

**Review Article**

# Protein Acetylation as an Integral Part of Metabolism in Cancer Development and Progression

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Acetylation of lysine is one of the major post-translational modifications of histone and non-histone proteins of eukaryotic cells. Acetylation has been indicated as an avenue for cellular response to environmental, nutritional and behavioral factors. At the same time, aberrant protein acetylation has been related to cancer as well as many other diseases. Abnormal expression of some classes of histone deacetylases and histone acetyl transferases has been documented for the majority of cancers. These observations have led to extensive efforts in the development of inhibitors for these enzymes for the treatment of cancer as well as other diseases as well as pathogen control.

Regulation of protein activities and gene expression by acetylation influences many processes relevant for cancer development, including metabolism. At the same time acetylation depends on a number of metabolic co-factors and a variety of metabolites act as inhibitors of acetylation proteins making acetylation enzymes an integral part of metabolism. Cancer metabolic phenotype is generally understood as one of the major hallmarks of cancer and thus the interplay between acetylation, anabolism and catabolism provides a very interesting forum for exploration of cancer development and for novel treatments. An ever increasing pool of publications shows relationships between the acetylation process and related enzymes with metabolites in cancerous and non-cancerous systems. In this review we are presenting previously established relationships between acetylation/deacetylation, metabolites and enzyme regulation particularly in relation to cancer development, progression and treatment.

**Keywords:** Epigenetics; metabolism; cancer; acetylation; deacetylation; metaboloepigenetics; Warburg effect**Peer Reviewers:** Kota V. Ramana, PhD, Dept. of Biochemistry & Molecular Biology, University of Texas Medical Branch, United States; Nallasivam Palanisamy, PhD, Department of Pathology, University of Michigan, United States**Received:** October 31, 2013; **Accepted:** January 27, 2014; **Published:** February 11, 2014**Competing Interests:** The authors have declared that no competing interests exist.**Copyright:** 2013 Cuperlovic-Culf M *et al.* This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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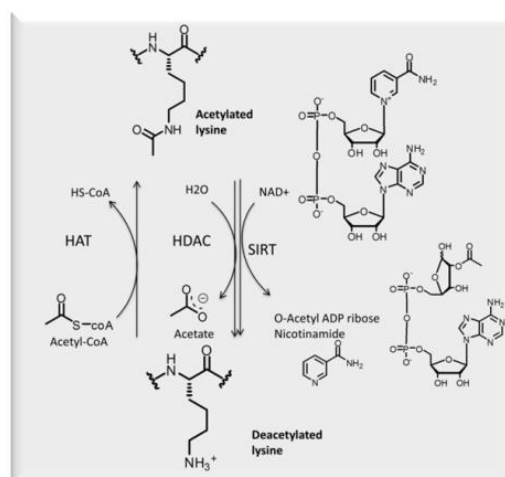
## Introduction

Metabolism comprises chemical processes that occur within living organisms and includes anabolism - construction of molecules from smaller units - and catabolism - breaking down of large molecules into smaller units. In healthy and pathological cells nutrient utilization, anabolism, energy metabolism, gene expression and protein regulation are associated through epigenetic modifications [1]. In fact, epigenetic modifications viewed as enzymatically driven chemical processes that construct or break down larger molecules fit directly within the definition of metabolism. A tight link between anabolism and catabolism of metabolites and epigenetic alterations and control including chromatin remodelling, histone modifications, DNA methylation and microRNAs is becoming increasingly apparent [1,2,3,4]. Protein posttranslational modifications including acetylation, which is the focus of this review, require metabolites as cofactors and substrates of enzyme function [1,2]. Metabolites can also act as inhibitors of epigenetic enzymes or epigenetic reactions [3,4]. At the same time, epigenetic changes regulate gene expression and protein activity thereby directly or indirectly controlling metabolism [1,2]. Outstanding question is how changes in the levels of cellular metabolites influence epigenetic modifications in general and acetylation in particular and also how changes in acetylation influence concentrations of metabolites and overall metabolism.

Acetylation has been known as a factor in transcription and protein activity regulation for almost 50 years [5]. Cancers' unique metabolism had been observed over 90 years ago [6]. Interestingly, both of these important avenues for cancer analysis and treatment have been largely overlooked for many years. Over the last decade, however, both acetylation and metabolism control are leading to major discoveries that are presenting new treatment avenues. At the same time, an increasing number of publications have been showing a close link between metabolism and acetylation of proteins leading to a

better understanding of these processes, their regulation and interplay, thereby opening a wide range of new possibilities in combined therapies, theragnostics or treatment follow-up.

Acetylation of lysine residues of proteins (Figure 1) is a reversible modification regulated by the antagonistic activity of two groups of enzymes – histone acetyl transferases (HAT) and histone deacetylases (HDACs and SIRT). HAT enzymes catalyze transfer of acetyl groups from acetyl-CoA to the  $\epsilon$ -amino group of the lysine residue. HDACs remove the acetyl group from acetylated lysine residues releasing an acetate molecule in the presence of water [7,8]. SIRT deacetylate lysine residues using nicotinamide adenosine dinucleotide (NAD<sup>+</sup>) as a cofactor, producing the deacetylated substrate, O-acetyl-ADP-ribose and nicotinamide [9,10].



**Figure 1** Acetylation and deacetylation process with substrates and by-products for HATs, HDACs and SIRTs.

Reversible acetylation is involved in regulation of a highly diverse proteins including histones as well as a number of nuclear and cytoplasmic proteins [5,8,11,12]. Chromatin modifications through acetylation and deacetylation of histone tails are directly involved in gene expression regulation. Positive charges on histone lysine residues are neutralized by acetylation leading to a relaxed conformation of chromatin. Relaxation of chromatin prevents generation of higher-order chromatin structures. These, more open chromatin chains, provide access for the transcription complex leading to gene expression [11]. Specifically, acetylated

histones have been shown as binding sites for bromodomain proteins which act as transcriptional activators [11]. Bromodomain specifically binds to acetylated lysine residues and is generally found in proteins that regulate chromatic structure and gene expression, *e.g.* HATs and ATPase. Bromodomain proteins also bind to acetylated non-histone protein that can acts as transcriptional activators (*e.g.* p53). Acetylation of histones has been indicated as a regulator of many cellular processes including for example the inflammatory response and metabolism [13].

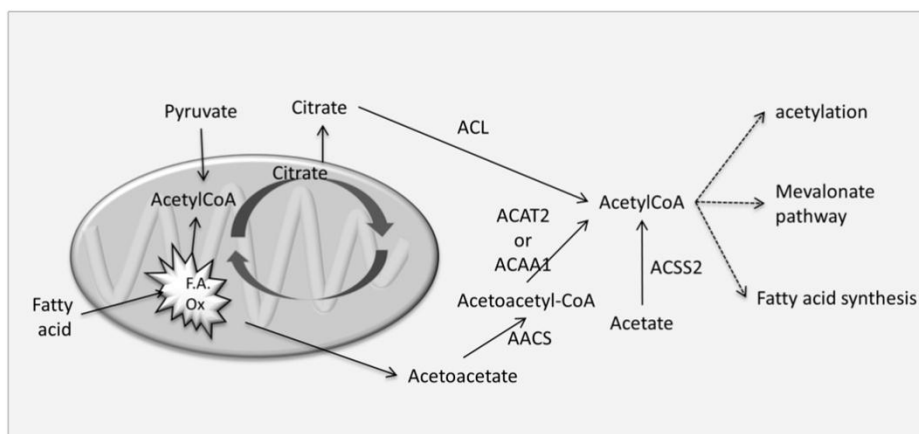
Acetylation is a significant factor in the regulation of function of non-histone proteins possibly rivalling phosphorylation in its importance in control of protein activity [15]. Recent proteomic studies have determined a large number of proteins that were acetylated in mammalian cells mostly in the cytoplasm but also in the mitochondria and the nucleus. These acetylated proteins ranged in their functions and include transcription factors, kinases, ubiquitin ligases, ribosomal and heat shock proteins, structural proteins and enzymes in the cytoplasm and organelles [16, 115]. Regulation of the activity of transcription factors through acetylation further controls gene expression making acetylation and therefore HDACs, SIRT6 and HATs both direct and indirect regulators of gene expression.

The majority of enzymes of intermediary metabolism are also regulated by acetylation [17], thus making reversible lysine acetylation a route for direct metabolism control through function of epigenetic factors. Additionally, virtually all enzymes of central metabolism appear to be acetylated. Acetylation and deacetylation processes are directly controlled by metabolism changes through substrates and co-factors for acetylation process, *e.g.* acetyl CoA and NAD<sup>+</sup>. All HATs require acetyl-CoA as a donor of acetyl group and are thus regulated by its concentration at the site of acetylation. SIRT6 require NAD<sup>+</sup> as a co-factor. Global reduction of nuclear acetyl-CoA levels decreases histone acetylation while reduction of NAD<sup>+</sup> levels inhibits histone deacetylation by SIRT [2]. The heterogeneous distribution of these and other metabolites as well as

competition between different enzymes utilizing the same cofactors and metabolites can lead to further changes in acetylation and deacetylation processes. However, there is still no data showing these effects. Pyruvate, a product of glycolysis and precursor for Krebs cycle as well as a number of biosynthesis processes is an ubiquitous HDAC inhibitor [18]. Similarly, L-carnitine which is involved in the transport of long-chain acyl groups from fatty acids into mitochondria appears to work as an endogenous HDAC inhibitor [19]. Nicotinamide inhibits the function of SIRT6 invoking SIRT6 regulation by the NAD<sup>+</sup>/nicotinamide balance in the cell [21]. In this review we will outline known relationships between acetylation and metabolism and explore the significance of these interactions in relation to the known changes in these processes in cancers.

## Metabolism and global acetylation

Enzymes involved in post-translational protein modification, including chromatin modifying enzymes, have been suggested as metabolic sensors regulating gene expression as a response to metabolism. Several metabolites are involved in this sensory system with acetyl-CoA and NAD<sup>+</sup>, directly related to acetylation. Acetyl-CoA, an acetate-thioester is an activated form of acetate critical for production of cholesterol, lipids, amino acids and a number of other components for cell growth. Acetyl-CoA is produced in several different processes in mammalian cells, both in the cytoplasm and mitochondria (Figure 2). An increase of acetyl-CoA concentration stimulates genes that promote cell growth in yeast and these genes closely match those that are induced by c-Myc oncoprotein in mammalian cells [4]. Acetyl-CoA levels influence acetylation with indications that a large, up to 10-fold, variation in the acetyl-CoA level in cells observed through the cell cycle profoundly and quickly affects acetylation levels [22]. Histone acetylation appears to be an extremely dynamic process with a half-life of histone acetylation possibly as short as 3 minutes [4, 14].



**Figure 2** Mechanisms for acetyl-CoA production. Major methods for acetyl-CoA production in cells are shown and include production from pyruvate of  $\beta$ -oxidation in mitochondria and from citrate in cytoplasm. In addition to these major routes acetyl-CoA can also be produced catabolically from threonine (mitochondrial threonine dehydrogenase enzyme), anabolically from acetate, ATP and CoA (acetyl-CoA synthase enzymes) or via anaplerotic pathway through reductive carboxylation of  $\alpha$ -ketoglutarate. Mitochondrial acetyl-CoA cannot enter cytoplasm and therefore cannot contribute to the acetyl-CoA pool for histone or cytoplasm protein acetylation.

Histone acetylation in mammalian cells depends on the expression and function of ATP citrate lyase enzyme (ACL) [23]. Following growth factor stimulation and during differentiation ACL converts glucose-derived citrate, obtained through partial Krebs cycle, into acetyl-CoA resulting in increased histone acetylation and gene expression. Therefore, glucose availability affects histone acetylation in an ACL-dependent manner. Increased histone acetylation promoted by amplified intracellular glucose levels leads to the expression of insulin-responsive glucose transporter (GLUT4), hexokinase-2 (HK2), phosphofructokinase-1 (PFK-1) and lactate dehydrogenase A (LDH-A), all significant regulators of glycolysis, leading to further increased glucose consumption and glycolysis as well as acetyl-CoA production [23]. As well as a factor in acetylation, acetyl-CoA is a major building block for biosynthesis of fatty acids and cholesterol and is involved in isoprenoid based protein modifications. In these roles acetyl-CoA and ACL are a link between glucose and glutamine metabolism, fatty acid synthesis and mevalonate pathways as well as histone and protein acetylation regulation, all of major importance in cancer cell development and

progression. ACL is up-regulated and activated in several cancer types and its inhibition has been shown to have an anti-proliferating effect on cancer cells [24]. Although histone acetylation is directly dependent on ACL, the acetylation of non-histone, cytoplasmic proteins, such as tubulin and p53, is not dependent on ACL function. It appears that acetyl-CoA for non-histone protein acetylation can be obtained from a variety of pathways and sources rather than from just citrate in the ACL catalyzed pathway (Figure 2). In cancer cells, Warburg effects leads to enhanced reliance on glucose for acetyl-CoA production. Donohoe and co-workers [25] have presented an interesting example dealing with cancer in colonocytes. Normal colonocytes used butyrate as the primary energy source and as a source of acetyl-CoA for acetylation as well as energy production. In cancerous colonocytes glucose becomes a primary energy source resulting in accumulation of butyrate whose high concentration results in HDAC inhibition. Therefore, in colonocytes, the presence of butyrate leads to enhanced histone acetylation in both normal and cancerous cells. However, different routes to histone acetylation – through acetylation or reduced deacetylation - affects

distinct sets of genes that either affect the proliferation of cancerous colonocytes undergoing the Warburg effect or stimulate proliferation of normal colonocytes.

Global histone acetylation levels change across tissues, biological systems and also across cancer types and grades. In a variety of primary cancer tissues it has been shown that lower cellular levels of histone acetylation, suggesting reduced gene expression, can be associated with more aggressive cancers and poorer clinical outcomes [26,27,28] although biological reasons for this observation are still not clear. The global histone acetylation levels also change with metabolic or physiological alterations in the cells such as changes in pH levels as was presented by McBrien *et al.* [26]. A decrease in intracellular pH leads to global hypoacetylation of histones in an HDAC dependent manner [26]. As a result there is an increased export of acetate anions and excess protons out of the cell leading to increased intracellular pH as well as decreased extracellular pH. This transport is performed by proton H<sup>+</sup> coupled monocarboxylate transporter (MCT). An increase in intracellular pH leads to the opposite effect with increased flow of acetate and protons into the cell and increased histone acetylation. In this process glucose, glutamine or pyruvate are required as metabolic sources for production of acetyl-CoA. At the same time, extracellular pH of cancer cells has been shown to be acidic, possibly benefiting tumor invasiveness [29,30]. The relationship between intracellular and extracellular pH and acetylation levels for both histone and non-histone proteins deserves further attention.

## HDACs, HATs and SIRT6 and cancer metabolic phenotype

HDACs are ancient enzymes that date back to the prokaryotes with highly significant sequence preservation across species from plants, animals, fungi as well as archaeobacteria and eubacteria (even in the absence of histones) [31]. Eighteen HDACs have been identified to date in human cells and they

are grouped into 4 classes (Table 1) based on their homology with yeast proteins. Class I, II and IV are referred to as “classical” Zn<sup>2+</sup> dependent HDACs. Class III HDACs - sirtuins - require NAD<sup>+</sup> as a cofactor. Although both groups of enzymes perform deacetylation, there is no sequence similarity between sirtuins and zinc dependent deacetylases.

Studies of yeast HDAC knockout strains have shown a division of tasks amongst classes of HDACs. In yeast HDA1 is primarily regulating genes involved in carbon metabolism and Rpd3 primarily controls cell cycle genes and Sir2 regulates mostly amino-acid biosynthesis [32]. HDAC1 in *Drosophila* appears to be the major “housekeeping” enzyme regulating diverse sets of genes most significantly those involved in cell proliferation and mitochondrial energy metabolism [12]. From studies on model systems as well as human cells an understanding of the functions and characteristics of various classes of HDACs have emerged. Some HDACs have been shown as ubiquitous and essential (class I) while others have more tissue specific functions. HDACs can be localized to nucleus, mitochondria or cytoplasm or can transfer between different cell compartments. Through deacetylation HDACs regulate and influence many physiological processes including some that are aberrantly controlled in tumor development and progression [5,7,13,15]. Metabolism is clearly one of the major processes partially controlled by acetylation. Distinct classes as well as individual HDAC enzymes have specific roles in the direct and indirect regulation of metabolism in mammalian and other cells. *In vivo*, HDACs are usually part of multi-protein complexes. Function of the whole complex can be regulated through several mechanisms including direct regulation of HDAC activity, regulation of complex structure and functionality or regulation of complex availability. These HDAC-containing complexes exist in the nucleus, mitochondria and cytoplasm and can also shuttle between different cellular compartments [33,34]. HDACs can be themselves directly regulated by acetylation, leading to reduced activity, or sumoylation, and phosphorylation, stimulating activity.

**Table 1** HDACs of human cell

Class (family)	Human protein	Yeast homolog	Major Localization
Class I	HDAC1	RPD3	Nucleus
	HDAC2		Nucleus
	HDAC3		Nucleus/cytoplasm
	HDAC8		Nucleus/cytoplasm
Class IIa	HDAC4	HDA1	Nucleus/cytoplasm
	HDAC5		Nucleus/cytoplasm
	HDAC7		Nucleus/cytoplasm/Mitochondria
	HDAC9		Nucleus/cytoplasm
Class IIb	HDAC6		Nucleus/cytoplasm
	HDAC10		Nucleus/cytoplasm
Class III	SIRT1	Sir2	Nucleus/cytoplasm
	SIRT2		Cytoplasm
	SIRT3		Mitochondria
	SIRT4		Mitochondria
	SIRT5		Mitochondria
	SIRT6		Nucleus
	SIRT7		Nucleus
Class IV	HDAC11		Nucleus

**HATs** catalyze the opposite process to HDAC, *i.e.* acetylation of lysine residues of histones as well as other proteins. Similarly to HDACs, HATs are affecting and regulating DNA transcription, protein-protein interaction as well as protein activity. The prime targets of the HATs in chromatin are the N-terminal tails of the core histones H2A, H2B, H3 and H4. Acetylation of specific lysine residues on these histones results in gene transcription. Therefore, histone acetyltransferase activity is combined in several examples with transcriptional activation. HATs are generally not promoter specific; rather they are part of the transcriptional complex, which is promoter specific. In addition to histones, HATs have been shown to acetylate non-histone proteins such as p53, E1A viral oncoprotein as well as a range of non-nuclear proteins [35]. Equivalently to HDACs, HATs regulate activity of many enzymes involved in glycolysis, fatty acid and glycogen metabolism, Krebs' cycle and the urea cycle to name a few.

However, only a subset of HATs has been identified due to the relatively low sequence similarity between members of this group. In fact, only the acetyl-CoA binding site seems to show structural similarity between different HATs [36,37]. Currently HATs are grouped into different families based on some, limited sequence similarity within the HAT domain. Table 2 shows seven different families, however there are other classifications resulting in four classes: Gcn5/PCAF, MYST, p300/CBP and Rtt109. HAT activity can be regulated through interaction with regulatory protein subunits, through concentration effects of acetyl-CoA and possibly other metabolites as well as through auto- or self-acetylation. A recent, very interesting example of function and regulation of HAT activity deals with the function of monocytic leukemia zinc finger (MOZ). MOZ functions as a co-activator for acute myeloid leukemia 1 proteins (AML1) and Ets family transcription factor PU.1 dependent transcription. MOZ is also an

acetyltransferase of p53 and its interaction with p53 enhances p21 expression [38]. MOZ is involved in acetylation and activation of p53, however it is a different HAT factor that regulates acetylation of histone at the p21 promoter. P300/CBP-associated factor (PCAF) catalyzes stress-responsive histone

acetylation at the p21 promoter and thereby regulates p53-directed transcription of p21 and the resultant growth arrest [39]. The extended complex of acetyl transferases, transcription factors as well as kinase proteins enhances and regulates the function of all members in a highly efficient manner.

**Table 2** HATs of human cells [112]

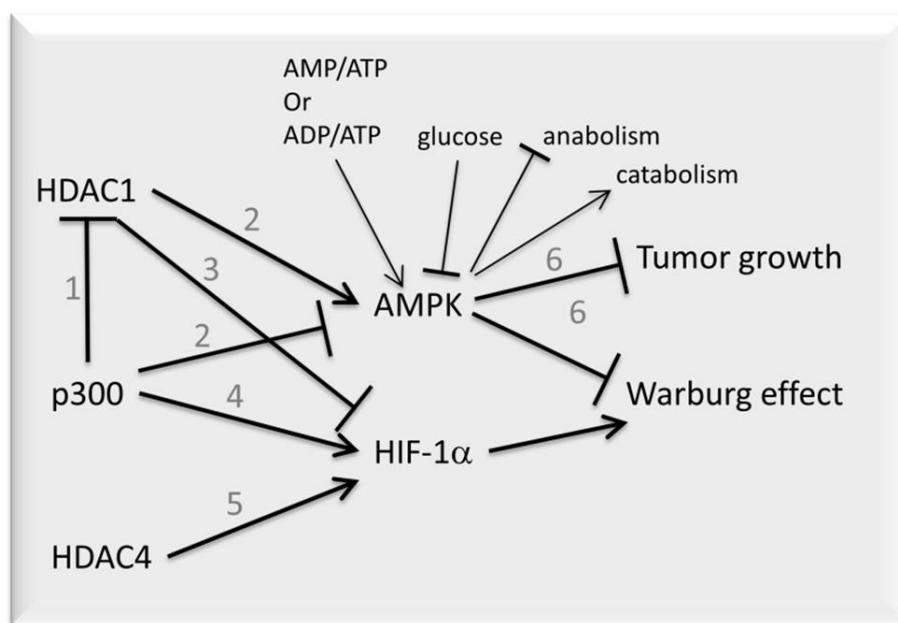
Class (family)	Members	Aliases	Major Localization
HAT	HAT1		Nucleus/cytoplasm
	HAT2		
GCN5	GCN5L1		Nucleus
	GCN5L2		
PCAF	PCAF		Nucleus
p300/CBP	P300		Nucleus
	CBP		
p160	NCOA1	SRC1	Nucleus
	NCO2	GRIP1	
		TIF2	
	NCOA3	AIB1	
		RAC3	
		ACTR	
		P/CIP	
		TRAM1	
MYST	MOZ		Nucleus
	MORF		
	HBO1		
	Tip60		
TF <sub>II</sub> D complex	TAF <sub>II</sub> 250	TAF2A	Nucleus
		CCG1	
		BA2R	
hTF <sub>III</sub> C90	hTF <sub>III</sub> C90		Nucleus

**Class I HDACs** are expressed in all tissues and include HDAC1, HDAC2, HDAC3 and HDAC8. They are primarily localized in the nucleus where they are part of multi-protein complexes together with transcription factors and co-repressors. Class I HDACs are important in survival and proliferation of normal cells. At the same time class I HDACs are up-regulated in a number of cancers [40]. Cyclin-dependent kinase inhibitor-1 (p21) is one of the most extensively studied targets of class I HDACs but many other target genes involved in cell growth,

apoptosis, tumorigenesis and angiogenesis have been shown [40]. A number of non-histone targets has also been determined for Class I HDACs and include number of transcription factors: p53, Stat1, Stat3 and Nf- $\kappa$ B to name just a few [40]. In addition, deacetylation enzymes of class I were recently shown as deacetylation catalysts of AMP-activated protein kinase (AMPK), which is a central energy sensor-regulating metabolism across all eukaryotes [41,42]. HDAC1 and HAT - p300 control deacetylation and acetylation of AMPK and its

activity [41]. Deacetylation of AMPK enhances its interaction with the upstream kinase that promotes AMPK phosphorylation and activation. A relation between AMPK and metabolism has been extensively studied although only recently has its role as a negative regulator of the Warburg effect and suppressor of tumor growth been shown *in vivo* [43]. Inactivation of AMPK promotes a metabolic shift to aerobic glycolysis and increased utilization of glucose for lipid biosynthesis and biomass accumulation. This transformation requires normoxic stabilization of HIF-1 $\alpha$  that is accomplished, in part through deacetylation catalyzed by HDAC4 (class IIa) [44]. At the same time more recent results also show that p300 acetyl transferase stabilizes HIF-1 $\alpha$  through acetylation of a different lysine residue [45]. From these results it appears that p300 is a crucial activator of the Warburg effect through its deactivation of AMPK and stabilization of HIF-1 $\alpha$ . It is therefore not surprising that p300 inhibitors such as curcumin have an anticancer effect [46]. Curcumin has also been indicated as a specific HDAC4 inhibitor leading to the same effect on HIF-1 $\alpha$  stabilization through inhibition of deacetylation as well [47]. HDAC1

appears to have the opposite role in this process. This is somewhat surprising as HDAC1 is known to be generally overexpressed in cancers. One possible explanation for this controversy is that p300 acetylates HDAC1 and thereby deactivates the protein [48]. Thus a high expression of HDAC1 in the presence of p300 might be irrelevant as the protein would not be active. At the same time clinical trials of specific HDAC1 inhibitors for treatment of solid tumors (where Warburg effect is a significant factor for tumor survival) were mostly disappointing [49] possibly, at least in part, due to the already deactivated HDAC1 through the action of p300 enzyme. Although the functions of AMPK is not a subject of this review, the reader should be aware that the role of AMPK in tumors is still not completely clear as there were reports showing that its activation and on the other side de-activation has an anticancer effect [41,43]. AMPK is activated also by an increase in ROS levels and this can be an additional factor in this analysis. The function and relevance of AMPK needs to be further explored prior to the development of anti- or pro-AMPK treatments for cancer (outlined schematically in Figure 4).



**Figure 4** Relationships between acetylation factors and AMPK and the development of Warburg effect. Reference correspondences on the figure are: 1-[47]; 2 – [41]; 3 – [117]; 4 – [45]; 5 – [44]; 6 – [43].



**Class-IIa HDACs**, which include HDAC4, HDAC5, HDAC7 and HDAC9, have low enzyme activity and have been suggested to act as controllers of low basal activities with acetyl-lysines processing restricted to sets of specific, albeit as of now unknown, natural substrates [40,50]. Unlike other HDACs, the class IIa group of enzymes can shuttle in and out of the nucleus depending on the phosphorylation status of only a few serine residues in the N-terminus regulated by various kinases [42]. Class IIa HDACs also interact with HDAC3 (from class I) leading to suggestions that class IIa enzymes in fact only bear minimal activity and that their major deacetylation activity comes from associated HDAC3 [51,52]. The function of HDAC4 in the Warburg effect has been discussed above.

**Class-IIb HDACs**, including HDAC6 and HDAC10, are primarily located outside of the nucleus and are mostly involved in regulating protein folding and turnover [40,53]. HDAC6 has been suggested as a homeostasis surveyor of the cell. Major substrates for HDAC6 are  $\alpha$ -tubulin and Hsp90. Hsp90 – heat shock protein 90 - is a chaperone protein involved in protein folding, stabilization and transport. Hsp90 targets a number of important proteins including many oncogenes. Specific inhibition of HDAC6 was shown to increase acetylation of heat-shock proteins such as Hsp90 and directly or indirectly leads to proteasomal degradation of a number of cancer related proteins including: HER2/neu, ERBB1, ERBB2, Akt, c-Raf, Bcr/Abl and FLT3 [reviewed in 40,113,114]. HDAC6 uniquely contains two deacetylase domains and three zinc ions in addition to an ubiquitin binding domain. HDAC6 has been determined as a cytoplasmic deacetylase and thus more directly involved in regulation of non-histone proteins [1, 54,55]. As a deacetylation enzyme for  $\alpha$ -tubulin, HDAC6 destabilizes dynamic microtubules and promotes cell motility [56,57,58]. It also regulates cortactin [59],  $\beta$ -catenin [60] peroxiredoxins I and II [61] and Ku70 [62], chaperones and IFN $\alpha$ R and has been implicated in regulating autophagy and hepatic metabolism [42,51,52,63]. All of these activities can be related to metabolism of cells as well as cancer development

and progression. In addition, it has also been indicated as a regulator of mitochondrial metabolism. Down-regulation of HDAC6 causes a reduction in the net activity of mitochondrial enzymes including respiratory complex II and citrate synthase [51]. HDAC6 knockdown cells have unchanged expression levels of mitochondrial enzymes as well as unchanged mitochondrial mass [51] suggesting that HDAC6 does not enter mitochondria. Instead, HDAC6 must regulate activity of mitochondrial enzyme from cytoplasm, possibly through its regulation of Hsp90. An abundant pool of Hsp90 localizes to mitochondria in various tumor cells and regulates mitochondrial integrity. Acetylated Hsp90 is inactivated leading to reduced mitochondrial activity. In this model HDAC6 controls mitochondrial activity through deacetylation and there-by activation of Hsp90. Ubiquitin binding activity of HDAC6 is also involved in the regulation of mitochondrial metabolism. HDAC6 is a known partner of p97/VCP protein, which is involved in mitochondrial protein quality control and degradation. It has been speculated that the loss of HDAC6 function can lead to stagnation of mitochondrial protein quality control [55]. With a critical role of HDAC6 in maintaining cellular homeostasis the reduction of mitochondrial metabolism may impose restrictions to energy consumption of cells and also possibly to biomolecule production routes. The observed reduction in cell motility due to HDAC6 inhibition in various cells [64, 65] could be a result of its control of mitochondrial function as well.

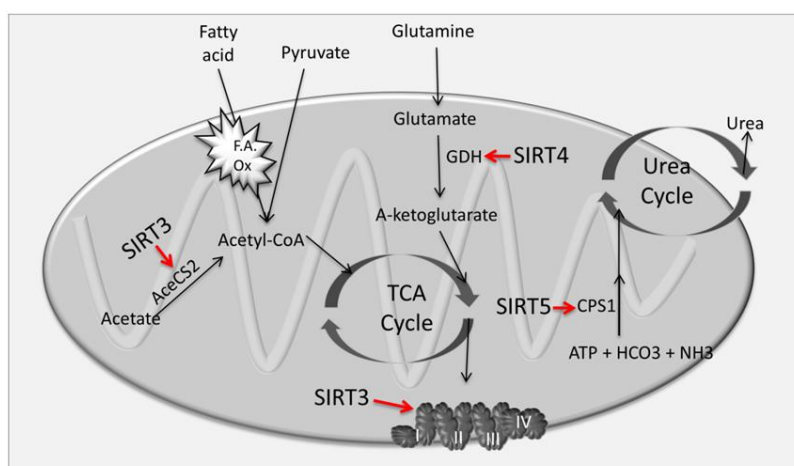
HDAC10 is one of the least studied HDACs [66, 67]. Similarly to HDAC6 it contains a unique second catalytic domain. It can be found in nucleus and cytoplasm but its targets both on histone and non-histone proteins are still unknown. Recent study has shown that inhibition of HDAC10 leads to release of cytochrome c and activation of apoptotic signal possibly through accumulation of reactive oxygen species. Although initial indications have been made that this process happens through HDAC10 regulation of thioredoxin [66, 116] further study is necessary.

**Class III HDACs called Sirtuins** (from the

founding member from budding yeast called silent mating type information regulation two protein) – SIRT (1 to 7) - show unique dependence for their catalytic activity on the presence of the oxidized form of nicotinamide adenosine dinucleotide ( $\text{NAD}^+$ ) [9,10]. Although SIRT and HDACs perform the same function, they are structurally unrelated. Furthermore, HDAC inhibitors do not affect SIRTs. Deacetylation reactions catalyzed by SIRT produces deacetylated substrates O-acetyl-ADP-ribose and nicotinamide. It is important to point out that SIRT activity does not lead to the production of acetate unlike the action of other classes of HDACs.  $\text{NAD}^+$  is a key electron carrier in the oxidation of hydrocarbons in cells and its concentration is directly related to cellular metabolic processes [68].  $\text{NAD}^+/\text{NADH}$  metabolism has been indicated as one

of the key determinants of cancer cell biology in part through its regulation of SIRT [69].

Seven thus-far identified sirtuins in human cells share a conserved catalytic core domain but through various amino and carboxyl terminal extensions have different subcellular localizations and somewhat different catalytic activities [70]. SIRT1 and 6 are located in the nucleus and function as histone deacetylases as well as the regulators of acetylation of transcription factors such as MyoD, FOXO, p53 and NF- $\kappa$ B. SIRT2 is in the cytoplasm and is known to associate with microtubules deacetylating  $\alpha$ -tubulin. SIRT7 is located in the nucleolus and acts as a positive regulator of RNA polymerase I transcription [70-76]. SIRT3, 4 and 5 are located in mitochondria and have a variety of roles described in detail in a recent review [70] and outlined in the Figure 5.



**Figure 5** Mitochondrial functions of SIRTs

Variations in the levels of  $\text{NAD}^+$  may affect distinct members of the SIRT family differently depending on the local concentration of  $\text{NAD}^+$  and also the relative activity of each SIRT protein. Therefore, kinetics of SIRT- $\text{NAD}^+$  interaction and function needs to be further determined for each member of this family. Apart from metabolic control,  $\text{NAD}^+$  levels are also regulated in a circadian manner presenting a direct link between cyclic rhythms and energy metabolism. Rhythmic changes in  $\text{NAD}^+$  levels can also provide clues into circadian fluctuation in HDAC activity even though expression levels of SIRTs are

noncyclical [77,78].

As is already stated, several SIRTs are localized in mitochondria (Table 1) and are directly involved in deacetylation and regulation of mitochondrial metabolism. With a dependence of SIRT function on  $\text{NAD}^+$  and, therefore  $\text{NAD}^+/\text{NADH}$  ratio it has been suggested that mitochondrial or cytoplasmic SIRTs might provide a link between extracellular nutrient levels and acetylation, *i.e.* function of various metabolic enzymes [17]. It has been reported that SIRT as well as  $\text{NAD}^+$  levels increase with caloric restriction although the effect of cell starvation on

NAD<sup>+</sup> levels is still not clearly proven [4]. During energy excess, NAD<sup>+</sup> is depleted due to its increased flux through the glycolysis pathway leading to production of NADH from NAD<sup>+</sup>, reducing SIRT1 activity [79, 80]. In several examples in animal models, fasting induced an increase in NAD<sup>+</sup> levels in the liver leading to elevated SIRT3 and SIRT5 activity [81, 82]. Enhanced activity of SIRT3 and SIRT5 increases cell survival [81, 82] and leads to increased activity of phosphate synthetase 1 (CPS1), an urea cycle enzyme which catalyzes the initial step of the urea cycle for ammonia detoxification. Analysis of various SIRT knock-out mouse models have shown that although mice with singular SIRT knock-outs are developmentally normal, deletions of either Sirt3, Sirt4 or Sirt5 leads to altered activity of several metabolic enzymes and processes including basal ATP levels, fatty-acid oxidation (Sirt3 null); abnormal increase of glutamate dehydrogenase (GDH) activity and insulin secretion (Sirt4 null) and decreased activity of CPS1 and reduced urea cycle function (Sirt5 null) [reviewed in 17].

SIRT1 deacetylates PGC1 $\alpha$  transcription co-activator. PGC1 $\alpha$  regulates expression of a number of genes, many of which are involved in the control of metabolism. The heavily acetylated form of PGC1 $\alpha$  is inactive. SIRT1 catalyzed deacetylation leads to activation of PGC1 $\alpha$  in the nucleus. A recent report shows an involvement of SIRT1 in the modulation of ubiquitin-like (SUMO) function [83]. SIRT1 regulated deacetylation serves as a dynamic switch for sumoylation and together, through a newly described SIRT1/Ubc9 regulatory pathway, acetylation and sumoylation regulate hypoxic response leading to the expression of genes such as VEGF and CITED2 that mediate survival of cancer cells.

Another key example of SIRT's regulation of metabolic processes is its regulation of acetyl-CoA synthase enzyme AceCS2. Under certain conditions AceCS2 is acetylated on a specific, known, lysine residue and this inhibits its ability to convert acetate into acetyl-CoA. SIRT3 mediates deacetylation of AceCS2 leading to its activation but without changing the concentration of acetate in the

mitochondria. Therefore, due to specific characteristics of SIRT's catalysis, deacetylation of AceCS2 is independent of acetate concentration in mitochondria. Recently, it has been shown that SIRT3 and SIRT6 act as tumor suppressors [84,85] and that SIRT6 targeted genes overlap significantly with genes codependent on the c-Myc oncoprotein and SAGA/GCN5 histone acetylase complex [4,85,86]. SIRT6 is a chromatin-bound factor which has previously been shown to control cellular senescence and telomere structure by deacetylating a particular lysine residue of histone H3 [87]. SIRT6 deficient mice also display an acute, severe and ultimately lethal metabolic abnormality accompanied with increased glucose uptake in a number of cell types [85]. SIRT6 regulates aerobic glycolysis in cancer cells. Results presented by Sebastian *et al.* [85] show that loss of SIRT6 function or expression leads to tumor formation even without activation of any known oncogenes. SIRT6 negative cells display increased glycolysis and tumor growth suggesting that SIRT6 plays roles in both establishing and in maintenance of cancer. SIRT6 also co-represses c-MYC activity and in this way functions as a regulator of ribosome metabolism. SIRT6 is also selectively down-regulated in several human cancers with low SIRT6 expression suggesting poor prognosis [85]. In fact, according to the analysis presented by Sebastian *et al.* [85] The SIRT6 gene region on the chromosome is deleted in a large percentage of cancers. Amongst these authors list 62% of pancreatic cancer cell lines with deleted SIRT6 and no pancreatic cell lines with an amplified SIRT6 gene. SIRT6, according to work of Sebastian *et al.* [85], acts as a tumor suppressor. In animal models the same authors have shown that tissue specific silencing of SIRT6 genes leads to the development of high-grade tumors that rely heavily on glycolysis and were manageable with dichloroacetate inhibition of PDK. However, another recent analysis of the function of SIRT6 has shown that the function of this gene in inflammatory response with a major role in promoting expression of inflammatory cytokines in pancreatic cancer cells, suggesting that in fact inhibition of SIRT6 might have

an anticancer effect in some subtypes, surprisingly again in pancreatic cancers [88]. This result has led to the development of novel SIRT6 deacetylation inhibitors [89]. It is clear however that prior to treating cancers with SIRT6 inhibitors it will be necessary to develop further understanding of its context-dependent role and also determine markers for cases that can benefit from SIRT6 inhibitor treatment as in some other cases SIRT6 inhibition can clearly lead to detrimental effects.

**Class IV HDAC – HDAC11** – has only recently been considered as possibly a very significant factor in cancer development and treatment. Initial experiments have shown that HDAC11 depletion is sufficient to cause cell death and to inhibit metabolic activity in several colon, prostate, breast and ovarian cancer cell lines [90]. Most significantly, HDAC11 inhibition in normal cells did not cause any change in metabolism or viability suggesting possible tumor selective treatment. These early results are very promising and should encourage further investigation of the relationship between HDAC11, metabolism and cancer.

## Regulation of HDACs, SIRTs and HATs by metabolites

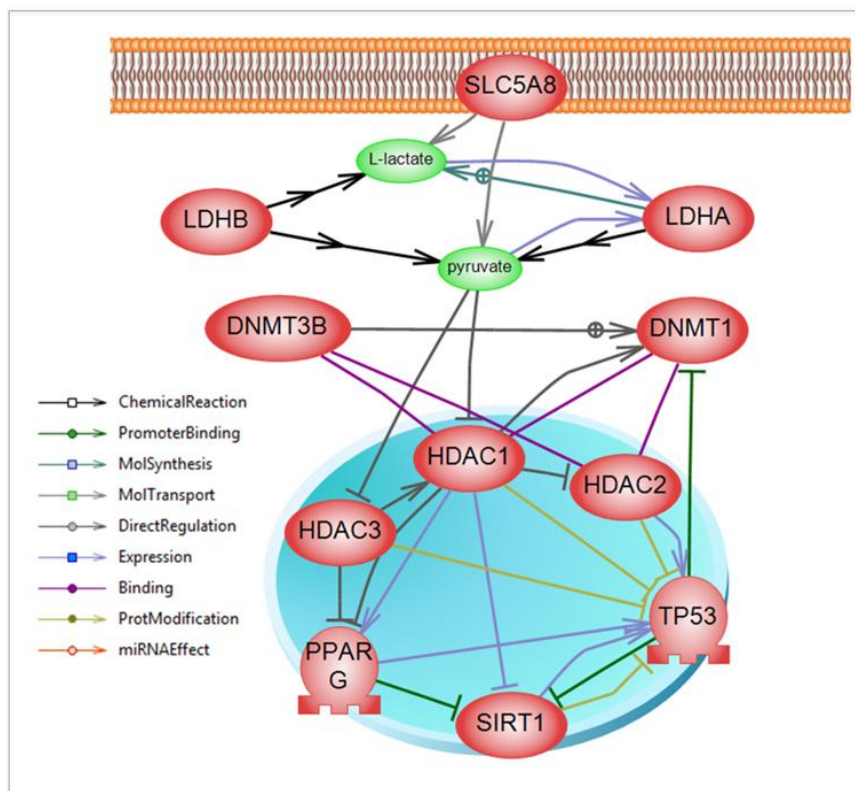
Reaction kinetics of acetylation and deacetylation should depend on the concentration of the acetyl group in the proximity of the enzyme (at least for non-SIRT enzymes). To this effect, an analysis of the influence of acetate supplement on brain cells has shown a significant increase of histone acetylation levels [91]. Acetate supplementation did not, however, have any effect on the function or kinetics of HAT catalyzed acetylation. Instead, acetate supplementation inhibited HDAC activity. The explanation provided by the authors of this work was that acetate supplementation leads to increase of acetyl-CoA levels, which reduces HDAC activity and expression leading to an amplified histone acetylation state. The authors also observed a 50% reduction of HDAC2 expression in brain tissue following acetate treatment. Both of these observed effects could

explain the observed increase in histone acetylation [91]. The work of Vogelaer *et al.* [92] however shows an opposite effect in class I HDACs, indicating that several coenzyme A (CoA) derivatives, such as acetyl-CoA, butyryl-CoA, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) and malonyl-CoA as well as NADPH act as allosteric activators of recombinant HDAC1 and HDAC2 *in vitro*, suggesting that the reduced HDAC2 expression, rather than kinetic effect of increased concentration of deacetylation product, was the major factor in the effect of acetate on brain histone acetylation.

Sodium butyrate, a short chain fatty acid formed by gut microflora, was the first natural product and metabolite indicated as an inhibitor of histone deacetylation [93]. With this HDAC inhibitory role sodium butyrate was suggested as a chemopreventive metabolite – a metabolite that can prevent the occurrence of colon cancer (outline of this effect and difference in the effect of butyrate on cancer and normal colon cells is provided earlier in the text). Similarly, a related molecule 3-hydroxybutyrate, which is one of the three ketone bodies produced in millimolar quantities as two enantiomers (D-3-hydroxybutyric acid and L-3-hydroxybutyric acid) after prolonged exercise or starvation inhibits the activity of class I HDACs. The results of Shimazu *et al.* [94] clearly show that this distinct metabolite is able to fuel metabolic adaptation to calorie reduction and to sustain a protective epigenetic state by inhibiting activities of HDACs [94]. Pyruvate, an ubiquitous metabolite, is also an HDAC inhibitor [95]. Pyruvate is a product of glycolysis that is in cancer cells largely converted into lactate (“Warburg effect”). Pyruvate, inhibits HDAC1 and HDAC3. HDAC1 and HDAC3, in turn, deactivate p53 [95]. Thus, by reduction of the concentration of pyruvate cancer cells reduce inhibition of HDAC1 and HDAC3, which deactivate oncosuppressor p53 and thereby reduces apoptotic signals in cells. Furthermore, tumor suppressor SLC5A8, a transporter of short-chain fatty acids (acetate, propionate and butyrate) as well as pyruvate, is down-regulated in cancer cells. Reduced input of pyruvate also promotes reduction of pyruvate levels, evading pyruvate HDAC inhibition and p53

induced lethality. Relationships between pyruvate and these and related proteins obtained from literature sources are shown in Figure 3. At the same time, lactate, appears to also be a weak inhibitor of HDAC

function [96]. In this case further analysis is needed to determine specific effects of different HDACs and their targets.



**Figure 3** Relationships between pyruvate and some major acetylation and metabolic enzymes obtained from literature using Pathway Studio 9.0. Type of relationship between proteins and metabolites (when known) represented.

HDAC inhibition in neuronal cells led to a decrease of total cholesterol content by up-regulating genes responsible for cholesterol catabolism (CYP46A1) and efflux (ABCA1) and down-regulating genes responsible for cholesterol synthesis (HMGCR, HMGCS and MVK) and uptake (LDLR) [97]. Although the specific route to cholesterol levels decrease through HDAC inhibition is not yet completely resolved, HDACs provide an interesting therapeutic avenue for targeting cholesterol accumulation in neurodegenerative conditions. Inhibitors used in the analysis of the relationship between HDACs and cholesterol in neuronal cells were non-specific and thus the role of individual HDACs is still unclear. At the same time it has been observed that in patients experiencing problems with cholesterol level in neuronal cells

there is an up-regulation of HDAC4, HDAC6 and HDAC11. HDAC3 has been suggested as a factor involved in down-regulation of cholesterol synthesis in HeLa cells through repression of lanosterol synthase expression, an enzyme of the cholesterol biosynthesis pathway [98]. The relation between HDAC, HATs and cholesterol synthesis through protein acetylation and activation as well as through the competition for acetyl-CoA needs further investigation.

Hait and co-workers [99] have shown one of the first examples of regulation of Class I HDACs by metabolites. According to the result of Hait *et al.* HDAC1 and HDAC2 are regulated by the endogenous lipid mediator sphingosine 1-phosphate (S1P). In nuclear extracts S1P inhibited HDAC activity by 50% relative to 90% inhibition by a

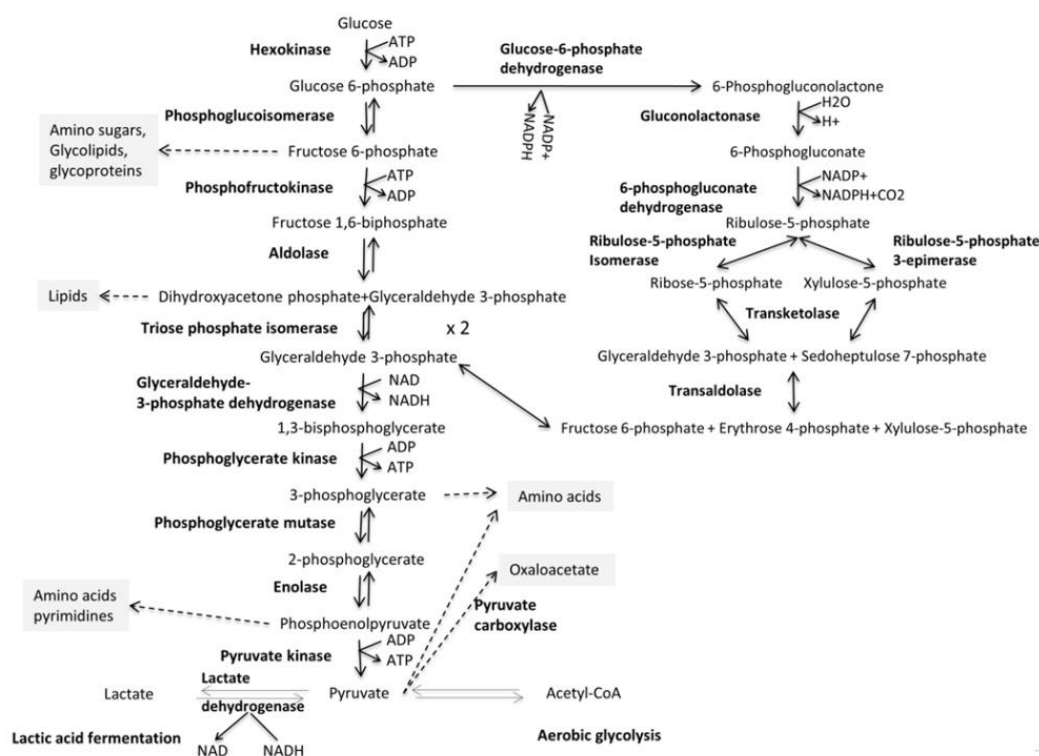
known, small molecule inhibitor (trichostatin A). HAT activity was not affected by S1P in these experiments. Interestingly, structurally related molecules sphingosine and lysophospholipid did not have any significant effect on HDAC1 or HDAC2. Also, out of all human HDACs, only HDAC1 and HDAC2 have been shown to bind to S1P. SphK2 – an enzyme that produces S1P in the nucleus from sphingosine is associated with HDAC1 and HDAC2 complexes providing proximal production of S1P to its nuclear target. Treatment of cells with an activator of protein kinase C that enhances activity of SphK2 led to nuclear export of SphK2 and transient inhibition of HDACs. This, in turn, increased acetylation of histone H3 and induced accumulation of p21 protein and mRNA as well as c-fos and possibly other genes. Thus, S1P metabolism appears to be a signaling route for HDAC controlled gene expression and response to a variety of signals. S1P formed by nuclear SphK2 in response to environmental signals affected histone acetylation and gene expression through HDAC inhibition presenting a link between sphingolipid metabolism in the nucleus and remodeling of chromatin and the epigenetic regulation of gene expression.

*In vitro* analyses of Vogelauer *et al.* [92] have shown that derivatives of CoA as well as NADPH stimulate the activity of class I HDACs on histones whereas free CoA inhibits HDAC activity. *In vitro* activities of HDAC1 and HDAC2 were increased by approximately 1.5-fold with addition of acetyl-, acetoacetyl- and succinyl-CoA. Adding butyryl-, isobutyryl-, glutaryl, HMG- or malonyl-CoA increased HDAC1 and HDAC2 activity approximately 2-fold. Finally, addition of methylmalonyl-, crotonyl- and  $\beta$ -methylcrotonyl-CoA led to 2-3 fold increases relative to the control compound. However, palmitoyl-CoA – a long fatty acid derivative - inhibited HDAC1 and HDAC2 activities. Although a number of CoA derivatives listed above increase activity of HDAC1 and HDAC2, coenzyme A by itself reduced the activities of these enzymes by approximately 60%. In the same work, the authors also showed that NADPH, but not NADP<sup>+</sup>, stimulates HDAC activity. Neither NAD<sup>+</sup>

nor NADH showed any significant effect. These effects were shown *in vitro* on recombinant HDAC proteins as well as in a natural molecular environment of HDAC from cell extracts. All of HDAC activating metabolites studied in [92] have an adenosine phosphate moiety. All of the reported metabolites are intermediates of cellular metabolic pathways. Acetyl-CoA is a central molecule in the intermediary metabolism generated from degradation of glucose, pyruvate, amino acids, as well as oxidation of fatty acids. NADPH is the main reducing agent for biosynthetic reactions. Glutaryl-CoA, crotonyl-CoA, and HMG-CoA are generated in the pathway of lysine degradation; methylcrotonyl-CoA and succinyl-CoA are degradation products of leucine and methionine, respectively. Malonyl-CoA and HMG-CoA, are the main precursors for fatty acid and cholesterol syntheses, respectively. Further investigation is certainly needed to explore the effects of cellular levels of these metabolites on HDACs.

## Acetylation regulation of enzymes

Analysis of a subset of 12 critical human HDACs (HDAC1-4, HDAC6-9, SIRT1-3 and SIRT5) using a genome-wide synthetic lethality screen in cultured human cells was used to identify enzyme-substrate relationships between individual HDACs and a number of substrate non-histone genes from a wide variety of biological processes. Interestingly amongst many processes represented by these genes the most enriched group within the HDAC interaction partners are genes involved in metabolism [41]. From the studies in liver tissues it has been found that amongst the 2,200 detected acetylated proteins there is the majority of enzymes involved in glycolysis, gluconeogenesis, Krebs' cycle, fatty acid oxidation, urea cycle and nitrogen metabolism, glycogen metabolism, oxidative phosphorylation and amino acid metabolism [100,101]. Amongst those are both enzymes localized in the cytoplasm and in mitochondria. Acetylation activates some proteins while deactivating others. A detailed review of these effects has been recently presented [10] and is represented schematically in Figure 6.



**Figure 6** Known acetylation of enzymes involved in glycolysis and PPP. Enzymes that are known to be regulated by acetylation are shown in bold [17].

Acetylation of lysine's in proteins can help in recruiting co-activator complexes *via* conserved modular domain or, alternatively, engage co-repressor complexes through lysine deacetylases. Acetylation can also directly modulate the activity of enzymes and influence their stability. Many proteins within the chromatin modifying complex are heavily acetylated. Also, HATs are themselves acetylated on lysine residues. In the case of p300, acetylation protects it from degradation thereby increasing its activity. Acetylation modulates nuclear transport of PCAF while also increasing its binding affinity for acetyl-CoA. Lysine acetylation of a single lysine residue reversibly inhibits the function of methyltransferase SUV39H1 [2 and references therein].

## Conclusions

Although many HDAC, HAT and SIRT inhibitors are being explored and even used for cancer treatment a

very limited attention has been paid to the effect of these compounds on metabolism. Some early experiments using the short chain fatty acid derivative valproate as HDAC inhibitor in rat liver mitochondria have shown inhibition of fatty acid  $\beta$ -oxidation. Utilization of valproate also resulted in general depression of cell oxidative metabolism leading to a decrease in the rate of  $O_2$  consumption, ATP synthesis as well as cytochrome oxidase activity [102-104]. In colorectal adenocarcinoma cell lines treatment with butyrate, an inhibitor of deacetylation, inhibited glucose uptake and oxidation as well as ribose synthesis and increased *de novo* fatty acid synthesis along with activation of the Pentose phosphate pathway (PPP). Similar results were obtained in cells treated with other pan-HDAC inhibitors [105, 106]. Treatment of myeloma cells with HDAC inhibitors once again resulted in the decrease in glucose uptake with down-regulation of GLUT1 and HK as well as an increase in amino acid catabolism [107]. In the lung cancer cell line, H460,

sodium butyrate treatment reduced lactate production, down-regulated GLUT1 and up-regulated expression and activity of GLUT3. Also, this treatment increased mitochondrial bound HK activity and expression (isoform HKI) while down-regulating HKII isoform and increasing activity of glucose 6-phosphate dehydrogenase, one of main regulators of PPP. Mitochondrial metabolism has also been shown to increase with sodium butyrate treatment [108]. As part of the Warburg effect in cancer cells there is an increased production of lactate from pyruvate by lactate dehydrogenase A (LDH-A). LDH-A is often overexpressed in tumor cells but with its activation is also regulated by acetylation. Acetylation of a specific, known, lysine residue of LDH-A inhibits its activity. Importantly, acetylation of LDH-A is reduced in pancreatic cancers thus stimulating its activity and increasing transformation of pyruvate into lactate [109]. Cancer cells with an acetylation mimic of LDH-A show reduced cell proliferation and cell migration [109]. Inhibition of HDAC in lung cancer cell lines revealed a reduction of lactate production. This can be explained through the effect on LDH-A as well as through changes in expression and activity of a number of other enzymes involved in glycolysis. Glycolysis related changes include: altered activity of HK bound to membrane and increased ADP recycling, modified G6PDH activity leading to increased PPP flux and NADPH supply; reduced GLUT1 and increased GLUT3 expression following HDACi [108].

Further work is needed to clarify metabolic changes as well as to determine specific functions of individual HDACs in these transformations. Although selective HDAC inhibition is very difficult due to high similarities between members of the same class it is becoming possible for at least some HDACs. For HATs, on the other hand, some selective inhibitors have been developed almost simultaneously with their discovery [110] due to very distinct structures of the proteins with HAT activity. Inhibitors of p300/CBP and PCAF/Gcn5 families have received extensive attention in the literature [111]. Detailed analysis of the metabolic effects of these inhibitors is still needed.

Aberrant metabolism is characteristic of cancers. Many drugs targeting these overall metabolic alterations independently are currently in clinical trials with mixed success. HDACs and HAT are direct intracellular targets of a number of metabolites and metabolism is both directly and indirectly regulated by HDACs and HAT in many examples. Understanding the interplay of these important processes may facilitate the search for safer and more effective cancer therapies targeting independently or in combination these major processes. Furthermore, better descriptions of the relationships between metabolism and epigenetic changes and cancer will allow the determination of true risk factors.

## References

1. Sassone-Corsi P. When metabolism and epigenetics converge. *Science*. 2013, 339:148-150
2. Katada S, Imhof A, Sassone-Corsi P. Connecting threads: epigenetics and metabolism. *Cell*. 2012, 148: 24-28
3. Lu C, Thompson C B. Metabolic regulation of epigenetics. *Cell metabolism*. 2012, 16:9-17
4. Kaelin WG, McKnight SL. Influence of Metabolism on Epigenetics and Disease. *Cell*. 2013, 153: 56-69
5. Allfrey VG, Faulkner R, Mirsky AE. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc Natl Acad Sci*. 1964, 51: 786-794
6. Warburg, Uber den Stoffwechsel der Carcinomzelle. *Klin Wochenschr Berl*. 1925, 4:534-536
7. Barneda-Zahonero B, Parra M. Histone deacetylases and cancer. *Molecular oncology*. 2012, 6:579-89
8. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modification. *Cell Res*. 2011, 21:381-395
9. Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity



- protein Sir2 is an NAD-dependent histone deacetylase. *Nature*. 2000, 403:795- 800
10. Landry J, Sutton A, Tafrov ST, Heller RC, Stebbins J, Pillus L, Sternglanz R. The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *Proc Natl Acad Sci*. 2000, 97:5807-5811
  11. Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. *Nature Rev Genetics*. 2009, 10:32-42
  12. Foglietti C, Filocamo G, Cundari E, De Rinaldis E, Lahm A, Cortese R, Steinkühler C. Dissecting the biological functions of histone deacetylases by RNA interference and transcriptional profiling. *J Biol Chem*. 2006, 281:17968-17976
  13. Villagra A, Sotomayor EM, Seto E. Histone deacetylases and the immunological network: implications in cancer and inflammation. *Oncogene*. 2010, 29:157-173
  14. Waterborg JH. Dynamics of histone acetylation in vivo. A function for acetylation turnover? *Biochem Cell Biol*. 2002, 80:363-378
  15. Kouzarides T. Acetylation: a regulatory modification to rival phosphorylation? *EMBO J*. 2000, 19:1176-1179
  16. Choudhary C, Mann M. Decoding signalling networks by mass spectrometry-based proteomics. *Nat Rev Mol Cell Biol*. 2010, 11: 427-439
  17. Guan KL, Xiong Y. Regulation of intermediary metabolism by protein acetylation. *Trends Biochem Sci*. 2011, 36:108-116
  18. Zhao SM, Xu W, Jiang WQ, Yu W, Lin Y, Zhang TF, Yao J, Zhou L, Zeng YX, Li H, Li YX, Shi J, An WL, Hancock SM, He FC, Qin LX, Chin J, Yang PY, Chen X, Lei QY, Xiong Y, Guan KL. Regulation of cellular metabolism by protein lysine acetylation. *Science*. 2010, 327:1000-1004
  19. Thangaraju M, Gopal E, Martin PM, Ananth S, Smith SB, Prasad PD, Sterneck E, Ganapathy V. Slc5a8 triggers tumor cell apoptosis through pyruvate-dependent inhibition of histone deacetylases. *Cancer Research*. 2006, 66:11560-11564
  20. Huang H, Liu N, Guo H, Liao S, Li X, Yang C, Liu S, Guan L, Li B, Xu L, Zhang C, Wang X, Dou QP, Liu J. L-Carnitine Is an Endogenous HDAC Inhibitor Selectively Inhibiting Cancer Cell Growth In Vivo and In Vitro. *PLoS one*. 2012, 7:e49062
  21. Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P. Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. *Science*. 2009, 324:654-657
  22. Phang JM, Liu W, Hancock C. Bridging epigenetics and metabolism. *Epigenetics*. 2013, 8:231-236
  23. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. ATP-citrate lyase links cellular metabolism to histone acetylation. *Science*. 2009, 324: 1076-1080
  24. Zaidi N, Swinnen JV, Smans K. ATP-citrate lyase: a key player in cancer metabolism. *Cancer Res*. 2012, 7:3709-3714
  25. Donohoe D R, Collins LB, Wali A, Bigler R, Sun W, Bultman SJ. The Warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation. *Mol Cell*. 2012, 48:612-626
  26. McBrien MA, Behbahan IS, Ferrari R, Su T, Huang T-W, Li K, Hong CS, Christofk HR, Vogelauer M, Seligson DB, Kurdistani SK. Histone acetylation regulates intracellular ph. *Molecular Cell*. 2013, 49:310-321
  27. Elsheikh SE, Green AR, Rakha EA, Powe DG, Ahmed RA, Collins HM, Soria D, Garibaldi JM, Paish CE, Ammar AA, Grainge MJ, Ball GR, Abdelghany MK, Martinez-Pomares L, Heery DM, Ellis IO. Global histone modifications in breast cancer correlate with tumor phenotypes, prognostic factors, and patient outcome. *Cancer Research*. 2009, 69:3802-3809
  28. Manuyakorn A, Paulus R, Farrell J, Dawson NA, Tze S, Cheung-Lau G, Hines OJ, Reber H, Seligson DB, Horvath S, Kurdistani SK, Guha C, Dawson DW. Cellular histone modification

- patterns predict prognosis and treatment response in resectable pancreatic adenocarcinoma: Results from rtog 9704. *Journal of Clinical Oncology*. 2010, 28:1358-1365
29. Robey IF, Baggett BK, Kirkpatrick ND, Roe DJ, Dosesco J, Sloane BF, Hashim AI, Morse DL, Raghunand N, Gatenby RA, Gillies RJ. Bicarbonate increases tumor pH and inhibits spontaneous metastases. *Cancer Research*. 2009, 69:2260-2268
  30. Cuperlovic-Culf M, Culf A, Touaibia M, Lefort N. Targeting the last hallmark of cancer: another attempt at a “magic bullet” drug targeting cancers’ metabolic phenotype. *Future Oncology*. 2012, 8:1315-1330
  31. Gregoret IV, Lee YM, Goodson HV. Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. *Journal of molecular biology*. 2004, 338:17-31
  32. Bernstein BE, Tong JK, Schreiber SL. Genome-wide studies of histone deacetylase function in yeast. *Proc Natl Acad Sci*. 2000, 97:13708-13713
  33. Wang AH, Kruhlak MJ, Wu J, Bertos NR, Vezmar M, Posner BI, Bazett-Jones DP, Yang X-J. Regulation of histone deacetylase 4 by binding of 14-3-3 proteins. *Molecular and Cellular Biology*. 2000, 20:6904-6912
  34. Grozinger CM, Schreiber SL. Regulation of histone deacetylase 4 and 5 and transcriptional activity by 14-3-3-dependent cellular localization. *Proc Natl Acad Sci*. 2000, 97: 7835-7840
  35. Yuan H, Marmorstein R. Histone acetyltransferases: Rising ancient counterparts to protein kinases. *Biopolymers*. 2013, 99:98-111
  36. Marmorstein R, Trievel RC. Histone modifying enzymes: Structures, mechanisms, and specificities. *Biochimica et Biophysica Acta*. 2009,1789:58-68
  37. Wallace D, Fan W. Energetics, epigenetics, mitochondrial genetics. *Mitochondrion*. 2010, 10:12-31
  38. Rokudai S, Laptenko O, Arnal SM, Taya Y, Kitabayashi I, Prives C. MOZ increases p53 acetylation and premature senescence through its complex formation with PML. *Proc Natl Acad Sci*. 2013, 110:3895-900
  39. Love IM, Sekaric P, Shi D, Grossman SR, Androphy EJ. The histone acetyltransferase PCAF regulates p21 transcription through stress-induced acetylation of histone H3. *Cell Cycle*. 2012, 11:2458-2466
  40. Spiegel S, Milstien S, Grant S. Endogenous modulators and pharmacological inhibitors of histone deacetylases in cancer therapy. *Oncogene*. 2012, 31:537-551
  41. Lin Y-Y, Kiihl S, Suhail Y, Liu SY, Chou YH, Kuang Z, Lu JY, Khor CN, Lin CL, Bader JS, Irizarry R, Boeke JD. Functional dissection of lysine deacetylases reveals that HDAC1 and p300 regulate AMPK. *Nature*. 2012, 482: 251-255
  42. Mihaylova MM, Shaw RJ. Metabolic reprogramming by class I and II histone deacetylases. *Trends in endocrinology and metabolism: TEM*. 2013, 24:48-57
  43. Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z, Dupuy F, Chambers C, Fuerth BJ, Viollet B, Mamer OA, Avizonis D, DeBerardinis RJ, Siegel PM, Jones RG. Ampk is a negative regulator of the warburg effect and suppresses tumor growth in vivo. *Cell Metabolism*. 2013, 17:113-124
  44. Geng H, Harvey CT, Pittsenbarger J, Liu Q, Beer TM, Xue C, Qian DZ. HDAC4 protein regulates HIF1 $\alpha$  protein lysine acetylation and cancer cell response to hypoxia. *J Biol Chem*. 2011, 286:38095-102
  45. Geng H, Liu Q, Xue C, David LL, Beer TM, Thomas GV, Dai MS, Qian DZ. HIF1 $\alpha$  protein stability is increased by acetylation at lysine 709. *J Biol Chem*. 2012, 287:35496-34505
  46. Marcu MG, Jung YJ, Lee S, Chung EJ, Lee MJ, Trepel J, Neckers L. Curcumin is an inhibitor of p300 histone acetyltransferase. *Med Chem*. 2006, 2:169-174

47. Lee SJ, Krauthauser C, Maduskuie V, Fawcett PT, Olson JM, Rajasekaran S., Curcumin-induced HDAC inhibition and attenuation of medulloblastoma growth in vitro and in vivo. *BMC Cancer*. 2011, 11:144
48. Luo Y, Jian W, Stavreva D, Fu X, Hager G, Bungert J, Huang S, Qiu Y. Trans-regulation of histone deacetylase activities through acetylation. *J Biol Chem*. 2009, 284:34901-34910
49. Wagner JM, Hackanson B, Lübbert M, Jung M. Histone deacetylase (HDAC) inhibitors in recent clinical trials for cancer therapy. *Clinical Epigenetics*. 2010, 1: 117-136
50. Lahm A, Paolini C, Pallaoro M, Nardi MC, Jones P, Neddermann P, Sambucini S, Bottomley MJ, Lo Surdo P, Carfi A, Koch U, De Francesco R, Steinkuehler C, Gallinari P. Unraveling the hidden catalytic activity of vertebrate class I histone deacetylases. *Proc Natl Acad Sci*. 2007, 104:17335-17340
51. Fischle W, Dequiedt F, Fillion M, Hendzel MJ, Voelter W, Verdin E. Human HDAC7 histone deacetylase activity is associated with HDAC3 in vivo. *J Biol Chem*. 2001, 276:35826-35835
52. Fischle W, Dequiedt F, Fillion M, Hendzel MJ, Voelter W, Verdin E. Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. *Mol Cell*. 2002, 9:45-57
53. Boyault C, Sadoul K, Pabion M, Khochbin S. HDAC6, at the crossroads between cytoskeleton and cell signaling by acetylation and ubiquitination. *Oncogene*. 2007, 26:5468-5476
54. Valenzuela-Fernandez A, Cabrero JR, Serrador JM, Sanchez-Madrid F. Hdac6: A key regulator of cytoskeleton, cell migration and cell-cell interactions. *Trends in Cell Biology*. 2008, 18:291-297
55. Kamemura K, Ogawa M, Ohkubo S, Y. Ohtsuka, Shitara Y, Komiya T, Maeda S, Ito A, Yoshida M. Depression of mitochondrial metabolism by downregulation of cytoplasmic deacetylase, HDAC6. *FEBS Lett*. 2012, 586 : 1379-1383
56. Matsuyama A, Shimazu T, Sumida Y, Saito A, Yoshimatsu Y, Seigneurin-Berny D, Osada H, Komatsu Y, Nishino N, Khochbin S, Horinouchi S, Yoshida M. In vivo destabilization of dynamic microtubules by hdac6-mediated deacetylation. *Embo Journal*. 2002, 21:6820-6831
57. Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, Yoshida M, Wang XF, Yao TP. Hdac6 is a microtubule-associated deacetylase. *Nature*. 2002, 417:455-458
58. Haggarty SJ, Koeller KM, Wong JC, Grozinger CM, Schreiber SL. Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. *Proc Natl Acad Sci*. 2003, 100:4389- 4394.
59. Zhang X, Yuan Z, Zhang Y, Yong S, Salas-Burgos A, Koomen J, Olashaw N, Parsons JT, Yang X-J, Dent SR, Yao T-P, Lane WS, Seto E. Hdac6 modulates cell motility by altering the acetylation level of cortactin. *Molecular Cell*. 2007, 27:197-213
60. Li Y, Zhang X, Polakiewicz RD, Yao TP, Comb MJ. HDAC6 is required for epidermal growth factor-induced  $\beta$ -catenin nuclear localization. *J Biol Chem*. 2008, 283:12686-12690
61. Parmigiani RB, Xu WS, Venta-Perez G, Erdjument-Bromage H, Yaneva M, Tempst P, Marks PA. HDAC6 is a specific deacetylase of peroxiredoxins and is involved in redox regulation. *Proc Natl Acad Sci*. 2008, 105:9633-9638
62. Subramanian C, Jarzembowski JA, Opipari AW, Jr., Castle VP, Kwok RPS. Hdac6 deacetylates ku70 and regulates ku70-bax binding in neuroblastoma. *Neoplasia*. 2011, 13:726-734
63. Winkler R, Benz V, Clemenz M, Bloch M, Foryst-Ludwig A, Wardat S, Witte N, Trappiel M, Namsolleck P, Mai K, Spranger J, Matthias G, Roloff T, Truee O, Kappert K, Schupp M, Matthias P, Kintscher U. Histone deacetylase 6 (hdac6) is an essential modifier of glucocorticoid-induced hepatic gluconeogenesis. *Diabetes*. 2012, 61:513-523
64. Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, Yoshida M, Wang XF, Yao TP.

- Hdac6 is a microtubule-associated deacetylase. *Nature*. 2002, 417:455-458
65. Cabrero JR, Serrador JM, Barreiro O, Mittelbrunn M, Naranjo-Suarez S, Martin-Cofreces N, Vicente-Manzanares M, Mazitschek R, Bradner JE, Avila J, Valenzuela-Fernandez A, Sanchez-Madrid F. Lymphocyte chemotaxis is regulated by histone deacetylase 6, independently of its deacetylase activity. *Molecular Biology of the Cell*. 2006, 17:3435-3445
  66. Fischer DD, Cai R, Bhatia U, Asselbergs FAM, Song CZ, Terry R, Trogani N, Widmer R, Atadja P, Cohen D. Isolation and characterization of a novel class ii histone deacetylase, hdac10. *Journal of Biological Chemistry*. 2002, 277:6656-6666
  67. Matthias P, Yoshida M, Khochbin S. HDAC6 a new cellular stress surveillance factor. *Cell Cycle*. 2008, 7:7-10
  68. Chalkiadaki A, Guarente L. Sirtuins mediate mammalian metabolic responses to nutrient availability. *Nature Reviews Endocrinology*. 2012, 8:287-296
  69. Chiarugi A, Dolle C, Felici R, Ziegler M. The nad metabolome - a key determinant of cancer cell biology. *Nature Reviews Cancer*. 2012, 12:741-752
  70. Huang J-Y, Hirschev MD, Shimazu T, Ho L, Verdin E. Mitochondrial sirtuins. *Biochimica Et Biophysica Acta-Proteins and Proteomics*. 2010, 1804:1645-1651
  71. Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin YX, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, Greenberg ME. Stress-dependent regulation of foxo transcription factors by the sirt1 deacetylase. *Science*. 2004, 303:2011-2015
  72. Luo J, Nikolaev AY, Imai S-i, Chen D, Su F, Shiloh A, Guarente L, Gu W. Negative control of p53 by sir2alpha promotes cell survival under stress. *Cell*. 2001, 107:137-148
  73. Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J*. 2004, 23:2369-2380
  74. North BJ, Marshall BL, Borra MT, Denu JM, Verdin E. The human Sir2 ortholog, SIRT2, is an NAD<sup>+</sup>-dependent tubulin deacetylase. *Mol Cell*. 2003, 11:437-444
  75. Michishita E, McCord RA, Berber E, Kioi M, Padilla-Nash H, Damian M, Cheung P, Kusumoto R, Kawahara TL, Barrett JC, Chang HY, Bohr VA, Ried T, Gozani O, Chua KF. SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin, *Nature*. 2008, 452:492-496
  76. Ford E, Voit R, Liszt G, Magin C, Grummt I, Guarente L. Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription. *Genes Dev*. 2006, 20:1075-1080
  77. Nakahata Y, Sahar S, Astarita G, Kaluzova M, P. Sassone-Corsi. Circadian control of the NAD<sup>+</sup> salvage pathway by CLOCK-SIRT1. *Science*. 2009, 324:654-657
  78. Ramsey KM, Yoshino J, Brace CS, Abrassart D, Kobayashi Y, Marcheva B, Hong H-K, Chong JL, Buhr ED, Lee C, Takahashi JS, Imai S-i, Bass J. Circadian clock feedback cycle through nampt-mediated nad(+) biosynthesis. *Science*. 2009, 324:651-654
  79. Imai S, Guarente L. Ten years of NAD-dependent SIR2 family deacetylases: implications for metabolic diseases. *Trends Pharmacol Sci*. 2010, 31:212
  80. Sassone-Corsi P. Physiology. When metabolism and epigenetics converge. *Science*. 2013, 339:148-150.
  81. Yang H, Yang T, Baur JA, Perez E, Matsui T, Carmona JJ, Lamming DW, Souza-Pinto NC, Bohr VA, Rosenzweig A, de Cabo R, Sauve AA, Sinclair DA. Nutrient-sensitive mitochondrial nad(+) levels dictate cell survival. *Cell*. 2007, 130:1095-1107
  82. Nakagawa T, Lomb DJ, Haigis MC, Guarente L. SIRT5 Deacetylates carbamoyl phosphate synthetase 1 and regulates the urea cycle. *Cell*. 2009, 137:560-570

83. Hsieh Y-L, Kuo H-Y, Chang C-C, Naik MT, Liao P-H, Ho C-C, Huang T-C, Jeng J-C, Hsu P-H, Tsai M-D, Huang T-H, Shih H-M. Ubc9 acetylation modulates distinct sumo target modification and hypoxia response. *Embo Journal*. 2013, 32:791-804
84. Kim H-S, Patel K, Muldoon-Jacobs K, Bisht KS, Aykin-Burns N, Pennington JD, van der Meer R, Nguyen P, Savage J, Owens KM, Vassilopoulos A, Ozden O, Park S-H, Singh KK, Abdulkadir SA, Spitz DR, Deng C-X, Gius D. Sirt3 is a mitochondria-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress. *Cancer Cell*. 2010, 17:41-52
85. Sebastian C, Zwaans BMM, Silberman DM, Gymrek M, Goren A, Zhong L, Ram O, Truelove J, Guimaraes AR, Toiber D, Cosentino C, Greenson JK, MacDonald AI, McGlynn L, Maxwell F, Edwards J, Giacosa S, Guccione E, Weissleder R, Bernstein BE, Regev A, Shiels PG, Lombard DB, Mostoslavsky R. The histone deacetylase sirt6 is a tumor suppressor that controls cancer metabolism. *Cell*. 2012, 151:1185-1199
86. Cai L, Sutter BM, Li B, Tu BP. Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. *Mol Cell*. 2011, 42:426-437.
87. Michishita E, McCord RA, Boxer LD, Barber MF, Hong T, Gozani O, Chua KF. Cell cycle-dependent deacetylation of telomeric histone H3 lysine K56 by human SIRT6. *Cell Cycle*. 2009, 8:2664-2666
88. Bauer I, Grozio A, Lasiglie D, Basile G, Sturla L, Magnone M, Sociali G, Soncini D, Caffa I, Poggi A, Zoppoli G, Cea M, Feldmann G, Mostoslavsky R, Ballestrero A, Patrone F, Bruzzone S, Nencioni A. The nad(+)-dependent histone deacetylase sirt6 promotes cytokine production and migration in pancreatic cancer cells by regulating ca2+ responses. *Journal of Biological Chemistry*. 2012, 287:40924-40937
89. Kokkonen P, Rahnasto-Rilla M, Kiviranta PH, Huhtiniemi T, Laitinen T, Poso A, Jarho E, Lahtela-Kakkonen M. Peptides and Pseudopeptides as SIRT6 Deacetylation Inhibitors. *ACS Medicinal Chemistry Letters*. 2012, 3:969-974
90. Deubzer HE, Schier MC, Oehme I, Lodrini M, Haendler B, Sommer A, Witt O. HDAC11 is a novel drug target in carcinomas. *Int J Cancer*. 2013, 132:2200-8.
91. Soliman ML, Rosenberger T. Acetate supplementation increases brain histone acetylation and inhibits histone deacetylase activity and expression. *Mol Cell Biochem*. 2011, 352:173-80.
92. Vogelauer M, Krall AS, McBrien M, Li JY, Kurdستاني SK. Stimulation of histone deacetylase activity by metabolites of intermediary metabolism. *J Biol Chem*. 2012, 287:32006-32016
93. Candido EP, Reeves R, Davie JR. Sodium butyrate inhibits histone deacetylation in cultured cells. *Cell*. 1978, 14:105-113
94. Shimazu T, Hirschey MD, Newman J, He W, Shirakawa K, Le Moan N, Grueter CA, Lim H, L.R. Saunders, R.D. Stevens, C.B. Newgard, Farese Jr RV, de Cabo R, Ulrich S, Akassoglou K, Verdin E. Suppression of oxidative stress by  $\beta$ -hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science*. 2013, 339:211-214.
95. Thangaraju M, Gopal E, Martin PM, Ananth S, Smith SB, Prasad PD, Sterneck E, Ganapathy V. SLC5A8 triggers tumor cell apoptosis through pyruvate-dependent inhibition of histone deacetylases. *Cancer Res*. 2006, 66:11560-11564
96. Latham T, Mackay L, Sproul D, Karim M, Culley J, Harrison DJ, Hayward L, Langridge-Smith P, Gilbert N, Ramsahoye BH. Lactate, a product of glycolytic metabolism, inhibits histone deacetylase activity and promotes changes in gene expression. *Nucleic Acids Res*. 2012, 40:4794-4803
97. Nunes MJ, Moutinho M, Gama MJ, Rodrigues CM, Rodrigues E. Histone deacetylase inhibition decreases cholesterol levels in neuronal cells by

- modulating key genes in cholesterol synthesis, uptake and efflux. *PLoS One*. 2013, 8: e53394
98. Villagra, N. Ulloa, X. Zhang, Z. Yuan, E. Sotomayor, E. Seto, Histone deacetylase 3 down-regulates cholesterol synthesis through repression of lanosterol synthase gene expression. *J Biol Chem*. 2007, 282:35457-35470
  99. Hait NC, Allegood J, Maceyka M, Strub GM, Harikumar KB, Singh SK, Luo C, Marmorstein R, Kordula T, Milstien S, Spiegel S. Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. *Science*. 2009, 325:1254-1257
  100. Choudhary, C. Kumar, F. Gnad, M.L. Nielsen, M. Rehman, T.C. Walther, J.V. Olsen, M. Mann Lysine Acetylation Targets Protein Complexes and Co-Regulates Major Cellular Functions. *Science*. 2009, 325:834-840
  101. Schwer B, Eckersdorff M, Li Y, Silva JC, Fermin D, Kurtev MV, Giallourakis C, Comb MJ, Alt FW, Lombard DB. Calorie restriction alters mitochondrial protein acetylation. *Aging Cell*. 2009, 8:604-606
  102. Ponchaut S, van Hoof F, Veitch K. Cytochrome aa3 depletion is the cause of the deficient mitochondrial respiration induced by chronic valproate administration. *Biochem Pharmacol*. 1992, 43:644-647
  103. Silva MF, Ruitter JP, Li IJ, Jakobs C, Duran M, de Almeida IT, Wanders RJ. Differential effect of valproate and its Delta2- and Delta4-unsaturated metabolites, on the betaoxidation rate of long-chain and medium-chain fatty acids. *Chem Biol Interact*. 2001, 137:203-212
  104. Turnbull DM, Bone AJ, Bartlett K, Koundakjian PP, Sherratt HS. The effects of valproate on intermediary metabolism in isolated rat hepatocytes and intact rats. *Biochem Pharmacol*. 1983, 32:1887-1892
  105. Alcarraz-Vizan G, Boren J, Lee WN, Cascante M. Histone deacetylase inhibition results in a common metabolic profile associated with HT29 differentiation. *Metabolomics*. 2010, 6:229-237
  106. Boren J, Lee WN, Bassilian S, Centelles JJ, Lim S, Cascante M. The stable isotope-based dynamic metabolic profile of butyrate-induced HT29 cell differentiation. *J Biol Chem*. 2003, 278:28395-28402
  107. Wardell SE, Ilkayeva OR, Wieman HL, Frigo DE, Rathmell JC. Glucose metabolism as a target of histone deacetylase inhibitors. *Mol Endocrinol*. 2009, 23:388-401
  108. Amo-âlo ND, Rodrigues MF, Pezzuto P, Galina A, Da Costa RM, De Almeida FCL, El-Bacha T, Rumjanek FD. Energy metabolism in H460 lung cancer cells: effects of histone deacetylase inhibitors. *PLoS One*. 2011, 6 :, e22264
  109. Zhao D, Zou S-W, Liu Y, Zhou X, Mo Y, Wang P, Xu Y-H, Dong B, Xiong Y, Lei Q-Y, Guan K-L. Lysine-5 acetylation negatively regulates lactate dehydrogenase a and is decreased in pancreatic cancer. *Cancer Cell*. 2013, 23:464-476
  110. Zheng Y, Thompson PR, Cebrat M, Wang L, Devlin MK, Alani R, Cole P. Selective HAT inhibitors as mechanistic tools for protein acetylation. *Methods in enzymology*. 2004, 376 :, 188-199
  111. Rekowski MVW, Giannis A. Histone acetylation modulation by small molecules: a chemical approach. *Biochim Biophys Acta*. 2010, 1799:760-767
  112. Mahlknecht U, Hoelzer D. Histone acetylation modifiers in the pathogenesis of malignant disease. *Molecular Med*. 2000, 6:623-644
  113. Kovacs JJ, Murphy PJM, Gaillard S, Zhao X, Wu JT, Nicchitta CV, Yoshida M, Toft DO, Pratt WB, Yao TP. HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. *Mol Cell*. 2005, 18:601-607
  114. Bali P, Pranpat M, Bradner J, Balasis M, Fiskus W, Guo F, Rocha K, Kumaraswamy S, Boyapalle S, Atadja P, Seto E, Bhalla K. Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: a novel basis of antileukemia activity

- of histone deacetylase inhibitors. *J Biol Chem.* 2005, 280:26729-26734
115. Kim SC, Sprung R, Chen Y, Xu Y, Ball H, Pei J, Cheng T, Kho Y, Xiao H, Xiao L, Grishin NV, White M, Yang XJ, Zhao Y. Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. *Mol Cell.* 2006, 23:607-618
116. Lee JH, Jeong EG, Choi MC, Kim SH, Park JH, Song SH, Park J, Bang YJ, Kim TY. Inhibition of histone deacetylase 10 induces thioredoxin-interacting protein and causes accumulation of reactive oxygen species in SNU-620 human gastric cancer cells. *Mol Cell.* 2010, 30:107-112
117. Kim SH, Jeong JW, Park JA, Lee JW, Seo JH, Jung BK, Bae MK, Kim KW. Regulation of the HIF-1alpha stability by histone deacetylases. *Oncol Rep.* 2007, 17:647-51