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REVIEW

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Lysine crotonylation in disease: mechanisms, biological functions and therapeutic targets

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Abstract

Lysine crotonylation (Kcr), a previously unknown post-translational modification (PTM), plays crucial roles in regulating cellular processes, including gene expression, chromatin remodeling, and cellular metabolism. Kcr is associated with various diseases, including neurodegenerative disorders, cancer, cardiovascular conditions, and metabolic syndromes. Despite advances in identifying crotonylation sites and their regulatory enzymes, the molecular mechanisms by which Kcr influences disease progression remain poorly understood. Understanding the interplay between Kcr and other acylation modifications may reveal opportunities for developing targeted therapies. This review synthesizes current research on Kcr, focusing on its regulatory mechanisms and disease associations, and highlights insights into future exploration in epigenetics and therapeutic interventions.

Introduction

The concept of epigenetics was first introduced in 1942 by Austrian developmental biologist Conrad Waddington [1]. Protein post-translational modification (PTM), an important mechanism of epigenetic regulation, participates in processes like DNA replication, transcription, cell differentiation, and metabolism by modulating protein activity, stability, or localization [2, 3]. Advances in high-resolution mass spectrometry have extensively characterized numerous novel PTMs on histone proteins, including lysine acetylation (Kac), butyrylation (Kbu), crotonylation (Kcr), propionylation (Kpr), malonylation (Kmal), glutarylation (Kglu), benzoylation (Kbz), 2-hydroxyisobutyrylation (Khib), β-hydroxybutyrylation (Kbhb), succinylation (Ksucc), lactylation (Kla) and many others [3–6]. Additionally, acylation modifications have been identified in non-histone proteins, indicating their widespread distribution in cells and critical roles in physiological and pathological processes [7, 8].

Kcr is an evolutionarily conserved and widespread non-acetyl histone acylation that transfers the crotonyl group onto lysine residues by using crotonyl-CoA as a substrate via crotonyltransferase [9]. Crotonyl-CoA is an intermediate metabolite involved in mitochondrial and peroxisomal fatty acid oxidation, as well as lysine and tryptophan metabolism [10]. The levels of Kcr often reflect changes in intracellular crotonyl-CoA concentrations, directly linking cellular metabolic states to target protein functions. In 2017, Wei et al. identified Kcr on non-histone proteins in the HeLa cell line [5]. Despite overlaps in regulators and sites between Kcr and Kac, the crotonyl group, a four-carbon planar moiety with a C=C bond, suggests unique biological functions for Kcr [11]. Overall, crotonylation is involved in physiological processes, including gene transcription regulation, chromatin remodeling, cell cycle and DNA damage response [5, 9, 12, 13]. This review summarizes past and recent advances

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in Kcr research, focusing on crotonylation mechanisms in histone and non-histone proteins across diverse physiological processes and diseases.

The research progress of Kcr

The research history of crotonylation is shown in Fig. 1. In 2011, Tan et al. at the University of Chicago identified crotonylation on histones as a novel PTM. They discovered 67 previously unreported histone marks using mass spectrometry-based proteomics. Subsequent structural and genomic analyses revealed that histone Kcr is a highly conserved PTM across evolution, functionally distinct from Kac. They confirmed that histone Kcr marks specific X-linked genes escaping sex chromosome inactivation in haploid cells after meiosis [9]. This study suggested that crotonylation plays a crucial role in gene activation. Sin et al. found that Kcr accumulates at transcription start sites of sex-linked genes in an RNF8 (an E3 ubiquitin-protein ligase)-dependent manner, enhancing RNF8-related gene expression and activating previously inactive sex chromosomes in post-meiotic spermatocytes [14]. Overall, the role of histone Kcr during meiosis and post-meiosis in male germ cells was elucidated. In 2017, Liu et al. reported that chromodomain Y-like transcription corepressor (CDYL) functions as a crotonyl-CoA hydratase, down-regulating histone Kcr during spermatogenesis [15]. Advances in high-resolution protein mass spectrometry have uncovered numerous crotonylation modifications on non-histone proteins, driving research into their functions and highlighting the broader significance of Kcr. Subsequently, Wei et al. pioneered discoveries in non-histone Kcr research. They identified 558 Kcr sites on 453 proteins in HeLa cell lines, confirming that Kcr regulates diverse protein functions and cellular pathways [5]. Concurrently, Zhang et al. identified 2,696 Kcr sites on 1,024 proteins in H1299 lung cancer cells. They discovered that acetyltransferases, such as CBP, PCAF, and hMOF, catalyze non-histone Kcr, whereas deacetylases like HDAC1 and HDAC3 mediate decrotonylation [12]. Subsequent studies identified numerous Kcr sites on proteins across species, including humans, yeast, zebrafish, tobacco, and rice [13, 16, 17]. Recently, Zheng et al. identified 4,187 Kcr sites on 1,533 proteins in pancreatic cell lines, revealing that methylenetetrahydrofolate dehydrogenase1 (MTHFD1) hypo-crotonylation impairs ferroptosis and promotes

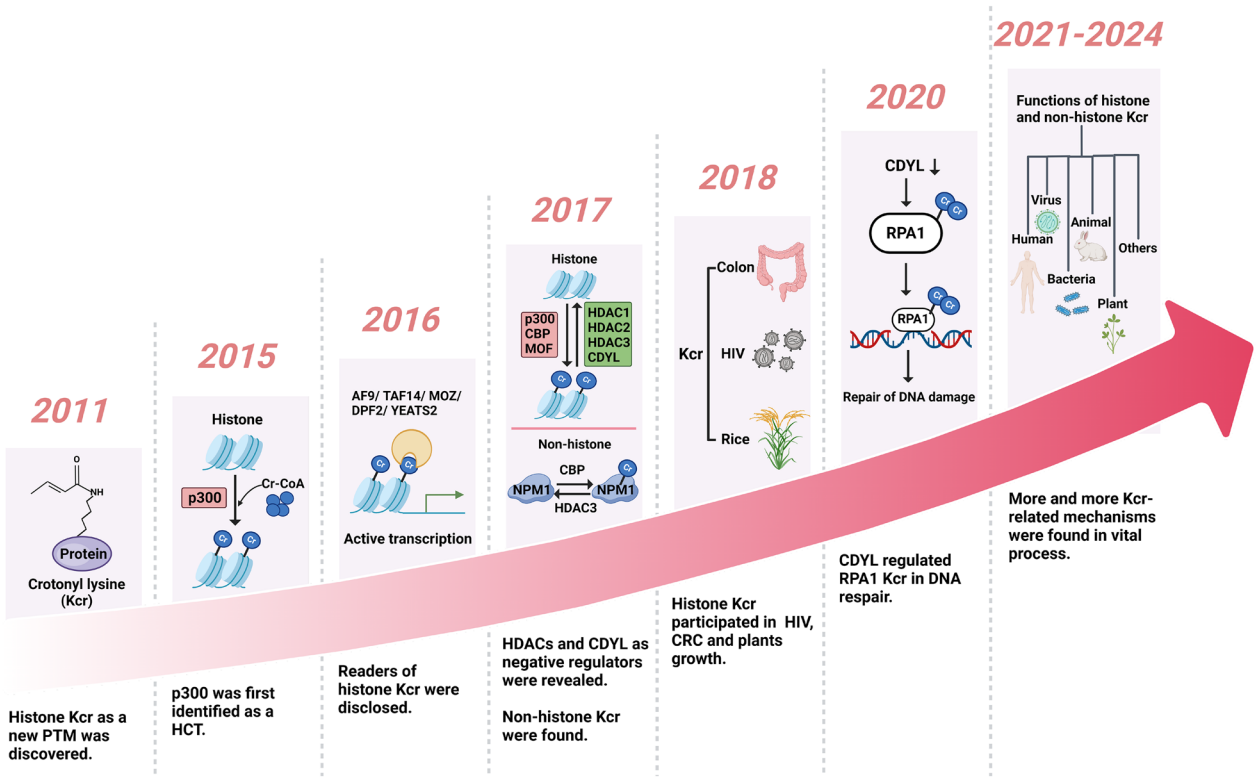

Fig. 1 The research history of protein Kcr

Pancreatic ductal adenocarcinoma (PDAC) progression [18].

The regulation mechanisms of Kcr

PTMs such as Kcr, Ksucc, Kmal, Kglu, and Kbhb are regulated by acyl-CoA metabolism, which fluctuates under specific physiological conditions [2, 8]. Kcr regulation resembles other acylation modifications, influenced by intracellular crotonyl-CoA concentration and dynamically controlled by crotonyltransferases and decrotonylases. Table 1 lists the regulatory factors of protein Kcr, and Fig. 2 depicts the modification process. Identifying and characterizing crotonyltransferases and decrotonylases are essential for elucidating the regulatory mechanisms of protein Kcr.

Kcr writers

Crotonyltransferases, often called the "writers" of Kcr, play a key role in this modification [4, 20]. With the well-characterized regulatory enzyme systems of acetylation in PTMs, histone acetyltransferases (HATs) were found to exhibit histone crotonyltransferase (HCT) activity [4]. Although it is noted in the literature that crotonyl-CoA has an unsaturated moiety, this would seem to make it unsuitable for most HATs [21]. The three major HAT families including p300/CREB-binding protein (p300/CBP), Gcn5-related N-acetyltransferases (GNAT) and MYST (MOZ, Ybf2/Sas3, Sas2, and TIP60), which have been reported as HCTs that use crotonyl-CoA as substrate to catalyze Kcr [2, 20, 22].

In 2015, Sabari et al. at Rockefeller University first reported that p300/CBP catalyzes H3 lysine 18 (H3K18) crotonylation. The authors noted that p300-catalyzed histone crotonylation directly stimulates transcription and does so to a greater degree than p300-catalyzed histone acetylation. They further confirmed that crotonylation promotes gene transcription activation more effectively than acetylation [20]. Another study identified p300 and CBP as the primary HCTs in mammalian cells [22]. Another study noted that p300's aliphatic back pocket limits its HCT activity, making it 62-fold lower than its HAT activity, and suggested auxiliary factors may enhance its function [23]. Similar to CBP and p300, MOF catalyzes crotonylation at H3K4, H3K9, H3K18, H3K23, H4K8, and H4K12 sites [22]. This catalytic activity is evolutionarily conserved; for instance, the yeast homolog Esa1 catalyzes histone Kcr at H4K5, H4K8, H4K12, and H4K16 [22, 24]. Interestingly, Esa1, human MOF (hMOF), and general control non-derepressible 5 (GCN5) lack HCT activity in vitro, whereas the Gcn5-Ada2-Ada3 (ADA) and Esa1-Yng2-Epl1 (Piccolo NuA4) HAT complexes exhibit this activity [25]. Histone acetyltransferase binding to origin recognition complex 1(HBO1) catalyzes H3K14cr and H4K12cr in vivo [26]. A recent study identified YjgM, a novel crotonyltransferase, which alters E. coli drug resistance by regulating PmrA K164cr and characterized its HCT activity through in vitro and in vivo experiments [27].

HATs not only regulate histone Kcr but also control numerous non-histone Kcr modifications that influence critical cellular pathways. For instance, CBP and hMOF strongly crotonylate non-histone NPM1, while PCAF exhibits moderate crotonylation activity [12]. CBP catalyzes non-histone DDX5 Kcr, whereas p300, PCAF, and hMOF lack this activity [12]. Lysine acetyltransferase 7 (KAT7) binds directly to calnexin, crotonylating its K525 site under leucine deprivation. This modification regulates calnexin translocation to lysosomes and controls mechanistic target of rapamycin kinase 1 (MTORC1) activity [28]. TIP60 showed new crotonyl transferase activity. TIP60 mediates crotonyltransferase at EB1 Lys66 site and participates in mitosis [29].

Kcr erasers

Decrotonylases, known as the "erasers" of crotonylation, are proteins capable of removing crotonylation modifications in living organisms. Histone deacetylases (HDACs) are categorized into four groups: the NAD^+-dependent sirtuin family (SIRTs [class III: Sirt1-7]) and the Zn^{2+}-dependent histone deacetylase family (HDACs [class I: HDAC1,2,3 and 8; class II: HDAC 4,5,6,7,9 and 10; class IV: HDAC11]) [30]. Researchers confirmed the histone decrotonylase (HDCR) activity of HDACs through functional assessments [4].

Peptide-based in vitro screening experiments first identified the HDCR activity of the HDAC3-NCoR1 complex. The HDCR activity of the HDAC3-NCoR1 complex was inhibited by HDAC inhibitors such as vorinostat and apicidin [31]. HDAC1 regulates crotonylation at H3K4, H3K9, H3K23, H4K8, and H4K12 [32]. HDAC2 regulates the modification levels of H2BK12cr [33]. Deleting HDAC1/2 in murine embryonic stem cells (ESCs) elevated global histone crotonylation and reduced total HDCR activity by 85% [34]. In addition to regulating histone crotonylation sites, HDAC1 and HDAC3 also decrotonylate non-histone NPM1 [12]. HDAC3 decreased nonhistone AKT1 Kcr levels during myogenic differentiation [35]. Besides, a study identified lamin A as a crotonylated protein at K265/270, regulated by HDAC6 [36]. Another study noted that HDAC7 affected Leu-deprivation-induced autophagy by regulating nonhistone 14–3–3ε K73 and K78 Kcr [37].

Class III HDACs, including SIRT1 and SIRT2, were shown to downregulate H3K9cr in vitro using radiometric thin-layer chromatography [38]. Kcr of H2AK119 is mediated by SIRT1 [39]. SIRT3 acts as an HDCR,

Table 1 The regulatory factors and substrate modification sites for histone and nonhistone Kcr

	Enzymes	Targets		References
		Histone	Nonhistone	
Writer	p300	H3K18	/	[20]
	CBP	H3K18	NPM1, DDX5, ENO1	[12, 20, 90]
	MOF	H3K4, H3K9, H3K18, H3K23, H4K8, H4K12	NPM1	[12, 22]
	GCN5	H3K9, H3K14, H3K18, H3K23, H3K27	/	[25]
	HAT1*	H3K9	/	[48]
	Rtt109*	H3K9	/	[48]
	yeast Esa1*	H4K5, H4K8,H4K12, H4K16	/	[25]
	PCAF	/	NPM1, DDX5	[12, 22]
	HBO1	H3K14, H4K12	CANX(K525)	[26, 28]
	TIP60	/	EB1	[29]
Eraser	SIRT1	H3K9, H4K8, H3K4, H3K18, H2AK119	SERCA2a(K120)	[32, 38, 39, 78]
	SIRT2	H3K9	ENO1(K420)	[38, 90]
	SIRT3	H3K4, H3K27	PRKACA	[40, 91]
	FOSIR5*	H3K18	/	[41]
	SIRT6	H3K27	ACSL5(K98/361/367)	[42]
	SIRT7	/	PHF5A(K25)	[43]
	HDAC1	H3K4, H3K9, H3K23, H4K8, H4K12, H3K18	NPM1	[12, 35]
	HDAC2	H3K9, H3K23, H4K8, H4K12, H3K18, H2BK12	/	[32, 33]
	HDAC3	H3K9, H4K8, H3K18	NPM1, AKT1	[12, 32, 35]
	HDAC6	/	Lamin A (K265/270)	[36]
	HDAC7	/	14–3–3ε (K73/78)	[37]
	HDAC8	H3K4, H3K9, H3K23, H4K8, H4K12, H3K18	/	[32, 33]
Reader	TAF14	H3K9	/	[48]
	YEATS2	H3K27	/	[45]
	AF9	H3K9, H3K18, H3K27	/	[46]
	MOZ, DPF2	H3K14	/	[11]
	TAF1	H3K9, H3K27	/	[46]
Other regulators	NEAT1	H3K27	/	[53]
	CDYL	H2BK12, H3K9, H3K27, H4K8, H3K18, H3K23, H3K79	RPA1	[13, 15]
	ACSS2	H3K9, H3K18	/	[20, 110]
	ACOX1	/	/	[10]
	ACOX2	H2BK86	Ehhadh (K329/344/572)	[19]

Table 1 (continued)

Enzymes	Targets		References
	Histone	Nonhistone	
		Crot (K69/384)	
ACOX3	/	/	[10]
ACADS	/	/	[10]
GCDH	/	/	[50]
ECHS1	H3K18, H2BK12	/	[76]
EPB41L4AAS	H3K27	/	[116]
BRD4	H3K18	/	[101]

CANX calnexin, *EB1* End Binding Protein 1

regulating histone Kcr dynamics and gene transcription in living cells [40]. FoSir5, a homolog of human SIRT5, reduces transcripts of aerobic respiration pathway enzymes by downregulating H3K18cr [41]. SIRT6 knockdown significantly increases H3K27cr levels in vitro [42]. SIRT7 was later identified to induce K25 decrotonylation of PHF5A, thereby regulating aging [43]. These findings indicate that SIRT1/2/3/5/6/7 serves as an efficient HDCR for proteins.

Kcr readers

Proteins that specifically recognize modifications and translate them into various functional outcomes within the cell are called "readers". In the cell nucleus, the recognition of histone modification sites by reader proteins is essential for physiological functions. For instance, when specific reader proteins recognize Kac, they recruit transcription factors to chromosomal regions, thereby promoting gene transcription. Currently, three types of domains that recognize Kcr have been identified: YEATS (YAF9, ENL, AF9, TAF14, and SAS5), double PHD finger (DPF), and bromodomain [16, 44–47]. DPF and YEATS domains preferentially recognize Kcr.

In 2016, the YEATS domain was identified as the first effective reader of Kcr at histone H3K9, H3K18, and H3K27 sites, playing a key role in gene transcription [48]. Researchers later confirmed that the YEATS domain preferentially binds longer acyl chains with the strongest affinity for Kcr [46]. The homologous gene of AF9 in YEATS, Taf14, was also found to recognize Kcr at the H3K9 site [46, 48]. The YEATS domain of the human YEATS2 protein specifically recognizes Kcr at histone H3K27 sites, but not at H3K9, H3K14, or H3K23 sites [16]. These studies demonstrate that YEATS domains preferentially read crotonyllysine.

Xiong et al. later demonstrated that the PHD domain, typically recognizing lysine methylation, can also recognize Kcr. They found that the hydrophobic binding region of the DPF domain in MOZ and DPF2 strongly recognizes H3K14cr but does not recognize H3K9cr, H3K18cr, or H3K27cr [11]. There is still much to explore regarding Kcr recognition factors and identifying them will provide a more precise understanding of Kcr function in cells.

Other regulators

Kcr is derived from crotonyl-CoA, which can directly regulate Kcr levels. A group of metabolic enzymes modulate Kcr levels by regulating the production, conversion, and stabilization of crotonyl-CoA. Below, we summarize these other regulators (Fig. 2). Acyl-CoA synthetase short-chain family member 2 (ACSS2) converts crotonate into crotonyl-CoA. Depletion of ACSS2 reduces cellular crotonyl-CoA and histone Kcr, suggesting crotonate as a potential endogenous source of crotonyl-CoA [20, 49]. Acyl-CoA dehydrogenase short-chain (ACADS) catalyzes the conversion of butyryl-CoA to crotonyl-CoA during fatty acid oxidation, while acyl-CoA oxidase (ACOX3) serves as a major crotonyl-CoA producer during endoderm differentiation [10]. Glutaryl-CoA dehydrogenase (GCDH) oxidizes glutaryl-CoA to crotonyl-CoA during the metabolism of lysine, hydroxylysine, and tryptophan [50]. CDYL, a crotonyl-CoA hydrase, converts crotonyl-CoA to β-hydroxybutyryl-CoA, negatively regulating histone Kcr [15]. CDYL was identified as a crotonyl-CoA hydratase that negatively regulates Kcr levels at H2BK12, H3K9, H3K27, and H4K8 sites [15].

The biology of protein Kcr

Gene regulation

Histone crotonylation plays a crucial role in regulating gene expression. Research has demonstrated that histone crotonylation in human somatic cells and mouse sperm cells is strongly associated with gene promoters and enhancers, indicating its role in transcriptional activation [9, 15]. Subsequent studies revealed that p300-catalyzed histone crotonylation enhances transcriptional activation

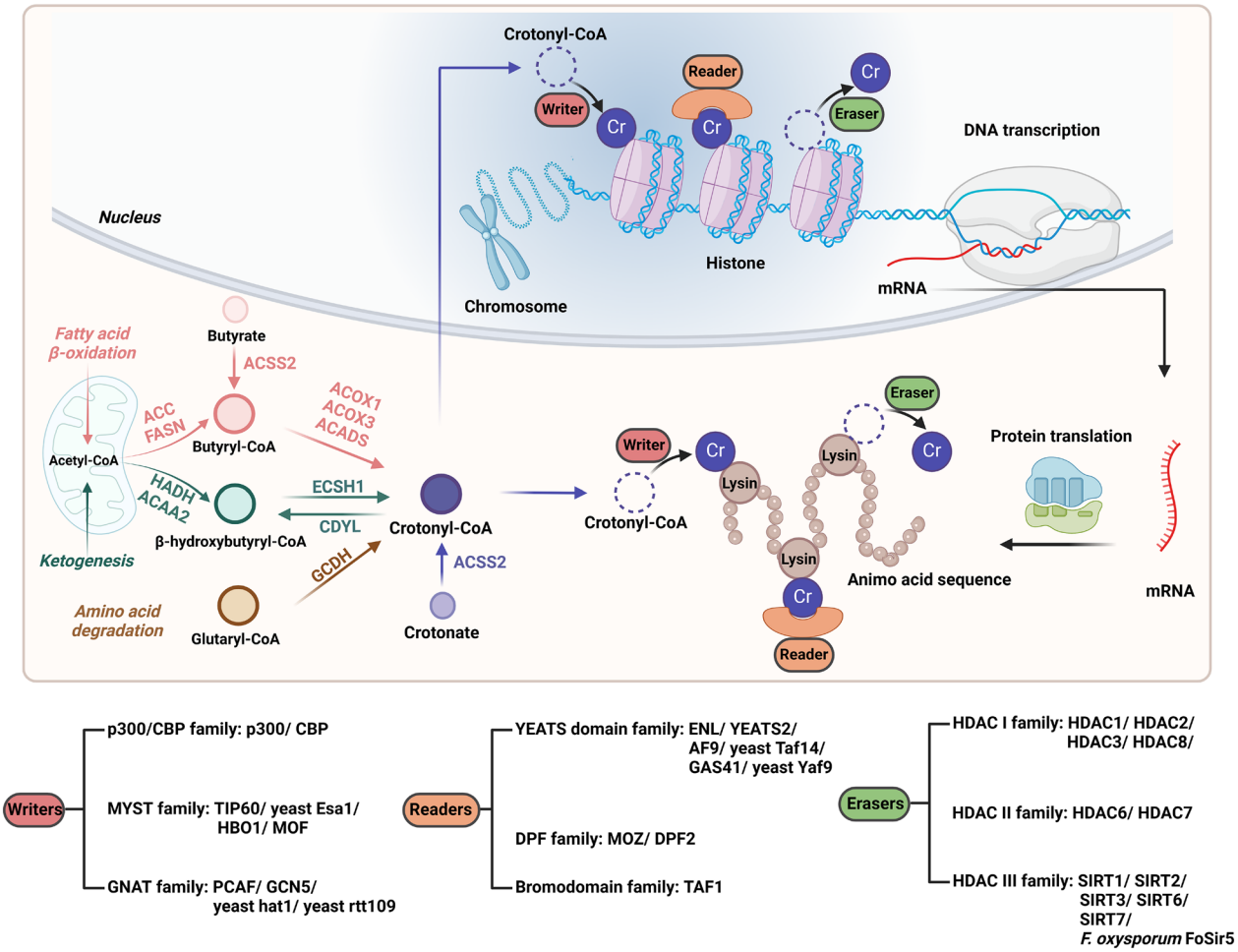

Fig. 2 The modulation of protein Kcr. Histone and non-histone Kcr are regulated by intracellular Cr-CoA levels, writers, erasers, and readers. Acetyl-CoA derived from fatty acid β-oxidation is converted to Butyryl-CoA by ACC and FASN, and subsequently to Cr-CoA. Similarly, acetyl-CoA from ketone body synthesis is converted to β-hydroxybutyryl-CoA by HADH and ACAA2, and further processed into Cr-CoA by ECSH1. Glu-CoA, produced via amino acid degradation, is converted to Cr-CoA by GCDH. Additionally, crotonate can be catalyzed by ACSS2 to form Cr-CoA. Writers, such as the p300/CBP, MYST, and GNAT families, catalyze the addition of crotonyl groups to lysine side chains on histones and non-histones. Crotonyl groups are removed by erasers, including the HDAC I and III families

more effectively than histone acetylation [20]. A study identifying the eraser of histone crotonylation revealed that SIRT3 suppresses the expression of PtK2, Tshz3, and Wapal. Histone crotonylation was found to co-localize at the transcription start sites of these genes, suggesting its role in promoting transcription [51]. Additionally, studies on acute kidney injury (AKI) showed that histone crotonylation in renal tissues activates PGC-1 and SIRT3 expression, protecting the kidneys from damage [52]. Overall, Histone crotonylation at residues H3K4, H3K9, H3K18, H3K27, and H2BK12 has been identified to promote transcriptional activation.

Beyond promoting transcription, elevated Kcr levels suppress growth- and endocytosis-related gene expression, indicating a potential negative regulatory role. Recent studies have shown that NEAT1 regulates p300 acyltransferase activity, modulating H3K27cr and H3K27ac near the transcription start sites of endocytosis-related genes, thereby repressing their transcription [53]. inhibition of NEAT1 reduces H3K27ac levels while increasing H3K27cr by suppressing acetyl-CoA production [53]. Another study on metabolic status and epigenetic regulation found that in yeast cells with elevated fatty acid oxidation, increased H3K9cr levels were accompanied by decreased H3K9ac levels, which inhibited the expression of growth-related genes activated by H3K9ac [16].

Chromatin remodeling

Kcr is a post-translational modification that significantly influences chromatin remodeling, thereby affecting gene expression and various cellular processes. For instance, crotonylation of histone H3 at lysine 18 (H3K18) and histone H4 at lysine 8 (H4K8) is associated with active transcription and an open chromatin state, facilitating access to transcriptional machinery [2, 20]. Recent studies have also highlighted the role of Kcr in non-histone protein regulation [5, 12], suggesting its involvement in broader aspects of chromatin dynamics and cellular function. The identification of "readers" [45, 46] that specifically recognize crotonylated lysines adds another layer of complexity to how Kcr influences chromatin remodeling and gene regulation.

DNA damage response

Recent studies suggest that Kcr plays a crucial role in the DNA damage response (DDR) by modulating chromatin accessibility and recruiting DNA repair factors [54]. The research indicates that GCN5 mediates the crotonylation of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), promoting the repair of DNA double-strand breaks through the non-homologous end joining (NHEJ) pathway and influencing cancer radiosensitivity [54]. Moreover, that crotonylation of replication protein A1 (RPA1) plays a key role in DNA damage repair. The crotonylation levels of RPA1 at K88, K379, and K595 increase upon DNA damage, promoting its binding to single-stranded DNA, enhancing its recruitment to DNA damage sites, and facilitating the homologous recombination repair process [13]. Metabolic cues influence Kcr-dependent DDR pathways. Since crotonyl-CoA availability governs Kcr levels, metabolic alterations (e.g., oxidative stress or nutrient deprivation) may affect DNA repair efficiency.

In summary, Kcr contributes to DDR by modulating chromatin states, recruiting repair proteins, and integrating metabolic signals. Further research is needed to clarify its interplay with other PTMs and its therapeutic potential in DNA repair-deficient diseases.

The relationship between Kcr and multiple diseases

As research on Kcr progresses, its links to various diseases have been revealed. These findings could provide potential drug targets for disease treatment. The link between Kcr and diseases is summarized in Table 2. The biological functions and molecular mechanisms of protein Kcr in diseases are shown in Figs. 3 and 4, respectively.

Neurological system disorders

Alzheimer's disease

Alzheimer's disease (AD) is a degenerative disorder of the central nervous system, primarily affecting older adults or those in the pre-senile stage. The key features of AD include progressive cognitive and behavioral impairments. Typical histopathological changes in AD include β-amyloid peptide (Aβ) deposition and neuronal fibrillary tangles, ultimately leading to neuronal atrophy or death, which results in cognitive dysfunction such as memory, language, calculation, and behavior. Wang et al. reported that paranuclear spot assembly transcript 1 (NEAT1), a long non-coding RNA, is downregulated in early AD [53]. The downregulation of NEAT1 leads to decreased expression of endocytosis-related genes, which subsequently triggers glial cell-mediated neuroinflammation via Aβ [53]. They also found that NEAT1 influences the HAT activity of p300 by binding to the p300/CBP complex, altering H3K27ac and H3K27cr near the transcription start sites (TSSs) of these genes. Interestingly, the inhibition of NEAT1 downregulates H3K27ac and upregulates H3K27cr by inhibiting acetyl-CoA production [53]. Additionally, evidence suggests that crocin, the main component of saffron, exerts neuroprotective effects in AD by downregulating Kcr [55]. Unfortunately, due to limited research on Kcr, its precise regulatory role in the pathogenesis of AD remains unclear and requires further investigation.

hypoxic-ischemic encephalopathy (HIE)

Hypoxic ischemic encephalopathy (HIE) is characterized by reduced oxygen and blood flow, leading to an inadequate nutrient supply to the brain due to a complex interplay of factors. He et al. observed that reduced H3K9cr in HIE rats downregulates the expression of neurotrophic genes related to HIE, resulting in pathological damage to the cerebral cortex and hippocampus [56]. Sodium butyrate (SB) can reverse and ameliorate HIE-induced brain injury via the gut-brain axis [56]. Clinical studies also indicate that SB alters the microbiota in patients with inflammatory bowel disease and may possess anti-inflammatory properties [57]. Based on current research, SB may soon be applied in the clinical treatment of HIE.

Depression

Major depressive disorder (MDD), often described as the "common cold" of psychiatry, is strongly influenced by stressful lifestyles, traumatic events, and regulated by epigenetic modifications. Liu et al. found that stress-susceptible rodents have lower histone Kcr levels in the medial prefrontal cortex, accompanied by selective

Table 2 The roles of Kcr in diseases

Disease category	Diseases	Biological context	Kcr level	Biological impact	References
Neurological system disorders	Alzheimer's disease	U251 cells, brain tissues from Alzheimer's disease mice	↑H3K27cr	Inhibit endocytosis	[53, 55]
	Hypoxic-ischemic encephalopathy	Cerebral cortex and hippocampus tissues from Neonatal hypoxic–ischemic brain damage rats	↑Pan-Kcr, ↑H3K9cr	SB ameliorates HIE	[56, 57]
	Depression	SH-SY5Y cells, prelimbic cortex tissues from chronic social defeat stress mice	↓Pan-Kcr	CDYL-mediated histone Kcr aggravated stress-induced depression	[58]
	Neuropathic pain	The trigeminal ganglia tissues from $Cx3cr1^{GFP}$ mice and complete freund adjuvant induced inflammatory pain mice	↑Pan-Kcr	Kcr induces macrophage activation and inflammatory	[59]
	Neurodevelopmental diseases	Human neural stem/ progenitor cells and P19 embryonal carcinoma cells	↓Pan-Kcr, ↑H3K9Cr	Activates bivalent promoters to stimulate gene expression in nspc	[64–66]
Dental disorders	Osteogenic differentiation of PDLSCs	Human periodontal ligament stem cells	↑Pan-Kcr	Treatment with sodium crotonate (NaCr) and silencing ACSS2 affected the activation of the PI3K-AKT signaling pathway	[67]
	Oral squamous cell carcinoma	CAL27 cells	↑HSP90AB1 Kcr	HSP90AB1 Kcr in hypoxic conditions may enhance the glycolysis regulation ability in oral squamous cell carcinoma	[68]
Respiratory system disorders	Chronic obstructive pulmonary disease	Human lung tissues	↓PanKcr	Kcr impacts chronic obstructive pulmonary disease	[70]
	Non-small cell lung cancer	PEM-resistant non-small cell lung cancer cells and H1299 cells	↓H3K18cr, ↓BEX2 K59cr	Reduce proliferation of PEMresistant non-small cell lung cancer cells	[12, 71]
Cardiovascular system disorders	Ischemic heart disease	Vascular smooth muscle cells from mice with cardiac I/R injury, volatile mycocytes from isoprenaline induced neonatal rats	↑IDH3a K199cr, ↑TPM1 K28/29cr	Preserve myocardial function	[72–74]
	Hypertrophic cardiomyopathy	Cardiomyocytes from Echs1 mutant mice and ECHS1-expressing/siechs1 neonatal rats	↑H3K18cr, ↑H2BK12cr,↑NAE1 K238cr	Kcr is involved in cardiac hypertrophy	[76, 77]
	Cardiac dysfunction and arrhythmia	Cardiomyocytes from SIRT1 cardiac specific knockout mice	↑SERCA2a K120cr	Kcr induces cardiac dysfunction	[78]
Digestive system disorders	Liver fibrosis	Liver tissues from CCl_4 induced liver fibrosis rats	↓Pan-Kcr, ↓H2BK12cr, ↓H3K18cr	Improve the hepatic structure and fibrotic progression	[72]
	Hepatocellular carcinoma	HepG2 cells, liver tissues from Acox2 knockout mice	↓Crot Kcr, ↓Scp2 Kcr, ↓Hsd17b4 Kcr	Regulate hepatic metabolic homeostasis	[83]
		Huh-7 cells, HepG2 cells, liver tissues from female balb/c nude mice	↑PanKcr, ↑LamininA K265/270cr	Promote liver cancer cell proliferation	[36]

Table 2 (continued)

Disease category	Diseases	Biological context	Kcr level	Biological impact	References
		HepG2 cells and MHCC97 cells, liver tissues from mice with liver tumor	↑PanKcr, ↑SEPT2	Promote cell invasive capability	[84]
		implantation	K74cr		
		Human hepatoma-derived cells	↑PanKcr	Inhibit the movement and proliferation of hepatoma cells	[85]
	Colorectal cancer	HCT-116 cells, DLD-1 cells, LoVo cells and SW480 cells	↓H3K27cr	The level of H3K27cr was reduced during DNA damage in colon cancer	[42, 88, 89]
		Human colorectal cancer tissues	↑ENO1 K420cr	ENO1 K420cr promotes growth, migration, and invasion of CRC cells	[90]
		Human PBMCs	↑H2BK12cr	H2BK12cr may serve as a biomarker	[92]
	Pancreatic cancer	PANC-1 cells	↓MTHFD1 K354/553cr	MTHFD1 Kcr inhibited the development of pancreatic cancer by increasing resistance to ferroptosis	[18]
Urogenital system disorders	Acute kidney injury	Proximal tubular cells from folic acid induced mice	↑PanKcr	Against inflammation and mitochondrial stress	[52]
	Renal fibrosis	Human distal tubular cells and tubular epithelial cells	↓PanKcr, ↑H3K9cr	Alleviate IL-1β-dependent macrophage activation and tubular cell senescence, and delay renal fibrosis	[49, 95]
	Autosomal dominant polycystic kidney disease	WT 9–12 cells, kidney tissues from CDYL transgenic mice and Pkd1 knockout mice	↑Pan-Kcr, ↑H3K18cr	Overexpression of CDYL reduces Kcr and slows cyst growth	[98, 99]
	Prostate cancer	Human prostate cancer cell lines (PC-3 cells, LNCaP cells, and C42B cells), human prostate cancer tissues	↓H3K18cr	Promote the function of Prostate cancer cells	[101]
Reproductive system disorders	Polycystic ovary syndrome	Ovarian tissues from mice	↑LONP1 K390cr	Kcr leads to mitochondrial dysfunction and oxidative stress	[102]
	Spermatogenesis disorder	Germ cells from mice	/	Regulate escape gene activation from inactive sex chromosomes in post-meiotic spermatids	[14]
		Spermatogenic cells from mice	↑PanKcr	Specifically mark testis specific genes	[9]
		Round spermatid tissues from CDYL transgenic mice	↓PanKcr, ↓H2BK12cr	CDYL-regulated histone Kcr regulates spermatogenesis and male fertility	[15]
	Cervical cancer	Human cervical cancer cells and the normal cervical epithelial cells	↑Pan-Kcr	Enhance cell proliferation, invasion, and migration of hela cells	[107]
Infectious disorders	AIDS	Human HIV latency cells	↑H3K4cr	Viral reactivation	[110]
		Human jurkat cells of HIV latency	↑PanKcr	Viral reactivation	[113]

Table 2 (continued)

Disease category	Diseases	Biological context	Kcr level	Biological impact	References
	Bacterial infection	Porcine alveolar macrophages after T. Gondii infection from mice	↓H2BK12cr	Activation of immune response	[36, 115]
Metabolic disorders	Type 2 diabetes mellitus	HK-2 cells, kidney tissues from diabetic (db/db) mice	↑H3K27cr	Glucose uptake	[116, 117]
	Obesity	White adipocyte tissues from B3-adrenergic receptor (CL316,243) agonist induced the white fat browning mice	↑PanKcr	Modulation of white fat browning	[125, 126]

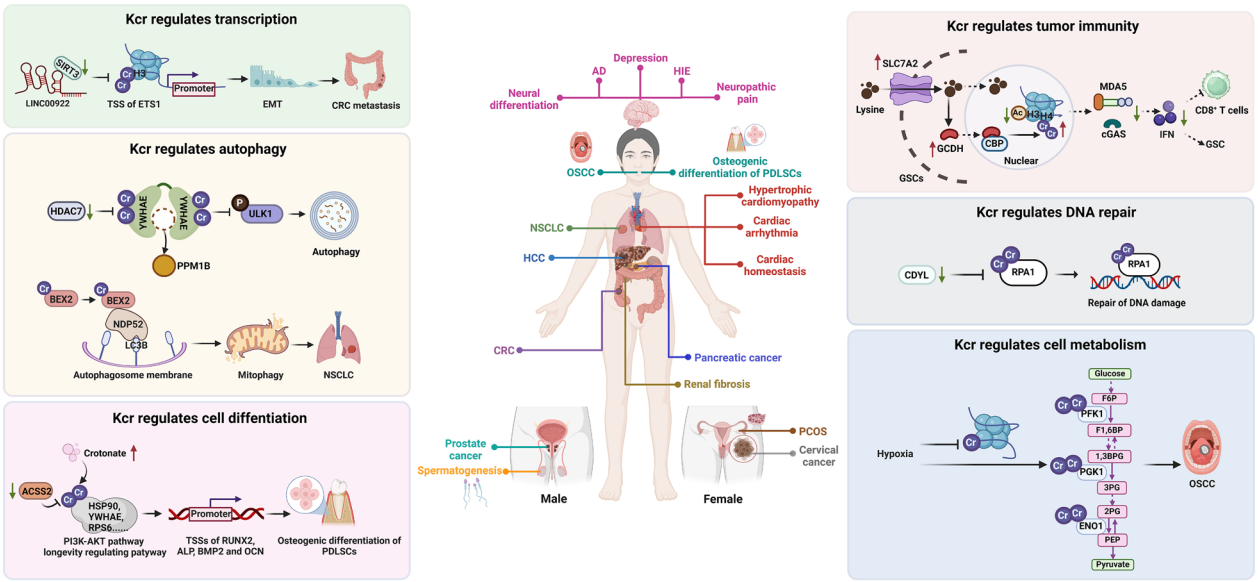

Fig. 3 Biological functions of protein Kcr in diseases. Kcr has been shown to regulate protein biological functions in various diseases, and abnormal Kcr levels closely linked to disease onset and progression. For instance, Kcr regulates gene transcription in colorectal cancer, mitophagy in NSCLC, and metabolic processes in OSCC. Furthermore, Kcr plays a key role in DNA damage repair, tumor immunity, and cell differentiation

upregulation of CDYL [58]. Overexpression of CDYL in the prelimbic cortex leads to increased social avoidance behaviors and anhedonia in mice. In contrast, the knockdown of CDYL in the prelimbic cortex prevented the decrease in depression-like behaviors induced by chronic social defeat stress. Mechanistically, CDYL inhibited structural synaptic plasticity mainly through transcriptional repression of the neuropeptide VGF nerve growth factor-inducible gene, with this activity dependent on its dual effect on histone Kcr and H3K27 trimethylation at the VGF promoter. Overall, the study demonstrated that CDYL-mediated histone Kcr plays a key role in regulating MDD, offering a potential therapeutic target for the disorder [58]. Unfortunately, few studies directly demonstrate the relationship between depression and Kcr, so more research is needed to explore this link.

Neuropathic pain

Abnormal regulation of Kcr in proteins may play a crucial role in neuropathic pain [59]. Zou et al. found that Kcr is widely present in various cell types, including macrophages, sensory neurons, and satellite glial cells of the trigeminal ganglia (TG). Peripheral nerve injury elevates Kcr levels in macrophages of the TG, while reducing Kcr levels in sensory neurons [59]. Administration of C646, which inhibits p300, significantly alleviated mechanical allodynia and thermal hyperalgesia induced by partial infraorbital nerve transection or spinal nerve ligation. Conversely, dose-dependent administration of crotonyl-CoA trilithium salt to upregulate Kcr induces mechanical allodynia and thermal hyperalgesia in mice. Mechanistically, they revealed that inhibition of p300 alleviates macrophage activation induced by partial infraorbital nerve transection and reduces the expression of pain-related inflammatory cytokines, including TNF-α, interleukin 1β (IL1β), and chemokines C–C motif chemokine ligand 2 (CCL2) and C-X-C motif chemokine ligand 10 (CXCL10) [59]. Correspondingly, exogenous crotonyl-CoA induces macrophage activation and the expression of TNF-α, IL1β, IL6, CCL2, and CCL7 which are repressed by C646 [59]. Therefore, targeting enzymes that modify Kcr to promote crotonylation or decrotonylation may offer a viable therapeutic strategy for neuropathic pain or diseases associated with neuroinflammation.

Neurodevelopmental diseases

Many studies have shown that histone methylation and Kac play key roles in neural development [60–63]. Dai et al. found that histone Kcr, which mainly localizes in active promoter regions, regulates genes involved in metabolism and proliferation of neural stem/progenitor cells (NSPCs). Moreover, elevated histone Kcr activates bivalent promoters, stimulating gene expression in NSPCs by increasing chromatin openness and recruiting RNA polymerase II (RNAP2). Functionally, these activated genes contribute to transcriptome remodeling and promote neuronal differentiation [64]. Dai et al. also disclosed that genome-wide changes in H3K9cr favor neural fate specification and identify potential co-factors binding H3K9cr in P19 embryonal carcinoma cells.

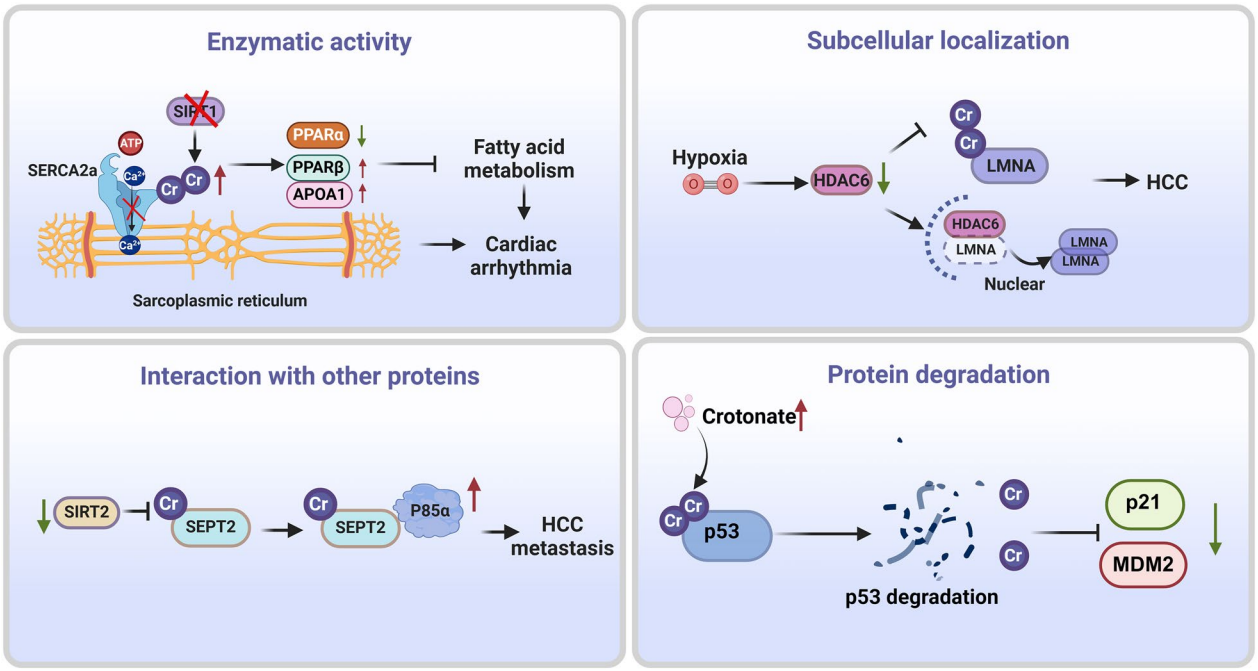

Fig. 4 Molecular function of protein Kcr in diseases. Kcr affects protein functions through diverse mechanisms, including by regulating enzymatic activity, subcellular localization, interaction with other proteins and crosstalk with other PTMs. Mouse double minute 2 (MDM2), Apolipoprotein A-I (APOA1)

In addition, they uncovered that H3K9ac, H3K9cr, and H3K18la, in combination with ATAC and RNA sequencing, are tightly correlated with chromatin state and gene expression and play extensive roles in transcriptome remodeling to promote cell-fate transitions in the developing telencephalon [65, 66]. These findings suggest that Kcr may be a potential target for treating neural development disorders and neurological diseases.

Dental disorders

Research on periodontal ligament stem cells (PDLSCs) revealed that Kcr levels, related to the PI3K-AKT signaling pathway, are significantly upregulated in PDLSCs after osteogenic induction. Treatment with sodium crotonate (NaCr) and silencing ACSS2 affect the activation of the PI3K-AKT signaling pathway [67]. This study demonstrated that Kcr promotes osteogenic differentiation of PDLSCs via the PI3K-AKT pathway, providing a novel therapeutic approach for bone tissue regeneration [67]. Another study revealed the role of Kcr in oral squamous cell carcinoma (OSCC) through large-scale identification of Kcr. They found that heat shock protein 90 kDA alpha, class B, member 1(HSP90AB1) Kcr under hypoxic conditions may enhance glycolysis regulation in OSCC, offering novel perspectives on the regulatory mechanism of crotonylation in hypoxic OSCC and potential therapeutic targets for OSCC treatment [68].

Respiratory system disorders

Chronic obstructive pulmonary disease (COPD)

Chronic obstructive pulmonary disease (COPD) is a common respiratory condition characterized by incomplete, reversible, and progressively developing airflow limitation [69]. A study identified 90 proteins modified by Kcr and differentially expressed in COPD combined with type II respiratory failure, which may contribute to the disease's development and could serve as markers for studying its molecular pathogenesis [70].

Non-small cell lung cancer (NSCLC)

Non-small cell lung cancer (NSCLC) is the most common subtype of lung cancer, and resistance to pemetrexed (PEM) limits its treatment options. A study identified 2,696 lysine crotonylation sites on 1,024 proteins in human lung adenocarcinoma H1299 cells for the first time, using high-resolution liquid chromatography-tandem mass spectrometry (LC–MS/MS)-based proteomics [12]. Mu et al. reported that Brain-Expressed X-Linked Gene 2 (BEX2) promotes mitophagy by facilitating the interaction between NDP52 and LC3B. BEX2 plays a crucial role in inhibiting chemotherapeutic agent-induced apoptosis by enhancing mitophagy in human lung cancer cells [71]. Moreover, BEX2 K59cr is critical for BEX2-mediated mitophagy in non-small cell lung cancer, and the K59R mutation of BEX2 inhibits

mitophagy by affecting the interaction between NDP52 and LC3B [71]. They also confirmed that BEX2 is overexpressed in lung adenocarcinoma and is associated with poor prognosis in lymph node metastasis-free cancer.

Therefore, combination treatment using pharmaceutical approaches targeting BEX2-induced mitophagy, along with anticancer drugs, may represent a potential strategy for NSCLC therapy [71].

Cardiovascular system disorders

Ischemic heart disease (IHD)

Ischemic heart disease (IHD) is a severe myocardial dysfunction and remains the leading cause of death worldwide. Vascular smooth muscle cells (VSMCs) undergo phenotypic transformation, leading to vascular remodeling in vascular diseases such as atherosclerosis, diabetic macroangiopathy, and restenosis [72, 73]. A bioinformatics analysis identified 2,138 crotonylation sites in 534 proteins involved in vital cellular pathways and functions in VSMCs, such as glycolysis/gluconeogenesis, vascular smooth muscle contraction, and the PI3K-Akt signaling pathway [72, 73]. Moreover, enrichment and PPI network analyses showed widespread interactions between crotonylated proteins and the clustering of their functions, such as ribosomes and spliceosomes [72, 73]. Additionally, Cai et al. revealed that Kcr is associated not only with disruption of cardiomyocyte mitochondria, sarcomere architecture, and gap junctions, but also with cardiomyocyte autophagy and apoptosis [74]. They also discovered that modulating Kcr on IDH3a (isocitrate dehydrogenase 3 [NAD^+] alpha) at K199 and tropomyosin alpha-1 chain (TPM1) at K28/29, or treatment with NaCr, not only protects cardiomyocytes from apoptosis by inhibiting Bcl-2 adenovirus E18 19-kDa-interacting protein 3 (BNIP3)-mediated mitophagy or cytoskeletal rearrangement but also preserves post-injury myocardial function by inhibiting fibrosis and apoptosis. These findings provide insight into a novel mechanism for Kcr in cardioprotection and may present a new therapeutic intervention for IHD.

Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy is the leading cause of sudden death in young people and can result in functional disability from heart failure and stroke [75]. Short-chain enoyl-CoA hydratase (encoded by enoyl-CoA hydratase short-chain 1, ECHS1) is an enzyme with the highest activity for hydrolyzing crotonyl-CoA, thereby reducing intracellular crotonyl-CoA and downregulating Kcr levels [76] A study reported decreased ECHS1 levels along with increased H3K18cr and H2BK12cr, suggesting the involvement of ECHS1 and histone Kcr in cardiac hypertrophy [76]. This group further explored that ECHS1 deficiency promotes the recruitment of the nuclear factor of activated T cells isoform C3(NFATC3) transcription factor to the promoter of myocardial hypertrophy genes, such as *Nppb*, leading to myocardial hypertrophy. Additionally, crotonate, which induces histone Kcr, can directly upregulate the expression of *Nppb*, while *Echs1* knockdown enhances the effects of crotonate on *Nppb* expression [76]. These findings elucidate the phenotypes and mechanisms underlying ECHS1 mutation-mediated cardiac defects in humans and suggest that histone Kcr may serve as a novel strategy for treating hypertrophic cardiomyopathy and heart failure. Moreover, research found that Kcr at the K238 site of NAE1 plays a crucial role in mediating cardiac hypertrophy through gelsolin (GSN) neddylation, providing potential novel therapeutic targets for pathological hypertrophy and cardiac remodeling [77].

Cardiac dysfunction and arrhythmia

Cardiac dysfunction, also referred to as "heart failure," is a condition in which the heart fails to pump blood effectively. Arrhythmia is an irregularity in heart rhythm or rate, resulting from abnormalities in the generation or conduction of electrical impulses in the heart. Arrhythmias are often a cause of cardiac insufficiency. One study revealed that Kcr at the K120 site of SR Ca^{2+}-ATPase 2 (SERCA2a) is significantly increased after cardiac-specific knockout of SIRT1 (ScKO). Consequently, the activity of SERCA2a decreases due to a reduced binding affinity between crotonylated SERCA2a and ATP. Changes in the expression of PPAR-related proteins suggest an alteration in energy metabolism. They concluded that SIRT1 knockout alters the ultrastructure of cardiac myocytes, induces cardiac hypertrophy and dysfunction, causes arrhythmia, and modifies energy metabolism by regulating the Kcr of SERCA2a in the heart [78].

Digestive system disorders

Liver fibrosis

Liver fibrosis occurs as a result of chronic liver injury, leading to inflammation and the activation of myofibroblasts, which secrete extracellular matrix proteins to form fibrous scars. The primary source of these myofibroblasts is the resident hepatic stellate cells (HSCs) [79]. Sorafenib, a multikinase inhibitor, prevents liver fibrosis by inhibiting HSC activation, epithelial-mesenchymal transition (EMT), and the transforming growth factor β1 (TGFβ1) signaling pathway [80–82]. Chen et al. investigated sorafenib's mechanism in a CCl4-induced rat model of liver fibrosis and found that it inhibits fibrosis by maintaining hepatic crotonylation-regulated enzyme and Kcr homeostasis. Further studies revealed that the expression of HDAC1, HDAC3, and CDYL was

significantly increased in fibrotic livers and reduced by sorafenib treatment [72].

Hepatocellular carcinoma (HCC)

In hepatocellular carcinoma (HCC), Kcr levels are correlated with the Tumor, Node, and Metastasis (TNM) stage [83]. A study found that HDAC knockout or HDAC inhibitors suppress hepatocyte migration and proliferation by increasing histone Kcr levels [83]. Another study found that HDAC6 was downregulated during hypoxia, leading to an increase in laminin A Kcr and HCC proliferation [36]. They also identified the Kcr sites of laminin A, K265/270, which are decrotonylated by HDAC6. Through bioinformatic analysis, Zhang et al. found that Kcr levels of Septin 2 (SEPT2) are significantly increased in highly invasive cells. The decrotonylated mutation of SEPT2-K74 impaired SEPT2 GTPase activity and inhibited HCC metastasis in vitro and in vivo [84]. Kcr levels are reduced in HCC, but the mechanism remains unclear. A study explored the role of GCDH in tumor suppression. GCDH inhibits HCC progression by decreasing Kcr levels, which suppresses the pentose phosphate pathway (PPP) and glycolysis, leading to cell senescence in HCC. Senescent cells further promote an anti-tumor microenvironment through the senescence-associated secretory phenotype (SASP). The GCDH ^{low} population showed responsiveness to anti-PD-1 therapy due to increased PD-1 + $CD8^+$ T cells [85].

Colorectal cancer (CRC)

Colorectal cancer (CRC) is among the most common malignancies and a leading cause of morbidity and mortality worldwide. Approximately 50% of CRC patients develop liver metastases during the course of their disease [86]. A recent study suggested that p300/CBP-mediated histone Kcr contributes to the hypoxic induction of autotaxin (ATX) in SW480 cells via a HIF-2α-dependent mechanism, promoting cancer cell migration through the ATX-LPA axis [87]. Furthermore, a study identified the YEATS domain-containing protein 4 (GAS41) as a previously unrecognized factor involved in regulating nuclear morphology. Mechanistically, GAS41 recruits BRD2 and the Mediator complex to the gene loci of these regulators, promoting their transcriptional activation. Disruption of GAS41-H3K27cr binding caused BRD2, MED14, and MED23 to dissociate from gene loci, leading to nuclear shape abnormalities [88]. In addition, Liao et al. found that H3K27cr levels decrease during DNA damage in CRC, with the reduction potentially mediated by SIRT6 [42]. Recently, a new study uncovered a novel regulatory function of H3K27cr, regulated by LINC00922, in facilitating CRC metastasis. The study found that LINC00922 promotes invasion and migration through H3K27cr-mediated cell adhesion molecules (CAMs) in epithelial cells. Notably, LINC00922 interacts with SIRT3 and obstructs its binding to the ETS1 promoter region, leading to an elevation in H3K27cr levels in this region and subsequent activation of ETS1 transcription [89]. Subsequently, a study found that CBP promotes ENO1 Kcr, while the deacetylase SIRT2 effectively reduces ENO1 Kcr levels, enriching the regulatory enzymes of non-histone Kcr. Additionally, ENO1 K420cr promotes growth, migration, and invasion of CRC cells in vitro by enhancing ENO1 activity and regulating the expression of tumor-related genes [90]. Interestingly, it has been reported that CBP and SIRT3 are crotonyltransferase and decrotonylase, respectively, for protein kinase cAMP-dependent catalytic-alpha (PRKACA) [91]. Blood-based tests are popular for cancer screening due to their minimally invasive nature, ability to integrate with other routine tests, and high patient compliance [92]. The authors found that H2BK12cr levels in peripheral blood mononuclear cells of CRC patients may serve as a biomarker for distinguishing CRC patients from healthy controls, offering advantages such as ease of operation and high diagnostic efficacy [92].

Pancreatic cancer

Pancreatic cancer is the fourth leading cause of cancer-related death in the USA, with an estimated 227,000 deaths annually worldwide [93]. A study revealed that decrotonylation of MTHFD1 at the K354 and K553 sites promotes pancreatic cancer development by increasing resistance to ferroptosis, suggesting that Kcr is a metabolic regulatory mechanism in pancreatic cancer progression [18].

Urogenital system disorders

Acute kidney injury (AKI)

Acute kidney injury (AKI) is a potentially fatal condition, characterized by limited therapeutic options, poor prognosis, and high mortality rates. Currently, apart from renal replacement therapy (RRT), few therapeutic options are available for AKI. Ruiz-Andres et al. induced AKI in mice using folic acid or cisplatin, observing significantly elevated levels of Kcr in kidneys from mice with AKI [52]. NaCr upregulates Kcr, the kidney protection factor proliferator-activated receptor gamma coactivator-1α (PGC-1α) and SIRT3, while downregulating CCL2 levels in cultured tubular cells and kidneys [52]. This finding suggests that increased histone Kcr may benefit AKI by blocking the tumor necrosis factor-like weak inducer of apoptosis-mediated upregulation of SIRT3 [52].

Renal fibrosis

Renal fibrosis is a common outcome of many chronic kidney diseases (CKD), regardless of the underlying etiology. Despite promising experimental data, current strategies only ameliorate or delay CKD progression, without reversing fibrosis [94]. Neuropilin-1 (NRP1), a co-receptor for various cytokines, including TGF-β, has been identified as a potential therapeutic target for fibrosis. However, its role in renal fibrosis remains unclear. A recent study showed that NRP1 is upregulated in distal tubular (DT) cells of patients with transplant renal insufficiency and in mice with renal ischemia–reperfusion (I-R) injury. Knockout of NRP1 reduces various endpoints of renal injury and fibrosis. The study also found that NRP1 facilitates the binding of TNF-α to its receptor in DT cells after renal injury. This signaling downregulates Kcr of glucose metabolic enzymes, decreasing cellular energetics and exacerbating renal injury. This negatively affects mitochondrial function, ultimately leading to various forms of cell death in distal renal tubular epithelial cells (TECs) and subsequent fibrosis [95]. Moreover, the crotonyl-CoA-producing enzyme (ACSS2) significantly increases histone H3K9cr levels without affecting H3K9ac in kidneys and TECs. Additionally, IL-1β levels induced by H3K9cr are suppressed by genetic and pharmacologic inhibition of ACSS2, alleviating IL-1β-dependent macrophage activation and tubular cell senescence, thus delaying renal fibrosis. Collectively, these findings suggest that H3K9cr plays a critical role in kidney fibrosis, with ACSS2 representing a potential drug target to slow the progression of fibrotic kidney disease [49].

Autosomal dominant polycystic kidney disease (ADPKD)

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disorder caused by mutations in the PKD1 or PKD2 genes, which encode polycystin-1 (PC1) and polycystin-2 (PC2), respectively[96, 97]. Mutations in PKD1 and PKD2 lead to dysregulation of various signaling pathways and activation of a pathological gene expression program [98]. Recently, Dang et al. found that CDYL is downregulated and accelerates cyst growth in ADPKD kidneys, accompanied by an increase in H3K18cr, identified as the major modification in human PKD1 mutant cell lines [99]. This suggests that CDYL nuclear condensation links histone Kcr to transcriptional responses and cystogenesis in ADPKD [99].

Prostate cancer (PCa)

Prostate cancer (PCa) is the most common malignancy in males worldwide and the second-leading cause of cancer-related death [100]. The level of Kcr is closely associated with the pathological grade of prostate cancer. A study found that Kcr, especially H3K18cr, which is regulated by the crotonyltransferases p300/GCN5, is elevated in PCa tissue [101]. Treatments with I-BET762, I-BET726, and CPI-203 can inhibit the proliferation, migration, and invasion of PCa, while also regulating histone Kcr and androgen receptor signaling pathways through modulation of BRD4 expression [101].

Reproductive system disorders

Polycystic ovary syndrome (PCOS)

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disorder characterized by metabolic abnormalities and ovulatory dysfunction. A study found that decrotonylation of Lon protease 1 (LONP1) at the K390 site is linked to mitochondrial dysfunction in PCOS. Furthermore, the LONP1 Kcr level in PCOS correlates with oxidative stress in ovarian tissue, androgen levels, and oocyte development [102].

Spermatogenesis disorder

In the absence of gene sequence changes, 5–15% of histone modification abnormalities in sperm are key factors contributing to male infertility [103]. Tan et al. were the first to identify that a novel histone Kcr is enriched on sex chromosomes, specifically marking testis-specific genes, including a significant proportion of X-linked genes that escape sex chromosome inactivation in haploid cells [9]. A subsequent study revealed that histone Kcr plays a critical role in maintaining the activity of X/Y-linked genes by conferring resistance to transcriptional repressors in post-meiotic male germ cells [104]. Some researchers found that Kcr accumulates at TSSs of sex-linked genes, which are activated in an RNF8-dependent manner. This RNF8-dependent epigenetic programming affects chromatin conformational changes. Notably, this RNF8-dependent pathway is distinct from the pathway that recognizes DNA double-strand breaks [14]. Furthermore, Liu et al. found that CDYL-mediated negative regulation of histone Kcr is intrinsically linked to its transcriptional repression activity and plays a role in the reactivation of sex chromosome-linked genes in round spermatids and genome-wide histone replacement in elongating spermatids [15]. Notably, the level of histone Kcr, along with epididymal sperm count and sperm motility, decreased significantly in mice overexpressing CDYL [15]. These studies demonstrate that histone Kcr also regulates spermatogenesis, providing an important theoretical basis for diagnosing and treating male infertility and certain genetic diseases.

Cervical cancer

Cervical cancer, primarily caused by human papillomavirus (HPV) infection, is one of the most common cancers in women. Additionally, various other genetic and epigenetic factors contribute to the underlying pathogenesis of cervical cancer [105, 106]. Recent studies have shown that p300-mediated Kcr plays a role in regulating HNRNPA1 in HeLa cell proliferation, invasion, and migration [107].

Infectious disorders

Acquired immunodeficiency syndrome (AIDS)

Acquired immunodeficiency syndrome (AIDS) is a systemic disease caused by infection with the human immunodeficiency virus (HIV). HIV transcription is regulated by several cell signaling pathways [108]. It is well established that epigenetic regulation of histone tails at the HIV long terminal repeat LTR is crucial for establishing latent reservoirs [109, 110]. A study found that reactivation of latent HIV occurs following the induction of histone Kcr through increased expression of the ACSS2, while pharmacologic inhibition or siRNA knockdown of ACSS2 reverses this effect [110]. Additionally, the protein nuclear factor-kappaB (NF-κB) is a potent inducer of HIV gene expression [111, 112]. Compared to the canonical NF-κB pathway, the noncanonical NF-κB (ncNF-κB) pathway has garnered more attention due to its gradual but persistent activation of NF-κB-driven transcription [111]. Another study clarified the relationship between Kcr and the ncNF- κB pathway, finding that Kcr enhances the active p52 subunit of ncNF-κB following AZD5582, further promoting HIV latency reversal in Jurkat and U1 cell line models of latency [113]. Additionally, researchers discovered that wogonin, a flavone isolated from *Scutellaria baicalensis*, inhibits the reactivation of latent HIV-1 by inhibiting p300 expression, thereby decreasing the Kcr of histone H3/H4 in the HIV-1 promoter region [114].

Bacterial infection

Streptococcus agalactiae is a common colonizer of the rectovaginal tract and causes infectious diseases in neonates and non-pregnant adults [115]. A study identified 241 Kcr sites from 675 screened proteins, enriched in metabolic, cellular, and single-organism processes, through proteome-wide profiling of Kcr in *S. agalactiae* [115]. Another study provided a starting point for further functional analysis of Kcr in *Brucella* survival within hosts, as well as for interpreting *Brucella* protein function and elucidating its pathogenic mechanisms [36].

Metabolic diseases

Type 2 diabetes mellitus (T2DM)

Type 2 diabetes mellitus (T2DM) is a chronic condition characterized by elevated blood sugar levels due to genetic and environmental factors, including insufficient insulin secretion and/or insulin resistance. Abnormal expression of lncRNAs has been reported to be associated with the progression of diabetes and plays a significant role in glucose metabolism [116]. Recently, it was found that LncRNA EPB41L4A-AS1 decreases glucose uptake by enhancing the endocytosis of glucose transporters GLUT2/4 through GCN5-mediated H3K27cr in the GLUT4 promoter region [116]. Diabetic kidney disease (DKD) is a common microvascular complication of diabetes, characterized by inflammation and fibrosis during its progression. Diabetic (db/db) mice and high glucose-induced human tubular epithelial cells (HK-2) were used to confirm that NaCr, which induces histone Kcr, has an antidiabetic effect by decreasing blood glucose and serum lipid levels and alleviating renal function and DKD-related inflammatory and fibrotic damage [117].

Obesity

Obesity has become a global epidemic. It leads to an increased risk of various diseases including insulin resistance, T2DM, fatty liver disease, cardiovascular disease and certain types of cancers [118, 119]. Brown adipose tissue (BAT) facilitates weight control, health, and provides an anti-obesity effect [120]. Therefore, increasing BAT activity could be a novel and effective therapeutic approach for preventing and curing obesity and its related diseases [121–124]. It was reported that dihydrolipoyl dehydrogenase (DLD) Kcr promotes white adipocyte browning by activating mitochondrial function through the RAS/ERK pathway, while DLD acetylation has the opposite effect [125]. Another study identified 7,254 Kcr sites in 1,629 proteins from a white fat browning model using proteomic sequencing analysis and LC–MS/MS. Further analysis found that the Kcr of glycerol-3-phosphate dehydrogenase 1, fatty acid-binding protein 4, adenylate kinase 2, triosephosphate isomerase 1, and NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 8 promote white fat browning, consistent with the sequencing results [126].

Therapeutic opportunities for targeting protein crotonylation

Kcr represents a promising therapeutic target for a wide range of diseases, including cancer, neurodegenerative disorders, metabolic diseases, and immune-related conditions. Targeting enzymes involved in the addition or removal of crotonylation marks, such as crotonyltransferases and decrotonylases, could provide novel

therapeutic strategies for cancer treatment. Inhibitors of crotonyltransferases like p300, which catalyze histone crotonylation, have the potential to reduce oncogene expression and slow down tumor growth [127]. Additionally, targeting crotonylation at non-histone proteins, such as metabolic enzymes involved in cell growth and survival, could provide another avenue for cancer therapy [13, 85, 127]. Enhancing crotonylation at specific neuroprotective genes or regulating crotonylation in combination with other PTMs could offer potential treatments for preventing neurodegenerations [58]. Moreover, enhancing crotonylation of specific metabolic pathways could also help in regulating insulin sensitivity and lipid metabolism, offering potential treatments for obesity and type 2 diabetes [116, 117]. Inhibiting crotonylation of NF-κB or other immune-related transcription factors could reduce chronic inflammation and immune cell activation [113].

By modulating the enzymes responsible for adding or removing crotonyl groups, it may be possible to restore normal cellular processes that are dysregulated in disease. As our understanding of crotonylation continues to grow, the development of novel therapeutic strategies targeting this modification holds great promise for improving patient outcomes across a range of conditions. Further research is required to identify specific crotonylation-based biomarkers, explore the molecular mechanisms underlying crotonylation, and develop effective clinical interventions targeting this modification.

Crosstalk of Kcr and other PTMs

Crosstalk among PTMs allows protein to integrate diverse regulatory signals, influencing various cellular processes (Fig. 5). Kcr shares modification sites with Kac on histones and non-histone proteins [4]. Both modifications are catalyzed and removed by the same enzymes, yet they exhibit distinct structural and functional properties. Kcr has a more rigid planar structure due to its C=C bond, whereas Kac is more flexible. Notably, YEATS and DPF domains show a higher affinity for Kcr than Kac [45, 46, 48]. Additionally, the balance between intracellular crotonyl-CoA and acetyl-CoA levels can affect the prevalence of Kcr and Kac, respectively. In the development of AD, NEAT1 interacted with the acetyltransferase complex P300 and CBP and that silencing NEAT1 expression not only downregulated H3K27ac but also upregulated the H3K27cr level, which might be caused by the NEAT1-mediated decrease of acetyl-CoA generation [53]. Reactivation of latent HIV was achieved following the induction of histone crotonylation through increased expression of the crotonyl-CoA–producing enzyme acyl-CoA synthetase short-chain family member 2 (ACSS2). This reprogrammed the local chromatin at the HIV long-terminal repeat through increased histone acetylation

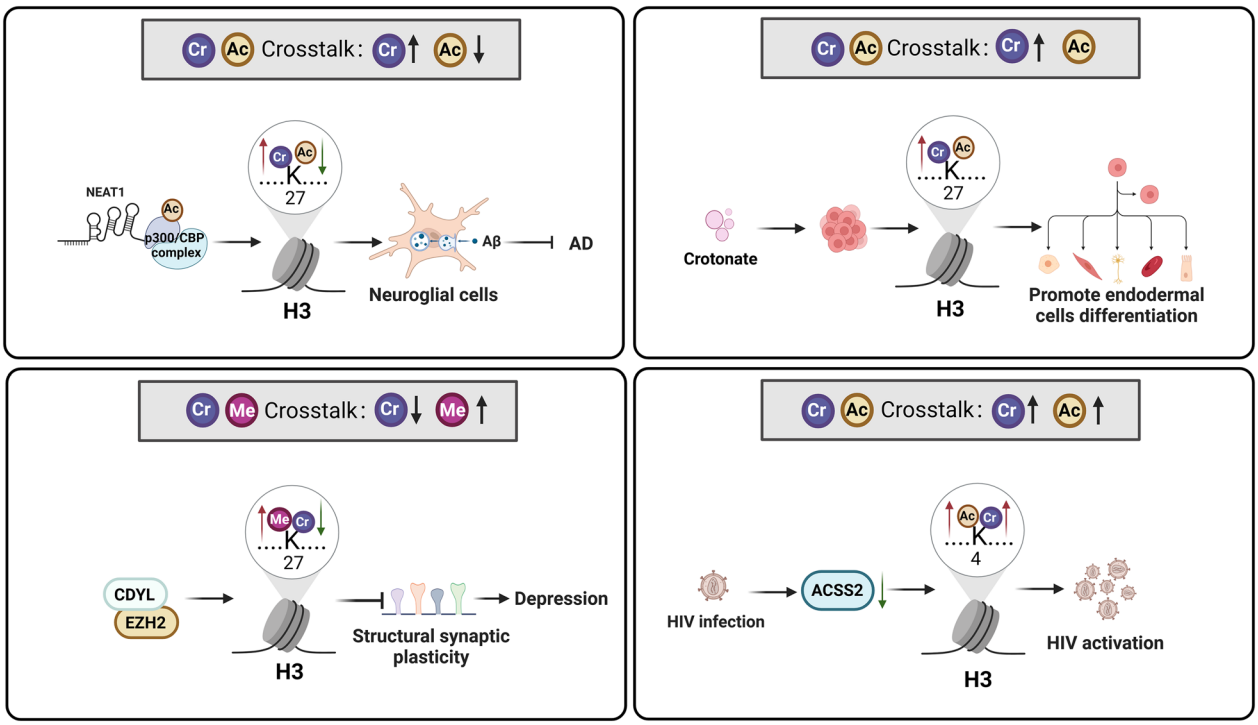

Fig. 5 Crosstalk of Kcr and other PTMs in diseases

and reduced histone methylation [110]. Histone Kcr is increased and enriched near endodermal genes upon endoderm differentiation, the levels of distinct histone Kac and Kbu sites were differentially altered after endoderm differentiation, indicating that histone Kcr is specifically induced upon endoderm differentiation of hESCs [10]. Under nutrient depletion, acetyl-CoA decreases while histone crotonylation persists, sustaining key gene transcription. During energy depletion, increased peroxisomal β-oxidation correlates with H3K9 crotonylation, reduced ATP and acetyl-CoA levels, and downregulation of ribosomal biogenesis genes.

Recent studies have expanded our understanding of Kcr's interplay with histone methylation. The coexistence of Kcr and methylation suggests a complex regulatory network in the progression of depression [58]. Furthermore, Kcr crosstalks with ubiquitination. Histone H2AK119 undergoes both crotonylation and ubiquitination. Under replication stress, H2AK119cr decreases while H2AK119ub increases, with SIRT1 and BMI1 controlling this switch. This process helps resolve transcription-replication conflicts, ensuring genome stability [39].

Understanding the intricate crosstalk between Kcr and other PTMs (such as acetylation, methylation, ubiquitination) is essential for deciphering complex cellular processes, particularly in epigenetic regulation and disease mechanisms. Future studies are needed to map the precise functional interdependencies between these modifications.

Conclusions

This review summarizes the latest research on the development of Kcr and its relationship with regulatory mechanisms and diseases. With the rapid advancement of high-resolution protein mass spectrometry (HPLC–MS), researchers have revealed that Kcr modifications are not limited to histones but are also prevalent on non-histone proteins, highlighting Kcr's broader significance than initially anticipated. Consequently, increasing studies have shown that protein Kcr has a significant impact on neurological, digestive, respiratory, reproductive, cardiovascular, and other systemic diseases, playing a crucial role in metabolic, tumor development [127] and infectious diseases. Some diseases involving Kcr have been partially elucidated; however, many studies remain limited, and the specific mechanisms linking Kcr to these diseases require further exploration. The overlap of Kcr regulators with other acylation modification regulators suggests that various epigenetic modifications are closely interconnected and collaborate to regulate gene expression. However, this overlap also complicates the study of the regulatory mechanisms and physiological functions of Kcr. To date, few specific regulators or targets of Kcr have been identified, posing a significant challenge to the study of epigenetics and related fields. Therefore, identifying the specific processes, regulators, and targets of Kcr in various diseases is essential.

In recent years, reliable research tools have been developed for the study of Kcr, alongside breakthroughs in HPLC–MS, site-specific antibodies, and bioinformatics databases. In addition to mechanistic research, well-designed clinical trials are needed to gain a deeper, more comprehensive understanding of Kcr, which will provide potential targets for the prevention and treatment of Kcr-related diseases.

Abbreviations

Kcr	Lysine crotonylation
PTM	Post-translational modification
Kac	Lysine acetylation
Kbu	Lysine butyrylation
Kpr	Lysine propionylation
Kmal	Lysine malonylation
Kglu	Lysine glutarylation
Kbz	Lysine benzoylation
Khib	Lysine 2-hydroxyisobutyrylation
Kbhb	Lysineβ-hydroxybutyrylation
Ksucc	Lysine succinylation
Kla	Lysine lactylation
CDYL	Chromodomain Y-like transcription corepressor
CBP	CREB-binding protein
hMOF	Histone acetyltransferase human males absent on the first
HDACs	Histone deacetylases
MTHFD1	Methylenetetrahydrofolate dehydrogenase 1
PDAC	Pancreatic ductal adenocarcinoma
ACSS2	Acyl-CoA synthetase short-chain family member 2
ACADS	Acyl-CoA dehydrogenase short-chain
ACOX	Acyl-CoA oxidase
GCDH	Glutaryl-CoA dehydrogenase
HATs	Histone acetyltransferases
HCT	Histone crotonyltransferase
GNAT	Gcn5-related N-acetyltransferases
H3K18	H3 lysine 18
GCN5	General control non-derepressible 5
ADA	Gcn5-Ada2-Ada3
Piccolo NuA4	Esa1-Yng2-Epl1
HBO1	Histone acetyltransferase binding to origin recognition complex 1
NPM1	Nucleophosmin 1
PCAF	P300/CBP-associated factor
DDX5	DEAD-box helicase 5
KAT7	Lysine acetyltransferase 7
MTORC1	Mechanistic target of rapamycin kinase 1
HDCR	Histone decrotonylase
ESCs	Embryonic stem cells
ACSL5	Acyl-CoA synthetase 5
NAFLD	Non-alcoholic fatty liver disease
PHF5A	Plant homeodomain -finger domain protein 5A
YEATS	YAF9, ENL, AF9, TAF14, and SAS5
DPF	Double plant homeodomain finger
AD	Alzheimer's disease
Aβ	β-Amyloid peptide
NEAT1	Paranuclear spot assembly transcript 1
TSSs	Transcription start sites
HIE	Hypoxic ischemic encephalopathy
SB	Sodium butyrate
MDD	Major depressive disorder
TG	Trigeminal ganglia
TNF-α	Tumour necrosis factor α
IL1β	Interleukin 1β

CCL2	Chemokines C–C motif chemokine ligand 2
CXCL10	C-X-C motif chemokine ligand 10
NSPCs	Neural stem/progenitor cells
RNAP2	RNA polymerase II
ATAC-Seq	Assay for transposase-accessible chromatin with sequencing
PDLSCs	Periodontal ligament stem cells
NaCr	Sodium crotonate
HSP90AB1	Heat shock protein 90 kDA alpha, class B, member 1
OSCC	Oral squamous cell carcinoma
COPD	Chronic obstructive pulmonary disease
NSCLC	Non-small cell lung cancer
PEM	Pemetrexed
LC–MS/MS	Liquid chromatography-tandem mass spectrometry
BEX2	Brain-expressed X-linked gene 2
IHD	Ischemic heart disease
VSMCs	Vascular smooth muscle cells
TPM1	Tropomyosin alpha-1 chain
BNIP3	Bcl-2 adenovirus E18 19-kDa-interacting protein 3
ECHS1	Enoyl-CoA hydratase short-chain 1
NFATC3	Nuclear factor of activated T cells isoform C3
NEDD8	Neural precursor cell expressed developmentally downregulated protein 8
NAE1	NEDD8-activating enzyme E1 regulatory subunit
GSN	Gelsolin
ScKO	Cardiac-specific knockout of SIRT1
SERCA2a	SR Ca^{2+}-ATPase 2
PPAR	Peroxisome proliferator-activated receptor
ATP	Adenosine triphosphate
AKT1	RAC-alpha serine/threonine-protein kinase 1
HSCs	Hepatic stellate cells
EMT	Epithelial-mesenchymal transition
TGFβ1	Transforming growth factor β1
HCC	Hepatocellular carcinoma
SEPT2	Septin 2
PPP	Pentose phosphate pathway
SASP	Senescence-associated secretory phenotype
CRC	Colorectal cancer
ATX	Autotaxin
GAS41	Glioma amplified sequence 41
CAMs	Cell adhesion molecules
ENO1	Alpha-Enolase 1
PRKACA	Protein kinase cAMP-dependent catalytic-alpha
MTHFD1	Methylenetetrahydrofolate dehydrogenase1
AKI	Acute kidney injury
PGC-1α	Proliferator-activated receptor gamma coactivator-1α
CKD	Chronic kidney diseases
NRP1	Neuropilin-1
DT	Distal tubular
I-R	Ischemia–reperfusion
TECs	Tubular epithelial cells
ADPKD	Autosomal dominant polycystic kidney disease
PC1	Polycystin-1
PC2	Polycystin-2
PCa	Prostate cancer
PCOS	Polycystic ovary syndrome
LONP1	Lon protease 1
BRD4	Bromodomain-containing protein 4
HPV	Human papillomavirus
AIDS	Acquired immunodeficiency syndrome
HIV	Human immunodeficiency virus
NF-κB	Nuclear factor-kappaB
ncNF-κB	Noncanonical NF-κB
T2DM	Type 2 diabetes mellitus
DLD	Dihydrolipoyl dehydrogenase
HPLC–MS	High-resolution protein mass spectrometry

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Y.J, S.L, and Y.Z. collected the related papers and drafted the manuscript. Y.Z, and S.L. drew the figures. C.G., Y.M., L.W., H.Y., and Z.Z. revised and commented on the paper. All authors read and approved the final version of the manuscript.

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Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

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Competing interests
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