Histone crotonylation in tumors (Review)

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Abstract. Lysine crotonylation (Kcr) refers to a type of modification in which crotonyl groups are transferred to lysine residues by histone crotonyltransferase (HCT) using crotonyl-coenzyme A (CoA) as a substrate. Kcr is distributed in core histones and in some nonhistone proteins. Histone crotonylation is a newly discovered epigenetic modification with a significant ability to regulate gene expression. Crotonylation occurs on the ε-amino group of lysine residues and results in a modification of the histone charge. Similar to acetylation, the substrate for crotonylation is a donor molecule, crotonyl-CoA, which is linked to the sulfhydryl group of CoA by a thioester bond. Crotonylation is involved in regulating a wide range of biological processes and diseases. With advances in detection technologies, the impact of histone crotonylation on tumors has been revealed. The present review examines the recent discoveries of histone crotonylation, its function in tumors and its regulatory mechanism, which will aid in elucidating the mechanisms of malignant tumor development and provide a theoretical foundation for the development of new targeted cancer therapies.

Contents

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1. Introduction

Histone modifications are crucial mechanisms in the regulation of gene expression (1). Lysine acylation is a form of post-translational modification (PTM) in which an acyl group is covalently attached to a lysine residue of a protein (2). Lysine acylation is a broad term that encompasses several different types of modifications that depend on the nature of the acyl group added. Since the development of highly sensitive mass spectrometry (MS) technology, various metabolites have been shown to covalently modify proteins via different forms of lysine acylation, including lysine acetylation, crotonylation, lactylation, succinylation, propionylation, butyrylation, malonylation, glutarylation, 2-hydroxyisobutyrylation and β-hydroxybutyrylation (3). Lysine acylations are a versatile and complex family of PTMs that play essential roles in the regulation of cellular processes. These modifications are involved in metabolic regulation, epigenetic regulation, and signal transduction (4,5). In addition to traditional histone modifications such as acetylation, methylation, and phosphorylation (6), a new modification called crotonylation has recently been discovered (7). Crotonylation is a short-chain fatty acid modification that was initially identified in yeast and was later confirmed to occur in human cells. Lysine, an amphiphilic residue with a hydrophobic side chain, can undergo acylation, which neutralizes the positive charge of the amino group and potentially alters protein conformation. Lysine crotonylation (Kcr) refers to the modification of lysine residues by histone crotonyltransferases (HCTs). Kcr reportedly plays a role in several physiological and pathological processes. In histones, crotonylation modifications have been shown to be closely associated with biological processes such as gene transcription regulation (7), spermatogenesis (8), acute kidney injury (9), depression (10) and HIV latency (11).

Increasing evidence has shown that histone crotonylation plays a critical role in tumorigenesis and tumor progression (12-15). The present review aims to systematically

summarize the research progress on histone crotonylation, explore its specific roles in tumors, and discuss its potential therapeutic applications, which may provide novel insights into cancer pathogenesis and therapeutic targets.

2. Discovery and mechanism of crotonylation

MS has become an ideal analytical tool for the qualitative and quantitative analyses of protein modifications, due to its unparalleled sensitivity and specificity (16). Currently, four strategies have been developed for the characterization of PTM sites via (liquid chromatography) LC-MS/MS: i) Tandem MS using one or more of several available fragmentation mechanisms; ii) removal of the modification between consecutive mass spectrometric analyses; iii) selective enrichment of modified proteins or peptides on the basis of the modified functional group prior to MS/MS; and iv) PTM-specific multistage MS strategies (16). LC-MS/MS has revolutionized the field of proteomics by providing a powerful platform for the identification and characterization of PTMs. The ability of MS to provide detailed insights into protein modifications underpins its critical role in advancing the understanding of cellular mechanisms and disease pathways. With the development and improvement of MS detection technology, increasing numbers of PTMs have been identified. These include acetylation, crotonylation, butyrylation, propionylation, and succinylation modifications, among others (Table I) (7,17-25). In 2011, Tan *et al* (7) from the University of Chicago discovered Kcr modification for the first time through an integrated MS-based proteomics approach and confirmed that it represents an evolutionarily conserved PTM of histone proteins. In 2017, Xu *et al* (26) at Peking University discovered that non-histone proteins can also be modified by crotonylation. Some acetyltransferases and deacetylases have also been shown to exhibit crotonyltransferase and deprotonylase activities (26). Kcr is closely related to but distinctly different from acetylation. Kcr occurs primarily on the ε-amino group of lysine, but its planar orientation and four-carbon length distinguish it from lysine acetylation (26). The results of quantitative proteomics also revealed that only 43% of the sites targeted by crotonylation overlap with those targeted by acetylation, which suggests differences in the substrate proteins targeted by these two modifications (27).

Crotonylation modifications are classified as reversible acylation modifications and are regulated by a variety of acylases and deacylases as well as by intracellular crotonyl-CoA substrate concentrations. The process and sites of histone crotonylation are shown in Fig. 1. Some studies have revealed that the process of intracellular crotonylation is in dynamic equilibrium, which is attributed to the presence of multiple regulatory proteins, such as crotonyltransferases, decrotonylases, and crotonylation recognition proteins (28,29). Research has found that the addition of exogenous crotonate significantly increased the abundance of crotonyl-CoA in cells, and the levels of crotonyl modification on global histones were also significantly upregulated, especially H3K18, confirming the close relationship between intracellular crotonyl modification levels and crotonyl-CoA (30). Sabari *et al* (30) found that H3K18 is the dominant site of both p300-catalyzed histone crotonylation.

Kcr writers. Crotonyltransferase, also known as a writer protein, can add crotonyl groups to substrate proteins. Sabari *et al* (30) reported that p300, a member of the lysine acetyltransferase family, can also catalyze Kcr via the use of crotonyl-CoA as a donor. p300 has been demonstrated to catalyze histone Kcr, which in turn stimulates transcription to a greater degree than does histone lysine acetylation (30). Liu *et al* (31) subsequently focused on the enzymes that catalyze histone crotonylation and demonstrated that among known histone acetyltransferases, in addition to CREB-binding protein (CBP) and p300, males absent on the first (MOF) proteins possess HCT activity and that this activity has been evolutionarily conserved (31). In addition, a previous study revealed that the acetyltransferases, CBP, p300/CBP-associated factor and human MOF act as crotonyltransferases for non-histone proteins (26). Kollenstart *et al* (32) identified the Gcn5- and Esa1-containing ADA and Piccolo NuA4 complexes as *bona fide* crotonyltransferases that promote crotonylation-dependent transcription in budding yeast (32).

Kcr erasers. Decrotonylases, also known as erasers, can remove crotonyl groups from proteins. Histone deacetylases (HDACs) comprise two main families: i) Zinc $(Zn)^+$-dependent HDAC family members, including class I HDACs (HDAC1-3, HDAC8), which are localized in the nucleus; class II HDACs (HDAC4-7, HDAC9-10); and class IV HDACs (HDAC11), which are localized in the nucleus and cytoplasm; and ii) The NAD^+-dependent deacetylase family including class III HDACs [sirtuin (SIRT)1-7]. HDACs have also been reported to exhibit histone decrotonylase activity (28). HDAC3 in complex with nuclear receptor corepressor 1 was shown to exhibit decrotonylase activity *in vitro* by systematic screening of the activities of eleven human Zn-dependent lysine deacylases (33). Bao *et al* (34) used a chemical proteomics approach to comprehensively profile 'eraser' enzymes that recognize a lysine-4 crotonylated histone H3 (H3K4Cr) mark and reported that SIRT1, SIRT2 and SIRT3 can catalyze the hydrolysis of lysine crotonylated histone peptides and proteins. However, among these three selective H3K4Cr binders, SIRT3 is likely a selective and relatively tight binding partner of H3K4Cr (34). Wei *et al* (35) presented evidence that class I HDACs, but not SIRT family deacetylases, are the major HDACs (35). Kelly *et al* (36) reported that genetic deletion of HDAC1/2 in embryonic stem cells increases the overall levels of histone crotonylation and results in an 85% decrease in total deprotonase activity and that HDAC1/2 regulates H3K18cr levels at active gene loci. However, its physiological effects have not been reported. In addition, it was revealed that the crotonylation level was increased by HDAC knockdown or by the addition of the HDAC inhibitor, TSA, which inhibited hepatoma cell motility and proliferation (12).

Kcr readers. Crotonylation recognition proteins, also known as readers, can recognize crotonylation sites on proteins. Certain specific structural domains were found to be involved in the transcriptional regulation process induced by crotonylation. Researchers have reported that YEATS, bromodomain, and double PHD finger (DPF) are important readers of Kcr modifications (37). The YEATS structural domain proteins constitute the first identified family of reader proteins that recognize

Table I. List of acylation modifications.

Modification	Abbreviation	Reported year	(Refs.)
Acetylation	Kac	1962	(17)
Propionylation	Kpr	2007	(18)
Butyrylation	Kbu	2007	(18)
Crotonylation	Kcr	2011	(7)
Succinylation	Ksucc	2012	(19)
Malonylation	Kma	2012	(19)
Glutarylation	Kglu	2014	(20)
2-Hydroxyisobutyrylation	Khib	2014	(21)
β-hydroxybutyrylation	Kbhb	2016	(22)
Benzoylation	Kbz	2018	(23)
Lactylation	Kla	2019	(24)
Isonicotinylation	Kinic	2021	(25)

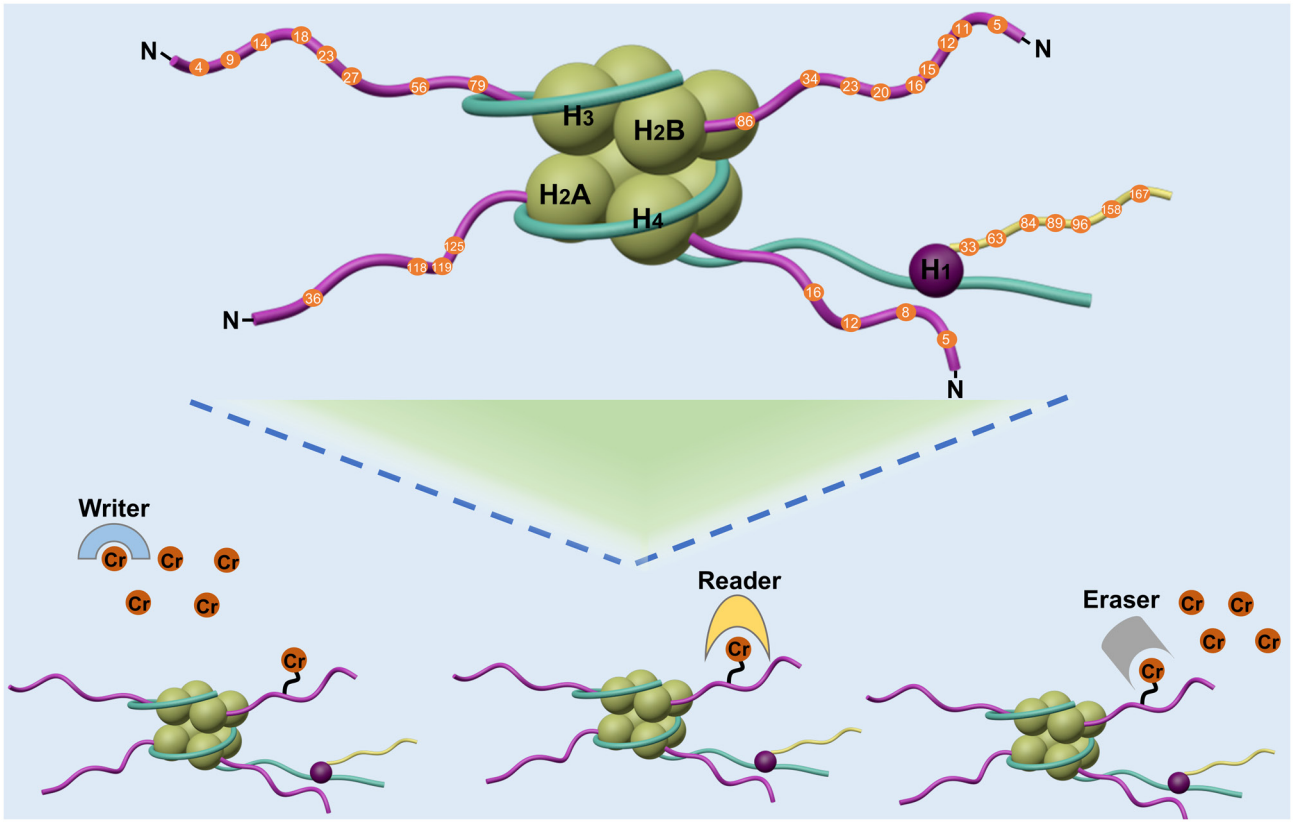

Figure 1. Process of crotonylation and illustrations of histone crotonylation sites in humans. Cr, crotonyl group modified by crotonylation.

crotonylation modifications. Researchers have shown that the Taf14 YEATS domain engages crotonyllysine via a unique πππ-stacking mechanism and that other YEATS domains have crotonyllysine-binding activity (38). Li *et al* (39) reported that the AF9 YEATS domain selectively displays increased binding affinity for crotonyllysine over acetyllysine. Structural studies revealed an extended aromatic sandwiching cage with crotonyl specificity that arises from π-aromatic and hydrophobic interactions between the crotonyl group and the aromatic rings (27,39). The YEATS2 protein efficiently reads H3K27cr and is essential for Kcr-mediated active transcription (40). The bromodomain has been reported to be involved in acetyllysine modification of histones, and it has also been shown to be involved in crotonyllysine modification. The second bromodomain of TATA-box binding protein associated factor 1 (TAF1) can bind crotonyl marks (41). Xiong *et al* (42) demonstrated that the histone acetylation-binding DPF domains of human monocytic leukemia zinc finger protein (MOZ) and D4 zinc finger domain containing protein 2 (DPF2) accommodate a wide range of histone lysine acylations with the strongest preference for Kcr. Crystal structures of the DPF domain of MOZ in a complex with H3K14cr, H3K14bu, and H3K14pr

Table II. Regulatory factors involved in histone crotonylation modification.

Enzyme family	Regulatory molecules	Crotonylation site	Reported year	(Refs.)
Writer	p300	H3K18	2015	(30)
	MOF	H3K4, H3K9, H3K18, H3K23, H4K8 and H4K12	2017	(31)
	GCN5	H3K9, H3K14, H3K18, H3K23 and H3K27	2019	(32)
	Esa1	H4K5, H4K8, H4K12 and H4K16	2019	(32)
Eraser				
$Zn2^+$-dependent HDACs	HDAC1,2,3,8	H3K4, H3K9, H3K23, H4K8, H4K12 and H3K23	2017	(35)
NAD^+-dependent sirtuins	SIRT1,2,3	H3K4	2014	(34)
Reader	Taf14	H3K9	2016	(38)
	AF9	H3K9, H3K18 and H3K27	2016	(39)
	MOZ	H3K14	2016	(42)

peptides reveal that these nonacetyl acylations are anchored in a hydrophobic 'dead-end' pocket with selectivity for crotonylation arising from intimate encapsulation and an amide-sensing hydrogen bonding network (42). A summary of regulatory factors involved in histone crotonylation is provided in Table II.

3. Crotonylation and malignant tumors

Protein PTM is a regulatory mechanism for activity modulation, localization, expression, and interactions of proteins with other cellular molecules (43). The PTMs of histones likely play pivotal roles in cancer development and progression, as they influence gene transcription, chromatin remodeling, and the organization of the nuclear architecture (44). Histone crotonylation modifications are also closely related to oncogenesis (12,27). The continuous advancement of various detection methods also provides strong support for the identification of crotonylation sites related to tumors (45). For the first time, Wan *et al* (28) suggested that the state of Kcr may be an important type of PTM that explains cancer progression. Using quantitative proteomics, researchers have found that p300-mediated Kcr and p300-targeted Kcr substrates are involved in the regulation of cancer (27). Huang *et al* (27) reported that 4.5% (20 out of 443) of the cancer protein biomarkers in the EDRN database are crotonylated. In addition, 32 Kcr proteins are related to cancer genes and account for 5.9% of all genes in the COSMIC cancer gene database. Notably, six p300 target proteins were identified as cancer gene-related proteins. It was revealed that some p300-targeted Kcr substrates are potentially linked to diseases such as cancer (27). A series of cancer samples was collected from patients with either liver, stomach, kidney, thyroid, esophageal, colon, pancreatic, or lung cancer. Wan *et al* (12) performed immunohistochemical staining with a pan anti-Kcr antibody. Kcr was detected in both the cytoplasm and nucleus, and its expression was downregulated in liver, stomach, and kidney carcinomas and upregulated in thyroid, esophageal, colon, pancreatic, and lung carcinomas (12). These findings suggest that Kcr may play diverse roles during cancer progression through the modulation of different key cancer-related proteins. In addition, Wan *et al* (12) collected 68 hepatocellular carcinoma (HCC) samples and performed immunohistochemical staining. The staining scores of Kcr expression levels were dichotomized into two groups, low and high. The correlations between Kcr expression and clinicopathological characteristics of HCC were investigated. The results revealed that Kcr is associated with the tumor, node, metastasis (TNM) stage. Through Transwell assays and WST-1 assays, it was found that the cell migration and proliferation abilities of Huh-7 cells decreased when HDAC1 or HDAC3 were knocked out or HDAC inhibitor TSA was added. Further *in vivo* xenograft tumor growth experiments revealed that the tumor growth rate and tumor weight in the TSA treatment group were lower than those in the control group. These findings indicated that crotonylation is also correlated with tumor progression (12).

Liver cancer. HCC is a common liver malignancy with high lethality and poor overall patient prognosis (46,47). Early-stage HCC is typically treated through liver resection and other forms of surgical intervention. For advanced HCC, treatment options include chemotherapy, immunotherapy, and oncolytic virotherapy. With the rise of nanotechnology-based drug delivery systems, these treatment approaches can be combined with nanotechnology to increase therapeutic efficacy and reduce side effects. Additionally, the combination of chemotherapy and immunotherapy can further improve treatment outcomes and overcome resistance (47). Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses revealed that in HCC tissues, Kcr proteins are extensively involved in various cellular processes, including signaling, metabolism, translation, acylation, and carcinogenesis (48). Zhang *et al* (49) investigated the correlation between crotonylation and HCC in 100 tumor tissues. Using amino acid analysis and LC-MS/MS for stable isotope labeling of HCC cells, it was reported that crotonylation was positively correlated with HCC metastasis and that high levels of crotonylation in HCC cells promoted cellular invasiveness (49). Researchers have revealed that the level of Kcr is correlated with TNM stage in HCC (12). Additionally, in a study by Zhang *et al* (48), Kcr protein levels were found to be positively correlated with HIF1α in tissue microarrays derived from a cohort of patients with liver cancer. These findings suggest that Kcr promotes liver cancer cell proliferation (48). Lamin A was previously

reported to be an oncogenic protein that enhances the proliferation of HCC (50). Zhang *et al* (48) even demonstrated that lamin A is a key Kcr protein that regulates the proliferation of HCC cells and that the crotonylation of lamin A occurs at K265 and K270. Zhang *et al* (51) reported that Acyl-CoA oxidase 2 (Acox2) expression levels are significantly lower in human HCC tissues than in normal liver tissues. In Acox2-knockout C57BL/6n mice ($Acox2^{-/-}$ mice), Acox2 loss damaged metabolic homeostasis by downregulating the level of crotonylation of several metabolic enzymes and peroxidases, which ultimately induced hepatocarcinogenesis in these mice. In that study, non-histone Kcr was partially downregulated in the liver tissues of $Acox2^{-/-}$ mice; however, histone Kcr was mildly upregulated, which suggests that histone and non-histone Kcr are differentially regulated in HCC (51). The potential mechanisms of Kcr in HCC progression remain unclear. Therefore, future research should focus on understanding how Kcr influences HCC progression and explore whether this process could provide a theoretical foundation for innovative treatments for liver cancer.

Glioblastoma (GBM). GBM is the most common and aggressive primary brain tumor in adults and has the highest grade according to the World Health Organization classification of brain tumors (52). Histone Kcr and lysine lactylation (Kla) are widely present in the brain and undergo significant changes during neural development. Furthermore, the dynamic genome-wide changes in H3K9cr and H3K18la are extensively involved in neural differentiation and cell proliferation, which highlights how the remodeling of histone acetylation coordinates changes in gene expression and cell fate transitions (53). Fellows *et al* (13) reported that proteins weighing ~70 kDa in brain extracts are recognized by antibodies against crotonyl lysine, which indicates the presence of crotonylated non-histone proteins in the brain (13). Yuan *et al* (54) discovered that glioblastoma stem cells (GSCs) reprogram lysine catabolism to propagate and transform into an immunosuppressive state and reported that reducing histone Kcr via genetic manipulation or lysine restriction impaired tumor growth. Lysine-restricted diets are more effective at slowing tumor growth and improving survival in immunologically active hosts. It has also been revealed that although Kcr is usually associated with increased gene transcription, H4 Kcr, rather than the well-characterized H3 Kcr, is enriched due to reprogrammed lysine catabolism in GSCs (55).

Lung cancer. Lung cancer is the leading cause of cancer-related deaths worldwide (56). Non-small cell lung cancer (NSCLC) is the most common type of lung cancer and accounts for ~85% of all lung cancer cases. Of the NSCLC cases, lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are the most prevalent subtypes (57). With continuous advancements in medicine, early diagnosis and personalized treatment are key to improving the survival rate of patients with NSCLC. The development of molecular targeted therapy, immune checkpoint inhibitors, and anti-angiogenic drugs has significantly improved patient prognosis (58). After A549 cells (NSCLC) were treated with suberoylanilide hydroxamic acid, an HDAC family inhibitor, 10,163 Kcr sites were identified on 2,445 proteins. Subcellular localization revealed that the sites were located mainly in proteins in the cytoplasm, nucleus and mitochondria (59). Proteomic analysis of H1299 lung adenocarcinoma cells revealed 2,696 crotonylation sites on 1,024 proteins (26). Recently, brain-expressed X-linked gene 2 (BEX2) was found to be localized in the cytosol and/or mitochondria and to regulate the apoptosis of cancer cells and tumor growth. Mu *et al* (60) reported that BEX2 is overexpressed in lung adenocarcinoma and is associated with poor prognosis in lymph node metastasis-free patients and clinical stage (I + II) patients (60). In addition, it was revealed that crotonylated BEX2 plays an important role in inhibiting chemotherapeutic agent-induced apoptosis by enhancing mitophagy in NSCLC cells. Combination treatment with mitophagy inhibitors and anticancer drugs that target BEX2 represent a potential strategy for NSCLC treatment (60). The advantage of using drugs to control protein crotonylation is that crotonylation is a reversible modification, which means that its levels can be dynamically regulated through drug intervention. Therefore, flexible therapeutic effects can be achieved. The disadvantage is that crotonylation plays a role in various cellular processes and different tissues, and drug regulation may lead to nonspecific effects and cause adverse side effects. In the future, drugs that target specific crotonylation enzymes (such as crotonyltransferases or decrotonylation enzymes) should be developed to achieve increased therapeutic specificity and reduce interference with other biological processes. The effective delivery of drugs to target cells or tissues also remains a technical challenge.

Colorectal cancer. Crotonylation is abnormally abundant in the epithelial tissues of the human small intestine, particularly in the crypts of the small intestine, and in the colon (13). This may be due to the production of crotonic acid (CA) resulting from the fermentation and degradation of food by the gut microbiota (13). Fellows *et al* (13) reported that histone H3K18cr is the most abundant histone crotonylation mark in the intestine. This site was characterized through chromatin immunoprecipitation sequencing (ChIP-seq). The analysis indicated that H3K18cr is associated with transcription start sites (TSSs). KEGG pathway analysis of genes with high levels of H3K18cr at their TSS highlighted several cancer-related pathways, which suggests that histone crotonylation may be involved in cancer (13). Further research revealed that the addition of short-chain fatty acids to the culture medium of human colon cancer cells (HCT116) and mouse small intestine organoids promotes the crotonylation of H3 and H4 histones (13). DNA damage plays a crucial role in the development and progression of colon cancer. Researchers have reported that H3K27cr levels are reduced in the setting of DNA damage in colon cancer and that changes in these levels may be mediated by SIRT6 (61). The regulatory mechanism of histone crotonylation in tumors with DNA damage should be further investigated. Liao *et al* (62) reported that H3K27cr expression is upregulated in metastatic colorectal cancer tissues and is positively correlated with clinical advanced stage disease. In this study it was reported that LINC00922 interacts with the protein SIRT3 and hinders its binding to the ETS1 promoter region, which leads to an increase in the level of H3K27cr in this promoter region and the subsequent activation of ETS1 transcription (62). These findings revealed

a novel regulatory function of H3K27cr in colorectal cancer metastasis and facilitate the discovery of new therapeutic strategies. The level of crotonylation of H2BK12 (H2BK12cr) was revealed to be significantly increased in peripheral blood mononuclear cells (PBMCs) from patients with colorectal cancer and was strongly associated with distant metastasis and advanced TNM stage (63). The H2BK12cr level provides a novel method for the diagnosis of colorectal cancer. Hou *et al* (14) reported that the Kcr of enolase (ENO1) is significantly elevated in human colorectal cancer tissues compared with that in paraneoplastic tissues and further identified K420 as the major Kcr site of ENO1; crotonylation at this site regulated the expression of tumor-associated genes and promoted the growth, migration, and invasion of colorectal cancer cells *in vitro* (14). Notably, researchers have reported that crotonylation occurs on a serine residue rather than on the more well-known lysine residue. CA was demonstrated to reduce p53 levels in human cells by inducing Ser46 crotonylation. CA increased p53-dependent glycolytic activity and promoted the proliferation of colorectal cancer cells (64). These findings provide a new perspective on the role of histone crotonylation in tumors.

Prostate cancer (PCa). The level of crotonylation modification was revealed to be greater in PCa tissues than in adjacent tissues, and the level of modification gradually increased with increasing PCa malignancy (15). This study also revealed that BRD4 inhibitors (I-BET762, I-BET726, and CPI-203) inhibit the migration and invasiveness of PCa cells, whereas histone crotonylation promotes the migration and invasiveness of PCa cell lines (15). Following BRD4 inhibition, the expression level of p300 and the overall crotonylation level within the cells decreased, which indicates that BRD4 may influence crotonylation via p300. The expression level of the HDAC family proteins was not significantly altered, which suggests that crotonylation in PCa is not regulated by HDACs.

Cervical cancer. Human papillomavirus (HPV) is the primary etiologic factor of cervical cancer (65), which is the leading cause of cancer-related deaths among women worldwide (66). Han *et al* (67) reported increased expression levels of heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1) in HPV-associated cervical cancer cells, including HeLa, Caski, and SiHa cells, but especially in HeLa cells (67). In addition, HeLa cell proteomics revealed that 14,311 sites of 3,734 proteins could be modified by crotonylation (68). HNRNPA1 is a p300-regulated Kcr protein (27). In the study by Han *et al* (67) it was demonstrated that p300-mediated Kcr enhances HNRNPA1 expression, which promotes the proliferation, invasiveness and migration of HeLa cells (67). These findings revealed the therapeutic potential of controlling crotonylation in cervical cancer. Several common crotonylation-regulated proteins, such as SIRT2 (69) and SIRT3 (70), have been confirmed to play regulatory roles in cervical cancer. However, the specific mechanisms through which crotonylation modifications contribute to the regulation of these proteins remain unknown. Therefore, further experimental validation is needed to explore the regulatory mechanisms of crotonylation in cervical cancer.

Head and neck squamous cell carcinoma (HNSCC). Most head and neck cancers are derived from the mucosal epithelium in the oral cavity, pharynx and larynx and are known collectively as HNSCC (71). Jiang *et al* (72) revealed that the expression of Kcr regulators is associated with the tumorigenesis and progression of HNSCC. Compared with early T-stage tumors, lysine acetyltransferase 2B (KAT2B) was downregulated in advanced T-stage tumors (72). Additionally, HDAC2 was upregulated in patients with HNSCC with lymph node metastasis compared with those without lymph node metastasis (72). Furthermore, most Kcr regulators, including DPF2, HDAC2, HDAC3, HDAC8, KAT8, MLLT3, SIRT1, TAF1, and YEATS2, were significantly upregulated in patients with HNSCC with high histological grades. Notably, in the aforementioned study (72), several independent interaction groups were detected among the 'writers', 'readers', and 'erasers' which indicates the existence of different functional pathways of various regulators. The study identified and validated a nine-gene signature for HNSCC on the basis of Kcr regulators (72). These results may contribute to prognostic stratification and treatment escalation in patients with HNSCC.

4. Prospects for tumor treatment

Although the mechanism of crotonylation in tumors requires further research, the known findings still offer hope for the development of new targeted cancer therapies. Reducing the level of crotonylation modification by inhibition of HDAC family proteins has become a concept for the clinical treatment of tumors. In addition, a series of specific inhibitors of crotonylated reader proteins have been used in clinical practice. The application of B029-2, a novel p300 inhibitor, has shown significant antitumor effects on HCC cells both *in vitro* and *in vivo* (73). Lao *et al* (74) reported that glutaryl-CoA dehydrogenase (GCDH) inhibits HCC progression through crotonylation-induced suppression of the pentose phosphate pathway and glycolysis, which leads to HCC cell senescence. Senescent cells further shape the antitumor microenvironment through the senescence-associated secretory cell phenotype. Due to the increase in $PD\text{-}1^{+}CD8^{+}$ T cells, the GCDH low-expression group exhibited a better response to anti-PD-1 therapy compared with the GCDH high-expression group (74). The YEATS domain is associated with the progression of various malignancies (75) and serves as a key domain for recognizing crotonylation modifications. Several studies have demonstrated the application of inhibitors targeting the YEATS domain in the treatment of cancers such as lung cancer (76) and leukemia (77), which provides the potential for further development of crotonylation-related cancer therapies.

5. Conclusion

Since the discovery of crotonylation, numerous studies have demonstrated its significant role. This process is involved in the regulation of a wide range of biological processes and diseases. As detection technologies advance, the impact of histone crotonylation on tumors will continue to be revealed. Histone crotonylation in tumors, an emerging epigenetic modification, is still in its early stages of research. Future studies should focus on and elucidate the following: i) The specific mechanisms of

crotonylation in gene regulation and its interactions with other histone modifications; ii) the specificity and universality of crotonylation markers in different types of tumors determined using large-scale clinical sample analysis; iii) the development of efficient crotonylation inhibitors and the assessment of their efficacy and safety in cancer treatment; and iv) the combination of MS analysis and gene editing techniques to promote multidisciplinary research on the function and therapeutic potential of crotonylation in tumors. Future investigations will help us better understand the mechanisms of malignant tumor development and provide a theoretical foundation for the development of new targeted cancer therapies. As research continues to expand, histone crotonylation is expected to become an important field in cancer treatment, which will offer more therapeutic options and hope for patients.

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Authors' contributions

All authors (XW, YQ, ZL and QX) contributed to the study conception and design, as well as performed the literature search and interpretation of the relevant literature. The first draft of the manuscript was written by XW and YQ and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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