EPIGENETIC MECHANISM IN HUMAN SIRTUIN 1 AND AGING

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INTRODUCTION

Sirtuins are a distinctive group of NAD+-dependent deacetylases, classified as class III Histone deacetylases (HDACs). It is a family of proteins that are highly conserved, both structurally and functionally. Sirtuin members are integrated into most life forms, including eukaryotes and prokaryotes (Wątroba et.al.2017). The catalytic activity of sirtuins relies on NAD+ and is managed by the changes in NAD+ levels and the NAD+/NADH ratio. NAD+ is essential as a co-substrate, suggesting that sirtuins are formed as energy sensors and redox status in the cell. Biosynthesis of NAD+ occurs in two pathways: de novo production and the salvage pathway (Grabowska, W., et al. 2017). As HDACs, sirtuins have the potential to remove acetyl groups from histones which can alter the functional or structural stability of proteins. Thus they have a significant role in cellular functions ranging from apoptosis, gene silencing, and age-related pathological conditions such as the deregulation of metabolism and cancer, etc. Sirtuins have been studied extensively in recent years, and their potential as therapeutic targets for various diseases, including cancer, metabolic disorders, and aging-related diseases, is an active area of research. SIRT1 (silent information regulator T1) is the most studied member of the sirtuin family, which shows the highest sequence similarity with SIRT2 in yeast. They play a significant role in aging and related

processes. Epigenetic modifications in sirtuin can regulate many cellular functions such as cellular metabolism, gene expression, DNA repair, apoptosis, cell cycle, development, immune responses, and neural protection (Zhao, L. et al. 2020) (Elibol, B. and Kilic, U. 2018).

Human Sirtuins

Human sirtuins were categorized into SIRT1-SIRT7, based on their function and cellular localization (Xiong, L. et al, 2021).

Table 1: Seven-member Human Sirtuins - Subcellular location, Function, and Structural features.

Sl No:	Sirtuin Type	Subcellular location	Functional activity	Molecular Weight	No: of Amino acids
1	SIRT1	Nucleus and cytoplasm	Deacetylase	81.7	747
2	SIRT2	Cytoplasm	Deacetylase	41.5	389
3	SIRT3	Mitochondria	Deacetylase	43.6	399
4	SIRT4	Mitochondria	ADP-ribosyl transferase	35.2	314
5	SIRT5	Mitochondria	Deacetylase	33.9	310
6	SIRT6	Nucleus	Deacetylase/ADP-ribosyl transferase	39.1	355
7	SRT7	Nucleolus	Deacetylase	44.9	400

Human sirtuins play a significant role in regulating metabolic activities and aging processes. SIRT1 (nucleus& cytoplasm) is involved in the regulation of metabolism, aging, other transcription factors, and co-activators such as p53, FOXO, and NF-kB, which are important for cell survival and apoptosis. SIRT2 (cytoplasm) is associated with cytoskeletal dynamics and cell division. SIRT3 (mitochondria) is associated with regulating antioxidant defense, metabolism, and aging. SIRT4 (mitochondria)involves in the regulation of insulin secretion and amino acid metabolism. SIRT5 (mitochondria) deals with the regulation of lipid metabolism and the urea cycle. SIRT6 (nucleus) is linked to the regulation of genomic stability, DNA repair, and the aging process. And SIRT7 (nucleolus) controls transcription and the aging process. In recent years, these seven protein classes in the sirtuin family have been extensively studied, as they are involved in multiple significant functions in humans (Grabowska, W., et al. 2017) (Zhao, L. et al. 2020) (Elibol, B. and Kilic, U. 2018) (Iljas, J.D. et al, 2020).

Sirtuin1 (SIRT1)

Sirtuin1, NAD+-dependent deacetylase is a multifaceted human protein encoded by the SIRT1 gene and a class III histone deacetylase member of the human sirtuin family, homolog to yeast (evolution from yeast to human). Human sirt1 has 747 amino acids in length (Davenport, A.M., et al. 2014). The major function of a sirtuin is to remove acetyl groups from lysine residues of histone protein with the help of NAD+. Hence they are known as "NAD+-dependent deacetylases". Sirtuins transfer an acetyl group from the protein to the ADP-ribose part of NAD+ to create O-acetyl-ADP-ribose (Rahman, S. and Islam, R., 2011). Thus deacetylasation takes place in various histone and non-histone proteins to maintain the long-term functioning of proteins and stabilize their shape, to conserve energy for other cellular processes. It deacetylates proteins that contribute to regulating cellular functions ranging from cell metabolism to cell survival. SIRT1 is also implicated in many human disorders and related pathways including obesity-related

metabolic diseases, aging, cancer, stress, cellular senescence, prion-mediated neurodegeneration, stimulates autophagy, development, and placental cell survival, environmental stress-related inflammatory signaling, etc (Davenport, A.M., et al. 2014) (Rahman, S. and Islam, R., 2011) (Zhao, L. et al. 2020).

The activity of sirtuin1 is uniquely regulated by the C-terminal regulatory segment (CTR). It has the effect of stimulating heterochromatin formation, cell survival, and resistance to oxidative stress (Davenport, A.M., et al. 2014). SIRT1 deacetylates an extensive range of substrates such as tumor suppressor protein p53, FOXO transcription factors, p300, NF-κB (nuclear factor kappa B), and PGC1α (PPARγ coactivator), etc (Weinhold, B., 2006). Small molecule activators, cellular metabolic status, interacting proteins as well as post-translational modifications are responsible for the activity regulation of SIRT1. SIRT1 has both activators and inhibitors. AROS (Active Regulator of SIRT1), lamin A, resveratrol, etc. are sirtuin1 activators and the SIRT1 inhibitors are DBC1, HIC1, tenovins, etc (Brooks, C.L. and Gu, W., 2009). It has been reported that Human Sirt1 has 136 direct interactions in interatomic research which are involved in different cellular activities. Sirtuins participate in the epigenetic modifications related to aging.

Sirtuin enzymes inactivate some genes that involve in aging-related processes such as inflammation, blood sugar management, fat synthesis, and storage (Davenport, A.M., et al. 2014). Acetyl groups were added to proteins under stress conditions, in order to respond against the changes caused due to inflammation and oxidation. SIRT1 can regulate the functions of chromatin by the deacetylation of histones and can enhance alterations in histone-DNA methylation, directing to transcriptional repression. It has a significant impact on controlling the strength (amplitude) and the time span of circadian gene activity in the retina by removing acetyl groups from BMAL1 and PER2, key circadian clock regulators. Moreover, SIRT1 actively promotes Human cell homologous recombination (HR), recombinational repair of DNA damage, and activation of Th17 cells involved in autoimmune disease. Moreover, SIRT1 actively promotes Human cell

homologous recombination (HR), recombinational repair of DNA damage, and activation of Th17 cells, which involves in autoimmune disease. However, the utilization of therapeutic activation of SIRT1 may also be relevant in treating autoimmune diseases (Rahman, S. and Islam, R., 2011).

AGING AND SIRTUINS

Aging is a universal process in all species and leads to an increased risk of dysfunction and even death when organisms get older. Longevity and the aging process are influenced and caused by various factors such as the shortening of telomeres, mitochondrial malfunction, nutrient sensing, oxidative stress, the decline in DNA repair and formation of DNA damage, alterations in protein homeostasis that leads to the formation and clustering of misfolded proteins, and modifications in epigenetic regulation (Wang, K. et al. 2022) (Elibol, B. and Kilic, U. 2018). The longevity effect of sirtuins has been reported in various organisms, but the mechanisms and effects vary based on the complexity of the organism. They play a significant role in slowing cellular senescence and prolonging the lifespan of an organism by regulating several cellular processes. Sirtuin restrains cellular senescence by delaying age-related telomere attrition, assisting genome integrity, and advancing DNA damage repair (Brooks, C.L. and Gu, W., 2020) (Zhao, L. et al. 2020).

The importance of sirtuins rises in 1999 when a study reports the overexpression of Sirt2 is responsible for increasing the lifespan of yeast by up to 70%. Further studies revealed that the overexpression of sirtuins also extends the lifespan of other model organisms such as *Caenorhabditis elegans* (worm) and *Drosophila melanogaster* (fruit fly)(Grabowska, W., et al. 2017). Some research reports that sirt1 deacetylates histone (H3, H4, and H1) proteins and over 50 non-histone proteins, namely DNMT1, transcription factors, and DNA repair proteins. Sirtuins were spotted in major longevity pathways, including AMP-activated protein kinase (AMPK), the target of rapamycin (TOR), insulin/IGF-1 signaling (IIS), and forkhead box O (FOXO). FOXO is the most exciting sirtuin target (Brooks, C.L. and Gu, W., 2020).

The amount of SIRT1 declines in the liver during aging due to less NAD+ availability, whereas DNA damage accumulation increases at the same time. Aging-dependent decrease in SIRT1 level noticed in arteries points to its significance in the aging of the cardiovascular system. And also, decreased levels of SIRT1 due to accelerated senescence of cord blood endothelial cells lead to the early onset of vascular dysfunction in low birth weight premature infants. And the lack of SIRT1 also hastens the activation of genes associated with aging (Grabowska, W., et al. 2017). Resveratrol is a SIRT2 activator, that enhances insulin sensitivity and motor function. Furthermore, it has been stated that resveratrol slows down the aging process of adipose stem cells by reducing the levels of 5-mC in DNA and changing the way mitochondria function. SRT1720 is recognized as a stimulator of sirtuin-1 (Wang, K. et al. 2022).

CALORIE RESTRICTION

Calorie restriction (CR) is the only strategy for prolonging lifespan that has been scientifically confirmed to work without using drugs or genetics. CR leads to some physical and behavioral changes such as Smaller body size, reduced growth factor, glucose, and lipid levels, as well as increased mobility and foraging activity. Also, CR leads to the increase of the majority of sirtuins, except SIRT4. However, many in vitro and in vivo studies reported that in addition to mediating the effects of CR, sirtuins play a critical role in fostering a species-specific lifespan (Grabowska, W., et al. 2017) (Watroba, M. and Szukiewicz, D. 2021).

A series of therapeutic stratergies have been modified based on aging-related epigenetic modifications. Various species have been tested with Geroprotective drugs that target longevity-related histone acetylation, incorporating NAD+ precursors, and STACs. In preclinical studies, drugs like metformin, rapamycin, etc have exhibited potential effects on age-related epigenetic changes and pathologic conditions. Further clinical research is necessary to determine the safety and efficacy of these medications.

EPIGENETICS

Epigenetics mechanism is the heritable phenotypic variations without altering the DNA sequence or genetic code. The nucleosome is the core functional unit in DNA, that stands alone in the nuclear context and determines gene expression. Thus all the elements that take part in nucleosome-chromatin regulation which determines gene expression are studied in epigenetics. It is a chemical modification in a specific gene (mitotically and meiotically) or gene-associated proteins of an organism. These genetic modifications can determine how the genes are utilized and expressed in cells (Simmons, D., 2008) (Bock, C. and Lengauer, T., 2008).

The word epigenetics in its contemporary usage was first coined by British embryologist Conrad Waddington (1942), by using the greek word “epi” as a prefix. Epi means “above or in addition to” (Holliday, R., 2006). So he coined the term to link developmental biology and genetics. He defined the field as, - “a branch of biology which studies causal interactions between genes and their products which bring the phenotype into being”.

Epigenetic changes may take place regularly and naturally but it also occurs by the influence of various factors including the health condition and age of an individual, living environment, lifestyle, etc. Naturally, epigenetic changes are essential to many organisms for their proper functioning, but if it occurs improperly it will cause many adverse effects on the health and behavior of the organism (Weinhold, B., 2006).

Epigenetics mechanisms include

- DNA methylation
- Histone post-translational modifications (PTMs)
- Interchanging of canonical histones with their variants.
- ATP-dependent chromatin remodeling

- Recruitment of long and short non-coding RNAs etc. (Wątroba et.al.2017) (Lundstrom, K., 2018.).

Among all these, methylation is the most significant epigenetic modification, in which a methyl group is added to the biomolecule (DNA/Protein). The process can be permanent or temporary and also it may change rapidly during the life span of a cell or an organism.

DNA methylation

The DNA methylation procedure involves attaching a methyl group to a cytosine residue at CpG locations, resulting in the creation of 5-methylcytosine (Simmons, D., 2008). CpG sites are the sites where cytosine is immediately in front of guanine. DNA methylation controls gene expression or activity of a gene. DNA 5-cysteine methylation is the most extensively researched epigenetic mechanism. In protein methylation, a methyl group is added to a lysine amino acid or an arginine residue and also controls gene expression by activating or deactivating a gene (Weinhold, B., 2006) (Simmons, D., 2008) (Wang, K. et al. 2022).

Post-translational modification of histone proteins

Post-translational modifications (PTMs) is a set of processing events that can change the function or property of a protein through the covalent addition of functional groups (methyl, acetyl, glycosyl, and phosphoryl to one or more acid residues), proteolytic cleavage, and complete degradation of proteins (Ramazi, S. and Zahiri, J., 2021.). PTMs include acetylation, methylation, ubiquitination, phosphorylation, lipidation, nitrosylation, proteolysis, and glycosylation. These modifications can influence the overall functioning of normal and pathological cells including assembly and function, interactions, lifespan, molecular trafficking, cellular localization, etc. Hence it is very important to identify and understand the PTMs occur in a cell or an organism for the study of cellular biology, pathogenesis, treatment, and prevention.

Post-translational modification in histone proteins

Generally, proteins that display significant structural and functional traits, such as histones, membrane proteins, and secretory proteins, have been observed to have PTMs. PTMs of a histone protein include methylation, acetylation, phosphorylation, ubiquitination, ADP-ribosylation, sumoylation, etc. These changes can affect how gene function or how they are expressed by changing their structure, leading to various biological processes such as chromosome arrangement, fixing or causing DNA damage, and activating or deactivating transcription. Histone H3 is the most modified histone (Simmons, D., 2008).

a) Histone methylation

The procedure involves the addition of one or multiple methyl groups from S-adenosyl-L-methionine to the arginine or lysine residues of histones through a covalent bond, facilitated by histone methyl transferase (HMTs) enzymes. This PTM modification influences the structure and arrangement of chromatin. During histone methylation, the associated genes may be activated or inactivated. Methylation in histones is categorized into three types - mono-methylation, di-methylation, and tri-methylation, which effects differently on gene expression. Mono-methylation results weakest effect, di-methylation lead to a moderate effect, and tri-methylation results in the strongest effect. Histone methylation is an important epigenetic modification that plays a significant role in the regulation of gene expressions and cellular function (Wang, K. et al. 2022).

b) Histone acetylation and deacetylation

Histone acetylation is a type of post-translational modification where an acetyl group from acetyl coenzyme A is attached to the lysine residues on the N-terminal end of histones. The process takes place with the help of an enzyme, Histone acetyltransferases (HATs). Histones are the

proteins that fabricate chromatin structures. So histone acetylation leads to structural change in chromatin by making it less closely packed and more receptive to transcription-related proteins, resulting in the regulation of gene expression. This process plays an important role in the regulation of DNA replication, cell cycle progression, DNA repair, gene silencing, cell differentiation, chromatin dynamics and transcription, neuronal repression, apoptosis, etc (Wang, K. et al. 2022).

On the other hand, histone deacetylation is the mechanism of removing the acetyl groups from lysine residues on the histones mediated by histone deacetylases (HDACs) enzyme. Thus histone deacetylation leads to structural change in chromatin by making it more closely packed and less accessible to transcription-related proteins, resulting in the repression of gene expression. A variation in the stability of histone acetylation was associated with cancer progression and tumorigenesis (Wang, K. et al. 2022).

c) Phosphorylation

The phosphorylation is the addition of a phosphoryl group ($-PO_3^{2-}$) from ATP to the tail ends of histone protein. This PTM serves as a critical intermediary stage that has a vital impact on chromatin remodeling by promoting connections between other histone modifications. Additionally, it acts as a platform for proteins that play a role in condensing chromosomes during cell division, cellular responses, controlling transcription, and fixing DNA damage (Holliday, R., 2006).

d) Ubiquitination

Ubiquitination or ubiquitylation is the process of adding ubiquitin to a substrate protein. Ubiquitin is a compact regulatory protein seen in most eukaryotic tissues. Ubiquitination changes various aspects of protein such as the protein location in the cell, function, protein degradation, enhance

or prevention of protein interactions. All these histone modifications can change the structure of chromatin which results in transcriptional activation or repression. Ubiquitination in protein can be either by a single ubiquitin protein (monoubiquitination) or a chain of ubiquitins (polyubiquitination) (Wang, K. et al. 2022).

ATP-dependent chromatin-remodeling

Epigenetic regulation through ATP-dependent chromatin remodeling involves utilizing the energy from ATP hydrolysis to reorganize the chromatin fiber with the aid of specific enzymes (Wang, K. et al. 2022). ncRNAs are RNA molecules that perform a function but are not turned into proteins as they are transcribed from DNA but not translated into a protein. They play an important role in gene expression at both the transcriptional and post-transcriptional stages. The ncRNAs can be classified into two groups based on nucleotide chain length - short ncRNAs (less than 200 nts) and long ncRNAs (over 200 nts). Some ncRNAs involved in epigenetics include miRNA, siRNA, piRNA, and lncRNA. MicroRNAs (miRNAs), short-interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs) are the three major classes of short non-coding RNAs. Both ncRNA groups play a significant role in DNA methylation targeting, histone modification, heterochromatin formation, and gene silencing in epigenetics. Gene silencing is the epigenetic regulation of a gene and It occurs at the level of transcription or translation, to prevent a particular gene expression in a cell (Lundstrom, K., 2018.). Gene silencing mechanism includes DNA methylation, RNA interference, and histone modification (Weinhold, B., 2006).

Epigenetics studies have revolutionized the scientific world, especially in the genetics and developmental biology fields. Scientists have discovered several credible chemical modifications to DNA and histones (proteins possessing abundant lysine and arginine amino acid residues) that are closely associated with DNA in the nucleus. These alterations can predict the time or whether the selected gene functions in a cell or an organism. It is an emerging keystone of modern

biology with great significance and promising practical application for the current and future medicinal world.

CONCLUSION

The protein Sirtuin 1 has a significant impact on controlling numerous cellular operations, including the aging process. The close functional relationship between NAD+-sirtuins and epigenetic modifications in sirtuins plays a significant role in regulating metabolic processes and contributing to aging in various organisms. Delaying aging can be achieved through a healthy diet and regular exercise, which are currently the most effective methods. Nevertheless, the option of using drugs that target molecular and cellular changes related to aging is still a desirable alternative. However, the interaction between sirtuins (SIRT1) and other biomolecules, epigenetic changes, and other factors such as genetics, the microbiome, and environmental factors remain unknown. More research is needed to monitor such modifications and related processes during the entire aging process.

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