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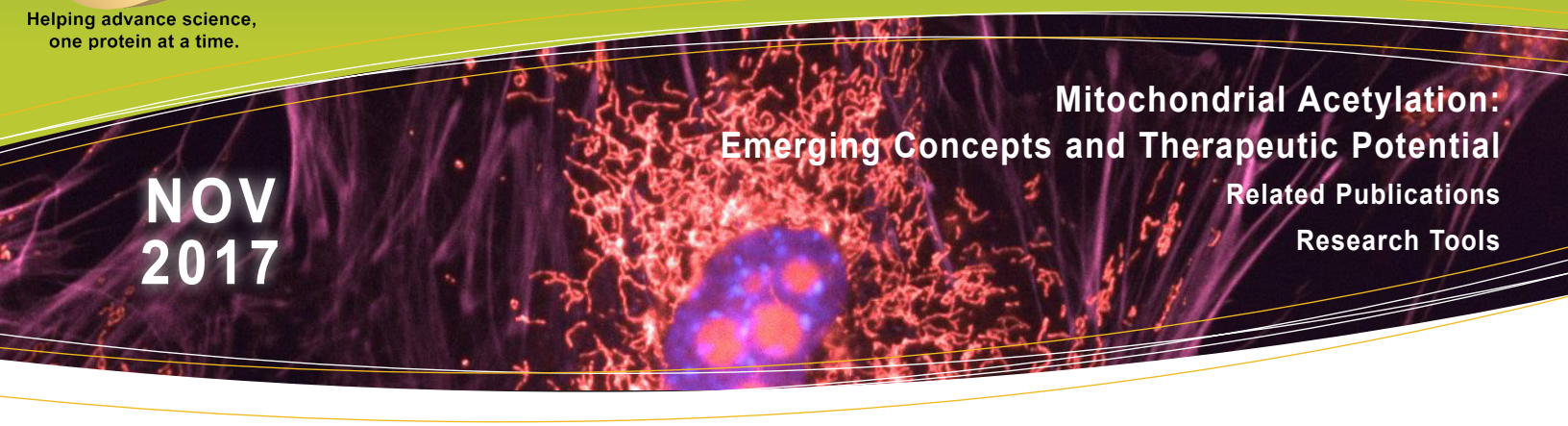

Mitochondrial Acetylation: Emerging Concepts and Therapeutic Potential

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Mitochondrial Acetylation: Emerging Concepts and Therapeutic Potential

General Overview of Lysine Acetylation in Health and Disease

Acetylation of the epsilon amino group of lysine residues (N^{ε}-acetylation) is an ancient, highly conserved post-translational modification (PTM)[1,2] that links acetyl coenzyme A (acetyl-CoA) metabolism and cellular signaling. This occurs largely through the opposing activities of lysine acetyl transferases (KATs) and lysine deacetylases (KDACs)[3]. In humans, there are 3 major KAT families (GCN5, CBP/p300, and MYST) that all use acetyl-CoA as an essential cofactor to donate an acetyl group to target lysine residues. There are two KDAC families, the zinc-dependent histone deacetylases (HDAC1-11) and the NAD+-dependent sirtuins (SIRT1-7)[1-3].

It is well documented that acetylation of nuclear histones plays a major role in regulating chromatin compaction and transcriptional activity wherein acetylation favors a more open, transcriptionally active chromatin[1]. Recent proteomic studies have identified over 4,500 non-histone proteins as targets of acetylation, thereby establishing lysine acetylation as a major global PTM. This PTM is present in many, if not all, cellular compartments, including the nucleus, cytoplasm, cell membrane, and mitochondria[4]. Functionally, reversible lysine acetylation has been shown to regulate enzyme activity[4-6], protein-protein interactions[7], and protein localization and stability[8,9]. In addition, it plays critical regulatory roles in many cellular processes, including gene expression, cellular metabolism, apoptosis, cytoskeleton regulation, and membrane trafficking[10].

In 2006, Vorinostat became the first FDA approved drug targeting histone epigenetics through HDAC inhibition for the treatment of cutaneous T-cell lymphomas (CTLC)[11]. There are now four FDA approved HDAC inhibitors for the treatment of CTLC, peripheral T-cell lymphoma, and multiple myeloma[12], which clearly demonstrates the potential of targeting lysine acetylation for therapeutic intervention. Clinical success, coupled to the vast expansion in identification of non-histone acetyl lysine targeted proteins, has enhanced interest in exploring the regulatory functions and therapeutic potential of this PTM beyond epigenetics. The remainder of this Newsletter focuses on lysine acetylation in the mitochondria and its possible value as a therapeutic target.

Mitochondrial Lysine Acetylation

Lysine acetylation is abundant in mitochondria, with an estimated 700 proteins (63%) undergoing this modification, three fold higher than phospho-modifications in this organelle[13]. Elucidating the mechanisms that regulate mitochondrial acetylation and identifying potential therapeutic targets is a new and rapidly growing area of investigation[10]. In recent years, some general concepts have begun to emerge which are briefly outlined below:

1) Lysine acetylation is highly prevalent in enzymes of the major mitochondrial metabolic pathways and many of these proteins are hyper-acetylated[4].

2) There is some sequence selectivity for mitochondrial protein acetylation as shown in a preference for negatively charged residues in the immediate vicinity of the acetylation site and a strong preference for hydrophobic residues at position +2[4].

3) Mitochondrial protein acetylation changes rapidly in response to cellular nutrient availability or energy status and is likely a major mechanism regulating energy homeostasis[14]. It also appears that the levels of mitochondrial KATs and KDACs are tightly controlled by nutrient availability[15]. The link between pathological disruption of energy homeostasis and mitochondrial acetylation holds great therapeutic potential[14].

4) Lysine acetylation has broadly been correlated with inhibition of mitochondrial enzyme activity, particularly in oxidative metabolism[13]. It is, however, noteworthy that of the 700 or so acetylated mitochondrial proteins, less than 40 have been characterized at the mechanistic level[13], and there are cases where acetylation may either activate or inhibit the same protein depending on the tissue type[6,15].

5) Several groups have proposed a non-enzymatic mechanism for mitochondrial lysine acetylation, and the relative contribution of enzymatic vs non-enzymatic regulation remains to be determined[16-18].

6) Low stoichiometry (< 1-5%) that translates to critical and regulated biological responses is a norm for PTMs and is one of the reasons that it is technically challenging to study these

modifications[19,20]. Low stoichiometry often reflects the highly dynamic nature and subcellular localization of the modified proteins and their role in cell signaling[19,20]. Stoichiometry of acetylation in mitochondrial proteins appears to follow this trend[23,24]. Understanding how sub-stoichiometric levels of PTMs can have significant effects on mitochondrial function remains an open question[13,23,24].

7) PTM crosstalk is recognized as a major cell regulatory mechanism[25]. Mitochondrial actetylation has been shown to participate in PTM crosstalk as exemplified by the regulation of the pyruvate dehydrogenase complex via the hierarchical coordination of kinase, phosphatase, acetyltransferase, and deacetylase activities[21].

8) Heart disease and heart failure are strongly correlated with increased acetylation of mitochondrial proteins in this organ[22], and multiple reports have shown that the major mitochondrial deacetylase, Sirtuin 3 (Table 1), exerts a protective function against heart disease[26,27]. Currently, there is intense interest in targeting SIRT3 and the mitochondrial acetylome for therapeutic intervention[28,29].

9) As shown in Table 1, the presence of mitochondrial KATs has only recently been described; however, they are considered to have strong therapeutic potential as targets for a variety of human diseases including cancer, heart disease, diabetes, and obesity (see references in Table 1).

Conclusion

Therapeutic exploitation of the mammalian mitochondrial acetylome offers great potential for the treatment of human disease; however, it is clear that much is still to be learned about the basic biology of this PTM beyond epigenetics. The role of lysine acetylation in the cytoplasm, plasma membrane, and other cellular compartments is also an area of high interest. In this regard, the development of an expanded toolset to aid in this research is urgently needed.

Table 1: Mammalian Mitochondrial Acetyltransferases and Deacetylases

Protein	Gene Name	Activity	Disease Linkage	Year Activity Described	Refs.
Acetyl-CoA acetyl-transferase 1	ACAT1	acetyl transferase	C	2014	21
Males absent on the first	MOF	acetyl transferase	H	2016	30
General control of amino acid synthesis 5-like 1	GCN5L1	Lacks catalytic domain, essential component of acetyl transferase complex	H, O, D	2012	31,32
Sirtuin 3	SIRT3	deacetylase	H, O, D	2002	33
Sirtuin 4	SIRT4	Deacylase and very low deacetylase	D	2014	34
Sirtuin 5	SIRT5	deacylase	na	na	35

C-cancer, H-heart disease, O-obesity, D-diabetes

Signal-Seeker™ PTM Kits, Antibodies, Beads, Etc

Kit or Affinity Beads	Type	Size	Cat. #
Acetyl-Lysine Antibody Mouse Monoclonal (7B5A1)	MAB	2 x 100 ul 1 x 25 ul	AAC02 AAC02-S
Acetyl-Lysine Antibody Mouse Monoclonal (19C4B2.1)	MAB	2 x 100 ul 1 x 25ul	AAC03 AAC03-S
Acetyl-Lysine-HRP Antibody Mouse Monoclonal (19C4B2.1)	MAB	1 x 100 ul 1 x 25 ul	AAC03-HRP AAC03-HRP-S
Acetyl-Lysine Affinity Beads	Beads	4 x 500 ul	AAC04-beads
Signal-Seeker™ Acetyl-Lysine Enrichment Kit	Kit	30 assays 10 assays	BK163 BK163-S

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