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REVIEW ARTICLE

Protein lysine crotonylation in cellular processions and disease associations

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Abstract Protein lysine crotonylation (Kcr) is one conserved form of posttranslational modifications of proteins, which plays an important role in a series of cellular physiological and pathological processes. Lysine ϵ-amino groups are the primary sites of such modification, resulting in four-carbon planar lysine crotonylation that is structurally and functionally distinct from the acetylation of these residues. High levels of Kcr modifications have been identified on both histone and non-histone proteins. The present review offers an update on the research progression regarding protein Kcr modifications in biomedical contexts and provides a discussion of the mechanisms whereby Kcr modification governs a range of biological processes. In addition, given the importance of protein Kcr modification in disease onset and progression, the potential viability of Kcr regulators as therapeutic targets is elucidated.

Introduction

Posttranslational modifications (PTMs) are among the main mechanisms involved in the regulation of protein functions and various biological processes under both pathological and physiological conditions.[1,2] Recent advances in high-resolution mass spectrometry and proteomic analyses have enabled the efficient and reliable detection of PTMs, expanding the spectrum of established PTMs. As an amphipathic amino acid with a hydrophobic side chain, lysine (K) is the target for many forms of PTMs, including lysine acetylation (Kac)[3] and other forms of acylation or alkanoylation on lysine such as crotonylation (Kcr),[4] formylation (Kfo),[5] butyrylation (Kbu),[6] succinylation (Ksucc),[7] propionylation (Kpr),[6] methacrylation (Kmea),[8] malonylation (Kmal),[9] benzoylation (Kbz),[10] glutarylation (Kglu),[11] isobutyrylation (Kibu),[12] 2-hydroxyisobutyrylation (Khib),[13] lactylation (Kla),[14] and β-hydroxybutyrylation (Kbhb),[9] most of the above acylations have been identified in both histone and

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non-histone proteins.[15,16] Based on the substrates to be modified, acylation types are categorized into two groups (Fig. 1), the histone group (HTG) and the non-histone proteins group (NHTG). Emerging evidence suggests that these acylations are similar to the archetypal lysine acetylation, but differ in hydrocarbon chain length, hydrophobicity, or charge, and can regulate chromatin remodeling, gene expression, cell cycle, and cellular metabolism.[17] However, the functional elucidation of these newly identified acylations remains to be explored.

Kcr was first detected as a form of lysine acylation present on core histones in HeLa cells.[4] However, following studies demonstrated that both histone and non-histone proteins can be subjected to Kcr modification.[18–20] The precursor of Kcr is crotonyl—coenzyme A (CoA), which is a metabolic intermediate containing a four-carbon acylchain with one double bond. The Kcr modification of histones has been reported in organisms ranging from yeasts to humans and, due to its enrichment in promoter and enhancer regions, it is mainly associated with active transcription.[4] The Kcr modification of non-histone proteins is associated with the regulation of cell cycle progression, organizational processes, and metabolic activity in mice and humans.[18,21] These studies indicate potentially critical and wide-ranging roles for crotonylation in multiple cellular functions, which has sparked interest in the regulation of Kcr on the activities and biological functions of both histone and non-histone proteins.

In the present review, we summarize recent advances in the regulatory mechanism of both histone and non-histone protein Kcr modifications and the mechanisms whereby this form of PTM influences diverse biological processes. In addition, we further discussed the functional importance of Kcr modification in the pathogenesis of various diseases and the viability of targeting these modification pathways to guide therapeutic interventions.

Protein crotonylation: An overview

Protein crotonylation was first identified by Tan et al in 2011 who used mass spectrometry to systematically assess the forms of histone PTMs.[4] Strikingly, both Kac and Kcr were identified as the form of hydrophobic acetylation associated with the extension of hydrocarbon chains, and both modified lysine e-amino groups. As Kcr modification harbors a unique C-Cπ-bond, it adopts a rigid planar structure distinct from that observed in other forms of histone acylation. Given Kcr is composed of a longer carbon chain, crotonyllysine can also more readily neutralize positive charge relative to acetyllysine.[22] Since the initial discovery of crotonylation, a series of studies, through using the pan anti-Kcr enrichment and mass spectrometry, have confirmed the existence of Kcr-modified core histones in humans,[4,23,24] mice,[25–27] yeast,[28] melanogaster,[29] and even in plants.[30,31] Consistent with its evolutionary

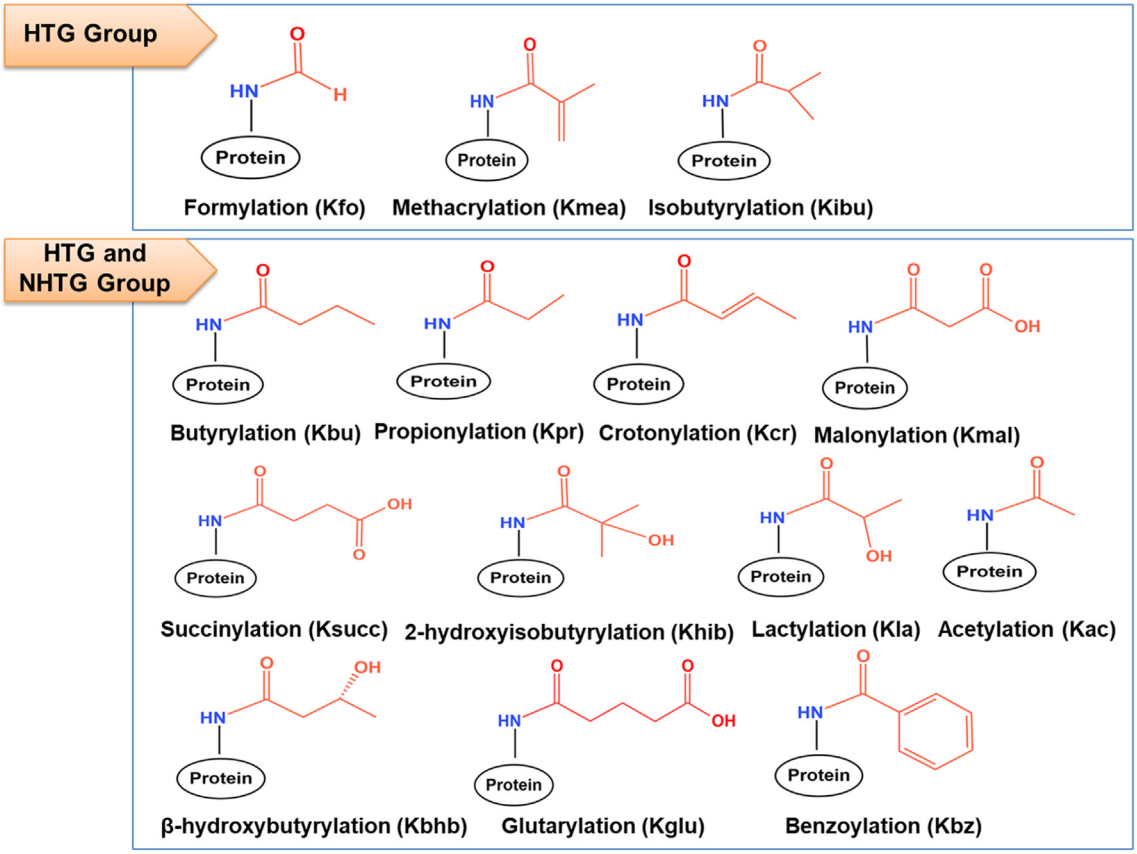

Figure 1 Chemical structures of protein lysine acylations. Acylation types are classified into two groups, HKcr group and NHKcr group, based on substrates of histone or non-histone proteins.

conservation and universal, histone Kcr modifications serve as important regulators of transcriptional activity and disease-related changes. More recent work employing more sensitive immunoaffinity purification and high-resolution liquid chromatography-tandem (LC-MS/MS) techniques and more robust bioinformatics strategies have identified the Kcr modification of a series of non-histone proteins, with modified proteins including important regulators of key processes such as cell cycle progression, chromatin remodeling, organization, and metabolic activity.[18,32–36] Several factors, including crotonyl-CoA levels, positive regulators (ACOX1, ACOX3, ACADS, and ACSS2), and negative regulators (CDYL and ECHS1), determine Kcr modification of proteins and the ability of this form of PTM to regulate cellular processes.[36–39] Efforts to measure intracellular and tissue crotonyl-CoA content can thus offer insight into the functional importance of Kcr in various physiological contexts.

Crotonylation-related writer, reader, and eraser proteins

Dynamic shifts in crotonyltransferase and decrotonylase within cells are important determinants of Kcr modification status.[40] Often referred to as writer proteins, crotonyltransferases function by catalyzing covalent Kcr modifications. To date, no specific crotonyl group writer proteins have been detected, although histone acetyltransferases (HAT) reportedly exhibit crotonyltransferase. Three major groups of HATs have been defined based on their organizational structure and sequence similarity, including the MYST (Moz, Ybf2, Sas2, and Tip60), p300/CREB-binding protein (p300/CBP), and GNAT (GCN5-related N-acetyltransferase) HAT families. The first protein with histone crotonyltransferase activity to be reported was p300, and while members of all these families can reportedly modify histones, only a limited subset thereof have been reported to play a role in non-histone modification (p300, PCAF, KAT7, Tip60, CBP, MOF) (Fig. 2 and Table 1).

For Kcr to impact protein function, specific proteins known as reader proteins must be able to recognize the Kcr modification. To date, however, no crotonyl-specific readers have been identified. Both Kcr and Kac are structurally similar in many respects, and three primary groups of histone acetylation and non-acetyl acylation reader proteins, including YEATS domain proteins, double plant homeodomain finger (DPF) proteins, and bromodomain proteins have been identified. Of these proteins, those harboring DPF and YEATS domains have been reported to

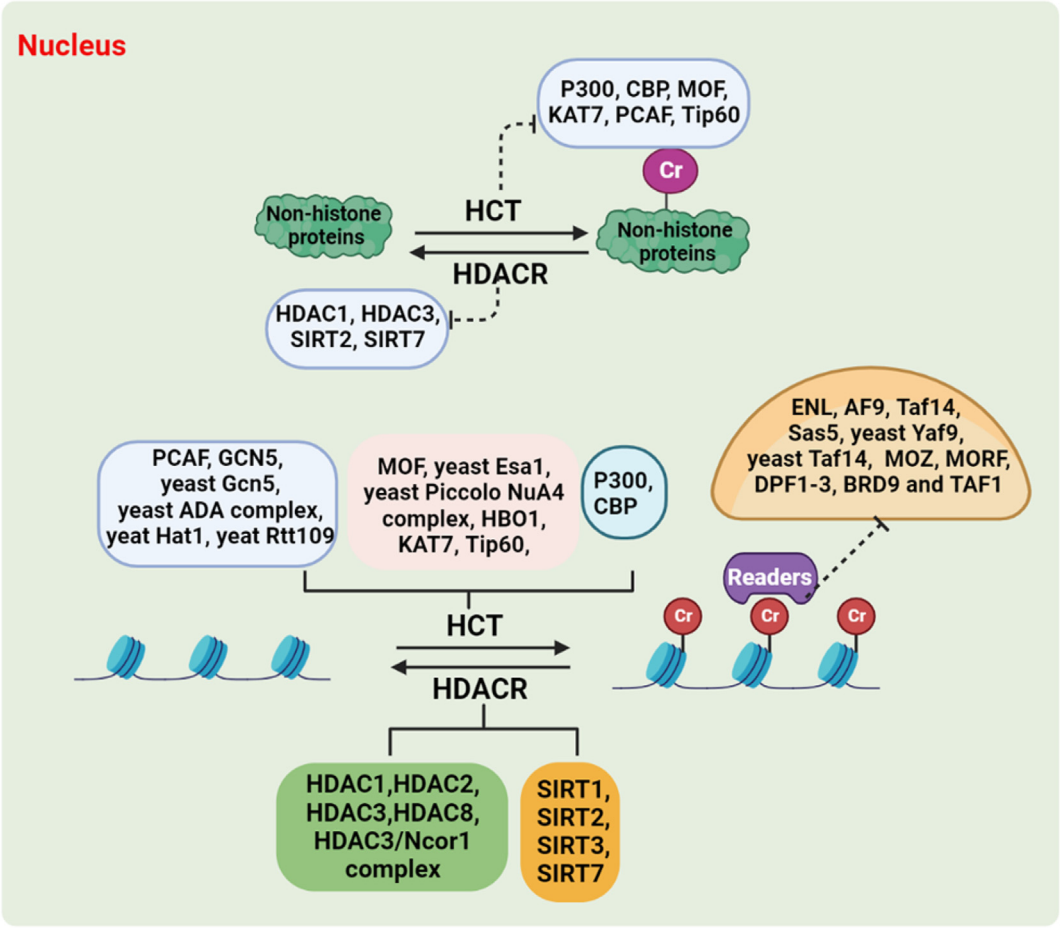

Figure 2 The regulatory factors of crotonyaltion. Readers, writers (crotonyltransferase, HCT), and erasers (decrotonylase, HDCR) in histone and non-histone proteins are shown in this review. The readers are recruited by protein Kcr. Protein Kcr is balanced by HCT and HDCR *in vitro* and *in vivo*.

Table 1 Lysine crotonyltranferases and decrotonylases of Kcr in histone and non-histone proteins.

	Family	Enzymes	Targets		References
			Histone	Non-histone	
Writers	p300/CBP family	P300	H3K9,H3K18, and H4K8	PHF5A	18,37,41,42
		CBP	H3K9,H3K18,and H4K8	DDX5,NPM1,ENO1, PHF5A	18,41—44
	MYST family	MOF	H3K4,H3K9,H3K18,H3K23,H4K8 and H4K12	NPM1	18,41
		yeast Esa1	H3	/	41,45
		yeast Piccolo NuA4 complex	H4K5,H4K8,H4K12 and H4K16	/	45
		HBO1	H3K14 and H4K12	/	43
		KAT7	/	CANX	20
		Tip60	/	EB1	19
	GNAT family	PCAF	/	NPM1	18
		GCN5	H3K27	/	43
		yeast Gcn5	H3K27 and H3K9	/	45—47
		yeast ADA complex	H3K9,H3K14,and H3K18	/	45
		yeat Hat1	H3K9	/	46
		yeat Rtt109	H3K9	/	46
Erasers	Zn^{2+}-dependent HDACI family	HDAC1	H3K4,H3K9,H3K18,H3K23,H4K8 and H4K12	NPM1	18,21,48,49
		HDAC2	H3K9,H3K18 and H4K8	/	21,48,49
		HDAC3	H3K9,H3K18 and H4K8	NPM1,EB1	18,19,21
		HDAC8	H3K4,H3K9,H3K18,H4K8 and H4K12	/	21
		HDAC3/Ncor1 complex	H3K18	/	48,50
	NAD^{+}-dependent HDAC III family	SIRT1	H3K4,H3K9 and H4K8	/	21,51,52
		SIRT2	H3K4 and H3K9	ENO1	44,51,52
		SIRT3	H3K4	/	52
		SIRT7	/	PHF5A	42

exhibit preferential binding to histone Kcr modifications compared with other forms of acylation.[22,53] While bromodomain proteins are capable of Kcr recognition, they exhibit substantially poorer affinity for crotonylated peptides compared with their ability to bind other proteins. Histone readers identified to date include the ENL, AF9, Taf14, Sas5, yeast Yaf9, yeast Taf14, and YEATS2 proteins in the YEATS domain family,[17,22,46,54] as well as the MOZ, MORF, and DPF1-3 members of the DPF family[53] and the TAF1 and BRD9 bromodomain proteins.[55] Reader proteins specifically refer to proteins capable of binding covalent histone modifications, and there is no evidence to date of readers for non-histone proteins (Fig. 2).

As eraser proteins, decrotonylases can remove covalent Kcr modifications from proteins. Given that the Kcr and Kac modifications share writer and reader proteins, they are also likely to exhibit shared erasers. The two major histone deacetylase (HDAC) families reported to date include NAD-dependent sirtuins (Sirt 1—7; class III) as well as zinc-dependent members of the Rpd3/Hda1 HDAC family, which are further subdivided into class I (HDAC1—3, 8), class II (HDAC4—7, 9, 10), and class IV (HDAC11). Class I and III HDAC proteins have been found to exert histone decrotonylase (HDCR) activity.[40] Moreover, HDAC1 and HDAC3 reportedly decrotonylate nucleophosmin-1 (NPM1), which is a non-histone protein, and this activity can be effectively suppressed through treatment with trichostatin A (TSA) or other HDAC inhibitors including LBH589 and suberoylanilide hydroxamic acid (SAHA).[18] One study also found HDAC3 to decrotonylate EB1, which is another non-histone protein.[19] In some recent reports, the SIRT2 and SIRT7 members of the sirtuin family have also been shown to serve as decrotonylases for non-histone proteins (Fig. 2 and Table 1).

The biology of protein crotonylation

DNA damage and repair

DNA serves as the primary mode of heritable information transition, and the preservation of DNA integrity during individual rounds of cell division. Both exogenous and endogenous factors can cause DNA damage, jeopardizing the viability and genomic stability of affected cells and organisms and contributing to oncogenic transformation. Accordingly, cells have evolved a diverse range of DNA damage response (DDR) pathways that help repair DNA lesions caused by genotoxic

exposure.[56] Protein PTMs, including Kcr, can play critical roles in facilitating the detection and repair of damaged DNA.

A study published in 2019 was the first to describe the importance of histone Kcr modifications in DDR-specific contexts.[57] Following exposure to ionizing radiation (IR), ultraviolet (UV) radiation, laser micro-irradiation, or exposure to etoposide to promote damage to the genome, a rapid but transient drop in H3K9cr levels was observed at sites of DNA damage within U2OS cells. Such damage-induced decreases in H3K9cr levels were independent of sirtuin activity but dependent on HDAC decrotonylase activity in the context of DNA damage. These results highlight a previously uncharacterized association between DDR and histone Kcr modification, confirming the ability of HDACs to dynamically remove these PTM groups from histones following DNA damage. Given that HDACs can target both Kcr and Kac modifications, there is a need to establish how these two modifications individually contribute to DDR regulation.

In another recent study, H3K9cr and Kcr levels were found to be significantly decreased at sites of AsiSI-induced DSB formation.[58] CDYL1 was shown to regulate this drop in H3K9cr levels in a study in which chromatin immunoprecipitation sequencing (ChIP-Seq) analyses were performed using cells deficient for CDYL1 expression. Through its crotonyl-CoA hydratase activity, CDYL1 has been found to serve as a negative regulator for Kcr modification.[38] By focusing on three genes exhibiting high levels of transcriptional activity that exhibited high levels of γH2AX enrichment (LYRM2, MIS12, and RBMXL1), these authors established a strong correlation between such enrichment and decreases in the levels of both Kcr and H3K9cr. Other cell lines were found to exhibit similar CDYL1-dependent drops in H3K9cr levels following DSB formation in response to VP16 and IR treatment. Functionally, the crotonyl-CoA hydratase activity of CDYL1 can counteract the establishment of Kcr modifications at DSB lesions. Although CDYL1 has also been found to interact with HDAC1/2, both of which can also counteract Kcr establishment following DNA damage,[57,59] these results suggest that reductions in Kcr modifications at sites of DSB formation are primarily mediated by the crotonyl-CoA hydratase activity of CDYL1 rather than by impaired HDAC recruitment to the sites of DSB formation. These results also offer insight into the functional association between DSB-induced transcriptional silencing and HR-based repair mechanisms.

DSB formation is the most cytotoxic form of DNA damage, serving as a primary mediator of radiotherapy- and chemotherapy-induced tumor cell killing.[1,60] DSBs may be repaired using either error-free HR or error-prone repair pathways such as classical non-homologous end joining (c-NHEJ), alternative end joining (alt-EJ), and homologous recombination.[56,61,62] Exogenous DSB formation can promote temporary silencing of transcriptional activity, thereby precluding interactions between transcription factors and repair proteins at the site of DSB formation, preventing the formation of truncated transcripts resulting from DSB formation within the body of a given gene.[63] In concert with important mediators of transcriptional silencing such as ATM, PARP1, and DNA-PK, histone PTMs including methylation, ubiquitylation, acetylation, and phosphorylation can also regulate such silencing.[62,64,65] Indeed, there is recent evidence supporting the ability of Kcr modifications to mediate the silencing of transcriptional activity at DSBs. Local CDYL1-dependent reductions in active transcriptional markers including histone Kcr and H3K9cr at AsiSI-induced DSBs are correlated with the suppression of transcription. PARP inhibition can reverse reductions in Kcr and alleviate this DSB-induced silencing activity. The transcriptional elongation factor ENL can serve as a Kcr reader, and RNA Pol II initiation and elongation are reduced following ENL chromatin displacement. The activity of CDYL1 crotonyl-CoA hydratase activity can counteract Kcr and H3K9cr modifications at sites of DSB formation, triggering the exclusion of ENL from these sites and thereby supporting the silencing of transcriptional activity. Silencing of transcription induced by DSBs is reported to occur at all stages of the cell cycle at HR- and NHEJ-prone DSBs. Kcr levels at HR-susceptible sites are higher than those at NHEJ-prone sites before DSB induction. Moreover, at sites of DSB formation, CDYL1-dependent decreases in H3K9cr levels are closely tied to the silencing of transcriptional activity without influencing HR integrity, highlighting the dispensability of DSB-induced silencing as a precursor for appropriate HR activity. Similarly, this DSB-induced silencing activity is nonessential for 53BP1 recruitment to the site of DSB formation in the context of NHEJ activity, and the activity is thus thought not to be required for NHEJ integrity. Overall, these data suggest that the silencing and repair activities of CDYL1 at sites of DSB formation can be uncoupled from one another. Additional research, however, will be essential to gain insight into functional interdependencies among various PTMs in the context of the silencing of transcriptional activity in response to DSB formation.

Following the clarification of the association between histones and DDR activity, further studies have elucidated the roles of non-histone proteins in this setting.[36] The eukaryotic single-stranded DNA (ssDNA) binding protein RPA (replicative protein A) is an important regulator of meiotic processes including HR, replication, and repair. In humans, RPA is a heteromeric complex comprised of RPA1, RPA2, and RPA3. Of these subunits, RPA1 plays an important role in binding to ssDNA and other DNA metabolism-associated factors. Several PTMs regulate the activity of RPA1 in the context of DNA metabolism including phosphorylation, ubiquitylation, SUMOylation, and acetylation.[36,66] RPA1 Kcr levels were recently found to increase following DNA damage as a result of exposure to UV radiation, IR, camptothecin (CPT), and hydroxyurea (HU), with CPT causing the most profound effect.[36] Treatment with CPT increases the overall levels of RPA1-K88 and RPA1-K379 Kcr, whereas the RPA1-K595 levels were only slightly increased in response to such treatment. CPT is a drug capable of disrupting topoisomerase I elongation activity within tumor cells, thereby promoting DSB generation, fork collapse, arrest in the S phase of the cell cycle, and HR in the context of DSB repair. Kcr modifications of RPA1 drive its enhanced ability to bind to ssDNA, in addition to promoting RPA1 recruitment to sites of DNA damage induced in response to CPT treatment, augmenting its ability to interact with HR-related factors including BLM, DNA2L, Mre11, NBS1, and RAD51, promoting ssDNA formation in response to CPT through interactions with resection-related proteins such as BLM, DNA2L, and the MRN complex, and facilitating the CPT-induced

formation of RAD51 foci. RPA1 knockdown in CPT-treated HeLa cells results in significantly increased rates of apoptotic cell death while overexpressing wild-type RPA1 but not RPA1 in which Kcr sites are mutated can reverse this effect. When subject to Kcr modification, the ability of RPA1 to interact with ssDNA and resection machinery components is altered, contributing to improved cellular survival following DNA damage. RPA1 is additionally a downstream substrate of CDYL, which can reduce the Kcr modification of this protein by targeting the K88, K379, and K595 residues. However, it is not possible to exclude the regulatory roles of HDCRs and/or HCTs in this context, as they may also dynamically influence RPA1 Kcr modification following treatment with CPT. The ability of multiple different PTMs to govern RPA1 functionality in the context of different forms of DNA metabolic activity also has yet to be fully clarified. The above results suggest that both histone and non-histone Kcr modifications play an important role in a range of DNA repair-related processes (Fig. 3).

Gene transcription

Transcription is an essential step in the process of gene expression, and a range of acylation-based modifications including Kcr play a role in this process. Histone Kcr modifications in promoter regions have generally been linked to enhanced transcriptional activity, although a few recent articles have suggested that they can also suppress gene expression in some settings.[67,68] The roles of histone Kcr in transcriptional regulation are discussed below (Table 2).

Histone Kcr-based transcriptional regulation was initially described in 2011 in a study wherein the transcriptional start sites and enhancer regions in murine germ cells and human somatic cells exhibited specific patterns of histone

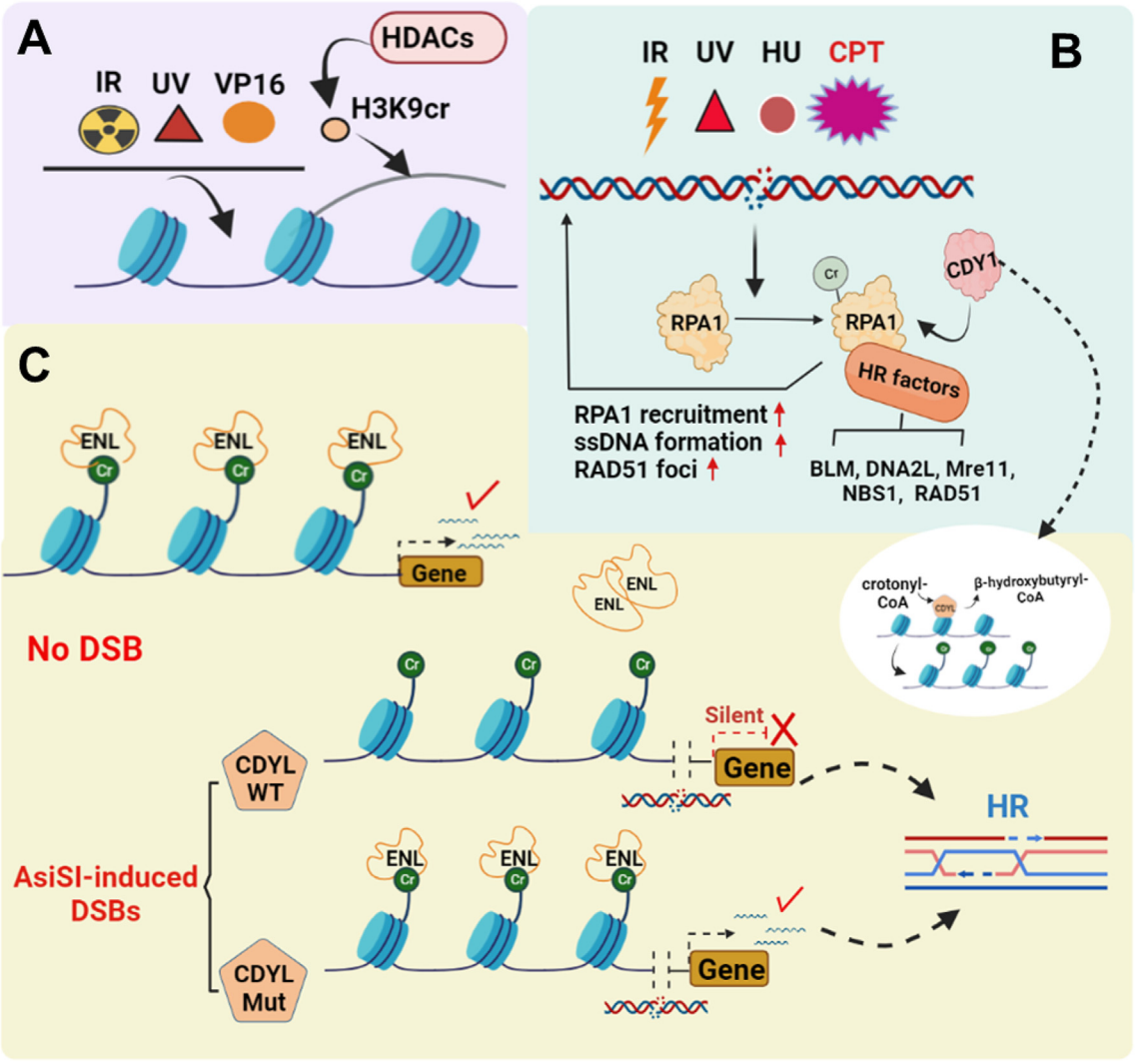

Figure 3 The function of histone and non-histone proteins Kcr in DNA damage and repair. **(A)** Ionizing radiation (IR), ultraviolet radiation (UV), or etoposide (VP16) decreases the levels of H3K9cr; during this process, HDACs are the major HDCR in U2OS cells. **(B)** The role of RPA1 Kcr in camptothecin (CPT)-induced DSB of DNA. CDYL negatively regulated Kcr of RPA1 at K88, K379, and K595 sites, which were involved in DNA damage. **(C)** CDYL1 crotonyl-CoA hydratase activity counteracts Kcr and H3K9cr at DSB sites, which triggers the eviction of the transcription elongation factor ENL and fosters transcriptional silencing.

Table 2 The regulatory role of histone crotonylation in gene expression.

Regulatory role	Histone crotonylation	Target	References
Promoting transcription	Histone Kcr	PGC-1α and sirtuin-3	68
	Histone Kcr	Ptk2, Tshz3, and Wapal	52
	H2BK12Cr	Pin4, Ccdc160, Tceal, Rnf138rt1, and 4933436I01Rik	38
		NFATc3	39
	H3K4Cr	HIV LTR	69
	H3K18Cr	Il6, Gbp2, Ift1, and Rsad2	37
		RANDP3L, AOX1, GRPR, and NCAM1	21
		HIV long-terminal repeat (LTR)	69
		NFATc3	39
	H3K27Cr	SLY, SOX30,BRD4, and BRDT, BORIS and CTCF	70
Inhibiting transcription	H3K9Cr	Pro-growth genes	67
	H3K27Cr	Endocytosis-related genes	68

Kcr modification. Such histone Kcr was particularly enriched on sex chromosomes, marking X-linked genes in male germ cells that evaded chromosomal inactivation following meiotic division.[4] Efforts to identify histone Kcr eraser proteins revealed that Sirt3 was able to reduce Ptk2, Tshz3, and Wapal expression while suppressing crotonylated histone enrichment at transcriptional start sites associated with these genes.[52] The increase in renal histone Kcr levels during acute kidney injury (AKI) protects against damage by up-regulating Sirt3 and PGC-1α levels, which regulates mitochondrial biogenesis via increased histone Kcr modification of these genes.[71] These data are consistent with the ability of histone Kcr to positively regulate gene expression. Consistently, certain sites of histone Kcr modification have been linked to the activation of particular genes, as in the case of H3K18cr modification of "de novo-activated" RSAD2, IL6, IFT1, or GBP2,[37] and H3K18cr modification of NCAM1, AOX1, RANDP3L, or GRPR.[21] Similar activity has been ascribed to the H2BK12Cr modification of post-meiotic genes that govern spermatogenic processes,[38] the H3K18cr and H2BK12cr modifications of NFATc3, which contribute to the impact of ECHS1 and histone Kcr,[39] and the H3K4cr and H3K18cr modifications of the HIV long-terminal repeat (LTR) gene, which plays a central role in establishing latent viral reservoirs and reactivating latent HIV.[69] Certain histone Kcr reader proteins are also involved in transcriptional regulation, as in the case of AF9, which influences p300-catalyzed Kcr and gene expression in the context of lipopolysaccharide (LPS)-induced inflammatory activity, colocalizing with H3K18cr modifications and positively impacting gene expression.[22] The human DPF2 and MOZ proteins have been shown to exhibit the highest level of Kcr preference, and MOZ colocalizes with H3K14cr in a DPF-dependent fashion, whereas H3K14cr modifications are reportedly enriched in MOZ target genes in HEK293T cells.[53] The histone acetyltransferase ATAC complex component YEATS2 is also capable of the specific in vitro recognition of H3K27cr residues.[54]

Apart from the roles of histone Kcr in active transcription, increased Kcr levels have been shown to inhibit the expressions of genes associated with growth and endocytic activities, consistent with a potential negative regulatory role. One article describes temporally distinct patterns of Kac and Kcr modification in the highly synchronized yeast metabolic cycle, with these patterns correlating with the expression of particular genes. This synchrony enabled the authors to determine that H3K9cr peaks were associated with the repression of genes favoring cellular growth, underscoring the link between H3K9 Kcr and the repression of these pro-growth genes.[67] Knocking down NEAT1 can also reportedly drive a global increase in H3K27cr levels while reducing global levels of H3K27ac modification. Exogenous crotonic acid supplementation can also suppress the expression of a range of endocytosis-associated genes such as TGFBR1, TGFB2, and CAV2 owing to increased H3K27cr and decreased H3K27ac modification of the corresponding promoter region, suggesting that H3K27cr modification served to mark endocytic genes that are subject to transcriptional repression.[68]

While most knowledge of transcriptional activity associated with protein Kcr pertains to histones, a limited number of studies have similarly evaluated the Kcr of non-histone proteins in this context. For example, the p300-mediated Kcr of other proteins can drive enhanced transcriptional activity.[10,37] Crotonyl-CoA can more readily stimulate transcription relative to acetyl-CoA. The I1395G mutant form of CBP/p300, which retains KCT but not KAT activity, is capable of crotonylating promoter regions and thereby enhancing transcription within cells, contributing to the more robust activation of SMAD7 and PAI1 in response to TGF-β1. LPS-mediated macrophage stimulation initiates a pronounced series of transcriptional changes necessitating the recruitment of p300 to many downstream target genes.[41] These prior data emphasize the crucial role that the Kcr of non-histone genes can play in the context of transcriptional regulation, and further work has the potential to highlight additional examples of such activity.

Cell cycle progression

During the cell cycle, certain protein has been dynamically regulated by modification, such as acetylation and phosphorylation.[72] Several recent studies have examined the impact of Kcr modifications in the context of cell cycle progression (Fig. 4). Through its conversion into crotonyl-CoA, sodium crotonate (NaCr) can enhance overall histone Kcr. Recently, NaCr treatment was found to dose-dependently enhance histone H3 Kcr and H3 Ser10

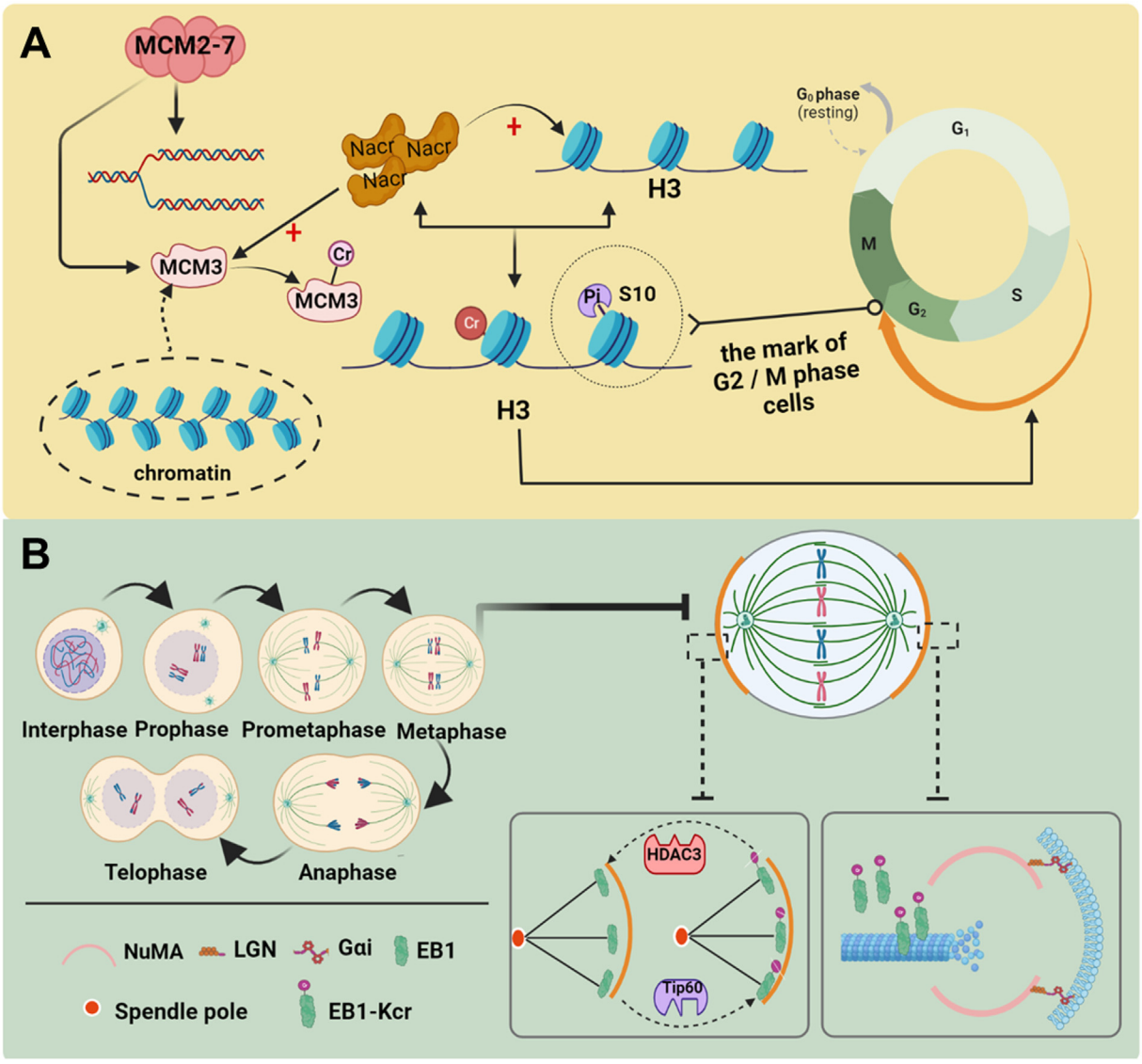

Figure 4 The mechanism of Kcr involves in cell cycle progression. **(A)** NaCr increases the Kcr of histone H3 and influences the phosphorylation of histone H3 at the Ser10 site in a dose-dependent manner, which is the mark of G2/M phase cells. NaCr treatment decreases the amount of S phase cells and raises cells in the G2 phase. **(B)** The role of EB1 Kcr in spindle positioning and orientation during mitosis. TIP60 controls the dynamic Kcr level of EB1 to carry out fine-tuning of mitotic spindle positioning and to facilitate accurate sister chromatids separation.

phosphorylation, which are markers for cells in the G2/M phase.[32] Flow cytometry studies have also revealed that the number of cells in the S phase fell following treatment with NaCr, with a concomitant increase in G2 phase cells. These data highlight a previously unrecognized role for nuclear protein Kcr in the context of cell cycle regulation, although further studies will be essential to clarify the underlying mechanisms.

In protein-protein interaction network analyses, crotonylated non-histone proteins such as MCM3 and CDK7 have been found to be enriched in the cell cycle protein network.[32] Members of the minichromosome maintenance (MCM) protein family (MCM2–MCM7) undergo hexamerization in metazoan species at the site of the DNA replication fork, facilitating the start of DNA replication.[73] After treatment with NaCr, chromatin-associated MCM proteins were found to be significantly suppressed, and MCM3 was subject to Kcr, indicating that protein Kcr may influence the initiation of DNA replication. Together, these effects may ultimately inhibit DNA replication and thereby influence cell cycle progression.

During mitotic division, cells proceed through prophase, prometaphase, metaphase, anaphase, and telophase in sequence.[74] The sister chromatids of the dividing cell are equally distributed between the two resultant daughter cells through a process that is strictly dependent on the approach regulation of spindle plasticity.[75] The evolutionarily conserved Gαi/LGN/NuMA network is particularly

important as a regulator of spindle positioning,[76] and during the early stages of mitosis, the dynamic interactions between kinetochores and microtubules are driven by p300/CBP-associated factor-mediated K220-EB1 acetylation.[77] In one recent report, a previously undiscovered form of mitosis-specific Kcr-controlled astral microtubule-EB1-NuMA interaction was detected,[19] wherein astral microtubule stabilization was regulated by EB1, which is a core and scaffold microtubule plus-end tracking protein (+TIP). In mitotic cells, EB1 undergoes K66 Kcr, and this K66 residue serves as a substrate for TIP60, with the TIP60-mediated Kcr thereof supporting accurate spindle positioning during mitotic division. Mechanistically, such TIP60-induced Kcr enables accurate astral microtubule attachment to the lateral cell cortex defined by NuMA-LGN enabling the fine-tuning of spindle positioning. Real-time analyses of the movement of chromosomes within HeLa cells in which EB1 was genetically encoded to be crotonylated highlighted the critical role that these Kcr dynamics play in accurately regulating the positioning of spindles during the transition from metaphase to anaphase. EB1 Kcr mediated by TIP60 thus enabled the formation of dynamic linkages between astral microtubules and the lateral cell cortex, allowing for the fine-tuning of spindle positioning.[19]

Vascular phenotypic remodeling

Vascular smooth muscle cells (VSMCs) compose the medial arterial layer and are required for normal arterial function, in addition to shaping pathological conditions affecting the arteries. In the context of vascular injury, VMSC transition from its quiescent contractile phenotype towards a more proliferative and migratory synthetic phenotype, with corresponding changes in contractility markers.[78] Phenotypic changes in these VSMCs contribute to the remodeling of the vasculature. In one recent report, several non-histone proteins were identified as targets of Kcr within VSMCs,[79] with 2386 Kcr sites (570 proteins) and 2138 Kcr sites (534 proteins) in VSMCs that were or were not treated using PDGF-BB. Of these targets, the Kcr modification of 5 markers of VMSC contractility was detected at multiple sites, including 16 and 15 sites in caldesman1 and myosin9, respectively. Three lysine residues of the transgelin protein were also subject to Kcr. These results highlight a potential role for crotonyl modifications as regulators of the contraction of VSMCs. Crotonylated proteins detected following PDGF-BB treatment were associated with key VSMC functions in pathway enrichment analyses, including key physiological processes such as glycolysis/gluconeogenesis, vascular smooth muscle contraction, and PI3K-Akt signaling. KEGG pathway analyses of these proteins revealed their association with VSMC phenotypic remodeling, underscoring the potential importance of Kcr as a regulator of the phenotypic changes that occur in PDGF-BB-treated VSMCs. Additional studies have suggested that crosstalk between ubiquitylation and Kcr in the regulation of glycolytic activity may play a previously unrecognized role in VSMC phenotypic remodeling.[79] More studies should investigate the physiological roles of crotonylated and ubiquitinated proteins.

Stem cell regulation

Relative to differentiated cells, murine embryonic stem cells (mESCs) harbor higher levels of histone Kcr, with the enrichment of these PTMs being essential for the self-renewal of these mESCs.[21] Consistently, overexpressing WT HDAC1 in ESCs has been reported to up-regulate differentiation-associated markers while down-regulating pluripotency markers, suggesting the ability of histone deKcr to drive the differentiation of ESCs.[21] In another recent report, histone Kcr was found to drive human ESC (hESC) endodermal differentiation.[23] Through genome-wide transcriptional and chromatin profiling efforts, histone Kcr modifications were found to be enriched on meso-endodermal gene regulatory elements. During endodermal differentiation, genes including ACOX3, ACSS2, and ACADS, which are involved in crotonyl CoA metabolism, were significantly increased in the endoderm. These crotonyl-CoA-generating enzymes can thus control both histone Kcr and endodermal differentiation. A recent study reported that the use of crotonate to treat hESCs was shown to readily promote endodermal differentiation in a cell line-independent fashion.[80]

A 2018 report defined the effects of histone Kcr on telomeric rejuvenation in the context of pluripotent stem cell (PSC) chemical reprogramming.[81] By adding crotonic acid to induce histone Kcr modifications, genes associated with the two-cell stage such as Zscan4 also increase the exchange of telomere sister chromatids, thus preserving telomeric length and protecting against chemical-induced telomere damage, enhancing induction efficiency. Systematic efforts to profile the murine PSC crotonylome revealed that most crotonylated target proteins were associated with pluripotency-related pathways including RNA biogenesis, central carbon metabolism, and proteasomal degradation.[82] The use of crotonic acid to increase crotonyl-CoA levels can also spur enhanced proteasomal activity in metastable PSCs, contributing to sustained pluripotency.[82]

A recent report revealed that increased levels of histone Kcr were linked to the activation of bivalent promoters and the up-regulation of gene expression in neural stem/progenitor cells. This was achieved by enhancing chromatin opening and promoting the recruitment of RNA polymerase II. The genes activated through this mechanism facilitated transcriptomic remodeling, which ultimately led to neuronal differentiation.[83] These data underscore the potential relevance of Kcr-based effects in the context of both neurological development and associated tissue pathology.

The regulation of spermatogenesis and ovarian development

Spermatogenesis is a tightly regulated and conserved process that entails spermatogonia proliferation, the differentiation of these cells into spermatocytes, the subsequent meiotic division of these spermatocytes to produce spermatids, round spermatid maturation, and mature spermatozoa release.[84] Histone Kcr modifications are reportedly

involved in controlling the expression of genes in both meiotic and post-meiotic male germ cells, in addition to shaping spermatogenic processes.[4,85] Through negatively regulating histone Kcr levels, CDYL can alter post-meiotic gene reactivation and histone replacement events.[35] During spermatogenesis in mice, Kcr enrichment at H3K27 residues has been reported relative to Kac.[70] Moreover, Kcr modifications can reportedly control spermiogenesis in E. sinensis through crosstalk with phosphorylation.[86]

Kcr modifications similarly play key roles in ovarian cells and tissues, thereby potentially impacting ovarian development and female fertility.[87] In H. axyridis, global analyses of Kcr profiles at the diapause stage revealed 3084 sites of Kcr modification across 920 proteins. These proteins were associated with several metabolic pathways and distinct subcellular localization. This study was the first to date examining ovarian Kcr profiles in any species, offering new insight into the potential mechanisms whereby this form of PTM may regulate female fertility and reproductive diapause in insects.

Other biological processes

Enrichment analyses focused on GO term annotations, KEGG pathways, and Pfam domain analyses have also highlighted the importance of Kcr modification in other biological contexts. HP1α, also known as CBX5, is a heterochromatin family member that binds to methylated histone residues and is enriched in the heterochromatin regions. Hp1α Kcr results in the nuclear redistribution of this protein and decreases its ability to bind to methylated H3K9 residues, which are highly abundant in heterochromatin regions, underscoring a possible relationship between Kcr and heterochromatin localization.[32] Telomeres are small complexes of DNA and protein that cap the ends of linear eukaryotic chromosomes, maintaining genomic integrity. Crotonic acid-induced Kcr can activate Zscan4, resulting in increased levels of T-SCE that can maintain telomeres and protect against chemical-induced damage to these structures.[81] Crotonlyated proteins are also associated with nucleic acid metabolism.[32] Besides, newly published research has demonstrated a critical role of YWHAE crotonylation in Leu deprivation-induced autophagy.[88]

The role of protein crotonylation in human disease

Cancer

Cancers are a leading cause of morbidity and mortality throughout the globe. H3K18cr has been identified as the most abundant form of histone Kcr modification in the intestines, wherein it was related to higher levels of expression for assorted cancer- and cell cycle-related genes.[48] HDAC2 is up-regulated in the context of colon cancer and other forms of tumor development and possesses decrotonylase activity resulting in histone Kcr reductions that may suppress tumorigenesis.[48] The YEATS domain, which exhibits greater affinity for Kcr modifications relative to other forms of acylation,[46,54,89] is also an important regulatory mediator in the context of leukemia, and inhibiting this domain can suppress the transcription of oncogenes in this cancer type.[90] The marked epigenetic impact that this YEATS domain has in leukemia may be partly attributable to crotonylated histone interactions. In one recent proteomics study examining the lysine crotonylome under the control of p300, certain p300-targeted Kcr substrates have been found to be tentatively associated with cancer.[10] In certain settings, Kcr may thus be conducive to tumor progression. Kcr modifications are also present at lower levels in prostate tumors and to be positively correlated with tumor grade. Inhibitors of bromodomain-containing protein 4 (BRD3) have demonstrated the ability to suppress cell migration and proliferation by inducing histone hypoKcr. This suggests that targeting Kcr modification may hold therapeutic potential for treating prostate cancer.[24] The K420Cr modification of ENO1 can also promote its more robust activation in colorectal cancer cells, driving their enhanced proliferation, invasivity, and migration.[44]

Crotonylated proteins are also widely distributed in tumor tissue. Global Kcr levels were reduced in gastric, liver, and kidney tumors and raised in lung, pancreatic, esophageal, thyroid, and colon cancers. Specifically, raised Kcr was found to reduce cell proliferation and migration in hepatoma.[91,92] Up-regulation of proteins with Kcr modifications in small cell lung cancer was associated with tumor metastasis, migration, and alterations of the tumor microenvironment, all features of malignant tumors.[93] In addition, as faulty DSB repair can result in genomic instability that may enhance tumorigenesis,[63] both Kcr-mediated repair of damaged DNA damage[36] and accurate spindle position during meiosis[19] contribute to the maintenance of genomic integrity and prevent tumorigenesis.

Neurological disorders

BTBR T Itpr3tf/J (BTBR) mice exhibit a range of disorders of the central nervous system and harbor a range of neuroanatomical structures. Relative to control C57BL/6 mice, BTBR mice present with higher global Kcr levels in the cerebral cortex consistent with a significant association between Kcr and neurological disease.[94] Depression is a psychiatric disease with a complex pathogenesis, and one recent report found CDYL-mediated reductions in histone Kcr modification to influence stress-induced depression.[95] In murine models of chronic social defeat stress and microdefeat stress, decreased histone Kcr levels have been detected in the medial prefrontal cortex (mPFC) that coincide with selective CDYL up-regulation. CDYL can also inhibit structural synaptic plasticity through repressing neuropeptide VGF nerve growth factor transcription through its dual impact on H3K27 trimethylation and histone Kcr modification of the VGF promoter. This CDYL-VGF axis can inhibit mPFC structural synaptic plasticity, ultimately contributing to behavioral alterations in susceptible individuals. Pan-Kcr antibodies are capable of recognizing 70 kDa proteins in extracts from murine brain samples, suggesting that non-histone proteins are subject to Kcr in the brain.[48]

Cardiovascular disease

Histone PTMs are closely related to the expression of genes and the regulation of a range of cardiovascular disease-related processes. Hypertrophic cardiomyopathy (HCM) is the most diagnosed form of genetic cardiovascular disease, resulting in unexplained non-dilated left ventricular hypertrophy. The ECHS1 gene encodes the short-chain enoyl-CoA hydratase (encoded by ECHS1), which exhibits high levels of crotonyl-CoA hydrolyzing activity, thereby lowering levels of crotonyl-CoA within cells and altering the Kcr of histones. ECHS1 down-regulation has been reported in the cardiac tissue of humans suffering from hypertrophic cardiomyopathy.[39] The down-regulation of ECHS1 coincides with increased H2BK12cr and H3K18cr levels consistent with a role for ECHS1 in the coordination of cardiac hypertrophy-related histone Kcr modification. ECHS1 deficiencies resulted in pronounced increases in the levels of NFATc3, H3K18cr, and H2BK12cr, contributing to increased hypertrophic fetal gene expression and promoting hypertrophic neonatal cardiomyocyte growth, suggesting that ECHS1 and the Kcr modification of histones are essential for the maintenance of cardiomyocyte maturity and homeostasis.[39] Histone Kcr may thus represent an important target for the treatment of individuals harboring mutations in the ECHS1 gene and hypertrophic cardiomyopathy patients.

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is a respiratory disease characterized by progressive restriction of airflow that is not fully reversible.[96] COPD patients often develop type I or type II respiratory failure (RF), with type II RF being the primary cause of COPD-related mortality. Diagnosing and treating COPD earlier is thus vital as a means of improving patient outcomes. In one recent study, Kcr expression levels were analyzed in the proteome of COPD patients suffering from type II RF.[97] This analysis led to the LC-MS/MS-based identification of 946 crotonylated sites across 318 proteins when assessing type II RF COPD patient and control samples, of which 32 sites across 23 proteins were up-regulated and the remainder were down-regulated. Functional enrichment analyses of up-regulated crotonylated proteins revealed them to be enriched in a range of mechanisms consistent with their roles in the pathogenesis of COPD and type II RF. Additional proteomics analyses revealed 190 and 151 up-regulated and down-regulated proteins, respectively. The 90 crotonylated proteins that were found to be differentially expressed in COPD patients suffering from type II RF may offer value as targets for the study of the molecular mechanisms governing this condition. However, these findings only provide a general foundation for efforts to understand the link between Kcr modification and type II RF in COPD patients, underscoring the need for further detailed studies.

Aging

Dynamic changes in the posttranslational modification of proteins can shape extracellular signaling responses to a range of intracellular conditions. Histone methylation and acetylation are the most frequently reported aging-related factors associated with chromatin remodeling. Various forms of histone methylation (H3K4me3, H3K9me3, H3K27me3, and H3K36me3) and acetylation (H3K9ac, H3K56ac, H4K12ac, and 4K16ac) can regulate aging-associated processes.[98] In one recent study of senescent fibroblast cells, 5149 sites of Kcr modification were identified on 1541 proteins.[42] PHF5A, one of the crotonylated proteins, is an alternative splicing (AS) factor that can undergo SIRT7-mediated K25 deKcr. This deKcr process reduces the levels of CDK2 expression by inducing abnormal AS via retained intron, ultimately driving more rapid senescence. Thus, PHF5A K25 deKcr plays a significant role in the aging process. Accordingly, drugs targeting this mechanism may offer value as a means of altering cellular aging processes and treating age-related disease.[42] In another report, global protein Kcr expression levels rise significantly with age in mice, underscoring a link between Kcr modifications and murine ovarian aging.[99]

Diabetes mellitus

Type 2 diabetes mellitus (T2DM) incidence rates have risen dramatically throughout the world in recent decades.[100] Long non-coding RNA (lncRNA) dysregulation has been reported to play a role in the regulation of glucose metabolism and diabetic progression. The lncRNA EPB41L4A-AS1 can regulate metabolic activity in cancer cells, inducing abnormal drops in energy metabolism. It has also been shown to control H3K27 Kcr at the GLUT4 promoter while also regulating PGC1β acetylation via interacting with GCN5, thereby suppressing GLUT4 expression and muscle cell glucose uptake. In the TXNIP promoter region, EPB41L4A-AS1 can conversely bind to GCN5, thus enhancing H3K27 and H3K14 acetylation in this region to promote transcriptional activation via facilitating MLXIP recruitment. This resulted in increased endocytic processing of GLUT2/4 and the additional suppression of glucose uptake.[45] These results offer new insight into the ability of this EPB41L4A-AS1/GCN5 complex to repress the uptake of glucose by targeting TXNIP and GLUT2/4 and through the regulation of the acetylation or Kcr of a range of target proteins, thereby influencing the pathogenesis of T2DM.

Infectious diseases

HIV is a retrovirus responsible for acquired immunodeficiency syndrome in humans, and HIV latency is regulated by a range of epigenetic histone modifications. Notably, histone Kcr modification at the site of the HIV LTR can regulate its transcriptional activity, facilitating the establishment of HIV latency.[69] The ACSS2 enzyme plays a role in fatty acid metabolism and can promote the histone Kcr modification of the HIV LTR, ultimately contributing to latent HIV reactivation and viral transcription *ex vivo* and *in vitro*, with the suppression of ACSS2 being sufficient to suppress the replication of HIV or the reactivation of latent forms thereof. High ACSS2 levels within the intestinal mucosa are associated with changes in fatty acid

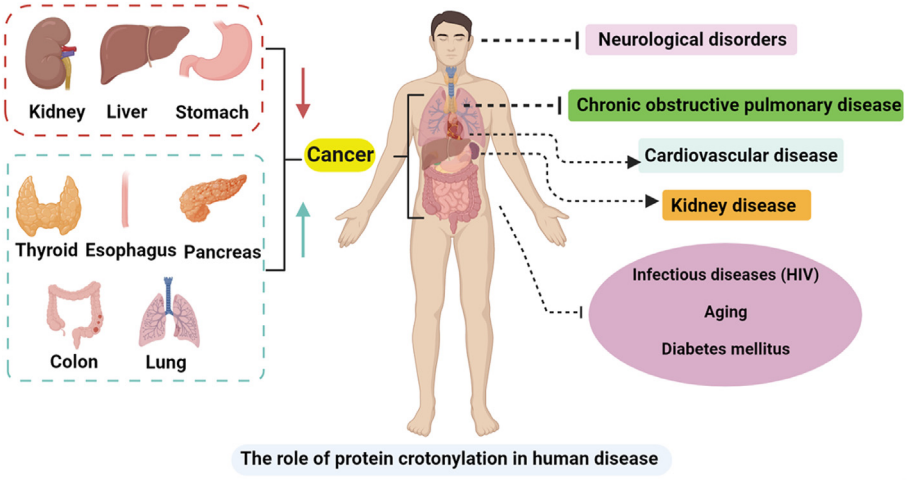

Figure 5 Non-histone and histone Kcr-associated diseases. The whole level of crotonylation is down-regulated in stomach, liver, and kidney cancers and up-regulated in lung, pancreatic, esophageal, thyroid, and colon cancers; BTBR T Itpr3tf/J (BTBR) mice present with higher global Kcr levels in the cerebral cortex, and CDYL-mediated reductions in histone Kcr modification to influence stress-induced depression. Functional enrichment analysis of up-regulated crotonylated proteins in samples of COPD patients with type II RF reveals that they enriched a range of mechanisms related to the pathogenesis of COPD and type II RF. The down-regulation of ECHS1 results in pronounced increases in the levels of NFATc3, H3K18cr, and H2BK12cr, promoting hypertrophic neonatal cardiomyocyte growth. Crotonlyation has the potential to impact a range of renal diseases, such as AKI, immunoglobulin A nephropathy, and hemodialysis. Histone Kcr modification at the site of the HIV LTR can regulate its transcriptional activity to facilitate the establishment of HIV latency. PHF5A K25 deKcr in senescent fibroblast cells can drive more rapid senescence, and global protein Kcr expression levels underscore a link between Kcr modifications and murine ovarian aging. The lncRNA EPB41L4A-AS1 can control H3K27 Kcr at the GLUT4 promoter to suppress GLUT4 expression.

metabolic activity in non-human primate models of acquired immunodeficiency syndrome infected with simian immunodeficiency virus. As such, ACSS2-mediated histone Kcr may represent a novel target for therapeutic efforts to eliminate HIV.[69]

Kidney disease

Crotonlyation also has the potential to impact a range of renal diseases. In a murine model of cisplatin- or folic acid-induced AKI, for example, higher levels of histone Kcr modification have been reported and found to be associated with increases in SIRT3 and PGC-1a together with reductions in CCL2.[71] Through its ability to increase histone Kcr modification, crotonate may offer therapeutic efficacy in the context of AKI by protecting against renal damage. Immunoglobulin A nephropathy is among the most prevalent glomerular diseases, and experiments comparing samples between IgAN patients and healthy controls revealed a close association between protein Kcr and the patient humoral immune response, with this association being particularly true for proteins related to antigen processing and presentation.[101] Hemodialysis remains a standard form of renal replacement therapy for patients suffering from acute or chronic kidney failure. Studies have combined LC-MS/MS and sensitive immunoaffinity purification techniques to compare the crotonylated proteome between maintenance hemodialysis patients suffering from kidney failure and healthy controls, leading to the detection of 96 and 253 proteins respectively exhibiting elevated and reduced levels of histone Kcr in those patients undergoing maintenance hemodialysis.[35] Further research is essential to completely define the correlation between kidney disease and Kcr. This can potentially unveil a fresh set of potential targets to steer the management of renal disease.

Overall, the misregulation of protein crotonylation is associated with a variety of human diseases, which may provide a new target for clinical therapy (Fig. 5).

Crosstalk of Kcr and other PTMs

Crosstalk means that proteins are modified by multiple PTMs and these PTMs can interact with each other. PTM crosstalk can integrate different signals, which increases their regulatory potential. Various PTMs engage in competitive crosstalk as they vie for the same lysine residue. Notably, Kcr exhibits similarities to Kac, another modification that takes place on the e-amino group of lysine. It is known that the Kcr and Kac modification sites overlap in histones and non-histones, and they are catalyzed by the same enzymes and removed by the same enzymes.[4,21,32,33,67,68] Nonetheless, Kcr has been established to possess unique structural and functional characteristics compared with Kac. One of the distinguishing factors between these two modifications is their respective carbon chain lengths and planar orientation.[102] Crotonyl group has a more rigid structure. However, the acetyl group is tetrahedral and rotatable. Indeed, YEATS and DPF domains had enhanced binding affinity for Kcr over Kac.[22,53] Moreover, while Kac and Kcr have overlapping enzyme regulators, Kcr may activate different regulatory modulators than Kac because of the C—C π-bond present in Kcr. Notably, when considering p300-mediated histone modifications, Kcr was observed to significantly enhance gene

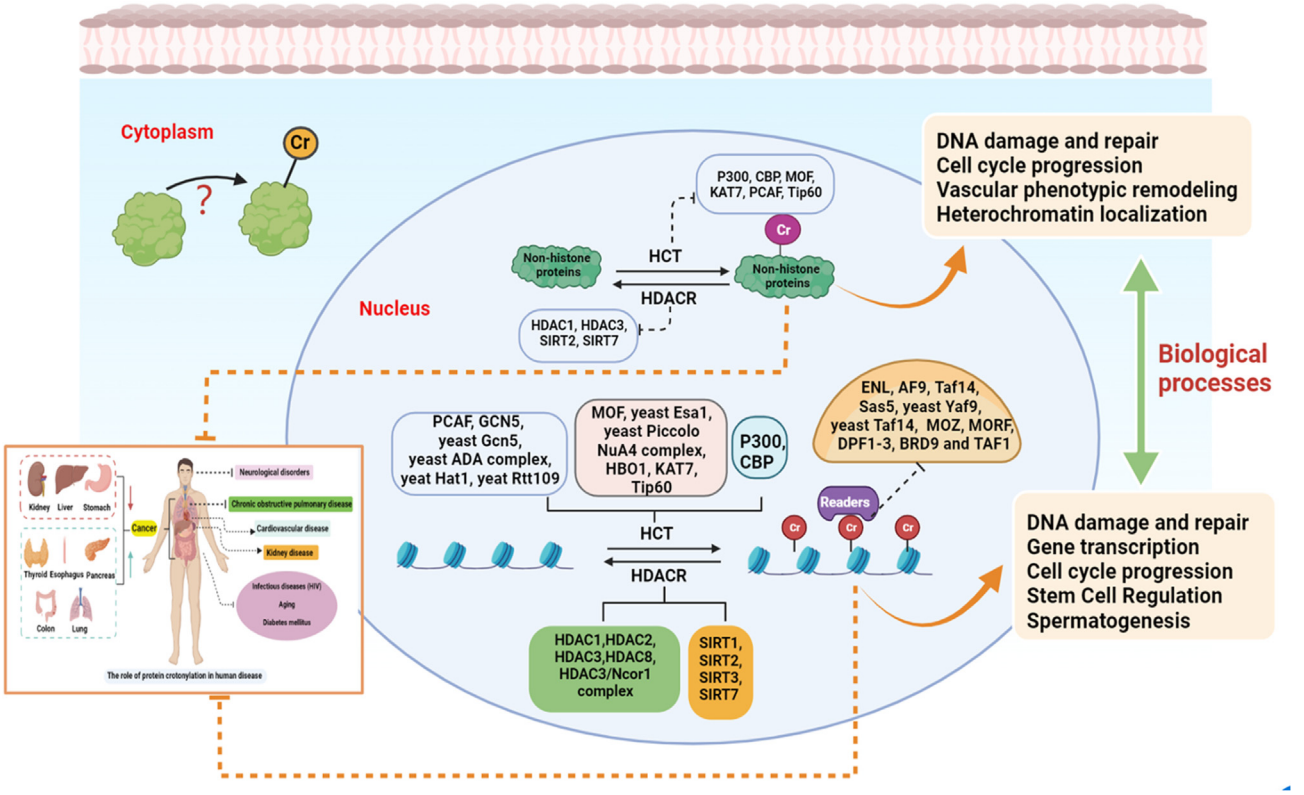

Figure 6 Regulation and functions of non-histone and histone Kcr. Kcr has been identified on lysine residues in histone and non-histone proteins. Protein Kcr was catalyzed by transferases (HCT) and decrotonylases (HDACR). Furthermore, Kcr acts as docking marks to recruit readers. The mechanism of Kcr involves many biological processes, such as DNA damage repair, gene transcription, and cell cycle. Protein Kcr plays roles in many diseases, such as cancer, neurological disorders, and cardiovascular disease.

transcription compared with histone Kac[37]; CBP/p300 mutants with deficient HAT and intact HCT activity[41] and HDAC1/3 mutants with impaired HDAC but intact HDCR activities[18] indicated different modulation patterns between Kac and Kcr. The crotonylation or acetylation of histone lysine relies on the balance of intracellular concentrations of crotonyl-CoA and acetyl-CoA.[18] In the case of nutritional exhaustion, the level of acetyl-CoA is significantly decreased, whereas the proportion of crotonylated histones may increase to preserve the transcription of key genes during starvation.[103,104] In the case of cell energetic exhaustion, peroxisomal fatty acid β-oxidation and H3K9 crotonylation increase and are accompanied by a decrease in the levels of ATP and acetyl-CoA, as well as a decrease in the expression of ribosomal biogenic genes.[105] Moreover, some literature suggests that the crotonylation of non-histone proteins is also modulated by the relative levels of crotonyl-CoA and acetyl-CoA in response to alterations in cellular energy status.[106]

In addition to crosstalk between Kcr and Kac, crosstalk between Kcr and ubiquitination has also been reported.[107] Histone H2A site 119 lysine has both crotonylation modification (H2AK119cr) and ubiquitination modification (H2AK119ub). Under DNA replication pressure, crotonylation modification was significantly reduced, while ubiquitination modification was significantly increased. Additionally, the deacylase SIRT1 and BMI1 molecules control the transformation of crotonylation/ubiquitination modification (H2AK119cr/H2AK119ub) on stalled replication forks. This process helps to resolve transcription-replication conflicts induced by replication stress and safeguard the stability of the genome.

Conclusions and future perspectives

Post-translational modifications are ubiquitous and play multifarious roles in the regulation of multiple cellar processes, enabling rapid responsivity to varying forms of cellular stress exposure. Increasingly advanced high-resolution MS and proteomics techniques have enabled the more detailed examination of PTM profiles in a range of physiological and pathological settings. Kcr is a recently detected form of PTM associated with a wide range of proteins in both prokaryotic and eukaryotic species. This article generalizes the related research on Kcr by discussing the research processes of Kcr, Kcr-related readers, writers, and erasers and their involved biological processes and diseases. Interestingly, Kcr and acetylation share certain writers, erasers, and readers. But, the regulatory mechanism of how the Kcr and acetylation activity orchestra of those enzymes is still obscure. The characteristics of many newly discovered

enzymes involved in regulating the Kcr process have yet to be fully defined. For example, it remains unknown as to whether these enzymes exhibit site-specific catalytic activity or shuttle between different parts of the cell. Therefore, identifying these specific enzymes for Kcr would be interesting. Due to the overlap between Kcr and other PTMs, such as Kcr and Kac, and ubiquitination, future work should aim to identify further evidence regarding their specific substrates and whether they have unique regulatory roles. Furthermore, exploring the interplay between Kcr and other PTMs in signal transduction and other intracellular processes, as well as investigating the stoichiometry of different acylations that occur on the same lysine residue, represent intriguing aspects for future studies.

Lysine histone and non-histone proteins Kcr have been shown to be involved in diverse biological processes from gene expression to protein stability and play important roles in many diseases, such as cancer, neurological disorders, and cardiovascular disease (Fig. 6). However, the underlying mechanism of Kcr in these biological processes and related diseases are still poorly understood. Besides, current crotonylation studies have mainly focused on histones, and the difference between histone and non-histone Kcr in the biological process or diseases has not been studied in depth. Published research has highlighted the significant roles of both histone and non-histone crotonylation in the regulation of DNA damage and repair, gene transcription, cell cycle progression, ovarian development, as well as the pathogenesis of various diseases, including cancer, neurological disorders, chronic obstructive pulmonary disease, and aging. In addition to participating in the above biological processes, histone crotonylation is involved in stem cell regulation, and the regulation of spermatogenesis, and plays important roles in cardiovascular disease, diabetes mellitus, infectious diseases, and kidney diseases. A growing number of studies have shown that non-histone protein crotonylation is involved in other major biological processes, including vascular phenotypic remodeling, heterochromatin localization, and metabolic pathways. Future work may focus on the in-depth mechanisms by which histone Kcr and non-histone Kcr play a role in biological processes or diseases, especially in non-histones. We performed an MS assay to detect the Kcr proteomic change upon DNA damage induced by irradiation. GO analysis of the result shows the cronolytion of lots of DNA damage-associated proteins is decreased upon DNA damage, which facilitates those proteins binding to DNA damage sites. Our data also shows the cronolytion is a ubiquitous modification in DNA damage- and transcription-associated proteins. This indicates protein Kcr functions mostly through inhibiting protein-DNA binding.

Crotonylation has been implicated in many human diseases and can mediate both protective and adverse functions in the development of different diseases. For example, the reduction of Kcr modification promotes the progression of stomach, liver, and kidney tumors, depression, T2DM, and AKI, but plays an inhibitory role in lung cancers, pancreatic cancers, esophageal cancers, thyroid cancers, colon cancers, neurological disease, hypertrophic cardiomyopathy, chronic obstructive pulmonary disease, aging, and HIV. Overall, the existing literature suggests that the status of lysine crotonylation may be a significant type of PTM that contributes to cancer progression. Nevertheless, crotonylation may exert diverse regulatory effects on disease development depending on the specific tissues, organs, cells, and cellular microenvironments involved. Thus, the common defective features of Kcr modification in disease onset and progression are not well understood. In the future, studies should conduct an in-depth understanding of the defective features of Kcr in disease. Besides, given the relationships between cronolytion and a range of human diseases, these crotonylated targets may also be amenable to pharmacological intervention. Thus, an in-depth understanding of the roles of Kcr in physiological processes and diseases will guide the development of drugs. In addition, researchers need to focus on developing anti-cancer drugs specifically targeting Kcr or other PTM.

Author contributions

H.Z.: conception and writing of manuscript draft; Y.H.: references preparation; P.Z. and S.G.: reading and revising manuscript; H.G.: revising and supervising submission.

Conflict of interests

The authors declare no conflict of interests.

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