Histone crotonylation-centric gene regulation

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Abstract
Histone crotonylation is a recently described post-translational modification that occurs at multiple identified histone lysine crotonylation sites. An increasing number of studies have demonstrated that histone crotonylation at DNA regulatory elements plays an important role in the activation of gene transcription. However, among others, we have shown that elevated cellular crotonylation levels result in the inhibition of endocytosis-related gene expression and pro-growth gene expression, implicating the complexity of histone crotonylation in gene regulation. Therefore, it is important to understand how histone crotonylation is regulated and how it, in turn, regulates the expression of its target genes. In this review, we summarize the regulatory factors that control histone crotonylation and discuss the role of different histone crotonylation sites in regulating gene expression, while providing novel insights into the central role of histone crotonylation in gene regulation.

Keywords: Histone crotonylation, Gene regulation, Writer, Eraser, Reader

Background
Lysine crotonylation is a histone modification first described in 2011 [1]. To date, multiple histone and non-histone lysine crotonylation (Kcr) sites have been identified in various organisms [2–7]. The majority of histone Kcr is enriched in transcriptional start sites (TSSs) and enhancer regions, suggesting a potential role for histone Kcr in gene regulation [1]. Most studies have reported that histone Kcr at the gene promoter facilitates the transcription of genes. However, we, as well as others, have shown that increased cellular crotonylation levels inhibit endocytosis-related and pro-growth gene expression [8, 9].

Increasing evidence has demonstrated that histone Kcr is associated with physiological and pathological processes, such as differentiation [1, 10], tissue injury [11], virus infection [12, 13], tumorigenesis [14], and neurodegenerative disease [8]. The first histone Kcr-related biological process to be discovered was germ cell differentiation. During spermatogenesis, histone Kcr is enriched in promoters of highly expressed testes genes, including a number of X-linked genes that function to maintain sex chromosome activation in haploid cells. This results in the differentiation of male germinal cells immediately following meiosis [1]. Histone Kcr was next found to be related to nephropathy, including acute kidney injury (AKI). Researchers found that increased histone crotonylation prevented AKI and a decrease in renal function via increasing PGC-1α and sirtuin-3 levels and decreasing CCL2 expression [11].

In addition, histone Kcr functions as an inducer for the reactivation of latent human immunodeficiency virus (HIV) by promoting viral gene transcription via the HIV long-terminal repeat (LTR) [12]. Moreover, histone Kcr expression is altered in a number of cancers, including liver, stomach, kidney, thyroid, esophagus, colon, pancreas and lung carcinomas. In hepatocellular carcinoma (HCC), the induction of Kcr through siRNAs targeting histone deacetylases (HDACs) or HDAC inhibitors inhibits hepatoma cell motility and proliferation [14]. Our recent study showed that induction of histone
crotonylation inhibits the expression of endocytosis-related genes, which are important for Aβ uptake during the development of Alzheimer’s disease (AD), through decreasing levels of histone acetylation in the promoters of these genes, suggesting that histone crotonylation is a potential therapeutic target against AD [8].

In this review, we summarize and discuss the molecules and regulatory patterns that modulate histone crotonylation. These molecules mainly function as crotonyltransferases, deacetylases, and readers. We also summarize and discuss the different roles of specific histone crotonylation in gene regulation.

Factors regulating histone crotonylation

Several factors are associated with histone crotonylation-mediated gene regulation. They function as writers, erasers, readers, or regulators for histone crotonylation (Table 1).

Writers

P300 was the first histone crotonyltransferase identified. Researchers found that only p300 showed measurable histone crotonyltransferase (HCT) activity relative to other histone acetyltransferases (HATs) including GCN5, TIP60, and MOF. Additionally, in vitro experiments showed that knockdown of p300 or its paralog CBP via siRNAs reduced the global levels of H3K18Cr [15]. However, compared to its acetyltransferase activity, the crotonyltransferase activity of p300 showed a nearly 62-fold decrease due to the restricted size of an aliphatic back pocket of p300 [16], suggesting other co-factors are required to enhance the catalytic efficiency of p300.

In addition, other reports have demonstrated that MOFs can also catalyze histone H3 crotonylation at lysine residues 4, 9, 18, and 23 and histone H4 at lysine 8 and 12 in HeLa cells [17], as well as that GCN5 can target lysine residues at positions 9, 14, 18, 23, and 27 in histone H3 for crotonylation in budding yeast [18]. In addition, Esa1, the homolog of human MOF, has been shown to catalyze crotonylation at lysine residues 5, 8, 12, and 16 in histone H4 [18].

Erasers

The first discovered histone decrotonylases were members of the sirtuin family of class III histone deacetylases: Sirt1, Sirt2, and Sirt3. Using a chemical proteomics approach, these three deacetylases were found to recognize the histone H3K4Cr mark by directly binding to the crotonylated histone peptide via a π–π interaction, indicating that they functioned as “erasers” of crotonylation, removing Kcr marks from histone proteins [19].

HDAC1, HDAC2, and HDAC3, members of the class I histone deacetylases, also mediate histone decrotonylation. Compared to class III histone deacetylases, class I histone deacetylases possess a greater regulatory effect on histone decrotonylation. HDAC1 is active at multiple crotonylated sites, including H3K4, H3K9, H3K23, H4K8, and H4K12, whereas HDAC2 and 3 are active at H3K23Cr; however, SIRT1 is the only eraser responsible for removing Kcr at H3K9Cr and H4K8Cr [20].

Readers

Recognition of histone modifications by “reader” modules constitutes a major mechanism of epigenetic regulation. The first discovered effective reader of histone crotonylation is Taf14, which specifically recognizes H3K18Cr [26].

Table 1 The regulatory factors for histone crotonylation

| Factor   | Regulatory pattern   | Crotonylation site          | References |
|----------|----------------------|-----------------------------|------------|
| P300     | Writer               | H3K18                       | [15]       |
| MOF      |                      | H3K4, H3K9, H3K18, H3K23, H4K8, and H4K12 | [17]       |
| GCN5     |                      | H3K9, H3K14, H3K18, H3K23, and H3K27 | [18]       |
| Esa1     |                      | H4K5, H4K8, H4K12, and H4K16 | [18]       |
| SIRT 1, 2, 3 | Eraser           | H3K4                       | [19]       |
| HDAC1, 2, 3 |                | H3K4, H3K9, H3K23, H4K8, H4K12 (HDAC1), and H3K23 (HDAC2, 3) | [20]       |
| Taf14    | Reader              | H3K9                       | [26]       |
| YEATS2   |                      | H3K27                       | [27]       |
| AF9      |                      | H3K9, H3K18 and H3K27       | [28]       |
| MOZ and DPF2 |                | H3K14                       | [29]       |
| NEAT1    | Regulator with unknown mechanism | H3K27 | [8] |
| CDYL     |                      | H2BK12, H3K9, H3K27, and H4K8 | [10]       |
| ACSS2    |                      | H3K4 and H3K18              | [12, 15]   |

NEAT1 and CDYL are associated with unknown mechanisms.
Crotonylation was the YEATS domain, which is an evolutionarily conserved gene regulatory factor found in species from human to yeast. YEATS family members can form a variety of important complexes that participate in transcriptional regulation, histone modification, histone deposition, and chromatin remodeling [21]. To date, several proteins have been reported to contain the YEATS domain, such as the transcription factor complexes TFID and TFIIH, chromatin-remodeling complexes INO80, SWI/SNF and RSC, and the histone acetyltransferase complex NuA3 [22–25]. The Taf14 YEATS domain was found to recognize H3K9Cr by adopting an immunoglobulin-like β sandwich fold containing eight anti-parallel β strands linked by short loops that form a binding site for H3K9Cr [26]. The YEATS domain of YEATS2 has the strongest affinity for H3K27Cr with lower binding efficiencies for H4K4Cr, H3K12Cr, H3K23Cr, and H3K9Cr via an end-open aromatic sandwich pocket for Kcr binding [27]. The AF9 YEATS binds strongly to H3K9Cr, H3K18Cr, and H3K27Cr via a pocket formed by the L1, L4, and L6 loops of AF9 [28].

The second discovered histone crotonylation reader was the double PHD finger (DPF) domain. The DPF domains of human MOZ (also known as KAT6A) and DPF2 (also known as BAF45d), two histone acetylation-binding proteins, recognize H3K14Cr via a hydrophobic “dead-end” pocket [29].

**Other regulators**

In our study on the role of the long non-coding RNAs in AD, we found that NEAT1 is associated with the acetyltransferase P300/CPB complex and that knockdown of NEAT1 increases histone Kcr and decreases acetylation at H3K27, suggesting that NEAT1 mediates histone acetylation and inhibits histone crotonylation at the same lysine sites [8].

Ring finger protein 8 (RNF8), a ubiquitin ligase (E3), has roles in the DNA damage response [30, 31] and cell cycle progression [32]. A study to determine the role of RNF8 in the development of spermatids found that RNF8 epigenetically regulates a set of sex-linked genes that tend to escape post-meiotic silencing and is activated in round spermatids through increased lysine crotonylated histone around TSSs of these genes, and thereby, an alteration of chromatin conformation. This suggests RNF8 acts as an important regulator of spermatogenesis through epigenetic programming in sex chromosomes [33].

In addition, acyl-CoA synthetase short-chain family member 2 (ACSS2) was found to promote the crotonylation at H3K4 and H3K18 by increasing the cellular levels of crotonyl-CoA [12, 15]. Chromodomain Y-like (CDYL), the chromodomain Y-like transcription corepressor, acts as a crotonyl-CoA hydratase and inhibits histone crotonylation at H2BK12, H3K9, H3K27, and H4K8 through its chromodomain and CoAP domains [10].

### The regulatory role of histone crotonylation in gene expression

Like other histone modifications, the main function of histone crotonylation is to regulate gene expression. In this section, we summarized and discussed the regulatory role of histone crotonylation in gene expression (Table 2).

**Transcriptional promotion**

The first evidence of histone Kcr regulating gene expression was found in male germinal cells immediately following meiosis. Kcr is enriched on sex chromosomes and specifically marks testis-specific genes, including a significant proportion of X-linked genes that escape sex chromosome inactivation in haploid cells [1, 34]. Then, an investigation to identify “erasers” of histone Kcr found that Sirt3 decreased the expression levels of Ptk2, Tshz3, and Wapal, as well as the enrichment of crotonylated histone at the transcription start sites of these target genes, suggesting that histone crotonylation might function as a positive regulator of the expression of these genes [19].

| Histone crotonylation | Target | Regulatory role | References |
|-----------------------|--------|----------------|------------|
| Histone Kcr           | PGC-1α and sirtuin-3 | Promoting transcription | [11] |
|                       | Ptk2, Tshz3, Wapal | Promoting transcription | [19] |
| H2BK12Cr              | Pin4, Ccdc160, Tceal, Rnf138rt1, and 4933436I01Rik | Promoting transcription | [10] |
| H3K4Cr                | HIV LTR | Promoting transcription | [35] |
| H3K9Cr                | Pro-growth genes | Inhibiting transcription | [9] |
| H3K18Cr               | Il6, Gbp2, fIt1, and Rsad2 | Promoting transcription | [15] |
|                       | RANDP3L, AOX1, GRPR, and NCAM1 | Promoting transcription | [20] |
|                       | HIV long-terminal repeat (LTR) | Promoting transcription | [35] |
| H3K27Cr               | Endocytosis-related genes | Inhibiting transcription | [8] |
In addition, during AKI, histone crotonylation in kidney tissue increases, which protects the kidney from AKI by increasing the expression of the mitochondrial biogenesis regulator PGC-1α and the sirtuin-3 decrotonylase by increasing the enrichment of histone crotonylation at these genes [11].

Specifically, several histone Kcr sites have been associated with gene activation, including H3K18Cr for “de novo-activated” genes such as Il6, Gbp2, Ift1, and Rsad2 [15]; H3K18Cr for RANDP3L, AOX1, GRPR, and NCAM1 [20]; H2BK12Cr for post-meiotic genes that play an important role in spermatogenesis [10]; and H3K4Cr and H3K18Cr for the HIV LTR, a key regulator for the establishment of latent reservoirs [35] for the promotion of LTR transcription and the reactivation of HIV from latency [12].

Transcriptional repression
Our previous study investigated the role of NEAT1, a long non-coding RNA that functions as an important regulator of gene expression [36, 37], in the development of AD. We found that NEAT1 knockdown increases global H3K27Cr and decreases global H3K27Ac levels. Further, exogenous addition of crotonic acid decreased the expression levels of endocytosis-related genes, including CAV2, TGFB2, and TGFBR1 through increasing H3K27Cr and decreasing H3K27Ac at these gene promoters [8]. This suggests that H3K27Cr functions as a marker of transcriptional repression of endocytosis-related genes.

In addition, a recent study illustrating the links between metabolic state and gene expression found that H3K9 crotonylation, which peaks at pro-growth genes, results in gene repression, indicating that H3K9 crotonylation is associated with transcriptional repression of pro-growth genes [9].

Conclusion and perspective
Histone crotonylation is a novel histone modification that participates in multiple biological and pathological processes. In this review, we summarized the factors, along with their regulatory patterns, which regulate histone crotonylation and are involved in histone crotonylation-mediated gene regulation. Moreover, crotonyltransferases, decrotonyltransferases, transcription factors, and regulators with unknown mechanisms participate in histone Kcr (Fig. 1). In addition, we discussed the role of histone crotonylation in gene regulation, highlighting that histone crotonylation functions as both an activator and repressor of gene transcription.

Overall, this review provides novel insights into histone crotonylation-centric gene regulation and highlights its potential therapeutic targets for the treatment of human diseases, although the underlying molecular
mechanisms still require further clarification. Specifically, to date, no single active or repressive TSS has been evidenced marked only with crotonylation and different histone acylations, such as crotonylation, acetylation, propionylation and butyrylation, seem to coordinate to regulate gene transcription [1, 38, 39]. Therefore, more approaches are needed in the future to characterize the role of histone crotonylation in gene regulation. Since a number of writers, readers and erasers for crotonylation were identified, there is a high chance that more specific regulators will have a major impact on understanding the interplay between crotonylation and other acylations, and the role of specific histone crotonylation sites in gene transcription.

Abbreviations
Kcr: Lysine crotonylation; TSSs: Transcriptional start sites; AKI: Acute kidney injury; HIV: Human immunodeficiency virus; LTR: Long-terminal repeat; HCC: Hepatocellular carcinoma; HDACs: Histone deacetylases; AD: Alzheimer’s disease; HCT: Histone crotonyltransferase; HATS: Histone acetyltransferases; DPF: Double PHD finger; NFR8: Ring finger protein 8; AC52: Acyl-CoA synthetase short-chain family member 2; CDYL: Chromodomain Y-like.

Acknowledgements
Not applicable.

Author contributions
K.L. prepared the manuscript. Z.W. reviewed and edited the manuscript. Both authors read and approved the final manuscript.

Funding
This work was supported by the National Natural Science Foundation of China (32000878) and the Natural Science Foundation of Shandong Province (ZR2020LZL008).

Availability of data and materials
Not applicable.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Both authors consent to publication.

Competing interests
There are no competing interests.

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Received: 13 December 2020 Accepted: 28 January 2021
Published online: 06 February 2021

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