4 Mitochondria Lysine Acetylation and Phenotypic Control

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Abstract

Mitochondria have a central role in cellular metabolism and reversible post-translational modifications regulate activity of mitochondrial proteins. Thanks to advances in proteomics, lysine acetylation has arisen as an important post-translational modification in the mitochondrion. During acetylation an acetyl group is covalently attached to the epsilon amino group in the side chain of lysine residues using acetyl-CoA as the substrate donor. Therefore the positive charge is neutralized, and this can affect the function of proteins thereby regulating enzyme activity, protein interactions, and protein stability. The major deacetylase in mitochondria is SIRT3 whose activity regulates many mitochondrial enzymes. The method of choice for the analysis of acetylated proteins foresees the combination of mass spectrometry-based proteomics with affinity enrichment techniques. Beyond the identification of lysine-acetylated proteins, many studies are moving towards the characterization of acetylated patterns in different diseases. Indeed, modifications in lysine acetylation status can directly alter mitochondrial function and, therefore, be linked to human diseases such as metabolic diseases, cancer, myocardial injury and neurodegenerative diseases. Despite the progress in the characterization of different lysine acetylation sites, additional studies are needed to differentiate the specific changes with a significant biological relevance.

Keywords

Mitochondria · Lysine acetylation · Sirtuin3 · Post-translational modifications · Proteomics

4.1 Introduction

Mitochondria have a central role in cellular metabolism considering that they harbour the main metabolic pathways. Urea cycle, tricarboxylic acid cycle, oxidative phosphorylation and fatty acid oxidation take place in these organelles. Therefore, since mitochondria provide the major source of ATP for cellular activity, defects in mitochondrial function contribute to pathologies. Indeed, mitochondrial dysfunctions have been associated with the pathogenesis of many diseases including cardiac diseases [45, 86], neurodegenerative diseases [51, 98], cancer [71, 79], and metabolic disorders such as obesity [23, 44] and type 2 diabetes [62, 84].

It is known that reversible post-translational modifications (PTMs) regulate activity not only

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of cytosolic and nuclear proteins, but also of mitochondrial proteins. Indeed, PTMs are involved in many cellular processes such as enzyme regulation, signal transduction, mediation of protein localization, interactions and stability [61]. Since genomic cannot be used for the analysis of modified proteins, proteomics is the method of choice in this area. Thanks to advances in mass spectrometry-based proteomics and affinity enrichment techniques, lysine acetylation has arisen as an important PTM that rivals phosphorylation and ubiquitination in its prevalence [15, 88]. Proteomics revealed that acetylation occurs in more than 2000 proteins involved in different cellular processes [15]. Moreover, lysine acetylation is a PTM more frequent than phosphorylation in bacteria. Thus, considering the endosymbiotic theory, it is not surprising that lysine acetylation is also more abundant in mitochondria of eukaryotic cells than other PTMs [17, 56].

Acetylation of proteins on lysine residues is a reversible PTM that was discovered more than 50 years ago on histones [2, 56] where it reduces their affinity with DNA. p53 was the first non-histone protein identified to be lysine acetylated [26]. Subsequently, in 2006, an extensive proteomics survey revealed, for the first time, that acetylation is a common posttranslational modification in the mitochondrion [42], and it has been assessed that nearly 35% of all proteins in mitochondria have one acetylation site, with an average of 5.6 sites per protein [4, 50]. Moreover, Lundby et al., by combining lysine-acetylated peptide immunoprecipitation and high-accuracy tandem mass spectrometric, discovered that, in muscle, mitochondria is the cells compartment which had the highest level of acetylation and the fraction of lysine-acetylated proteins is approximately three-fold greater than phosphorylated proteins [50].

4.2 Lysine Acetylation

4.2.1 General Aspects

The lysine acetylation levels are reflective of the balance between lysine acetyl-transferase (KATs) and lysine deacetylase (KDACs) activity, the specific enzymes which regulate acetylation and deacetylation, respectively (Fig. 4.1a). However, non-enzymatic acetylation can occur in mitochondria (Fig. 4.1b), where the physiologic pH (pH $\geq$7.5) in the matrix is sufficient to cause enzyme-independent acetylation [85]. During enzymatic acetylation an acetyl group is covalently attached to the epsilon amino group in the side chain of lysine residues using acetyl-CoA as the substrate donor. Therefore the positive charge is neutralized, and this can affect the function of proteins thereby regulating enzyme activity, protein interactions, and protein stability [35]. The proteins with acetyl-lysine residues can be specifically recognized by bromodomain-containing proteins which are mostly nuclear and cytosolic. Even if bromodomains have not yet been identified in mitochondria, unknown proteins might have similar role. Conversely, KDACs catalyze deacetylation by using Zn^{2+} as a cofactor (Fig. 4.1c), and are mainly localized in the nucleus and cytosol, whereas sirtuins (SIRTs) are a family of NAD^{+}-dependent deacetylases which release *O*-acetyl-ribose and nicotinamide (NAM) as by-products of the deacetylation [35] (Fig. 4.1d). This specific group includes seven members (SIRT 1-7) which are involved in the regulation of many cellular activities. Sirtuins 3, 4 and 5 are the three SIRTs located in mitochondria. In particular, SIRT 3 is the main deacetylase in mitochondria whose deregulation has been implicated in the development of diseases such as diabetes, myocardial injury, and cancer [90].

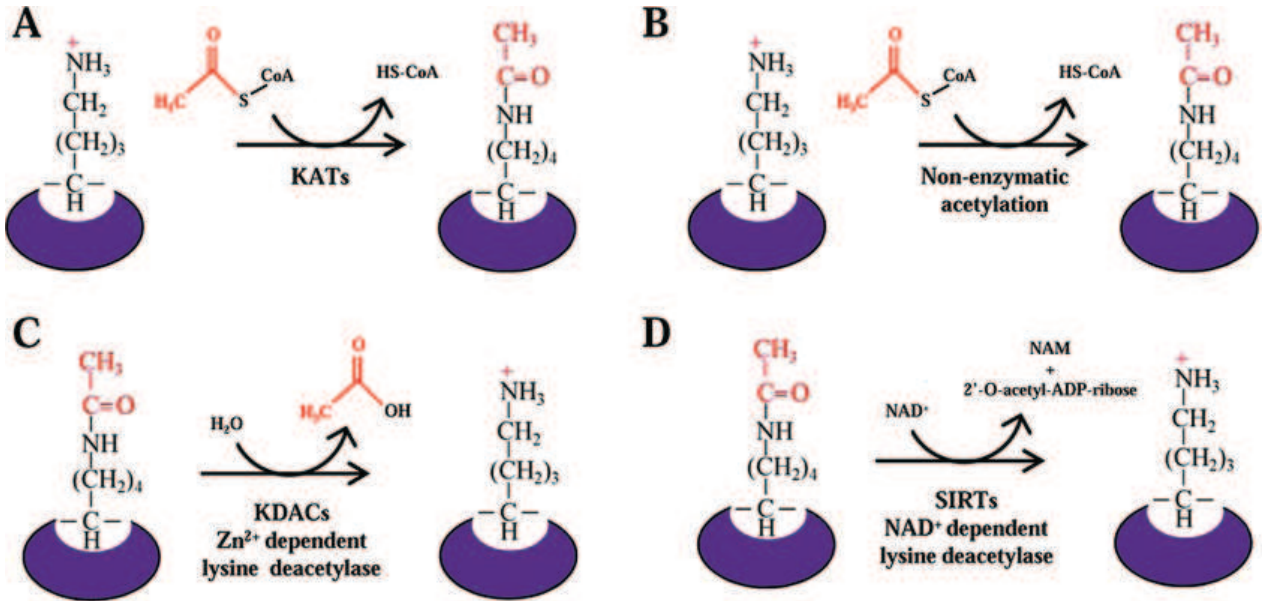

Fig. 4.1 Scheme of acetylation and deacetylation. (**a**) Enzymatic transfer of an acetyl group from acetyl-CoA to the ε-amino group of a lysine residue, catalyzed by lysine acetyl-transferase (KATs). (**b**) Non-enzymatic acetylation of the ε-amino group. (**c**) Deacetylation of acetyl-lysine residues catalyzed by lysine deacetylases (KDACs) by using Zn^{2+} as a cofactor. (**d**) Deacetylation of acetyl-lysine residues catalyzed by sirtuins (SIRTs), NAD^+ dependent deacetylases which release *O*-acetyl-ribose and nicotinamide (NAM)

4.2.2 Proteomics Analysis

So far, phosphorylation and ubiquitylation have been the most studied PTMs which are involved in the regulation of many cellular processes. Indeed, acetylation is generally less abundant, and its low stoichiometry nature complicates the identification of this modification [6]. Recently, analysis of acetylation has been propelled by progresses in proteomics approaches and it has been demonstrated that the regulatory role of lysine acetylation is wide and comparable with that of other major PTMs [15]. The method of choice for the analysis of acetylated proteins foresees that proteins are extracted from the biological samples. The isolation of subcellular fractions, such as mitochondria, enables the enrichment of acetylated peptides specifically present in different cell compartments. By this way it can be enhanced the sensitivity of the analysis when focused on a specific subset. Subsequently, proteins are digested into peptides, typically using trypsin. Peptides can be fractionated prior to enrichment, usually by ZIC®-Hydrophilic Interaction Liquid Chromatography (HILIC) or Strong cation exchange (SCX) based HPLC. Thereafter, the enrichment of acetylated peptides is required to decrease sample complexity since not all the proteins are acetylated. For this purpose, specific anti-acetyl-lysine antibodies are used for the immunoaffinity purification. Seeing the low specificity of the most commercially available antibodies, the use of at least two different antibodies is suggested [75]. Sample complexity can be further reduced by peptide fractionation methods (*e.g.* isoelectric focusing, reversed-phase and SCX chromatography) [35].

Fractions are then analyzed by nano-liquid chromatography tandem mass spectrometry (LC-MS/MS) using reversed-phase chromatography under acidic conditions. MS and MS/MS spectra are then computationally processed to acquire

peptide sequences, including the presence and location of PTMs [25]. Top-down proteomics might be suitable but the previous described bottom-up approach is the most common for the analysis of lysine-acetylated proteins.

4.3 Roles of Lysine Acetylation in Mitochondria

The three mitochondrial SIRTs (SIRT3, SIRT4 and SIRT5) mediate mitochondrial protein acetylation levels. However, SIRT4 and SIRT5 only display weak deacetylase activity [83], besides, SIRT5 seems to have demalonylase and desuccinylase activities instead of deacetylase activity [18]. Thence, SIRT3 is recognized as the major deacetylase in mitochondria. Indeed, when it is absent, mitochondrial proteins become hyperacetylated, whereas the lack of either SIRT4 or SIRT5 does not entail significant changes in acetylation [1, 48]. Interestingly, mitochondrial acetyltransferases (MATs) have not yet been characterized, therefore the issue how mitochondrial proteins can be acetylated has emerged [4]. One hypothesis is that high acetyl-CoA levels in mitochondria could promote a non-enzymatic acetylation mechanism seeing that non-enzymatic acetylation of histones with acetyl-CoA can occur in vitro [55]. Otherwise, MATs are probably acetyltransferases dissimilar from the known enzymes present in nucleus and cytosol that might be awaiting discovery [4].

As expected, the majority of mitochondrial proteins that have been identified as acetylated take part to some aspects of energy metabolism: e.g. TCA cycle (malate dehydrogenase, isocitrate dehydrogenase, fumarate hydratase, succinate dehydrogenase); antioxidant system (superoxide dismutase); lipid pathway (long-chain acyl-CoA dehydrogenase, enoyl-coA hydratase/3-hydroxyacyl-CoA dehydrogenase acetyl-CoA synthetase); amino acid metabolism (glutamate dehydrogenase, aldehyde dehydrogenase); ketone body metabolism (3-hydroxy-3-methylglutaryl-CoA synthase 2, 3-hydroxybutyrate dehydrogenase); urea cycle (carbamoyl phosphate synthetase 1, ornithine transcarbamoylase); oxidative phosphorylation (NADH dehydrogenase, ubiquinol-cytochrome c reductase, ATP synthase) [30, 42, 48, 53, 72, 96].

As a consequence, it was predictable and to date it is well known, that lysine acetylation on mitochondrial protein is sensitive to metabolic states and dietary conditions. Indeed, high-fat diet, fasting and feeding have been linked to acetylation of mitochondrial proteins, even if data are controversial. In order to characterized the proteins which are acetylated under diverse dietary and metabolic conditions, proteomic studies have been performed [15, 42, 66, 87, 96].

The proteomics survey by Kim et al. was the first analysing lysine acetylation of proteins in liver mitochondria from fasted and fed mice. After tryptic digestion, peptides from samples were purified by using agarose beads bearing immobilized anti-acetyl lysine antibody. The enriched peptides were then analyzed by nano-HPLC/MS/MS in an LTQ mass spectrometer. Among the mitochondrial acetylated proteins, 62% were identified in both fractions, 14% were specific to fed mice, and 24% were unique to fasted mice [42]. This could suggest the increase of acetylation during fasting. Actually, with another large-scale mass spectrometry screening, it was described an increase in mitochondrial protein acetylation in mice fed a calorie-restricted diet [66] similar to the acetylation patterns observed during fasting. But with the raise of researches on lysine acetylation, a paradoxical data came out.

SIRT3 expression is upregulated during fasting, modulating mitochondrial intermediary metabolism and fatty acid utilization. For examples the fatty acid oxidation enzymes long-chain acylCoA dehydrogenase (LCAD) and medium-chain acylCoA dehydrogenase (MCAD) are deacetylated and therefore activated by SIRT3 [8, 33]. Another study [67] showed that SIRT3 deacetylates and increases the enzymatic activity of 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2). This mitochondrial liver enzyme catalyses the conversion of acetoacetyl-CoA and acetyl-CoA into 3-hydroxy-3-methylglutaryl-CoA. This is a step in ketone body synthesis, the pathway which is up-regulated during starvation.

To corroborate this interpretation, under dietary restricted conditions, many fatty acid oxidation enzymes show increased lysine residue deacetylation in control mice versus SIRT3 knockout, and SIRT3 null mice have increased accumulation of acylcarnitines, a finding consistent with reduced fat oxidation [30, 49]. On the other hand, although there is this pervasive observation that SIRT3 is down-regulated in high fat feeding [41], the rate of mitochondrial protein acetylation and fat oxidation are invariably enhanced in this dietary milieu [3, 49]. These apparently controversial remarks suggest that any altered metabolic state, such as nutrient lack or excess, lead to mitochondrial protein acetylation, considering that hyperacetylation of mitochondrial protein is also detected with the ethanol supplementation in diet [57].

Up to now, although thousands of mitochondrial acetylation sites have been characterized, the role of acetylation on the majority of these proteins is still unknown. The first work that described the functional role for acetylation on a mitochondrial protein was by Schwer et al. [65]. They reported that mitochondrial acetyl-CoA synthetase 2 (AceCS2) was reversibly acetylated at Lys-642 in the active site of the enzyme. SIRT3 deacetylates Lys-642 of AceCS2 both in vitro and in vivo activating its acetyl-CoA synthetase activity. Since then, some findings have been reached. Still and collaborators applied multiplexed quantitative mass spectrometry for detecting alterations in the mouse liver mitochondrial acetylproteome after acute and chronic alterations in diet [72]. Their findings suggested that SIRT3 usually tends to enhance the activity of its target enzymes, especially in the refed state and early during the onset of obesity, showing an adjustable target preference under different nutritional states. For example, among the mitochondrial proteins with dynamic acetylation sites, they demonstrated that acetylation of acetyl-CoA acetyltransferase 1 (Acat1) inhibits its activity by disrupting CoA binding. Another important mitochondrial substrate of SIRT3 is superoxide dismutase 2 (SOD2) which, once deacetylated, has increased activity and as a result increases the detoxification of ROS [76, 77]. The ROS reduction is also fostered through the increase of the expression of SOD2 and of catalase via the activation of the transcription factor Foxo3a [59]. This is trigger by the interaction of SIRT3 with FoxO3a in mitochondria which support FoxO3a DNA-binding to the SOD2 promoter [37].

Table 4.1 Mitochondrial enzymes whose activity is regulated by SIRT3

Protein	Effect	References
Aldehyde dehydrogenase 2	Inhibition	[91]
Aconitase	Inhibition	[19]
F1F0-ATPase subunit	Activation	[7]
Malate DH	Activation	[32]
NADH dehydrogenase 1a subcomplex 9	Activation	[1]
Very long chain acyl-coa DH	Activation	[94]
ATP synthase	Activation	[81]
Succinate DH	Activation	[16, 20]
AceCS2 acetyl-CoA synthetase 2	Activation	[65, 29]
Complex I	Activation	[1]
Mitoribosome	Inhibition	[92]
Complex IIa	Activation	[16, 20]
Complex V ATP5E and ATP5O	Activation	[7, 89]
HMGCS2 (3-hydroxy-3-methylglutaryl-CoA synthase 2	Activation	[67]
Long-chain acyl-CoA dehydrogenase	Activation	[33, 8]
Pyruvate dehydrogenase	Activation	[39, 54]
Glutamate dehydrogenase	Activation	[48, 64]
Isocitrate dehydrogenase 2	Activation	[70]
Superoxide dismutase 2	Activation	[13, 58, 76]
MPTP cyclophilin D	Inhibition	[27]
8-oxoguanine-DNA glycosylase	Activation	[14]

Table 4.1 lists mitochondrial enzymes whose activity has been found to be regulated by SIRT3.

4.4 Mitochondrial Protein Acetylation in Human Diseases

Beyond the identification of lysine-acetylated proteins, many studies are moving towards the characterization of acetylated patterns in differ-

ent diseases. Modifications in lysine acetylation status can directly alter mitochondrial function and, therefore, be linked to human diseases. In this section, we will focus on the description of lysine acetylation in metabolic diseases, cancer, myocardial injury and neurodegenerative diseases.

4.4.1 Metabolic Diseases

The two major features of type 2 diabetes are insulin resistance and the impaired mitochondrial function in muscle. Altered mitochondrial lipid oxidation and glycolytic capacity have also been detected in subjects with type 1 diabetes obesity [38]. Jing and collaborators have demonstrated that the expression of SIRT3 is modified both in type 1 and type 2 diabetes models. This change regulates mitochondrial metabolism and production of ROS, which finally alters insulin signaling. This proves the importance of the balance between acetylation and deacetylation levels in mitochondria but also their probable relation with insulin resistance and metabolic disorders. Moreover, this could suggest that factors increasing SIRT3 activity might potentially reverse some of the adverse effects of type 2 diabetes [38].

An adverse effect of both type 1 and 2 diabetes, is cardiomyopathy. Diabetic cardiomyopathy is characterized by decreased glucose oxidation, mitochondrial bioenergetics, and cardiac function and increased fatty acid oxidation, lipid storage, and myocardial fibrosis [10, 82]. The quantitative analysis, by proteomics approach, of mitochondrial lysine acetylation revealed that the extent of this PTM is higher in diabetes compared with the control [82]. Deficiencies of SIRT3 and compromised acetylation balance have been found to be related to the development of the diseases of the metabolic syndrome. The metabolic syndrome is defined by metabolic abnormalities, including obesity, insulin resistance, hyperlipidemia, hyperglycemia, and hypertension [34, 60]. Sedentary lifestyles and high-fat diets are implicated in the increase of metabolic syndrome. It has been discovered that SIRT3 deficiency and the associated hyperacetylation of mitochondrial proteins cause mitochondrial dysfunction that leads to metabolic syndrome [34].

Therefore, specific deacetylation/acetylation regulation of mitochondrial proteins could be used as a therapeutic tool in diabetes and insulin resistance, as well as other mitochondrial diseases.

4.4.2 Cancer

Cancer is one of the leading causes of mortality, characterized by cellular metabolic alteration (e.g. the Warburg effect) and cells with excessive proliferation, resistant to apoptosis. The origin of these cellular behaviors has always been the target of extensive studies. PTMs are well known mechanisms involved in tumorigenesis, by which signaling and pathways are fine regulated. In particular, SIRT3 exhibits a tumor suppressor role. This protective role against cancer is related to the depletion of ROS. Indeed, SIRT3 reduces ROS levels by activating, via deacetylation, the mitochondrial SOD 2 [58, 76, 90], isocitrate dehydrogenase 2 [70], and FoxO3a [73]. To corroborate this role, a study from Haigis et al., with mice lacking SIRT3, provided a model connecting aberrant ROS, the Warburg effect, and carcinogenesis [28]. Murine without Sirt3 had abnormal levels of elevated ROS which lead to genomic instability and causes carcinogenesis in various cell types [21, 28, 90]. Programmed cell death or apoptosis is another well-known hallmark of cancer cells, and SIRT3 modulates pro-apoptotic but also antiapoptotic members of the Bcl-2 family. It is worth to mention that some studies have reached different conclusion, foreseeing a role of SIRT3 in promoting tumorigenesis. In esophageal cancer, the high expression of SIRT3 is associated with a poor outcome [97]. In addition, elevated levels of SIRT3 expression are related with poor prognosis in patients with grade 3 breast cancer [78]. However, it must be considered that different works can obtained diverse results since SIRT3 has a role in numerous cellular conditions on many substrates.

4.4.3 Myocardial Injury

Cardiac hypertrophy is a usual myocytes response to different pathologic and physiologic stimuli [73]. After birth, mammalian cardiomyocytes lose their proliferation ability; hence the only way for them to deal with an increased workload on the heart is to undergo hypertrophy [31]. However, prolonged hypertrophy and a continuous growth signal lead to malfunction and sudden cell death. By this way, the workload of the remaining cells increases which further cause heart failure and sudden death due to arrhythmias [22]. The molecular mechanism of myocyte death during heart failure is not yet fully understood [40, 74]. During stress, SIRT3 is highly expressed, probably because the modification in cellular NAD/NADH ratio may contribute to its increase. Therefore, increased NAD content elevates the deacetylase activity of SIRT3, contributing to protect cells against stress-mediated cell death. It has been demonstrated that SIRT3 is capable of blocking the cardiac hypertrophic response by reducing cellular ROS levels. This effect is mediated by the activation of Foxo-dependent antioxidants, catalase and manganese SOD, but also by suppressing ROS-mediated Ras activation and the downstream MAPK/ERK and PI3K/Akt signaling pathways [73]. Moreover, Ku70 has been identified as a new target of SIRT3. Deacetylation of Ku70 by SIRT3 promotes Ku70/Bax interaction, and this makes cells resistant to Bax-mediated cell damage [73].

Mitochondria are also believed to be the key organelle for cardioprotection against ischemia/reperfusion injury. Some works have demonstrated that caloric restriction (CR) restores mitochondrial dysfunction and attenuates oxidative damage in mitochondria [52, 69, 80]. A comprehensive proteomics analysis was undertaken to clarify the effect of aging and CR on mitochondrial proteome; and the findings showed that CR has a minor effect on age-related changes in proteins [12]. Subsequently, the DIGE system was used to identify changes in the expression levels and acetylated state of mitochondrial proteins [68]. Authors identified many deacetylated proteins, among them they found NDUFS1 and Rieske subunit of cytochrome bc1 complex, which belong to complexes I and III, respectively. These proteins were deacetylated in the CR heart, reflecting the decrease in mitochondrial ROS production after ischemia/ reperfusion in the CR heart. Hence, they proposed that the beneficial effect of CR on mitochondrial function is mediated by deacetylating specific mitochondrial proteins [68].

4.4.4 Neurodegenerative Diseases

Neurons consume about 20% of the body's energy, therefore they require the delicate maintenance of mitochondrial function [24]. Considering the critical role of SIRT3 as a regulator of mitochondrial protein function, it is not surprising that this enzyme is most probably involved in neurodegenerative diseases.

Alzheimer's Disease (AD) and Parkinson's disease (PD) are the most common neurodegenerative pathology in adult population.

AD is characterised by dementia, starting initially with the loss of short term memory and damaged cognitive abilities. The aggregation of abnormally folded amyloid β (Aβ) in amyloid plaques and tau proteins (hyper-phosphorylated tau; p-tau) in neuronal tangles, are directly related to neurodegenerative processes in patients' brains. Deficiency in mitochondrial metabolism, in particular, defects in Complex I and Complex IV activity, have been found in the early stage of AD [36]. Mitochondrial damage may derive from the accumulation of amyloid β in these organelles, which could lead to apoptotic cell death [11]. Another hypothesis is that the lack of SIRT3 can play a key role in AD. Expression pattern and mRNA of SIRT3 are altered in the cortex of double transgenic APP/PS1 mice [93]. Physical training of these animals decreases DNA damages and ROS production while improved mitochondrial metabolism increasing the activity of Complex I, Complex IV and ATPase. All of these effects may resulted from the exercise-induced expression of SIRT3 which implies the activation, via deacetylation, of SOD2 [9, 63]. Actually, SIRT3 expression has been found increased in

both human and mouse AD pathology. Since ROS marker analysis revealed the prevalence of oxidative stress in AD, SIRT3 level may increase in response to increased ROS synthesis [46].

PD is a gradual neurodegenerative disease whose symptoms aggravate with time. These comprise bradykinesia, resting tremor, postural instability. Whereas the mechanisms underlying the clinical and pathological features of PD remain to be defined, the characteristic sign is the aggregation of α-synuclein, a protein with dopaminergic neurotoxicity. It has been demonstrated that SIRT3 null mice do not have motor and non-motor defects respect to wild-type controls. Moreover, SIRT3 deficiency intensified the degeneration of nigrostriatal dopaminergic neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mice [47]. This effect is probably due to the role of SIRT3 in preserving free radical scavenging capacity in mitochondria. In line with these observations, Zhang et al. demonstrated that Sirt3 protects against DAergic neuronal damage. They used immunoprecipitation and LC-MS/MS to identify the interacting proteins of SIRT3 [95]. With this study, they found that SIRT3 reduction leads to increased mitochondrial protein acetylation and dysregulation of two critical acetylation substrates: SOD2 and ATP synthase β, which are implicated in the regulation of ROS elimination and ATP production [95]. Hence, SIRT3 prevents ROS accumulation and ATP depletion, contributing in reducing DAergic neuronal death upon MPTP treatment.

Amyotrophic lateral sclerosis (ALS) is a rare, lethal, progressive neurodegenerative disease which perturbs corticospinal tract and conducts to the death of motor neuron in spinal cord, cortex, and brainstem. The presence of SIRT3 together with peroxisome proliferator-activated receptor-c coactivator-1a (PGC-1a) defends from neuronal cell death and mitochondrial fragmentation [5, 43]. SIRT3 promoter region has an estrogen-related receptor (ERR)-binding element (ERRE) [43]. PGC-1a recruits ERRa to the ERRE when there is an increase of oxidative stress. The overexpression of SIRT3 lowers ROS levels by deacetylating SOD2.

Thus, considering that SIRT3 is deeply involved in many aspects of mitochondrial metabolism, it can be expected its role in neurodegenerative disorders. However, further studies are desirable in order to clarify the putative role of SIRT3 as a mediator of neuronal damage.

4.5 Concluding Remarks

In the last years protein lysine acetylation has emerged as an important PTM in mitochondria. The improvements in the sensitivity, mass accuracy and mass resolution of mass analyzer instruments have helped in the characterization of different lysine acetylation sites. Despite this progress, the subsequent step is to differentiate the specific changes with a significant biological relevance from adventitious modifications. The improvement of affinity-based quantitative acetyl-proteomics together with optimized alternative specific anti-acetyl lysine antibodies, in a robust workflow, can help to differentiate specific changes in the acetylation status. Indeed, the balance between acetylation and deacetylation may be critical in metabolic pathways and additional studies are needed to unveil the fine molecular mechanisms that regulate this balance in physiological and pathological conditions.

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