure for cardio vascular disease approved by FDA, recently it is considered as a surrogate endpoint with limitations [12]. Similarly, for type 2 diabetes mellitus (DM) Metabolic-endocrine division has approved HbA1c% and blood glucose as endpoints [13]. Diabetes mellitus (DM) is a metabolic disorder related to aging caused due to defective insulin secretion/ action or both [14]. Classic and gold standard markers are fasting and post prandial Plasma glucose levels and HbA1c%. Recently, mathematical formulae have emerged indicating insulin sensitivity such as, homeostasis model assessment of insulin resistance (HOMA IR), quantitative insulin- sensitivity check index (QUICKI) etc,. Plasma glucose do not give accurate result hence Impaired glucose tolerance by Oral Glucose Tolerance Test (OGTT) is considered a better test to perform [15]. However, OGTT is lengthy and cumbersome, since it takes two hours to complete [16]. Recently, biomarkers are implemented in metabolomics.

Metabolomics is an emerging area next to proteomics for accurate interpretation of cause and consequence of diabetes. Metabolomics include genetic profiling supporting correct diagnosis and treatment to be given for all types of diabetes and their future complications [14]. Kolberg *et al*. in the year 2009 conducted inter99 longitudinal population study to identify a better test or biomarker than OGTT. They emerged with Diabetic risk score (DRS) model comprising of C-reactive protein (CRP), ferritin heavy chain 1 (FTH1), detection antibodies for ADIPOQ (Adiponectin gene), interleukin 2RA, glucose and insulin. They further concluded that, testing only glucose and insulin will give false positive result since they are increased in obesity and inflammation also [17]. Study conducted by Wong TY *et al*., on diabetes and its microvascular complication diabetic retinopathy considered three different populations who showed different stages in occurrence of retinopathy. Authors further appealed that, subjects with lower and controlled fasting blood sugar (FBS) were also affected by retinopathy. Hence, Wong TY *et al*., concluded patients with diabetes are accountable for FBS as a biomarker is inappropriate in diagnosis of disease and in staging of diabetic retinopathy [16].

Other major microvascular complication is diabetic nephropathy (DN) or Diabetic kidney disease (DKD) known to affect approximately one third of the affected diabetic population worldwide [18]. Gold standard serum markers and urinary marker for early detection for DN are urea and creatinine with microalbuminuria respectively. Since, serum and urinary markers have wide diagnostic applications, lower sensitivity and specificity and many other limitations there is a need for novel specific biomarker for DN. In contrast, a component closely related to structural integrity of nephrons or kidney produce a valid tool in the diagnosis and management of diabetes and DN.

Studies related to renal biomarkers such as urinary, podocytes, cystatin C, type IV collagen, neutrophil gelatinase associated-lipocalin (NGAL), kidney injury molecule 1 etc are considered appropriate in the study of structural integrity of nephrons and kidney [18- 23]. Recently, combined equation of serum creatinine and cystatin C for calculating eGFR has emerged as an efficient early marker for detection of DN [20]. Since type 2 DM is considered to occur due to decreased insulin sensitivity and resistance by target cells Hyperinsulinemic euglycemic clamp (HEC) is considered gold standard for measuring them. Due to various flaws in HEC methodology many mathematical models are proposed for measuring insulin sensitivity/ resistance such as:

HOMA-IR [24]: Fasting insulin (I_0 in µU/L) x Fasting glucose (G_0 in nmol/L)/ 22.5
QUICKI [25]: 1/ log G_0 (mg/dL) + log I_0 (µU/mL)
Mc Auley index [26]:
Mffm/I= e [2,63- 0,28 ln (I_0)- 0,31 (fasting TAG)]
Matsuda index using OGTT values [27]
$ISI_{(matsuda)} = 1000/\sqrt{G_0 I_0 G_{mean} I_{mean}}$.

Amongst all indices HOMA- IR and QUICKI are considered clinicians friendly formulae since it is simple and accurate method for calculating insulin sensitivity/ resistance. However, all the researchers in general proposed to calculate insulin performance in individuals using fasting plasma insulin levels.
Sirtuin Need of the hour is to find a marker which will be a single solution for diagnosis, prognosis, and further management of diabetes and its microvascular complication; diabetic nephropathy. Sirtuin: a histone III deacetylase enzyme which mediates enormous pathophysiological processes is gaining momentum in management of DM and DN. A four member family of silent information regulator (Sir) gene in prokaryotes has significant role in regulating the important key processes such as metabolism, aging etc. Sir2 in Prokaryotes is a homologue of sirtuin proteins of eukaryotes particularly in mammals.

Sir2 was first discovered in the year 1986 by Kim *et al*. in, *Saccharomyces Cervesiae* (Baker's yeast) [28]. Ivy JM *et al*., has documented vital role of Sir2 in extending life- span in isolated *S. Cervesiae* [29]. Sirtuin is an NAD- dependent class III histone deacetylase and mono ADP- ribosyl transferase enzyme [30]. Further, studies during late 1990's in multicellular organisms investigated the role of sirtuins in higher organisms. However, longevity studies are proved only in lower organism and worms [32].

Sirtuins are highly conserved ancient protein of all phyla including viruses, archea, fungi, metazoan and recently the mammals [33, 34]. Mammalian sirtuin share almost similar catalytic core composition with other phyla, constituting about 250- 270 amino acids [35]. Mammalian sirtuin is a seven member family of sirtuin 1- 7 (Sirt1- 7), based on their function, composition and localization [35]. Phylogenetic analysis of core classified the protein into 4 different subclasses as Sirt I- Sirt IV by Frye *et al*. [35].

Class I: Sirt1, Sirt2 and Sirt3
Class II: Sirt4
Class III: Sirt5
Class IV: Sirt6 and Sirt7, well studied among the researchers is Sirt1 due to its diversification.
Sirt1 is the closest Homologue of Sir2 and with wide range of interest hence considered for research [36].

Localisation and Functions of Sirtuins: [37, 38]
Sirt1: Nucleus, Mediates Deacetylation
Sirt2: Cytoplasm does Deacetylation and Ribosylation
Sirt3 and sirt5: Mitochondria, catalyze Deacetylation
Sirt4 & Sirt6: Mitochondria and Nucleus (Heterochromatin) respectively helps in ADP- Ribosylation
Sirt7: Nucleolus, enhances Deacetylation

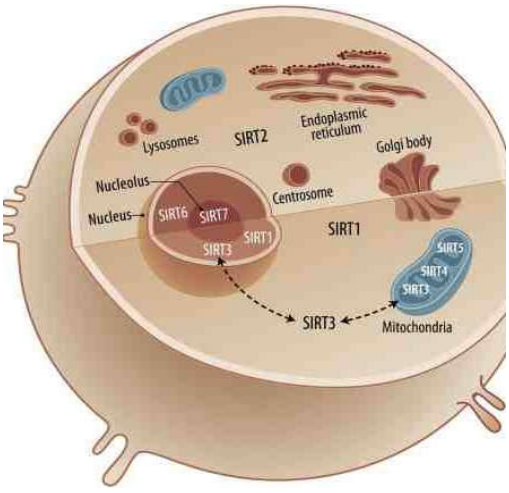

Fig 3 Sirtuin localization

Source: Turki Y Alhazzazi, *et al*,. Sirt3 and cancer: tumor promoter or suppressor? Biochemical et Biophysica acta. 2011; 1816 (1): 80- 88.

Shuttling of sirtuin within organelles and its compartmentalization justifies that there is a need to rescue cell from oxidative stress imbalance or other damages [39]. Studies have created controversies on sirtuin involvement in age- related disorders such as, diabetes, cancer, inflammation, apoptosis etc, particularly those sirtuins which are localized in nucleus [40].

Mechanism of anti- aging is by decreasing the extrachromosomal DNA and inactivating telomeres formed during Mitosis demonstrated in yeast by Sinclair and Guarente [41]. However, this needs to be proved in humans. Silencing of genes and chromatin are carried out either by deacetylation or by ribosylation modification of the protein or substrates [35].

Sirtuin an unnoticed molecule with clinical significance and a dilapidated molecule is involved in many significant processes supporting major metabolisms and recently it is considered as one of the important molecule of interest to develop and study DM and its related Microvascular complications.

Amongst all isoforms of sirtuin, sirt1 involve in key processes and functions such as, development of organs, metabolism, DNA repair and replication, transcription, oxidative stress, age- related diseases, neurodegenerative disorders, cardio vascular diseases (CVD) etc, [42]. Sirtuin site specific and organ specific actions are on, pancreas, kidney, skeletal muscle, adipose tissue, brain and liver [43- 48]. Sirt1 helps initiate and activate response during pathogenesis. It is evident from animal studies that calorie restriction increases lifespan by 15- 30% by Sirt6 overexpression involved in aging disorders and metabolism [37, 49]. The perquisite characteristic of Sir2 is the asymmetric inheritance related to nuclear damage in mother cell which will be maintained without inheriting to the daughter cells during cytokinesis for which sirtuins are responsible. However, this characteristic of sirtuin1 needs to be confirmed in humans [50]. Since, sirtuin, the molecule of interest, neglected till date, is gaining importance off late needs to be studied in depth. Reference ranges for sirtuin1 has to be established considering geography, genetics, population, diet, disease condition, limitations, quality conformance, confounders, diurnal variation, drugs action, gender and various other factors. Nevertheless, sirtuin1 is considered as therapeutic target for various Physiological and Pathological disorders indicating its pharmacogenetic importance in Health.

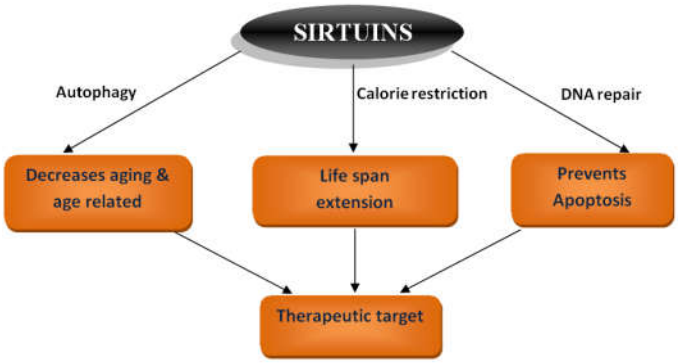

Fig 4 Overview of function and effect of sirtuins in Human

Biochemistry of Sirtuin

General reaction catalyzed by Sirtuin and Sir2 are:
Produce 2'-O- Acetyl Adenosine DiPhospho Ribose (AADPR)
Non- enzymatically converts 2'- AADPR to 3' -O- Acetyl ADPR spontaneously [53]

Fig 5 General reaction of sirtuin

Studies conducted by Chang J.H *et al*., observed that the product AADPR acts as sirtuin inhibitor by feedback inhibition mechanism [54]. Other inhibitor formed inhibiting sirtuin is Nicotinamide (NaM), derived from Nicotinamide Adenine Dinucleotide (NAD^+).

There are three documented pathways for NAD synthesis in mammals [55]:
Denovo synthesis by NaM as precursor
Kynurinine pathway from Tryptophan
From nicotinamide riboside provided externally.

Major difference in conversion of NaM to Nicotinamide nucleotide (NaMN) between yeast and mammal is the enzyme Pnc1p (pyrazin-amide/nicotinamide hydrolase), which converts Nicotinic acid to NaMN in two steps and in mammals it is the enzyme Nampt (nicotinamide phosphoribosyltransferase) which directly converts NaM to NaMN in single step [56].

It is evident from the above studies that presence of NAD^+ plays a major role in sirtuin regulation, which in turn is regulated by Pnc1p and Nampt enzymes in yeast and Mammals respectively.

Few unanswered questions on sirtuin are:

1. Its mystery in involvement of NAD^+ for deacetylation reaction.
2. Why not all deacetylase enzymes utilize NAD^+ as cofactor?

Why is this molecule studied extensively in recent days?

First reaction to be catalysed by sirtuin was ribosyltransferase in CobB *Salmonella typhimurium*, for de novo synthesis of cobalamine. This discovery also led to discovery of NAD^+ dependent deacetylation in the same organism [57]. Novelty to be noted with respect to sirtuin is that, it couples catalysis of ADP-ribosylation and protein deacetylation. Recent studies have demonstrated that sirtuins not only mediates deacetylation and ribosylation reaction but also deacylation of substrates such as: formylation, propionylation, butyrylation, succinylation and myristoylation [58, 59]. Ribosylation and deacylation are the major pathways for signaling and metabolic process regulation of sirtuin [53]. The mechanism involved in this process of sirtuin catalysis is nucleophile base exchange mechanism involving both SN_1 and SN_2 mechanisms [53, 60]. As a result, it is clear that sirtuins are noncompetitively inhibited by nicotimamide products in sirtuin mediated reaction [53].

Structure of sirtuin

Sirtuin is highly conserved protein in its structure and functions. Enzyme structure is divided into central core region, Carboxy (C) and Amino (N) terminal regions amongst which the enzyme core constitutes approximately 250 amino acids and terminal region length varies species to species, phyla to phyla and between the isoforms. Catalytic structural conformation of sirtuin includes a NAD^+ Rossmann fold which comprises a zinc binding module wherein the zinc forms coordination complex with four cysteines and helical domain

forming a flexible loop. Terminals are extended as flanking sequence which generates polarity on enzyme. Protein with the NAD^+ substrate binds between these domains forming a non polar cleft [53]. Acetylated protein when bound to sirtuin forms hydrophobic C and N terminals anchored by hydrogen bond. The helical domain is converted to three stranded β-sheets like conformation as a result of interaction between enzyme- substrate- flanking domains of enzyme [53].

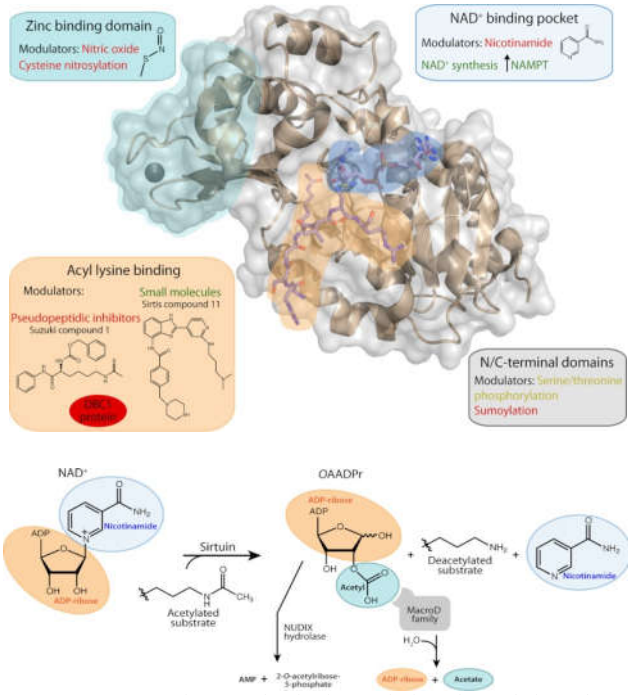

Fig 6 Source: Jessica L et al,. Sirtuin Catalysis and Regulation. The Journal of Biological Chemistry.

2012; 276 (51): 42419- 42427.

Structure of enzyme takes different conformations upon NAD^+ binding. Binding of acylated proteins in the protein binding tunnel forces NAD^+ to burry at specific site forming a dubbed C- pocket configuration [53]. Most studied, sirt1 apoform exhibits an open conformation wherein the smaller sub domain undergoes rotation proportionate to the larger NAD^+ binding sub domain. Substrate bound closed enzyme conformation with NAD^+ forms a hydrophobic shield around the active site. Uniqueness of sirtuin is, its activity regulated by C terminal regulatory segment (CTR) constituting 641- 655 amino acids [61].

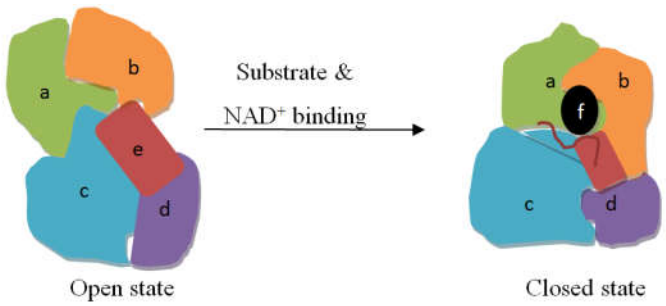

Fig 7 Source: Andrew M. Davenport *et al*., Structural and Functional Analysis of Human SIRT1. J. Mol. Biol. 2014; 426: 526- 541.

a. Zn^{2+} binding module, b. Helical module,
c. NAD^+ binding domain, d. C- terminal regulatory sequence, e. NAD^+ binding site,
f. active site

Omics of Sirtuin Structure- Highly conserved characteristics of sirtuins across all phyla of life distinguishes it as an ancient protein as described by Constantini *et al* [62]. Genomics and proteomics studies conducted revealed that sirt1 is the most ancient among all the isoforms and hence has many significant functions. Omic studies further confirmed that phosphorylation sites, GC content and amino acid disorderness at terminals play an important role in enzyme regulation [63]. Selection and specificity of the molecular partner for reaction to occur is mainly dependant on terminal stretches and charged sequences [62]. Charged and uncharged sequences affect the neighboring amino acids in sequence causing disorderness in enzyme. Charged sequences in terminals contribute to molecular recognition [62]. Protein folding is affected by disorderness, higher the disorderness lower the protein folding with increased molecular partners. Globular proteins are the major ones for reacting with such proteins [64]. Due to these features, it is found that sirtuins are involved in many signaling and regulatory processes which are yet to be addressed. Amongst all mammals, dogs, mouse, wild boar and human beings possess all seven isoforms of sirtuin. Sirt1 and Sirt2 are abundantly found in all phyla [62].

It is evident from studies that sirtuin1 structure (747 amino acid containing enzyme) has a unique two long disordered sequences in the termini indicating poor structural organization leading to higher disorderness and increased molecular partners [65]. This property creates more interest in studying sirtuin1 and its physiology.

Sirtuin activators

Demand for sirtuin activators plays a role during aging because of the fact that availability of NAD^+ decreases as the age advances due to which sirtuin linked repair mechanisms are hindered [66]. It is believed that small molecule such as resveratol activates sirtuin1 but not all isoforms, but supplementation with NAD^+ and the substrate NaM will upregulate all the mammalian sirtuins [67]. NAD activation is substrate independent type of activation since it is activated by altering sirtuin rate mechanisms [68]. Other endogenous activator is free fatty acid in the body which enhances sirtuin action on short chain acylated fatty acid along with the usual activity on long chain fatty acids [69]. Thus, NAD, resveratol and free fatty acids are notable specific activators of sirtuin1.

Sirtuin inhibitors

Sirtuins are inhibited by competitive and feedback inhibition. Intermediate product formed during deacylation AADPR inhibits sirtuin when over expressed without affecting its normal mechanism of protection [70]. Metabolite for synthesis of NAD^+ that is nicotinamide (NaM) inhibits sirtuin competitively with pocket of NAD^+ binding site leading to depression in base exchange to form the first intermediate; imidate complex by sirtuin [53, 42]. Isonicotinamide counter acts against NaM by preventing binding of NaM to sirtuin by reverting base exchange reaction selectively performed by NaM previously for sirtuin inhibition [71]. Thus, major sirtuin inhibitors documented till date are: AADPR, NaM and Isonicotinamide (iNaM)

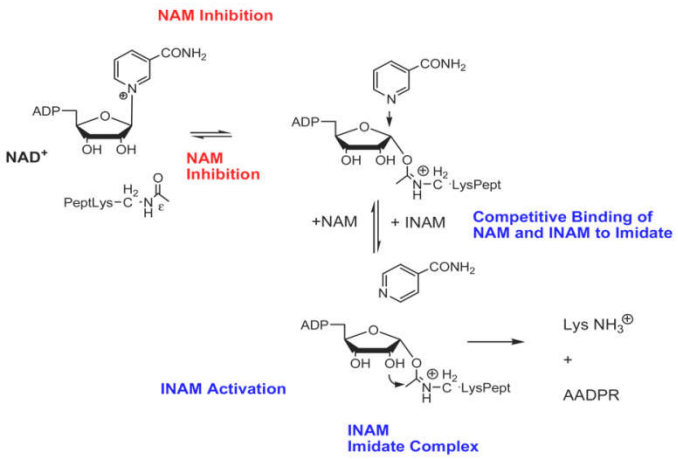

Fig 8 Source: Sauve AA *et al*., Chemical activation of Sir2-dependent silencing by relief of nicotinamide inhibition. Mol Cell 2005; 17: 595- 601

disorders are discovered, inventions of biomarkers and treatments must be carried out to avoid outbreak of disease Advanced biomarkers must also be taken into routine investigations with already available gold standard markers for better understanding of disease prognosis and diagnosis.

Diabetes mellitus and diabetic nephropathy Globally, Diabetes, Hypertension, Cardio vascular disease, Cancer and other age-related disorders are increasing day on day and needs to be addressed immediately to prevent their ill effects in future generations Since Diabetes is taking a lead globally, India is labeled the capital of diabetes is an alert alarm on recent trends and findings documented to slow its progress Role of biomarkers and therapeutics for diabetes may help in solving these problems.

Methods for estimation of Sirtuin1

Method	Sample specificity	Sensitivity	Sample/ tissue type & Sample volume	Detection wavelength (nm)	Minimum assay limit	Maximum assay limit	Dilution	Storage	Merit	Demerit
Fluorometric assay (71)	Sirtuin1	Intra- assay %CV 2.5 Inter- assay %CV 4.3	Yeast cells/ tissue extracts & 50 µL	460 ± 10	75 µM	200 µM	10x with distilled water	-80° C (<2months) - 20° C (<1month)	Safe, versatile, cost- effective and no specialized equipment required	Not suitable to measure NAD^+ activity against sirtuin
HPLC- MS (50)	Sirtuin1	Intra- assay %CV 13.1 Inter- assay %CV 0.3	e- coli/ cell lysate & 25 µL	280 (Indole chromophore) Or 326 (Coumarin moiety)	100 µM	400 µM	2x with distilled water	-80° C (<2months) - 20° C (<1month)	Coumarin chromophore is best used due to its non-overlapping results of NAD^+ absorbance compared to fluorometric assay	Mass of enzyme measurement is not precise
ELISA (73)	Sirtuin1	<0.32 ng/ mL	Body fluids, tissue homogenate, cell lysate, cell culture & 90 µL	450 ± 10	0.78 ng/ mL	50 ng/ mL	100x with distilled	-80 ° C (<2months) - 20° C (<1month)	Measurement of enzyme activity with mass & sensitivity is possible	Measurement of enzyme activity is poor compared to other methods

Methods of Estimation of Sirtuins

Sirtuins are estimated in biological samples such as; serum, plasma and urine. It is also estimated in sub- cellular organelles. Sirtuin analytical ready available, fluorometric assay [50], high performance liquid chromatography- Mass spectrophotometry (HPLC- MS) and Enzyme Linked Immuno Sorbent Assay (ELISA) are available. Sirtuin is not considered as a diagnostic molecule, may be because of lack of knowledge, Evidence based medicine, accuracy, specificity, sensitivity (AUC) and precision of methodology, no defined cut- off ranges, lack of population studies, genetics, internal quality control (IQC), external quality control (EQC), trueness, total error allowable etc., needs to be established and validated with a pilot study followed by multicentric large population studies. As on date, the most accurate and effective method of estimation of sirtuin is by Fluorometric assay with high sensitivity and precision. However, the activity of sirtuin is measured accurately by HPLC- MS [51].

CONCLUSION

With the current review following remarks can be made regarding:

Biomarkers

Play a vital role to assess development of a disorder and/or disease Since many new disorders and combinations of

Sirtuin

A diverse ancient protein family has its role as a marker as well as a metabolite essential for day to day wear and tear of cells in the system of organisms

Being the conserved protein sirtuin is of interest in research to reveal its mystery and mechanism of action in various physiological and pathological conditions Pace of research on sirtuin needs to be accelerated to acknowledge this enzyme in all spheres of research and consider it as a therapeutic target to assess its precise pathological condition where the molecule plays an important role

Detail study of sirtuin by in vivo experiments on human cells may give a clear picture about its mechanism in action of defense which aids in pharmaceutical research for therapeutic utilization and intervention.

List of abbreviations

AADRP: Acetyl adenosine diribose phosphate, ADP: adenosine diphosphate, AUC: area under curve, DNA: deoxyribonucleic acid, FTH1: Ferritin heavy chain1, GLP: good laboratory practice, G_0: Fasting glucose, I_0: fasting insulin, NAD: nicotimamide adenine dinucleotide, NaM: nicotinmide, NIH: national institute of health, PCR: polymerase chain reaction, QC: Quality control, VS: validation samples.

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