Biochemistry and Biophysics Reports 38 (2024) 101694

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Protein crotonylation: Basic research and clinical diseases

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ARTICLE INFO

Keywords:
Post-translational modifications
Protein crotonylation
Cancer
Diseases

ABSTRACT

Crotonylation is an importantly conserved post-translational modification, which is completely different from acetylation. In recent years, it has been confirmed that crotonylation occurs on histone and non-histone. Crotonylated Histone primarily affects gene expression through transcriptional regulation, while non-histone Crotonylation mainly regulates protein functions including protein activity, localization, and stability, as well as protein-protein interactions. The change in protein expression and function will affect the physiological process of cells and even cause disease. Reviewing previous studies, this article summarizes the mechanisms of histone and non-histone crotonylation in regulating diseases and cellular physiological processes to explore the possibility of precise regulation of crotonylation sites as potential targets for disease treatment.

1. Introduction

With the development of human understanding on protein function and biological mechanisms, the importance of post-translational modifications (PTM) of proteins has increased greatly. Various modifications of different amino acid residues have been reported on histone, such as histone methylation [1], formylation, acetylation, butyrylation, lactylation [2], and crotonylation [3]. Histone crotonylation is conserved in eukaryotes such as yeast [4] and mammals, but there are few reports about crotonylation in rice [5], tea plants [6], and papaya [7]. Histone crotonylation usually occupies a similar position as acetylation, but it is mechanistically and functionally distinct from histone lysine acetylation (Kac) because of the extended hydrocarbon chains and *C*–C π-bond in Kcr chemical structures [8]. This dynamic process is regulated by crotonyltransferases ("writer") and decrotonylases ("eraser") together [8], and the function of proteins to recognize Kcr are identified by "reader". Crotonylation of histone occurs mainly on lysine residues, and affects gene expression. Studies have reported that histone crotonylation plays vital roles in human physiological developments such as gene expression [8,9], cell cycle [10], DNA damage [11], aging [12], and spermatogenesis [13,14]. In addition, in recent years, it has been found that crotonylation occurs on histone as well as on non-histone proteins [15]. This modification can affect protein functions including protein localization, activity [16], and stability, as well as protein-protein interactions. Furthermore, crotonylation affects protein activity by modifying the serine of proteins [17]. Protein crotonylation is very important for the growth and development of organisms, participating in embryonic cell differentiation and division [18,19], maintaining metabolic homeostasis [20], and other physiological processes. Crotonylation of protein could also affect the development and progress of diseases, such as tumors, kidney diseases, heart diseases, etc.

To better understand the biological function of crotonylation in physiological processes and its roles in disease development, this review will focus on the diseases and biological processes related to histone and non-histone crotonylation, and elaborate its mechanism in physiological processes and diseases, which may provide new insights into the

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https://doi.org/10.1016/j.bbrep.2024.101694
Received 7 December 2023; Received in revised form 18 March 2024; Accepted 19 March 2024

epigenetic research of related diseases.

2. Regulation of crotonylation

Crotonylation is a histone post-translational modification first reported by high-sensitivity mass spectrometry in 2011 [8]. Protein crotonylation level in cells was precisely regulated by the activity of crotonyltransferases (writer) and decrotonylases (eraser), and can be influenced by the concentration of crotonyl-CoA in cells, therefore, enzymes that regulate crotonyl-COA metabolism are also involved in the regulation of crotonylation (Fig. 1).

2.1. Crotonyl-CoA

Protein crotonylation, is derived from crotonyl-CoA, an intermediate metabolite during fatty acid oxidation or lysine and tryptophan metabolism. Intracellular crotonyl-CoA could directly regulate Kcr [21], and is crucial for mesoderm/endoderm differentiation [22]. Crotonyl-CoA can be maintained at a relatively suitable concentration through various metabolic pathways: (1). Crotonate can be effectively converted to crotonyl-CoA by ACSS2 (acyl-CoA synthetase short chain family member 2), which is related to the level of crotonyl-CoA and crotonylation in the body [23], knock down ACSS2 in cultured cells notably changed the level of histone crotonylation[9]. (2) In amino acid metabolism, lysine, hydroxylysine, and tryptophan are metabolized to glutaryl-CoA, then glutaryl-CoA is oxidized to crotonyl-CoA, and this process is catalyzed by GCDH (glutaryl-CoA dehydrogenase). (3) The short-chain fatty acids (SCFAs) produced by microbiota are transported into cells via membrane receptors MCT or SMCT, and then converted into butyryl-CoA through β-oxidation pathway, which is then transformed into crotonyl-CoA by BCDH, moreover, ACADS [24], ACOX3 [22] and ACOX1 involved in crotonyl-CoA metabolism synthesis. ACOX2 is a indirect regulator of lysine crotonylation through interacts with methyl crotonyl coA carboxylase (MCCC1/2) and inhibits its enzyme activity [20]. MCCC1/2 a heterodimer and catalyze the carboxylation of 3-methylcrotonyl-CoA to 3-methylglutaconyl-CoA [25]. (4) ECHS1 (Short-chain enoyl–coenzyme A hydratase) has the highest activity for hydrolyzing crotonyl-CoA, and reduce intracellular crotonyl-CoA [26]. The chromodomain protein CDYL converts crotonyl-CoA to β-hydroxybutyryl-CoA, which is a negative regulator of histone crotonylation [21].

Several factors are associated with the process of crotonylation, namely writers, readers and erasers (Fig. 2).

2.2. Writer

Biochemically, histone acetyltransferase p300 induces histone crotonylation [27], which generates the crotonyllysine mark on histone H3 Lys18 (H3K18). Thus the connection between crotonylation and acetylation was clarified. One study reported that the acetyltransferases hMOF [28] PCAF [18] and CBP [27]act as crotonyltransferases for non-histone proteins [29]. Another study revealed that MOF is also the histone crotonyl transferase (HCT) that catalyzes the crotonylation of histones at multiple sites on H3 and H4, and MOF possesses potent HCT activity. KAT2B (PCAF) was also identified as an HCT [30]. Furthermore, the study also generated novel CBP/p300 mutants that were defective in histone acetyltransferase but maintained normal HCT activity. Using the CBP I1432G and p300 I1395G mutants, the study not only verified the HCT of mutants but also demonstrated that the mutants correlate with the recruitment of the promoter histone crotonylation and the crotonylation reader [30]. Recently, Gcn5 and Esa1 were found to possess crotonyltransferase activity due to the lysine of Gcn5-Ada2-Ada3 (ADA) and Esa1-YnG2-Epl1 (PiccoloNuA4) being crotonylated at the *N*-terminal tail of H4 and H3 [31].

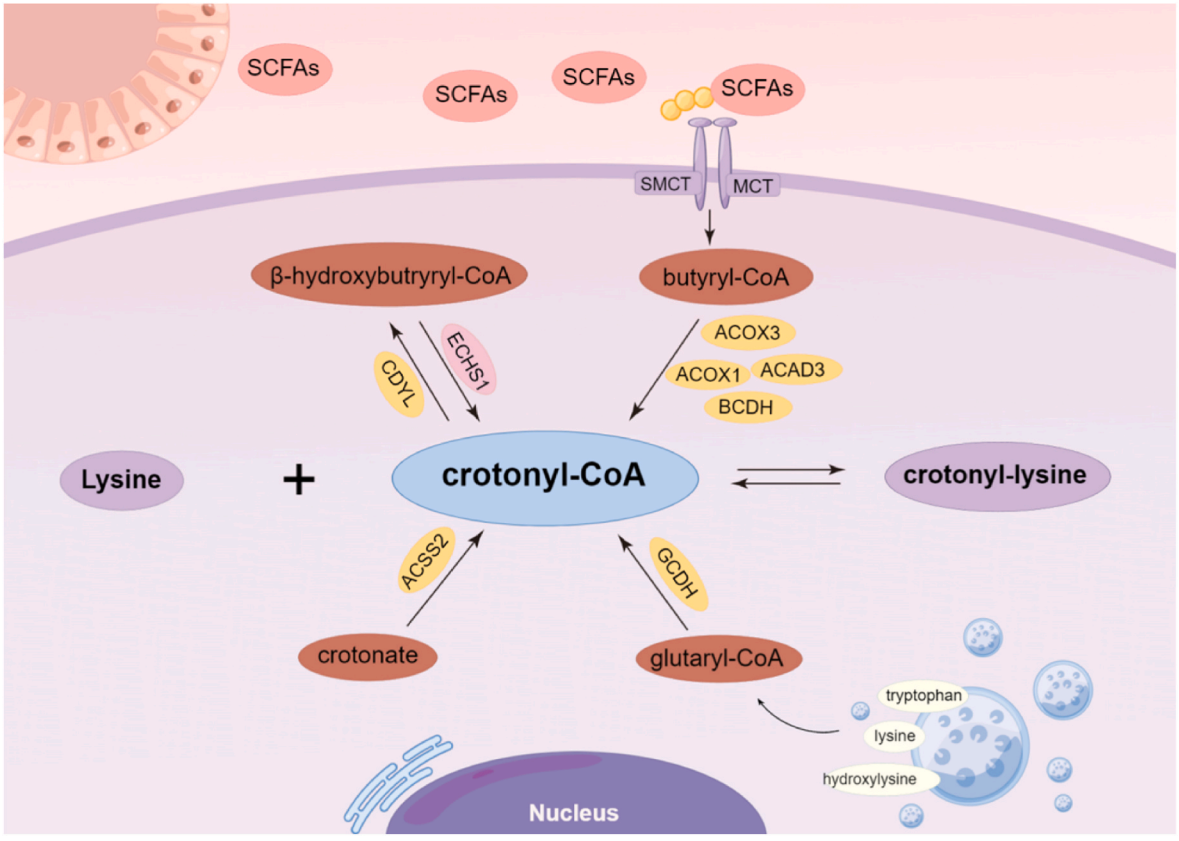

Fig. 1. Schematic representation of the chemical formula of crotonylation and the biosynthesis of crotonyl-CoA.

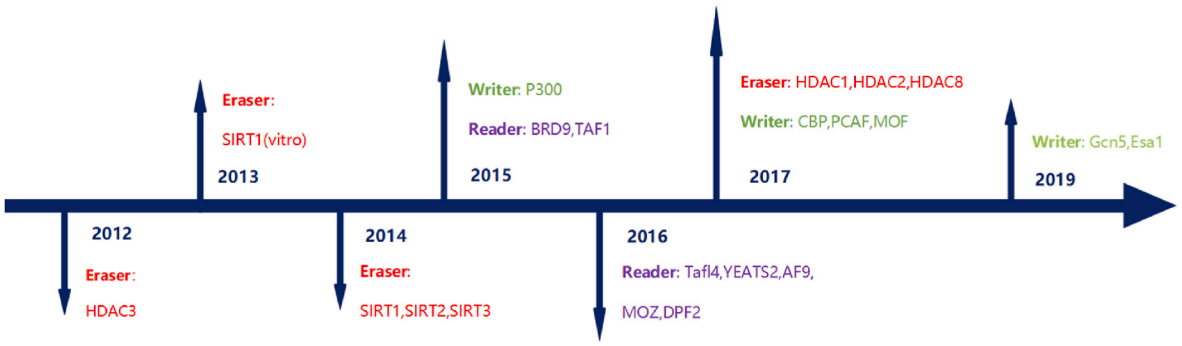

Fig. 2. Timeline depicting the discovery and characterization of crotonylation regulators.

2.3. Eraser

Eraser refers to the enzymes that can remove specific residues in proteins. In humans, histone deacetylases (HDACs) are classified into two families: the histone deacetylase family and the sirt regulator family [32]. The activity of histone deacetylase (HDCR) was found to be specific for class I histone deacetylation but not for class II and IV histone deacetylation by Rnai inactivation assay and in vitro histone deacetylation assay. For example, HDAC1 is active in decrotonylation of many proteins, such as H3K4, H3K9and H4K12. Moreover, it was confirmed to be an active HDCR enzyme [33,34]. Experiments found that HDAC2, HDAC3, and HDAC8 also could exhibit depyruvase activity in vitro [29, 35]. Moreover, SIRT1, SIRT2, and SIRT3 were all confirmed that they have the ability of histone decrotonylases in vitro [36,37]. HDAC1/2 deficiency increases histone crotonylation expression while reduces total decrotonylase activity by 85% in embryonic stem cells [38]. These results suggest that "eraser" is vitally important in crotonylation, and the underlying mechanisms need to be further explored.

2.4. Reader

The reader is to identify the functions of Kcr modification in physiology and pathology. It can be recognized by three classes of domains: Double PHD finger (DPF), Bromodomain, and YEATS domain [39]. Bromodomains weakly with a crotonylated peptide, BRD9 and TAF1 bind more tightly to acetylated peptides [40,41]. Studies have verified that Bromodomains was capable of binding to acetylated lysine residues [42]. H3K9cr was shown to be a selective target of the Yeats domain of Taf14, which binds crotonyllysine through a unique π-π-π superposition mechanism. This implies that Taf14 is an H3K9 crotonylation reader [33,43]. Another study identified YEATS2 as a histone crotonylation resolving reader with H3k27 site specificity and revealed an aromatic sandwich pocket with open ends within YEATS2 for Kcr binding [44]. Further, AF9 was found to positively regulate gene expression in the YEATS domain and colocalize with the crotonylated H3 [45]. Additionally, MOZ and DPF2 with DPF domains are specific readers of H3K14cr [46].

3. Related diseases of histone crotonylation and non-histone crotonylation

Similar to other modifications, crotonylation is closely associated with many diseases and physiological processes. And a substantial body of research has revealed protein crotonylation modifications in numerous diseases through analysis. However, only a few diseases have established a clear association between crotonylation and disease progression, and in-depth investigations into the underlying mechanisms remain limited. In this section, we primarily summarize the confirmed crotonylation in diseases (Table 1, Table 2), and the importantly crotonylated proteins and their sites (Table 3). Although clinical research on the impact of crotonylation sites on diseases is lacking, multiple animal and cell experiments have demonstrated their significance.

3.1. Cancer

With the development of medical technology, modern medicine can cure many diseases, but cancer is still the most concerned public problem. Fortunately, many recent studies have indicated that lysine crotonylation has a positive effect in the treatment of cancer [47] Report found that the lysine crotonylation expression increases in the esophagus, thyroid, pancreas, colon, and lung cancer, but decreases in the stomach, liver, kidney cancer [48]. From this, it can be seen that there is a significant correlation between crotonylation modification and tumors. However, due to the lack of clinical research data, further in-depth studies are still needed in the future. Here, we will mainly introduce the potential crotonylation modification sites have been identified in tumors.

A recent study found that HDACs inhibitor increased the crotonylation level and lysine crotonylation can affect the migration of hepatocellular carcinoma (HCC) cells and reduce the proliferation ability of hepatoma cells [48]. Furthermore, histone crotonylation in paracancerous tissues was lower than PCa tissues and Kcr increased with the enhanced malignancy of PCa [49]. Crotonylation was also observed in small cell lung cancer (SCLC) tissues, providing a new angle to understand the mechanisms of SCLC malignancy, such as metastasis, immunosuppression, and chemoradiotherapy resistance [50].

Compared with histone crotonylation and its relationship with cancer, non-histone crotonylation is much under-explored. Recently, the study reported that K420 was the main Kcr site of ENO1 (α enolase), ENO1 K420 crotonylation can enhance ENO1 activity and regulate the expression of tumor-related genes, thus promoting the growth, invasion, and migration of colorectal cancer cells (CRC) in vitro [27]. And the crotonylation of BEX2 at the K59 site is found to be critical for mediating mitophagy in lung cancer cells [51]. In addition, by modifying the serine 46 sites of p53, crotonic acid (CA) can negatively regulate the transcriptional level and activity of p53, increasing resistance of cancer cells to chemotherapy drugs [17]. High levels of SEPT2-K74 crotonylation promoted HCC metastasis both in vitro and in vivo, and predicted poor prognosis and a high recurrence rate in HCC patients [52]. And the crotonylation of MTHFD1 at Lys354 and Lys553 could inhibit the development of pancreatic cancer [53].

One of the regulators of lysine crotonylation is Acyl-CoA oxidase 2 (Acox2), its main point of action is Kcr and its deletion leads to liver cancer in mice [54]. H4K77cr and H4K91cr are two Kcr sites and critical for endoderm differentiation [22]. And studies have found that the occurrence and development of the disease can be better controlled if the cancer stem cell program is activated [55]. Both studies indicated that crotonylation-meditated mechanism may be important for cancer related stem cell therapy.

Although seldom known about the field of non-histone

Table 1
Diseases associated with crotonylation.

Diseases	Protein crotonylation	Regulatory mechanism	References
Colorectal cancer	ENO1	ENO1 K420 crotonylation promoted the growth, migration, and invasion of colorectal cancer cells (CRC) in vitro by enhancing the activity of ENO1 and regulating the expression of tumor-associated genes.	[27]
Non-small-cell lung cancer cells	BEX2	The crotonylation of BEX2 at the K59 site is found to be critical for mediating mitophagy in lung cancer cells.	[51]
Hepatocellular carcinoma	SEPT2	Crotonylation facilitates cell invasion through the crotonylated SEPT2-K74-P85α-AKT pathway	[52]
Pancreatic cancer	MTHFD	The activation of MTHFD1 by decrotonylation at Lys354 and Lys553 promotes pancreatic cancer the development of by increasing resistance to ferroptosis	[53]
Acute kidney injury	–	Cell stress increases affecting the TWEAK, it then decreased PGC1a and Sirt-3 expression and with increased CCL2 expression.	[60,61]
Autosomal dominant polycystic kidney disease	H3K18	CDYL increases the level of Kcr, and overexpression of CDYL decreased histone Kcr, inhibited the expression of cyst-related genes, and slowed the growth of cysts.	[64]
Depression	H3K27	Increased H3K27 content and decreased the level of Kcr to inhibit the transcription of a group of genes such as neuronal VGF, then it leads to promote depression.	[85,86]
Alzheimer's disease	H3K27	NEAT1 inhibition influences H3K27 acetylation (H3K27Ac) and H3K27 crotonylation (H3K27Cro) located nearby to the transcription start site of many genes, including endocytosis-related genes.	[81]
Hypertrophic cardiomyopathy	H3K18 and H2BK12	Short-chain enoyl-CoA hydratase (ECHS1) downregulation was accompanied with the upregulation of H3K18cr and H2BK12cr in human hearts with hypertrophic cardiomyopathy.	[30]
HIV latency	H3K4	Viral infection or addition of crotonyl-CoA induces the expression of the fatty acid metabolic enzyme ACSS2, and ACSS2 increased H3K4 crotonylation which leads to regulation of HIV latency/transcription.	[89]
Ischemic heart disease	IDH3a	IDH3a K199 and TPM1 L28/29 crotonyalation could not only protect cardiomyocytes but also preserve myocardial function after injury.	[72]
Immunoglobulin A nephropathy	–	Identified 353 crotonylated proteins	[68,119]
Chronic renal failure	–	Identified 1109 lysine modification sites	[69,120]
Hemodialysis	–	Identified total 1109 lysine crotonylation sites on 347 proteins	[57]
COPD Combined with Type II RF	–	Identified 32 sites of 23 proteins were upregulated and 914 sites of 295 proteins were downregulated	[121]

Table 2
Diseases associated with crotonylation metabolite.

Diseases	Metabolite	Regulatory mechanism	References
Hepatocellular carcinoma	HDACs	By adding HDACs inhibitor, it founded that lysine crotonylation can affect the Hepatocellular carcinoma (HCC)cell migration, and decrease the proliferation ability of hepatoma cell.	[48]
Wilms tumor	YEATS domain of MLLT1	Mutations in the YEATS domain of MLLT1 have been shown to be functionally relevant in Wilms tumor.	[122]
Hepatocellular carcinoma	ACOX2	ACOX2 is a regulator of Kcr. Compared with normal mice, experiments on ACOX2 mice have uncovered that the level of non-histone Kcr was dowenregulated while the level of histone (H2b) K86cr was upregulated.	[54]
Ovarian cancer	HDACs	HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) could inhibit growth of cancer cells, inducing expression of tumor suppressor genes, apoptosis, G2/M arrest, and autophagy.	[123]
inflammation	AF9 YEATS domain	AF9 YEATS domain has selectively higher binding affinity on crotonyllysine than acetyllysine, and AF9 YEATS Links the inflammatory genes' response to histone crotonylation.	[45]

crotonylation, non-histone proteins occupy important positions in numerous vital biological processes. Consequently, delving deeper into the study of crotonylation modifications on non-histone proteins holds immense prospects for future cancer treatments.

3.2. Kidney diseases

Kidney is one of the main organs of the human body, and many diseases will cause damage to kidney function. Commonly known kidney diseases include Acute kidney injury (AKI), Autosomal dominant polycystic kidney disease (ADPKD) and others. And studies have shown that crotonylation is closely related to these diseases [56]. By the liquid chromatography tandem mass spectrometry (LC-MS/MS) with highly sensitive immune-affinity purification, many studies have revealed the

Table 3
Proteins and their Kcr sites associated with crotonylation.

Protein Names	Enter Name	ID	Sites	Diseases and Biological Processes	References
Histone 2b	H2b	–	K12	Hypertrophic cardiomyopathy (HCM)	[30]
Histone 2b	H2b	–	K86	Metabolic homeostasis, Hepatocellular carcinoma	[54,100]
Histone H3	H3	Q6NXT2	K18	Autosomal dominant polycystic kidney disease (ADPKD), HCM	[30,64]
Histone H3	H3	Q6NXT2	K27	Depression, Alzheimer's disease (AD)	[81,85,86]
Histone H3	H3	Q6NXT2	K4	HIV	[89]
Histone H4	H4	P62805	K77, K91	Stem cell endoderm differentiation	[22]
Enolase 1, chloroplastic	ENO1	Q9C9C4	K40	colorectal cancer	[27]
Protein ENL	ENL	Q03111	S46	Wilms tumor	[122]
Cellular tumor antigen P53	P53	P04637		Render the tolerance of cancer cells to chemotherapeutics and drugs	[17]
Platelet glycoprotein 4	CD36	P16671		Chronic renal failure (CRF)	[69]
Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	IDH3a	Q9D6R2	K199	Ischemic heart disease (IHD)	[72]
Microtubule-associated protein RP/EB family member 1	EB1	Q8WQ86	K66	Spindle positioning	[94]
Tropomyosin alpha-1 chain	TPM1	P58771	K28	Ishemia-Reperfusion Injury	[72]
Brain expressed X-linked gene 2	BEX2	Q9BXY8	K59	Non-small lung cancer	[51]
Septin2	SEPT2	Q15019	K74	Hepatocellular carcinoma (HCC)	[52]
methylenetetrahydrofolate dehydrogenase, cyclohydrolase and formyltetrahydrofolate synthetase 1	MTHFD1	P11583	K354, K553	Pancreatic cancer	[53]

widespread presence of crotonylation modifications on proteins and sites in various kidney disease [57]. However, there is limited research on the specific protein targets and ways of action. Only few studies specified the accurate crotonylation affect kidney diseases' mechanism. There is still need more basic research to elucidate the proteins and specific sites of crotonylation, providing new insights for future clinical studies.

3.2.1. Acute kidney injury

AKI has high morbidity and mortality and is a major public health burden [19]. Research found the expression of histone crotonylation in experimental nephrotoxic AKI. Further experiments indicate that this increase in histone crotonylation is associated with the activation of the inflammatory cytokine TWEAK [58,59], and TWEAK promotes the crotonylation of cultured tubular cells. Additionally, cellular stress affects TWEAK expression, leading to reduced expression of Sirt-3 and PGC1a, as well as increased expression of CCL2. Regarding this matter, adding exogenous crotonic or HDAC inhibitor, resulted in increased expression of kidney SIRT3 and PGC-1α in both in vivo and cultured cell settings, and decreased expression of CCL2, providing protection against AKI [60,61]. Overall, both cellular stress and crotonic promote histone crotonylation and thus the protective effect of crotonylation against AKI.

3.2.2. Autosomal dominant polycystic kidney disease

ADPKD is the most common hereditary kidney disease that can impair kidney function and eventually lead to kidney failure [62,63]. A recent study revealed that crotonylation might have a crucial role in ADPK. Animal experiments, and statistical analysis found that the expression of chromodomain Y-like protein (CDYL) [21], was inhibited in ADPKD, whereas the level of Kcr was increased. Overexpression of CDYL can decrease histone Kcr, suppress the expression of cyst-related genes, and slow down cysts growth. Furthermore, H3K18 has been identified as a CDYL targeted crotonylation site in ADPKD cells [64].

3.2.3. Other kidney diseases

Immunoglobulin type A nephropathy (IgAN) and chronic renal failure (CRF) are severe kidney diseases [65–67]. Study analyzed lysine crotonylation in patients with IgA nephropathy (IgAN), chronic renal failure (CRF), and healthy controls. The results identified numerous crotonylated proteins and modification sites [68,69]., associated with processes like inflammation, oxidative stress [68] and fibrosis [69]. Also, crotonylation levels were slightly lower in maintenance hemodialysis patient group [61]. Although the main mechanisms of crotonylation affected these diseases are still unknown, they open up new ways for the development of treatment strategies and interventions patients, and even identifies crotonylation as a potential therapeutic target.

3.3. Cardiac diseases

Ischemic heart disease (IHD) is a serious condition that causes complex and multifaceted pathophysiology [70,71]. New research shows that when IHD ischemia-reperfusion injury triggers cardiac muscle cell contraction, the required protein is first crotonylated by lysine. Specifically, mitochondrial protein IDH3a (isocitrate dehydrogenase 3 [NAD+] alpha) at K199 and cytoskeletal protein TPM1 (tropomyosin alpha-1 chain) at K28/29 were selected for the regulation of crotonylation, adding addition of exogenous sodium crotonate to enhance crotonylation, and the results indicated that the two proteins crotonylation could not only protect cardiomyocytes from apoptotic structural rearrangement through inhibiting mitophagy or cytoskeleton mediated by BNIP3 (Bcl-2 adenovirus E18 19 kDa interacting protein 3), but also maintain myocardial function after injury through inhibiting fibrosis and apoptosis [72].

Hypertrophic cardiomyopathy (HCM) is a prevalent inherited cardiovascular disease with unknown etiology. Recent studies found that HCM patients exhibit a downregulation of short-chain enyl-coA hydrase (ECHS1) along with an up-regulation of H3K18cr and H2BK12cr [73]. Additionally, the hydratase ECHS1 could maintain the maturity and homeostasis of cardiomyocytes, as well as regulating the intracellular crotonyl-CoA through histone crotonylation and other ways [26]. It mentions the association between hemodialysis (HD) and crotonylation, suggesting that HD-induced stress and injury to the cardiovascular system may be linked to crotonylation changes [61,74].

Vascular smooth muscle cells (VSMCs) are essential components of tissue structure constituting to the maintenance of vascular tone [75,76]. And the LC-MS/MS assay identifies 2138 lysine crotonylation sites among 534 proteins. these non-histone crotonylated proteins involve in multiple essential biological processes, including glycolysis, cellular skeleton modulation, and VSMC contraction [77].

3.4. Neuropsychiatric diseases

Alzheimer's disease (AD) is characterized by severe cytoskeletal

alterations in only a few neuronal types in the human central nervous system [78]. A study revealed that the repression of Nuclear Paraspeckle Assembly Transcript 1 (NEAT1) [79,80] in early AD mediates the clearance of Aβ by inhibiting the expression of endocytosis-related genes [81]. NEAT1 has a complex relationship with P300/CBP, and its inhibition influence the H3K27 crotonylation (H3K27Cr) and H3K27 acetylation (H3K27Ac) of various genes, including toxic-related genes [81]. Whether NEAT1 could influence H3K27Cr to meditate Aβ still need further research.

Depression is a major mental illness, affecting a vast number of individuals. [82]. Despite its prevalence, the pathogenesis of depression remains elusive, and our understanding of the disease is limited. Compared with B6 mice, BTBR mice had higher levels of lysine crotonylation, which could contribute to neuropsychiatric disorders, like depressive disorder and Alzheimer's disease [83]. But this study did not identify the exact histone or non-histone lysine crotonylation [84]. By the dual effects of increased H3K27 and decreased level of Kcr, CDYL inhibits the transcription of a group of genes such as neuronal VGF, leading to the promotion of depression [85,86].

In addition to the aforementioned neuropsychiatric disorders, Kcr is widely present in macrophages, sensory neurons, astrocytes, and microglia of the medulla oblongata. Furthermore, histone crotonylation plays a crucial role in regulating neuroinflammation and neuralgia pain, although the precise mechanism remains unclear [87]. Although the findings suggested that the Kcr may greatly affect the progress of the neuropsychiatric diseases, the crotonylation modifications in neuro-psychiatric disorders is not yet widely explored, and more foundational research data is needed to validate this.

3.5. Acquired immunodeficiency syndrom (AIDS)

AIDS is caused by Human immunodeficiency virus (HIV) infection. Approximately a decade ago, HDACs inhibitors trohostatin and trapoxin were found to reactive latent HIV transcriptionally [88]. Recently, a study uncovered that induced by crotonyl-CoA or viral infection, ACSS2 could increase crotonylation of H3K4 as well as acetylation of H3K4 and acetylation of H3K18 (H3K18Ac), leading HIV latency/transcription [89]. More importantly, combined with PKC agonists PEP005, vorinostat, or JQ1 in T cell cultures in vitro and/or CD4+T cells, histone crotonylation was found to significantly enhance latent HIV reactivation in HIV-infected patients, suggesting a synergistic effect of histone crotonylation with PKC agonists [89]. These findings highlight the potential for investigating the combination of histone crotonylation and HIV latency therapy to improve treatment outcomes.

3.6. Inflammation

Inflammation serves as an adaptive response to harmful stimuli such as infection and tissue damage [90–92]. A study found that the AF9 YEATS structure domain has higher binding compatibility on lysine crotonylation and AF9 YEATS junction inflammatory gene response to histone crotonylation. AF9 was also found to be recruited to LPS-stimulated genes, and its recruitment can be further augmented through crotonate pre-treatment in a YEATS-dependent manner [45]. Additionally, inflammation in osteoarthritis chondrocytes could lead to increased HDACs expression and activity [93]. Although studies have indicated the presence of crotonylation in inflammation, no research has provided a specific mechanistic explanation to elucidate the connection between the two.

4. Physiological processes related to crotonylation

In addition to the research on crotonylated diseases, multiple studies have revealed the link between crotonylated diseases and physiological processes. Specifically, histone crotonylation have been shown to impact spindle positioning [94], metabolic homestasis [54], and stem cell endoderm differentiation [22]. Furthermore, non-histone crotonylation has been linked to subcellular organelles [72].

4.1. Spindle positioning

The accurate spindle localization is crucial physiological functions, including cell passage and development, as well as a significant prerequisite for proper mitotic and meiotic cell divisions [95]. Previous work has shown that P300/CBP-associated factor-(PCAF) regulates EB1K220 acetylation to make sure dynamic interaction between kinetochore and microtubule in early mitosis [96,97]. A recent study revealed that dynamic crotonylation of EB1 during mitosis, mediated by TIP60, plays a crucial role in ensuring precise spindle localization [94]. Specifically, TIP60 primarily catalyzes the crotonylation of EB1 over Lys66, rather than acetylation. The synergistic effect of HDAC3 enhances TIP60-catalyzed crotonylation. Further analysis showed that tip60 mediated crotonylation of EB1 by regulating the positive end dynamics of astral microtubules to ensure the orientation of mitotic spindles [94]. The investigation of the relationship between crotonylation and spindle positioning provides new avenues for the studying physiological functions both in vivo or ex vivo.

4.2. Metabolic homeostasis

ACOX2 (acyl-CoA oxidase 2) is an important peroxisomal enzyme that could impact the oxidation of branched-chain fatty acids and the metabolism of bile acid, and various physiological processes [98,99]. Importantly, ACOX2 has been implicated in the development of hepatocellular carcinoma [100] and primary malignant cardiac tumors (PMCTs) [101,102]. Recently, ACOX2 is identified as a regulator of Kcr and contributes to metabolic homeostasis via crotonylation. And ACOX2 mice have down-regulation of non-histone crotonylation levels and up-regulation of histone 2B K86cr levels. Together, these results underscore the significance of ACOX2 crotonylation in metabolic homeostasis and liver cancer [54].

4.3. Stem cell endoderm differentiation

Acetyl-coenzyme A (CoA) is an important cofactor for post-translational modifications, particularly in Kcr [26]. A recent study found that the crotonylation of human embryonic stem cells (hESCs) promotes mesodermal differentiation both in vitro and in mice embryos. The only sites detected in differentiated endodermal cells were H4K77cr and H4K91cr [22]. Endothelial differentiation is associated with increased expression of the crotonyl-CoA-producing enzyme in vivo and cultured cells (Fig. 3). Crotonate significantly enhances hESCs' endoderm differentiation efficiency [103]. Embryonic stem cells (ESCs) were enriched in histone crotonylation and the lack of HDAC1/2 affected the activity of the decrotonylase [34,38]. These findings have important physiological relevance and clinical implications. For example, short-chain acyl-CoA dehydrogenase deficiency (SCADD) is a disease mainly characterized by elevating butyryl-COA and its by-products in muscle and liver [104–106]. Combing these results, investigating histone crotonylation and other possible short-chain fat acylation in patients could help manage SCADD. Furthermore, as many organs including the pancreas, liver, digestive tract, and lung originate from the endoderm [107], these findings could potentially lead to new treatments for these organ diseases.

Furthermore, histone crotonylation can activate Zscan4, leading to a decrease in telomere damage and maintenance of telomere length in chemically induced pluripotent stem cells (CiPSCs) [12], Combined with great potential of CiPSCs in stem cell-based therapy [108]. These results highlight the potential significance of histone crotonylation in shaping the future of stem cell research.

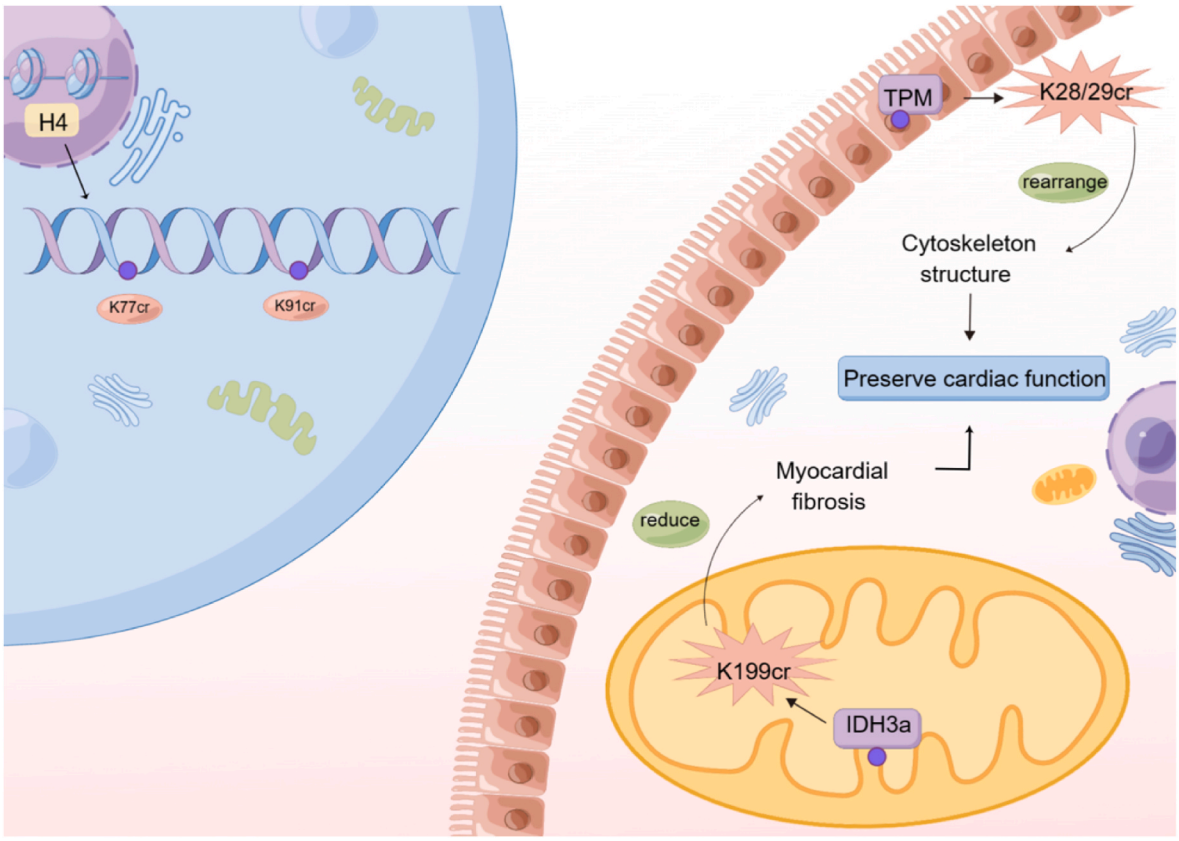

Fig. 3. The function of crotonylation in stem cell endoderm differentiation (Left). crotonylation was observed during the differentiation of hESCs into mesondodermal and endodermal cells. Two crotonylation sites were identified on endodermal: H4K77cr and H4K91cr. **Function of non-histone crotonylation in subcellular organelles (Right)**. crotonylation at the K28/29 site of TMP1 protein was found to rearrange the cytoskeleton structure, and crotonylation at the IDH3a K99 was observed to reduce myocardial fibrosis and protein BNIPS, thereby significantly preserving cardiac function.

4.4. Autophagy and the MTORC1 pathway

Autophagy usually refers to the process that involves lysosomes invading and degrading the substrates directly. Previous studies have suggested that leucine can affect this process, but the precise mechanism remains unclear. However, a recent study identified the relationship between lysine crotonylation levels and autophagy induced by leucine-deprivation [109]. Combined with previous findings on the interaction between HDAC7 and 14-3-3 proteins [110–112], leucine deprivation inhibits HDAC7 increases the 14-3-3ε crotonylation level, and releases the protein phosphatase 1B (PPM1B) from its interaction with 14-3-3ε. This dephosphorylates ULK1 and activate autophagy. Furthermore, quantitative crotonylomic profiling identified two lysine crotonylation sites, K73 and K78, on 14-3-3ε, which have a significant effect on leucine-deprivation-induced autophagy.

Regarding MTORC1 pathway, CANX (calnexin) is an essential regulator for the leucine-stimulated MTORC1 (mechanistic target of rapamycin kinase complex 1) pathway [113]. Specifically, in response to leucine deprivation, CANX lysine undergoes 525 crotonylation, which is mediate by KAT7(lysine acetyltransferase 7). The combination of CANX and LAMP2 (lysosomal associated membrane protein 2) further decreased the MTORC1 pathway activity. Together, these components playing a role in the interplay between lysine deprivation and the MTORC1 pathway [114].

4.5. Non-histone crotonylation in subcellular organelles

Previous studies have investigated the relationship between PTMs and myofilament proteins, such as short-term phosphorylation at multiple sites in the myosin light chain (MLC) [115]. Lysine crotonylation has been observed in myofilm and ribosomal proteins in zebrafish embryos. Biological process analysis revealed that Kcr was mainly involved in metabolic processes (22%) and cellular processes (28%), and the detected Kcr proteins were primarily localized in the cytoplasm (58%) and mitochondria (11%). These findings suggest the possible Kcr sites and modification in human [116]. Furthermore, proteins containing Kcr sites have been detected and predicted to localize in mitochondria [4].

Crotonylation also exists in subcellular organelles and is closely related to cellular physiological functions. Multiple cytoskeletal proteins have Kcr sites, K28/29 site of the TMP1 protein was found to preserve cardiac function and reduce myocardial fibrosis [117]. To detect Kcr in mitochondrial, the K199 site of IDH3a protein was selected because it participates in multiple physiological processes, and regulate mitochondrial function [118] (Fig. 3). The findings elucidate the role of non-histone crotonylation in suborganelles and cell physiological processes, greatly expanding the research scope of crotonylation [72].

5. Conclusion and perspectives

In conclusion, this review has provided a comprehensive overview of the recent research of Kcr, focusing on the role of histone and non-histone crotonylation in various diseases and physiological processes, and the potential for Kcr-targeted treatment. The review also summarizes the subcellular locations of Kcr in several diseases and future directions for the development of novel therapeutic approaches to combat diseases.

Kcr had an important impact on gene expression, aging, DNA damage and repair [8–14]. Despite the significant progress made in the study of Kcr, much remains to be understood about its precise and complete mechanism of Kcr in diverse diseases. Consequently, there is a pressing

need for further research in this area, with a particular emphasis on identifying the targeted sites of Kcr, and fully elucidating the physiology of this post-translational modification. Additionally, in-depth research on readers, writers, and erasers is essential to advance our understanding of this critical regulatory mechanism.

The clarification of the Kcr mechanism has the potential to yield practical therapeutic modalities for the treatment of several diseases, including those that are difficult to cure, such as cancer and kidney diseases. Furthermore, the identification of Kcr sites in cytoskeletal proteins and mitochondria indicates that Kcr sites may be the target for the treatment of muscle and mitochondrial diseases. Moreover, Kcr play a crucial role in spindle positioning and stem cell endodermal differentiation, which are the basis of multiple physiological processes and have broad implications for the treatment of many diseases. Therefore, future studies are warranted for developing drugs that can specifically target protein crotonylation to maximize the therapeutic potential of Kcr.

Overall, this review underscores the pivotal role of Kcr in disease and highlights the need for continued research in this field. By deepening our understanding of the mechanisms underlying Kcr, more effective treatments can be developed to combat diseases.

Findings

This work was supported by the National Natural Science Foundation of China under Grant 82003046, the Natural Science Foundation of Chongqing Municipal Science and Technology Bureau under Grant CSTB2022NSCQ-MSX0561 and CSTB2022NSCQ-JQX0010 , the Chongqing Municipal Health Commission under Grant 2024GDRC011, and Chongqing University under Grant 2023CDJYGRH-YB10.

CRediT authorship contribution statement

Dongling Li: Writing – original draft, Visualization. **Ling Lin:** Writing – original draft. **Fan Xu:** Data curation. **Tianlin Feng:** Data curation. **Yang Tao:** Supervision. **Hongming Miao:** Funding acquisition. **Fan Yang:** Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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