Acylations in cardiovascular biology and diseases, what's beyond acetylation

Xin Sun,[a,b,e] Yang Zhang,[c,e] Xiao-Feng Chen,[d,e] and Xiaoqiang Tang[a,*]

[a]Key Laboratory of Birth Defects and Related Diseases of Women and Children of Ministry of Education, State Key Laboratory of Biotherapy, West China Second University Hospital, West China Hospital, Sichuan University, Chengdu, 610041, China
[b]State Key Laboratory of Medical Molecular Biology, Department of Biochemistry and Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, 100005, China
[c]Department of Cardiology, Zhongshan Hospital, Fudan University, Shanghai Institute of Cardiovascular Diseases, Shanghai, 200032, China
[d]Department of Biochemistry and Molecular Biology, Basic Medical College, Chengdu University of Traditional Chinese Medicine, Chengdu, 611137, China

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Summary

Metabolism regulates cardiovascular biology through multiple mechanisms, including epigenetic modifications. Over the past two decades, experimental and preclinical studies have highlighted the critical roles of histone modifications in cardiovascular development, homeostasis, and diseases. The widely studied histone acetylation is critical in cardiovascular biology and diseases, and inhibitors of histone deacetylases show therapeutic values. In addition to lysine acetylation, a series of novel non-acetyl lysine acylations have recently been recognized. These non-acetyl lysine acylations have been demonstrated to have physiological and pathological functions, and recent studies have analyzed the roles of these non-acetyl lysine acylations in cardiovascular biology. Herein, we review the current advances in the understanding of non-acetyl lysine acylations in cardiovascular biology and discuss open questions and translational perspectives. These new pieces of evidence provide a more extensive insight into the epigenetic mechanisms underlying cardiovascular biology and help assess the feasibility of targeting acylations to treat cardiovascular diseases.

Keywords: Cardiovascular biology; Cardiovascular disease; Epigenetics; Non-acetyl acylation; Metabolism; Metabolite

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death globally, representing one third of all global deaths.[1] CVDs are closely associated with individual metabolic status and intracellular metabolic profile. Obesity, diabetes, and related dysregulation of the gut microbiota are key risk factors for CVDs. Metabolic reprogramming of cardiovascular cells controls cell function and the development of CVDs, partially via metabolite accumulation and imbalanced epigenetic modifications. However, the mechanisms underlying the metabolic regulation of CVDs via epigenetic modifications are still not completely understood, which has delayed the development of effective therapeutics.

Intracellular metabolites not only serve as substrates to support adenosine triphosphate (ATP) generation, but also act as donors for post-translational modifications (PTMs) of histones and non-histone proteins, which are critical mechanisms controlling the transcriptome, proteome, metabolome, and phenomics.[2,3] For instance, alpha-ketoglutarate from the tricarboxylic acid (TCA) cycle regulates DNA and histone methylation, whereas acetyl-coenzyme A (acyl-CoA) contributes to histone acetylation.[2] During the last two decades, several studies have demonstrated the critical roles of histone methylation and acetylation in the development of CVDs, and some inhibitors of histone deacetylases (HDACs) have been proven to be effective for treating CVDs, such as heart failure.[4,5] However, previous studies have unduly focused on histone methylation/acetylation, and the contributions of other types of PTMs have been ignored.

In recent years, long-chain and short-chain fatty acids (SCFAs), as well as other metabolites such as ketones from the gut microbiota or intracellular metabolism, have been shown to regulate health status.[2,6] Some of these new findings have highlighted the critical roles of SCFAs and ketones in CVDs and CVD-related risk factors, such as diabetes and obesity. Besides, SCFAs and other metabolites significantly regulate cell function by acting as donors for PTMs of proteins

*Corresponding author.
E-mail addresses: tangxiaoqiang@scu.edu.cn, txiaoqiang@yeah.net (X. Tang).
[e]These authors contributed equally to this work.

or inhibiting/activating enzymes.[3] For instance, recent biochemical studies have identified a series of short-chain fatty acylations, such as propionylation (Kpr), butyrylation (Kbu), isobutyrylation (Kibu), crotonylation (Kcr), methacrylation (Kmea), malonylation (Kmal), lactylation (Kla), succinylation (Ksucc), 2-hydroxyisobutyrylation (Khib), β-hydroxybutyrylation (Kbhb), glutarylation (Kglu), and some other types of modifications such as myristoylation and aminoacylation.[7] Some of these non-acetyl acylations share writers and erasers with acetylation; however, many of their functions are much different from acetylation.[3,8] For instance, histone acetylation and crotonylation are enriched at different regions of the chromatin to differentially regulate metabolic status such as glycolysis, oxidative phosphorylation, and fatty acid oxidation.[9,10]

Recent findings suggest that these non-acetyl acylations may play significant physiological and pathological roles in cardiovascular biology and disease risk factors, such as obesity, diabetes, and systemic inflammation. This review summarizes recent advances in the understanding of non-acetyl acylations in cardiovascular biology and discusses open questions and perspectives.

Acyl-CoA, acylations, and their writers and erasers

Protein acylation requires the participation of writers and erasers, as well as direct donors, namely acyl-CoAs (Table 1). Many types of acyl-CoAs exist in the cells and contribute to cellular metabolism and PTMs. These acyl-CoAs were generated by microbial products, such as acetate and propionate, or the catabolism of energy-rich substances such as glucose, fatty acids, amino acids, and ketones.[7] Acylations are important for gene transcription, metabolism, protein stability, and function (Fig. 1).

Acetylation

Lysine acetylation (Kac) of histones and non-histones was first reported decades ago.[8] Notably, acetyl-CoA is the most abundant acyl-CoA in cells. Thousands of proteins have been reported to be acetylated, and lysine acetylation participates in nearly all physiological and pathological processes. Protein acetylation is mediated by lysine acyltransferases (KATs) and removed by HDACs or sirtuins.[3]

Propionylation and butyrylation

Lysine propionylation (Kpr) and butyrylation (Kbu) extend hydrocarbon chains beyond that of Kac, which increases the hydrophobicity and bulk of the lysine residue.[8] Histone propionylation and butyrylation were detected via labeling and peptide mapping a decade ago, and non-histone substrates (p53, p300, and CREB-binding protein [CBP]) of lysine propionylation in eukaryotic cells were subsequently identified.[8] P300/CBP is the typical writer of Kpr and Kbu, while GCN5 (member of the GNAT family, also known as KAT2A) and MYSTs are the specific writers of Kpr. SIRT1–3 can remove propionyl-/butyryl-residues from histone lysine.[8,44]

	PTM types	Donors	Writers	Erasers	Substrates	Ref
C1	Formylation	Formyl-phosphate	NA	NA	Histones	7
C2	Acetylation	Acetyl-CoA	KAT	HDACs, Sirtuins	Widespread	4
C3	Propionylation	Propionyl-CoA	P300/CBP, GCN5, MYSTs	SIRT1-3	Histones, TPM3, MnSOD, P53	11–13
C3	Malonylation	Malonyl-CoA	GCN5(KAT2A),	SIRT2, SIRT5	Histones, mTOR, GAPDH, MCD	14–18
C3	Lactylation	Lactyl-CoA	P300/CBP, MOF, GCN5	SIRT1, SIRT2, HDACs	Histones, YY1, HMGB2	19–23
C4	Butyrylation	Butyryl-CoA	P300/CBP	SIRT1-3	Histones, P53	24
C4	Isobutyrylation	Isobutyryl-CoA	P300/CBP, KAT1	NA	Histones	25
C4	β-hydroxybutyrylation	β-hydroxybutyryl-CoA	P300/CBP	SIRT3	Histones, P53	26,27
C4	2-hydroxyisobutyrylation	2-hydroxyisobutyryl-CoA	TIP60, TmcA P300/CBP	HDAC1-3	Histones, ENO1	28–30
C4	Crotonylation	Crotonyl-CoA	P300/CBP, GCN5, and MYSTs	HDAC1-3, SIRT1-3	Histones, P53, ENO1, Septin2, IDH3a, TPM1	31–33
C4	Methacrylation	Methacryl-CoA	HAT1	SIRT1-2	Histones	34
C4	Succinylation	Succinyl-CoA	P300/CBP, GCN5	SIRT5	Widespread	35,36
C5	Glutarylation	Glutaryl-CoA	P300/CBP	SIRT5, SIRT7	Histones, IDH2, G6PD	37,38
C7	Benzoylation	Benzoyl-CoA	NA	SIRT2	Histones	39
C14	Myristoylation	Myristoyl-CoA	NMT1	SIRT6	Histones, AMPK, EZH2	40–42
-	Aminoacylation	Aminoacyl-CoA	Aminoacyl-tRNA synthetase	SIRT1, SIRT3	tRNAs, RagA, ASK1	7,43

KAT, lysine acyltransferase; HDACs, histone deacetylases; CBP, CREB-binding protein; GNATs, GCN5-related N-acetyltransferases; MYST, Moz, Ybf2, Sas2, and Tip60; TPM3, tropomodulin-3; MnSOD, manganese superoxide dismutase; N/A, not available; ENO1, Enolase 1; mTOR, mammalian target of rapamycin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MCD, malonyl-CoA decarboxylase; IDH2, isocitrate dehydrogenase 2; G6PD, glucose-6-phosphate dehydrogenase; YY1, Yin Yang 1; HMGB2, high-mobility group box 2.

Table 1: **The acylations and their writers, erasers, and donors.**

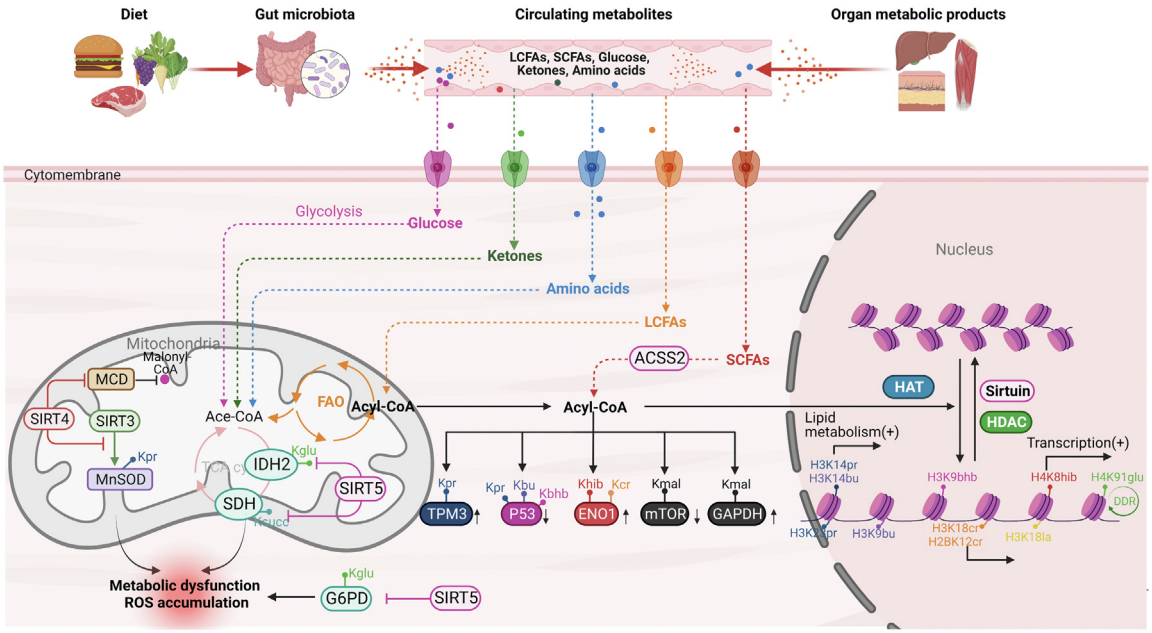

***Fig. 1:* Origin of acyl-CoAs and functions of lysine acylations.** Diet digestion, fermentation of dietary fibers by the gut microbes, and intracellular energy metabolism (fatty acid oxidation and amino acid catabolism) can generate various metabolites, such as LCFAs, SCFAs, amino acids, glucose, and ketones, which can enter cells and generate acyl-CoAs. Intracellular acyl-CoAs not only serve as substrates to support ATP generation, but also act as donors for PTMs of histones and non-histone proteins, which are critical mechanisms controlling the transcriptome, proteome, and metabolome. ACSS2 can convert SCFAs into cognate short-chain acyl-CoAs, which provide direct donors for short-chain lysine acylations of histone or non-histone proteins. PTMs can regulate transcription, signal transduction, and protein function, and these acylations are closely associated with the development of CVDs and CVD-related risk factors. LCFA, long-chain fatty acid; SCFA, short-chain fatty acid; PTM, post-translational modification; DDR, DNA damage repair; ACSS2, Acyl-CoA Synthetase Short Chain Family Member 2.

β-hydroxybutyrylation, 2-hydroxyisobutyrylation, and lactylation

Polar lysine acylations include β-hydroxybutyrylation (Kbhb),[26] 2-hydroxyisobutyrylation (Khib),[28] and lactylation (Kla),[19] which have a hydroxyl group that enables the modified lysine to form hydrogen bonds with other molecules.[8] P300 can add intracellular *β*-hydroxybutyryl-CoA to histones in a concentration-dependent manner,[8,44] and SIRT3 can reverse this process.[45] 2-hydroxyisobutyrate, the isomer of *β*-hydroxybutyrate, is derived from the microbial degradation of dietary proteins.[2] It is converted into 2-hydroxyisobutyryl-CoA, which is the donor of lysine 2-hydroxyisobutyrylation (Khib). Tip60, TmcA, and p300 can mediate Khib, while HDAC1–3 act as vital enzymes for Khib removal.[28–30,46] For protein lactylation, lacyl-CoA serves as the donor, but the source of lactyl-CoA is still under debate. Class I HDACs (HDAC1-3), SIRT1, and SIRT2 are histone lysine delactylases, and P300/MOF/GCN5 serves as the lactyltransferase.[19–21,47]

Malonylation and succinylation

Lysine malonylation (Kmal) and succinylation (Ksucc) are the acidic acyl groups that change the charge at the lysine residue from +1 to –1 at physiological pH, which is comparable to the change caused by protein phosphorylation (from 0 to –2).[8] Kmal is widely found in mammalian cell lines, liver tissues, and non-mammalian species on histones and non-histone.[3,8] Malonylation and succinylation of mitochondrial proteins have been reported for a long time, which is mediated by GCN5 and the deacylase SIRT2/5.[14,48] Succinyl-CoA is one of the most abundant acyl-CoA molecules in the heart and other tissues. P300/CBP or GCN5 can add a succinyl group to lysine residues and SIRT5 acts as desuccinylase.[49,50]

Crotonylation and methacrylation

Zhao et al. identified histone crotonylation (Kcr) in 2011, and 10 years later, histone methacrylation (Kmea) was identified by the same group.[8,34] Crotonylation and methacrylation contain a C–C π-bond that results in a rigid planar conformation, which is unique among histone acylations. Similar to propionylation and butyrylation, Kcr and Kmea can neutralize the positive charge of the amine group of the lysine residue, enhance the hydrophobicity, and increase the size of the lysine side chain.[8] Kcr is regulated by the level of crotonyl-CoA, and

short-chain enoyl-CoA hydratase (ECHS1) and chromodomain Y-like protein (CDYL) act as crotonyl-CoA hydratases to modulate the intracellular levels of crotonyl-CoA and control histone crotonylation.[51] In addition, P300/CBP, GCN5, and MYSTs mediate histone crotonylation, whereas HDAC1-3 and SIRT1–3 function as major executors of histone decrotonylation.[52] Histone Kcr chiefly enriches at the promoter, enhancer, or transcription start site of genes,[51] which may activate the transcription of specific genes. Kmea, corresponding to a structural isomer of crotonyl-lysine, is a dynamic marker, which is controlled by HAT1 as a methacyltransferase and SIRT1/2 as a demethacrylase.[34]

Glutarylation

Amino acid catabolism produces glutarate, which can be converted into glutaryl-CoA, providing the donor for protein lysine glutarylation (Kglu). Kglu was identified in 2014, and P300/CBP promotes protein glutarylation while SIRT5 and SIRT7 function as lysine deglutarylase.[37] Histone H4 lysine 91 glutarylation, a new histone marker, is enriched at promoters of highly expressed genes and can regulate chromatin structure and dynamics in response to DNA damage.[53]

Other types of acylations

Other types of acylations have been reported, including formylation, benzoylation, myristoylation, and aminoacylation. SIRT1-3 have been reported as deacylases of benzoylation and amino acylations, whereas SIRT6 serves as an eraser for myristoylation.[7,39,40] However, the regulators and physiological/pathological functions of these types of acylations are largely unknown.

Acylations in cardiovascular biology and diseases - an update beyond acetylation

Acetylation

During the past two decades, many studies have reported the roles of histone and non-histone acetylation in cardiovascular diseases. The roles of related enzymes such as sirtuins and HDACs have been fully investigated. Many reviews have summarized these advances.[4,5,54–56] Thus, this review focuses on the function of non-acetyl lysine acylations in cardiovascular diseases.

Propionylation

Histone H3 lysine 14 (H3K14) is a common lysine site of propionylation (H3K14pr) that is predominantly enriched at gene promoters to drive high transcriptional outputs. Enrichment of H3K14pr across transcription start sites has a significant effect on lipid metabolism pathways in mice, which is associated with the physiological and pathological progress of CVDs (Fig. 1).[57] Accumulation of propionylation donor propionyl-CoA causes metabolic disorder and contributes to dilated cardiomyopathy during childhood, resulting from the deficiency of propionyl-CoA carboxylase, a mitochondrial enzyme that metabolizes propionyl-CoA.[58,59]

Recently, Yang et al. discovered that BRPF1-KAT6 complexes can catalyze H3K23pr, and that H3K23pr deficiency in humans could lead to cardiac anomalies such as dilated ascending aorta,[11] which frequently leads to significant aortic valvular insufficiency, even in the presence of an otherwise normal valve (Fig. 2). However, further studies are required to investigate the role of histone propionylation (H3K14pr or H3K23pr) in the development of dilated ascending aorta in humans. Another recent study involving 21 diabetic patients and animal models showed that tropomodulin-3 propionylation in platelets was correlated with platelet hyperactivation and promoted thrombosis risk, which resulted from the elevated level of branched-chain amino acids (BCAA) in patients' plasma (Fig. 2).[12] Besides, increased protein propionylation contributed to mitochondrial respiration dysfunction, and propionate-induced propionylation of manganese superoxide dismutase (MnSOD), a reactive oxygen species (ROS) scavenger, could repress CVDs.[13,60,61]

These findings indicate that the propionylation of histone and non-histone proteins has potential roles in cardiovascular diseases, and further studies are needed to investigate the therapeutic value of targeting propionylation for the treatment of specific CVDs, such as dilated ascending aorta and thrombosis-related diseases.

Malonylation

Malonyl-CoA is the direct donor of lysine malonylation (Kmal), which is degraded into acetyl-CoA by malonyl-CoA decarboxylase (MCD) (Fig. 2). Elevated malonyl-CoA and protein malonylation impair mitochondrial function and fatty acid oxidation in MCD-deficient cells.[62] MCD deficiency is a rare congenital defect of metabolic disorder characterized by a variable phenotype of developmental delay, seizures, cardiomyopathy, and acidosis.[2] Inhibition of MCD increases the production of myocardial malonyl-CoA, an endogenous inhibitor of mitochondrial fatty acid uptake, which can increase the oxidation rate of pyruvate, ultimately decreasing toxic metabolic byproducts (lactate and protons) to augment cardiac function and cardiac efficiency and preventing myocardial ischemic injury in animal models.[2,63,64] The pro-hypertrophic factor SIRT4 controls the level of malonyl-CoA,[61,63] which may contribute to its role in regulating cardiac remodeling.

Malonylation is a key mechanism of malonyl-CoA in physiological and pathological processes. Demalonylase is critical for maintaining cardiovascular homeostasis. SIRT5 functions as a nicotinamide adenine dinucleotide (NAD^+)-dependent protein, demalonylase. Upon cardiac

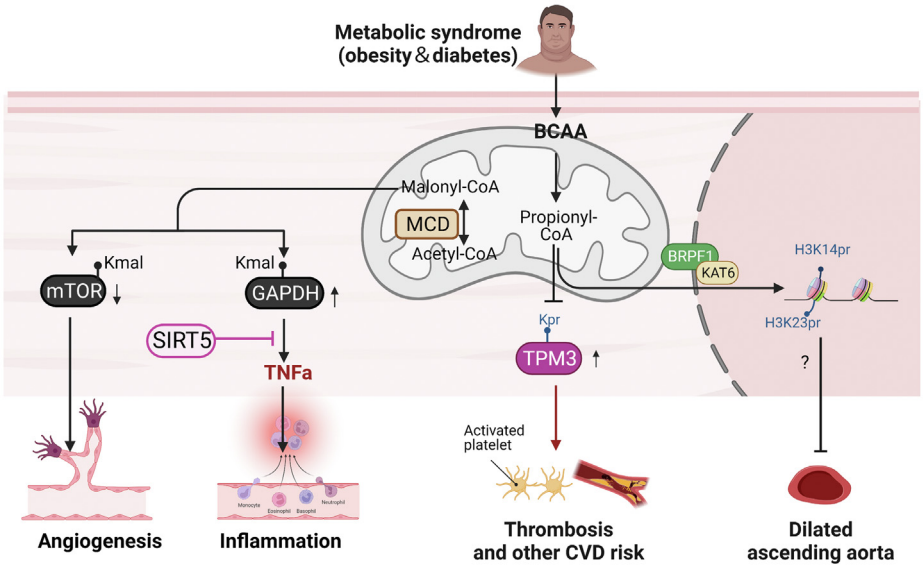

***Fig. 2:* Histone propionylation and malonylation regulate cardiovascular homeostasis.** Metabolic syndromes, such as diabetes, induce an increase in circulating BCAA, which contributes to increased propionyl-CoA via unknown mechanisms. Propionyl-CoA promotes propionylation and activation of TPM3, thereby increasing platelet activation and the risk of thrombosis. Histone propionylation is mediated by the BRPF1-KAT6 complex, the deficiency of which induces a dilated ascending aorta. In the cytoplasm, malonylation of mTOR regulates its role in angiogenesis, while malonylation of GAPDH modulates its role as an RNA-binding protein in inflammation regulation. BCAA, branched-chain amino acids; TPM3, tropomyosin 3; BRPF1, bromodomain and PHD finger-containing protein 1; KAT6, lysine acetyltransferase 6; mTOR, mammalian target of rapamycin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TNFa, tumor necrosis factor-alpha.

injuries such as pressure overload or ischemia, SIRT5 in myocardial tissues is reduced, leading to the hypermalonylation of both cytosolic and mitochondrial proteins to regulate metabolic reprogramming, and sequentially facilitating the development of CVDs, including cardiac hypertrophy and myocardial infarction in mice.[50,63,65,66] In endothelial cells, fatty acid synthase deficiency can elevate malonyl-CoA levels, causing malonylation of the mammalian target of rapamycin (mTOR) at lysine 1218 (K1218). mTOR K1218 malonylation impairs mTOR complex 1 kinase activity, eventually leading to angiogenic defects,[15] an event involved in myocardial infarction and tissue regeneration. Furthermore, malonylation of immune cells may contribute to the development of CVDs. A recent study documented that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) undergoes malonylation on lysine 213, leading to its dissociation from *tumor necrosis factor-alpha* (*TNFα*) mRNA, and promoting translation, thus contributing to macrophage activation and inflammation.[16] Since macrophage activation and inflammation are key mechanisms involved in cardiovascular remodeling, GAPDH malonylation may be one of the mediators.

Taken together, these findings suggest that malonyl-CoA and protein malonylation may be involved in the development of CVDs, such as angiogenic defects, cardiac hypertrophy, and myocardial infarction, by regulating malonylation within myocytes, endothelial cells, and immune cells (Fig. 2). Interestingly, accumulating evidence has shown that malonyl-CoA donor, malonate, acts as a cardioprotective factor by improving cardiomyocyte proliferation and regeneration in myocardial infarction and reperfusion injury in animal models.[67–69] Thus, malonate may have some therapeutic value for cardiac diseases; however, clinical evidence is limited. Interestingly, the cardioprotective functions of malonate rely on its inhibition of succinate dehydrogenase,[67–69] and whether malonylation contributes to this potential function of malonate remains elusive because protein malonylation is generally considered an unfavorable factor for CVDs.

Lactylation

Metabolic reprogramming during cardiovascular remodeling generally augments glycolysis in cardiovascular cells, which is a hallmark feature of cardiomyocytes in failing hearts and smooth muscle cells in aortic diseases.[70,71] Augmented glycolysis and lactate uptake lead to increased intracellular lactate, which is converted into lactyl-CoA via an unknown mechanism and contributes to both histone and non-histone lactylation (Fig. 3). Although the source of lactyl-CoA is still under debate, current studies suggest that lactylation may contribute to the effects of histone lactyltransferases and delactylases in cardiovascular biology.

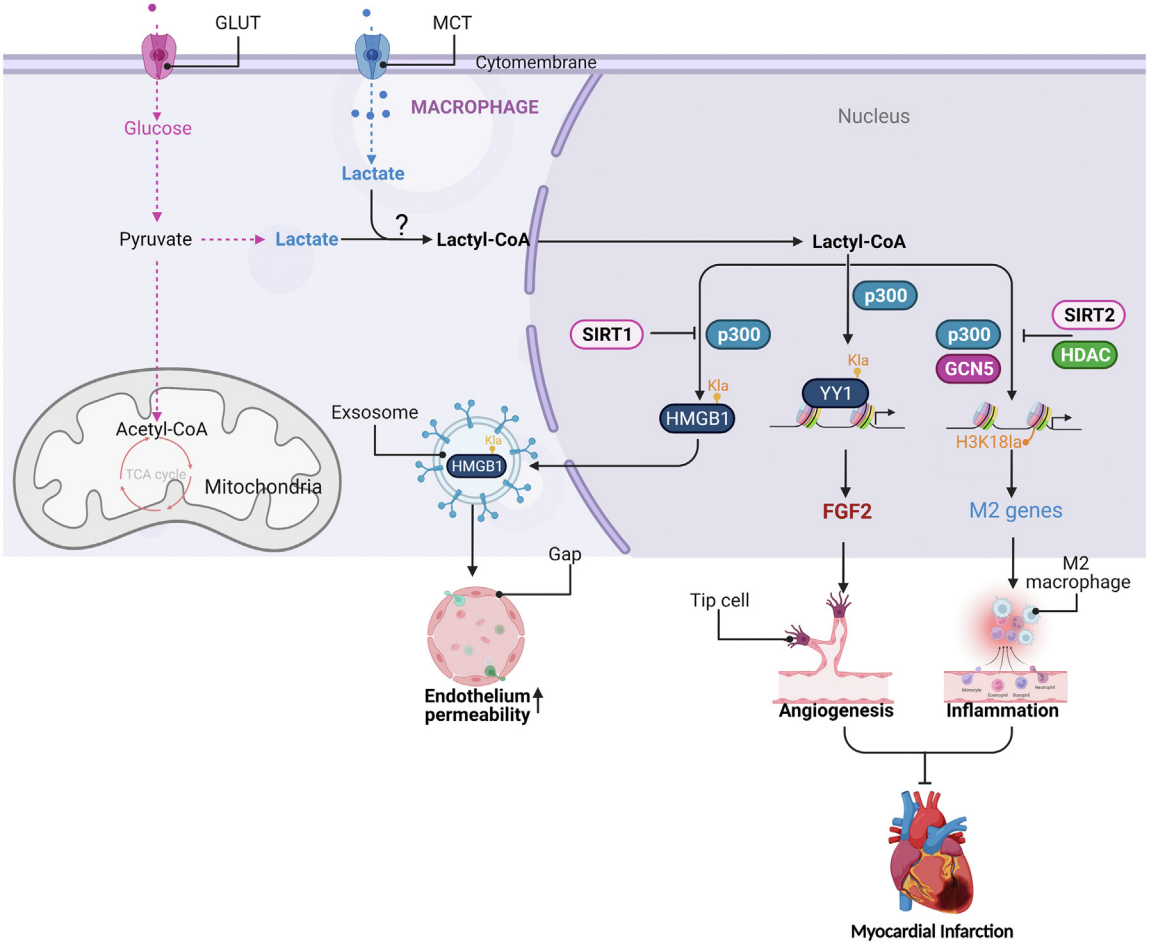

***Fig. 3:* Lactylation regulates vascular function and inflammation.** Lactate from glycolysis and excellular transportation leads to the increase of lactyl-CoA via an unknown mechanism. Lactyl-CoA contributes to lactylation of histone and non-histone proteins in macrophages, reprogramming paracrine factors (FGF2), exosomes (HMGB1), and transcriptome (M2-like gene signature) of macrophages to regulate vascular function (angiogenesis, permeability) and chronic inflammation (M2 macrophages), which may contribute to cardiovascular injury and regeneration. GLUT, glucose transporter; MCT, monocarboxylate transporter; FGF2, fibroblast growth factor 2; YY1, Yin Yang 1; HMGB1, High-mobility group box 1.

Lactylation critically participates in immune cell functions, such as macrophages. During macrophage polarization, histone H3 lysine 18 lactylation (H3K18la) catalyzed by P300/MOF/GCN5 is enriched in promoter regions and stimulates M2-like gene transcription.[19,21] Most genes marked by increased H3K18la are specific, and H3K18la-specific genes are different from H3K18ac.[19] Histone lactylation activates reparative gene activation in macrophages to boost cardiac repair post–myocardial infarction.[21] Lactylation of non-histones is also important. Under inflammatory conditions, lactylated HMGB1 is released from macrophages via exosome secretion to increase endothelial permeability in mice,[22] which may contribute to inflammatory vascular diseases. In the microglia (macrophages in the brain) of injured brains, lactylation of the transcription factor Yin Yang-1 (YY1) at lysine 183 (K183) triggers the transcription of fibroblast growth factor 2 (FGF2) to promote the angiogenesis of endothelial cells, which is critical for retinal neovascularization in animal models.[72]

In addition, lung myofibroblasts can secrete lactate into the extracellular milieu, and lactate-induced histone lactylation in the promoters of profibrotic genes in macrophages contributes to lung fibrosis.[73] Thus, histone lactylation may also cause the immune system and fibroblasts to participate in the development of inflammatory CVDs such as heart failure and atherosclerosis. Moreover, H3K18la directly stimulates the transcription of p53, which is a key regulator of senescence of endothelial cells and vascular smooth muscle cells (VSMCs) in CVDs.[19]

Taken together, via multiple mechanisms, high-level lysine lactylation regulates histones (*e.g.*, H3K18) and

non-histones (*e.g.*, YY1 and HMGB1) to modulate macrophages, fibroblasts, and endothelial cells to regulate vascular function (angiogenesis, permeability) and chronic inflammation (M2-like macrophages), consequently contributing to cardiovascular injury and regeneration. Thus, the repression of lactylation by inhibiting lactate uptake with monocarboxylate transporter 1 (MCT1) inhibitors or inhibiting intracellular generation with pyruvate dehydrogenase (PDH) inhibitors may serve as potential strategies for treating CVDs. However, several questions remain unanswered. An example is whether lactylation writers and erasers contribute to CVDs. For instance, whether the cardioprotective role of SIRT2, a histone delactylase,[20] relies on its delactylase activity is also interesting.

Butyrylation and isobutyrylation

A recent study found that H3K9bu is a major histone butyrylation that is enriched at the transcription start sites. Butyrate-induced higher levels of histone Kbu and H3K9bu antagonize diabetic renal inflammation and fibrosis. Inhibition of histone Kbu with the histone modification enzyme p300 inhibitor A485 reversed the anti-inflammatory and anti-fibrosis effects of butyrate.[74] Besides, higher levels of H3K9bu modulate transverse aortic constriction-induced reprogramming of gene expression to inhibit hypertrophic growth of the heart and protect cardiac ejection fraction in mice.[24] Since a high-fat diet has been reported to reduce the H3K9bu levels in the heart,[24] obesity may contribute to the development of CVDs by reducing H3K9bu abundance. The semisynthetic derivative LTK-14A of garcinol specifically inhibits histone butyrylation (H4K5bu) without affecting acetylation in adipocytes and leads to a decrease in body weight in high-fat-induced obesity mice.[75] The roles of histone butyrylation in CVDs and risk factors are complex, and further studies are needed to determine the biological functions of individual butyrylation sites in certain cell types and organs to elucidate how histone butyrylation plays complex roles in CVDs.

In addition to butyrylation, lysine isobutyrylation was recently identified as a novel histone modification marker. RNA-Seq profiling revealed that isobutyryl-CoA donor isobutyrate affects the transcriptional profile and CVD-associated pathways, such as extracellular matrix (ECM) remodeling and cardiac muscle contraction,[25] strongly suggesting that histone isobutyrylation may participate in the development of CVDs. However, further direct evidence is needed to elucidate whether and how histone butyrylation and isobutyrylation participate in cardiovascular biology.

The therapeutic value of butyrate, which may elevate butyrylation, is also of interest. Butyrate, by repressing inflammation, could inhibit vascular diseases such as arterial injury, atherogenesis, and pulmonary hypertension in preclinical models.[76–79] Further studies are needed to validate this finding in large animal and human populations and to test the involvement of protein butyrylation.

β-Hydroxybutyrylation and 2-hydroxyisobutyrylation

Histone lysine β-hydroxybutyrylation (Kbhb) is a marker enriched in the promoters of active genes, and H3 lysine 9 β-hydroxybutyrylation (H3K9bhb) production is significantly induced under conditions of starvation or diabetic ketosis, which is associated with the upregulation of genes involved in metabolic pathways, such as amino acid catabolism, peroxisome proliferator-activated receptor (PPAR) signaling pathway, and oxidative phosphorylation.[26] Therefore, H3K9bhb may regulate metabolic pathways involved in the development of CVDs. Furthermore, p53 β-hydroxybutyrylation catalyzed by CBP attenuated its activity, resulting in lower levels of p53 acetylation and reduced expression of the p53 downstream genes p21 and P53 up-regulated modulator of apoptosis (PUMA), as well as reduced cell growth arrest and apoptosis.[27] Thus, dysregulated p53 β-hydroxybutyrylation may be involved in the uncontrolled proliferation of smooth muscle cells in vascular diseases, such as pulmonary arterial hypertension.

β-Hydroxybutyrate can be transformed into β-hydroxybutyryl-CoA and contributes to β-hydroxybutyrylation. Furthermore, β-hydroxybutyrate can repress oxidative stress and inflammation, preventing heart failure with preserved ejection fraction (HFpEF) in a 3-Hit mouse HFpEF model induced by combining age, long-term high-fat diet, and desoxycorticosterone pivalate challenge.[80] β-hydroxybutyrate can modulate different types of histone acylations (β-hydroxybutyrylation and acetylation) or serve as an inhibitor of HDAC to repress oxidative stress,[80–82] thus β-hydroxybutyrate may repress HFpEF via multiple mechanisms. Overall, β-hydroxybutyrate may be a promising drug for treating CVDs such as HFpEF. β-Hydroxybutyrate-mediated β-hydroxybutyrylation is also involved in postnatal heart development by promoting cardiomyocyte mitochondrial maturation and metabolic reprogramming.[83] Thus, β-hydroxybutyrate and related β-hydroxybutyrylation are essential for cardiac development and disease prevention.

Approximately half of newly identified histone Khib residues are not known to be modified by Kac and Kcr, and 2-hydroxyisobutyrylation of histone H4 lysine 8 (H4K8hib) is associated with active gene transcription in meiotic and post-meiotic cells.[28] A total of 6548 Khib sites on 1725 non-histone proteins have been identified, and Khib was found to be closely associated with transcription, translation, protein degradation, and energy metabolism.[46] P300-catalyzed, Khib-specific modification of metabolic enzymes (*e.g.*, ENO1) regulates cellular glucose metabolism,[29,84] suggesting that p300 may modulate cellular metabolic homeostasis through Khib.

In rats with vascular dementia, donepezil treatment improves cognitive function, possibly by reducing aberrant acylations, such as 2-hydroxyisobutyrylation.[85] Protein lysine 2-hydroxyisobutyrylation contributes to the development of end-stage renal disease,[86] suggesting its potential role in the regulation of kidney dysfunction and related hypertensive syndrome. Overall, protein 2-hydroxyisobutyrylation is important for fundamental cell function; however, their involvement in cardiovascular biology and diseases remains unclear.

Crotonylation and methacrylation

Histone Kcr has been reported to participate in multiple biological processes and diseases, including nephropathy, depression, HIV latency, cancer, spermatogenesis, neurobiology, and fibrosis.[52,87,88] The role of Kcr in the pathophysiological processes of CVDs has been identified.

Mutations in the crotonyl-CoA regulator ECHS1 (enoyl-CoA hydratase, short chain 1) in humans can cause cardiomyopathies (>60%), including hypertrophic and dilated cardiomyopathies.[31] Our current work revealed that the ECHS1 expression level was reduced in human hypertrophic hearts, which was coupled with elevated histone hypercrotonylation (*e.g.,* H3K18cr and H2BK12cr). In rodents, the deficiency of *Echs1* markedly increased the H3K18cr and H2BK12cr levels, promoting the recruitment of the transcription factor nuclear factor of activated T-cell C3 on the promoters to activate the transcription of pro-hypertrophic genes, such as B-type natriuretic peptide.[32] *Echs1* deficiency also led to the activation of myofibroblast and cardiac fibrosis in aged mice.[89] Thus, histone Kcr critically contributes to cardiac development and hypertrophic remodeling and may serve as a potential target for the treatment of cardiac hypertrophy and heart failure (Fig. 4).

In addition to histone Kcr, non-histone protein Kcr is required for cardiomyocyte contractility, including mitochondrial and cytoskeleton proteins involved in cardiac ischemia-reperfusion (I/R) injury in mice. Modulating site-specific Kcr of selected mitochondrial protein isocitrate dehydrogenase 3 [NAD+] alpha (IDH3a) at K199 and cytoskeletal protein tropomyosin alpha-1 chain (TPM1) at K28/29 protects cardiomyocytes from apoptosis induced by isoprenaline and cardiac function after I/R injury.[33] It is important to consider that the conclusion largely depends on the mutation of substrate proteins. Whether the mutated lysine sites of IDH3a or TPM1 can be acetylated remains elusive, which is important for excluding the contribution of IDH3a and TPM1 acetylation to the phenotypes observed in this study. Further studies are needed to test whether the therapeutic effects of crotonate on I/R injury require protein crotonylation because of the complex metabolism of crotonate within cells.

Methacrylation is a recently identified crotonylation-derived acylation. Histone lysine methacrylation has been reported recently, and SIRT2 has been reported to serve as a demethacrylase[34]; however, the physiological and pathological functions of protein methacrylation are not clear. Our previous study reported that SIRT2 prevented cardiac remodeling and aging,[90] thus it is interesting to test whether SIRT2 acts as a demethacrylase to perform its cardioprotective role.

Succinylation

Histone and non-histone succinylation have been observed.[49,50] Succinylation is more likely to occur in mitochondrial proteins in some eukaryotic organisms because succinyl-CoA is an essential intermediate metabolite at the central nodes of the TCA cycle.[2,63,91] The protein Ksucc is regulated by SIRT5 and is involved in energy metabolism (Fig. 5). SIRT5 mediates the Ksucc of cytoplasmic and mitochondrial proteins, contributing to oxidative phosphorylation, fatty acid oxidation, ketogenesis, and branched-chain amino acid catabolism.[35]

With the development of the newborn's heart, the level of succinylation gradually increases, which may play an important role in controlling the shift from glycolysis to fatty acid oxidation.[92] SIRT5 may contribute to the maintenance of protein succinylation. In mice, succinyl-CoA and protein succinylation accumulate predominantly in SIRT5-deficient hearts. SIRT5-deficient mice exhibit defective fatty acid metabolism, decreased ATP production, and hypertrophic cardiomyopathy with aging.[50] In addition, SIRT5 deficiency increases the succinylation of succinate dehydrogenase, which increases its activity and the incidence of ischemia-reperfusion injury.[35] Notably, SIRT5-overexpressing mice showed a decrease in the total Ksucc levels in the heart, which can preserve cardiac function and suppress fibrosis in response to pressure overload.[50] In the brain, SIRT5-mediated desuccinylation restored energy metabolism and protected the mouse brain against subarachnoid hemorrhage, a risk factor for increased intracranial pressure (ICP) and reduced cerebral perfusion.[48] Thus, inhibition of protein succinylation with SIRT5 activators may serve as a promising strategy to improve metabolism and treat cardiac diseases such as cardiac hypertrophy and ischemic injury in the heart and brain.

Interestingly, the myofibril protein Ksucc levels decrease due to decreased succinyl-CoA production and increased succinyl-CoA turnover in patients with ischemic heart failure,[36] Further preclinical studies are needed to elucidate the roles of the myofibril protein Ksucc and their underlying mechanisms. Recently, serum protein succinylation has been identified, and the succinylation level of serum protein is decreased in humans and rats with acute myocardial infarction.[17] Serum biomarkers are important for the diagnosis and prognosis prediction of many diseases; thus, the

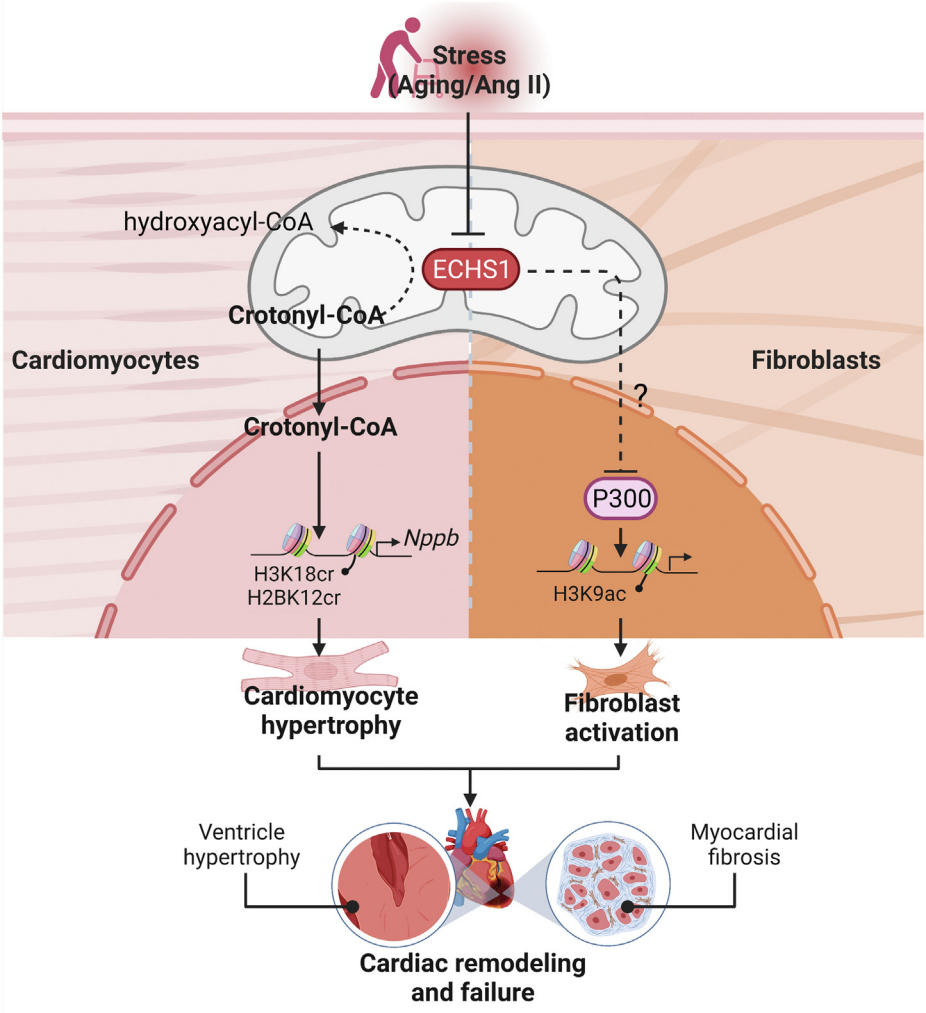

***Fig. 4:* Histone crotonylation contributes to cardiac remodeling and heart failure.** Aging and other stress such as angiotensin II (Ang II) decrease the expression of mitochondrial ECHS1, which is a short-chain enoyl-CoA hydratase participating in the catabolism of short-chain fatty acids. The deficiency of ECHS1 in cardiomyocytes increases the level of crotonyl-CoA, which promotes histone crotonylation and transcription of genes involved in cardiomyocyte hypertrophy. In fibroblasts, ECHS1 deficiency promotes the nucleus translocation of P300, leading to hyper-acetylation of histone and activation of fibroblast. ECHS1 deficiency induces cardiomyocyte hypertrophy and fibroblast fibrosis contributes to cardiac remodeling and failure. ECHS1, enoyl-CoA hydratase, short chain 1; Nppb, B-type natriuretic peptide.

sensitivity of serum protein succinylation should be tested as a biomarker of acute myocardial infarction.

In addition to desuccinylation, SIRT5 also deglutarylates isocitrate dehydrogenase 2 and glucose-6-phosphate dehydrogenase, thus activating both nicotinamide-adenine dinucleotide phosphate (NADPH)-producing enzymes to maintain cellular NADPH homeostasis and redox potential during oxidative stress[38]; This suggests that Kglu may be one of the mechanisms underlying the function of SIRT5 in the development of CVDs.

Therefore, succinylation and related enzymes (*e.g.*, SIRT5) play important roles in cardiovascular homeostasis. Further studies are required to explore critical functional targets regulated by succinylation during the development of CVDs.

Outstanding questions

Individual and systemic functions of different acylations in cardiovascular biology

A variety of novel PTMs (>10 types) have recently been identified[7]; however, most studies on PTMs in CVDs are phenotype-descriptive, and the underlying mechanisms of PTMs in CVDs remain unknown. Further studies are required to elucidate the functions and intrinsic mechanisms of individual PTMs. For instance, lysine

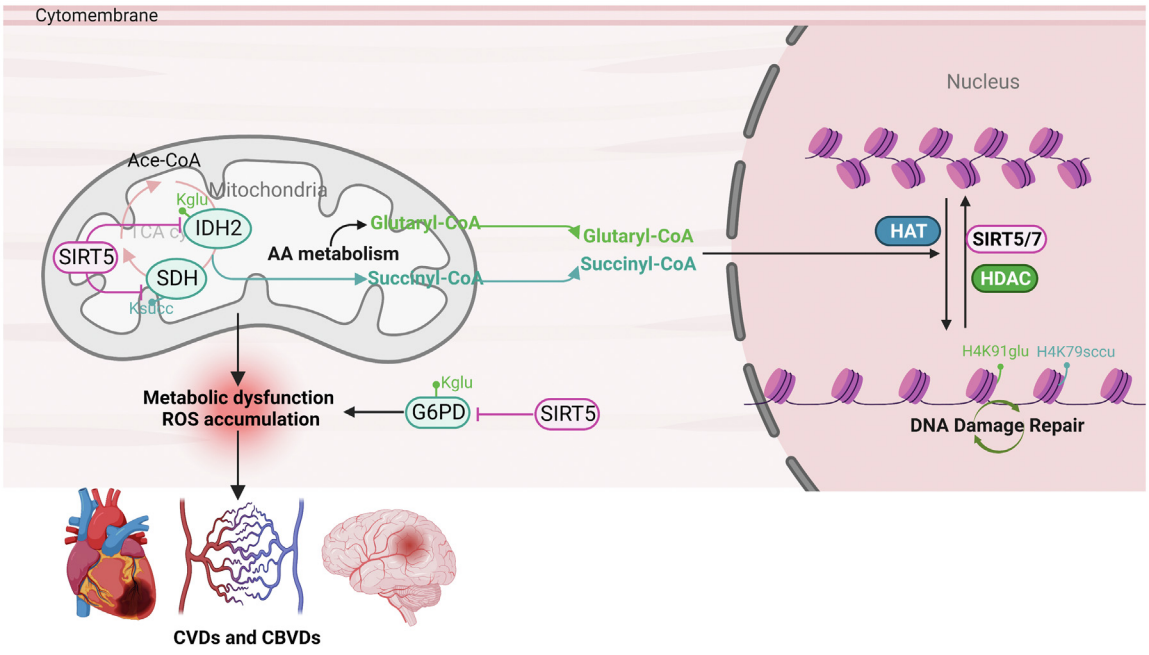

***Fig. 5:* Succinylation and glutarylation in cardiovascular diseases.** Succinylation and glutarylation were regulated by mitochondrial sirtuins. In animals, the deficiency of mitochondrial enzyme SIRT5 causes hyper-succinylation/glutarylation of mitochondrial metabolic enzymes (*e.g.,* SDH, IDH2, G6PD) and antioxidants, leading to metabolic dysfunction and ROS accumulation in cardiomyocytes and vascular cells, subsequently resulting in the development of cardiovascular (CVDs) and cerebrovascular diseases (CBVDs). MCD, malonyl-CoA-decarboxylase; SDH, succinate dehydrogenase; IDH2, isocitrate dehydrogenase 2; G6PD, glucose-6-phosphate dehydrogenase.

benzoylation is a histone marker controlled by SIRT2.[39] However, the physiological and pathological functions of this modification and its roles in cardiovascular biology remain largely unknown. Previous studies have highlighted the core contributions of acetylation of histone and non-histone proteins to CVDs; it remains unknown whether different types of acylation contribute equally to the development CVDs.

In addition, there are many types of acylations and PTMs, such as methylation, O-GlcNAcylation, ADP-ribosylation, and glutathionylation. However, it remains unknown which of these are functionally important. If functional importance is identified, it is essential to explore how these different PTMs act synergistically or antagonistically in cardiovascular homeostasis and diseases in a spatiotemporal manner. For instance, histone acetylation and crotonylation differentially regulate cell metabolism by binding to different regions of the chromatin in stem cells,[9,10] and crotonylation of histones may regulate their methylation by recruiting EZH2 (enhancer of zeste homolog 2) to function in the medial prefrontal cortex.[93] These synergistic mechanisms may also be involved in cardiovascular biology and diseases.

Studies of the individual and systemic functions of different acylations in cardiovascular biology are largely limited by the methodologies used to study these non-acetyl PTMs. For instance, the low endogenous level of benzoylation makes it difficult to study their functions in cardiovascular biology.[39] Besides, the established ChIP-grade anti-histone crotonylation antibodies do not work well for ChIP-seq analysis,[32,94] and ChIP-grade antibodies for many types of histone acylations (such as site-specific benzoylation antibodies) have not been developed. It is difficult to identify specific PTM residues that are crucial for cardiovascular physiology and pathology *in vivo* because of the fast-changing nature of some types of modifications such as O-GlcNAcylation.[95] Therefore, advances in experimental methodologies would promote studies of acylations in cardiovascular biology.

Functions of acylations in different niche cells and organs

Metabolic dysfunction often occurs during the development of CVDs, which results in changes in cellular metabolites and acyl-CoA, the local microenvironment, and circulation. Although several studies have investigated the role of acylations in cardiomyocytes and vascular cells, the role of these acylations in niche cells remains largely unknown. Fibroblast activation and immune cell recruitment and polarization are critically involved in cardiac remodeling.[96] The role of acylation in the regulation of these stromal cells remains unclear.

Covering these gaps would provide an extensive understanding of niche cells in response to local and systemic metabolism in cardiovascular biology and disease.

In addition, cardiovascular biology and diseases are regulated by other organs, such as the lung, kidneys, liver, and gut microbiota. The gut may regulate the intake of SCFAs (e.g., propionate and butyrate) to regulate CVDs and their risk factors such as diabetes. Therefore, it would be interesting to test how these organs regulate CVDs by targeting acylations in cardiovascular parenchymal cells and niche cells.

Mitochondrial control of epigenetics in cardiovascular biology

Mitochondria are the core factories of metabolism and are responsible for the production of metabolites that serve as donors of PTMs, such as acylations. Mitochondrial dysfunction may lead to the accumulation of metabolic intermediates that can be transported out of the mitochondria and into the nucleus, where they serve as donors for unbalanced histone and non-histone modifications. Interestingly, non-classical TCA cycle and TCA cycle-associated enzymes have been identified in the nucleus, which is implemented mainly to generate metabolic intermediates to maintain the homeostasis of histone modifications in cancer cells and cardiomyocytes.[97,98]

Cardiomyocytes have a high volume of mitochondria, which are critical for cardiac function and disease progression. Even low-mitochondria-containing endothelial cells are critically regulated by mitochondrial signals.[99] Monitoring the extent to which mitochondria determine the epigenetic and transcriptomic reprogramming that occurs in the nucleus in cardiovascular cells would help in understanding mitochondria-associated PTMs in cardiovascular parenchymal cells and niche cells.

Therapeutic targets and clinical translation

Which acylation to target and how to target it for treating CVDs remain important considerations. Phosphorylation is not dependent on the levels of the substrate (*e.g.,* ATP), but on the activity of kinases or phosphatases. In contrast to phosphorylation, some studies have suggested that acylations mainly depend on the substrate level rather than the enzyme activity of acyltransferases and deacylases, suggesting that these writers/erasers are not sufficiently important.[3,8] On the other hand, many acylations share the same writers, readers, and erasers, and some different acylations have the same modified residues and effects on the functions of proteins and cells, indicating that these enzymes are functionally important. This situation has confused the scientific community. The pathological events during the development of CVDs in humans, including whether and how donors and enzymes contribute to CVDs, require further investigation. These studies may help to identify effective targets.

Based on our previous findings and those of other studies, either changing substrates such as crotonyl-CoA or intervening with epigenetic modifiers such as sirtuins, can lead to significant changes in the disease phenotypes in patients and preclinical animals with CVDs. These facts suggest that PTMs can be targeted to treat CVDs; pragmatically, some drugs have been developed (*e.g.,* SIRT1 activator [SRT1720], SIRT6 activator [MDL-800], and MCD inhibitor [CBM-301106]).[54,100,101] In addition, metabolites that regulate acylations may have cardiovascular protective effects. Examples include malonate, butyrate, and β-hydroxybutyrate.[2,68,80] Thus, it is meaningful to test their translational value for treating CVDs.

An increasing number of studies have identified the potential involvement of new acylations in CVDs in humans and animals. However, clinical evidence seems limited, and further large cohorts are needed to test the prognostic and therapeutic values of protein acylations in each type of CVDs. Meanwhile, there are no drugs that can affect specific acylations; thus, it is valuable to test pan-activators/inhibitors that may change acylation omics to treat CVDs. Achieving this goal may appear difficult, but we have started the first step in this expedition of clinical applications.

Conclusion

Increasing evidence has shown that non-acetyl acylations are involved in cardiovascular biology and disease. These acylations not only affect the progression of CVDs, but are also involved in CVD-associated syndromes, such as obesity, insulin resistance, diabetes, inflammation, thrombosis, and other processes. These facts have challenged the current paradigm of acetylation in CVDs. The significance of these acylations in CVDs suggests that targeting these modifications to treat such diseases is reasonable. Therefore, frontiers in the acylation-mediated regulation of CVDs are timely; however, much work is still needed to investigate the functions, mechanisms, and therapeutic values of protein acylations in CVDs. A better understanding of protien acylations is needed to guide the design of acylation-targeted therapeutics for CVDs.

Search strategy and selection criteria

Data for this review were obtained from PubMed and Google Scholar using the search terms “formylation”, “acetylation”, “propionylation”, “malonylation”, “lactylation”, “butyrylation”, “isobutyrylation”, “β-hydroxybutyrylation”, “2-hydroxyisobutyrylation”, “crotonylation”, “methacrylation”, “succinylation”, “glutarylation”, “benzoylation”, “myristoylation”, “aminoacylation”, “acylation”,

"hypertension", "ischemic cardiomyopathy", "cardiac hypertrophy", "cardiac fibrosis", "angiogenesis", and "inflammation". Articles published between 2005 and 2023 were included with particular emphasis on those published in the past three years.

Contributors

Xin Sun, Yang Zhang, and Xiao-Feng Chen analyzed all literature and prepared the manuscript. Xiaoqiang Tang conceived the study and revised the manuscript. All authors read and approved the final version of the manuscript.

Declaration of interests

The authors declare that there is no conflict of interest.

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