Crosstalk between epigenetics and metabolism—Yin and Yang of histone demethylases and methyltransferases in cancer

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Abstract

Histone methylation is an epigenetic modification of chromatin undergoing dynamic changes and balancing tissue-specific demands of proliferation and differentiation. In cancer, aberrant histone methylation can facilitate oncogenic and tumor suppression programs by modulating gene expression. Histone remodelers such as lysine methyltransferases and lysine demethylases are seemingly opposite or contrary forces but may be part of an interconnected network complementing each other. We identify several layers of molecular communication where epigenetic master regulators engage in crosstalk between tumor metabolism and histone remodeling. Epigenetic master regulators have the ability to cooperate with members of the transcriptional machinery, DNA methyltransferases, as well as other histone modifiers. High-throughput sequencing and omics data in combination with cancer systems biology analysis have the power to prioritize regulatory events epigenome-wide.

Key words: cancer systems biology; melanoma; cancer metabolism; epigenomics; epigenetics; epigenetic drug; precision medicine; histone demethylase; histone methyltransferase; driver; master regulator; oncometabolite; oncogene; tumor suppressors; KDM; KMT; EZH2; JMJD; jumonji

Introduction

One of the great mysteries of biology is how the human genome is programmed in each cell to activate only the relevant genes. Eukaryotic chromatin is structurally organized in nucleosome particles, which are composed of a histone octamer core around which 147 base pairs of DNA are wrapped in 1.65 turns of a tight superhelix [1, 2]. Chromatin is not an inert structure, but rather an instructive DNA scaffold that can respond to external cues to regulate the many uses of DNA. The methylation of histone proteins at specific residues plays a major role in the maintenance of active and silent states of gene expression in developmental processes and disease (Figure 1). Pioneering experiments by Allfrey and Mirsky >50 years ago identified methylation and acetylation of histones by isotope incorporation and showed that histone modification can influence whether RNA synthesis of genes is switched on or off [3].

Histone methylation mainly occurs on the side chains of solvent-accessible lysines and arginines of termini of histone H3 and H4. Unlike acetylation and phosphorylation, however, histone methylation does not alter the charge of the histone protein but its hydrophobicity. Hydrophobic CH3- groups may be sequentially added and result in mono-, di- or trimethylated ε-lysine (Figure 1A) or mono- or symmetrically or asymmetrically dimethylated arginine [4]. Such distinct patterns of covalent histone marks introduced the idea of a histone code, a language edited and read by proteins and communicated in addition to the four-letter base code of DNA [5, 6]. Epigenetic control via histone modification is largely operated by lysine methyltransferases (KMTs) [7] and lysine demethylases (KDMs) [8–10], which were...
Epigenetic master regulators use reversible chemical modifications of chromatin, histone or nucleotide marks, and affect gene activity without altering the core DNA sequence. In cancer, such epigenetic master regulators are found at the top of regulatory hierarchies, particularly in pathways related to cellular proliferation, survival, fate and differentiation. For the manifestation of a genomic or non-genomic aberration of an epigenetic master regulator, it is a necessity that its own activity is affected by somatic mutation, copy number alteration, expression levels, protein cofactors or methylation status. Epigenetic master regulators often accomplish target specificity of their phenotypic program by cooperation with members of the transcriptional machinery and therefore may depend on tissue-specific expression of such auxiliary factors. In cancer, an epigenetic master regulator populates an extreme state and is either permanently switched on or off. An epigenetic master regulator will become a cancer driver, if it is not functionally neutral but contributes to tumorigenesis or disease progression in its hyperactive or deactivated state. A defined challenge in the field of epigenetic master regulators is to identify cancer-specific vulnerabilities in gene targets and biological pathways that are frequently and consistently perturbed under the control of an epigenetic driver.

Looking at the distribution of somatic alterations of epigenetic modifiers in cancer, trends of protein up- or down-regulation are not clearly established. The idea that aberrant histone methylation can throw tissue homeostasis off balance is a simple resolution to this conundrum (Figure 2). Comparable with Yin and Yang, where complementary forces keep each other in check, activity of histone modifiers has to be maintained in dynamic steadiness and otherwise result in fatal transcriptional outcomes [10, 17]. On the one hand, hyperactive KMT or inactive KDM can cause repression of tumor suppressor genes owing to accumulation of histone lysinemethylation marks [18]. On the other hand, inactive KMTs or hyperactive KDMs can contribute to transcriptional activation of oncogenes. The idea of balanced epigenetic homeostasis explains why extreme, epigenome-wide histone hypo- or hypermethylation have been observed in cancer (Table 1) [19].

Recent multiomics data have shown that the H3K27-KMT6 family member, EZH2, is hyperactivated in many cancers including prostate cancer, lymphomas and melanomas with poor prognosis owing to immune evasion and repression of tumor suppressors [20, 21]. In synergy with EZH2, the counteracting demethylases KDM6A and KDM6B show frequent loss-of-function mutations as well as gene deletions resulting in accumulation of repressive H3K27 marks [17, 22–24]. KDM3 and KMT1 family members focused on remodeling of H3K9 marks function mutations as well as gene deletions resulting in accumulation of repressive H3K9 marks. KDM3 and KMT1 family members focused on remodeling of H3K9 marks display a great range of dysregulation in cancer. In prostate cancer, sarcoma, lung cancer and melanoma, copy number and transcriptional upregulation of KDM3A take a predominant role as amplifier by transcriptionally activating oncogenic target genes [9, 25]. Other KDM3 family members, KDM4C and KDM4D, are equally up- and downregulated in cancer specimen and may have diverse targets in oncogenic or tumor suppressor functions [10, 26–28]. The H3K9-KMT1 family member SETDB1 has been identified in malignant melanoma to be amplified and to increase the aggressiveness of the disease [29]. SETDB1 forms a multimeric complex with SUV39H1 and other H3K9 methyltransferases to maintain gene silencing of tumor suppressor genes [30]. Taken together, precision medicine in combination with cancer systems biology has the ability to elucidate genome- and epigenome-wide alterations and identifies molecular pathways suitable for rational drug targeting [31].

In addition to being carbon sources for covalent histone modifications, metabolites play key roles as signaling molecules...
Systematic nomenclature of histone lysine (HK) methylation and gene symbols of their modifiers. Predominant structural aberrations of histone methyltransferases and demethylases and deregulation in cancer are classified by somatic mutations (MUT), transcriptional regulation of messenger RNA (mRNA), somatic copy number amplification (AMP) or deletion (DEL) in The Cancer Genome Atlas [23, 31]. Transcriptional upregulation (UP) and downregulation (DOWN) display consistent trends of gene expression in tumor progression from normal to primary tumor to metastatic cancer.

1 Also H3K27 and H3K36 activity reported.
2 Also H3K9 activity reported.
3 Also H3K36 activity reported.
4 Also H3K27 activity reported.

*Significant enrichment with deviation from mean based on 95% confidence interval.
#Enrichment of nonsense mutations indicative of tumor suppressor function.
$Enrichment of hotspot mutations.
ˆEnrichment in some cancer tissues.

| HK     | KMT    | SYMBOL | ALIASES | me₁ | me₂ | me₃ | MUT | mRNA | AMP | DEL | KDM     | KDM     | SYMBOL     | ALIASES | me₁ | me₂ | me₃ | MUT | mRNA | AMP | DEL |
|--------|--------|--------|---------|-----|-----|-----|-----|------|-----|-----|---------|---------|------------|---------|-----|-----|-----|-----|------|-----|-----|
in the regulation of epigenetics (Figure 3). KDMs are tightly linked to tricarboxylic acid cycle metabolites by their cofactor requirements and ability to bind organic ketoacids. Demethylation by jumonji-domain containing dioxygenase enzymes requires oxygen and 2-oxoglutarate (2OG) as substrates; complexed iron(II) and ascorbate as enzyme-cofactors; and generates formaldehyde and succinate as products. Following the oxidative decarboxylation of 2OG to succinate, a highly reactive ferryloxo species is generated that hydroxylates the methyllysine protein substrate. Because the intermediate, carbinalamine, is unstable, subsequent release of formaldehyde from the reaction intermediate produces demethylated lysine. The metabolic requirements for the 2OG-dependent dioxygenase cycle provide clues as to the physiological conditions that might affect KDM activity. Another complication arises in cancer cells where somatic active site mutations cause isocitrate dehydrogenase to increase an enzymatic sleeper-activity and to produce predominantly the oncometabolite 2-hydroxyglutarate (2HG) [32, 33]. Numerous organic acids, in particular 2-oxoketoacids including 2HG, pyruvate, oxaloacetate, malate, fumarate or succinate, have the ability to populate the enzymatic pocket of KDMs and act as competitive inhibitors (Figure 3A). Several metabolic conditions such as mitochondrial dysfunction, nutrient limitation, oxidative stress or hypoxia typical for cancer cells can modulate the activity of KDMs [34, 35]. Metabolic regulation of histone methylation is induced by low 2OG as a result of glutamine depletion, ascorbate or iron deprivation, high succinate, high fumarate or high 2HG, and potentially leads to globally altered histone lysine methylation. Importantly, such direct metabolic regulation impacts a wide range of effectors and is not restricted to a specific transcriptional program or a distinct histone lysine demethylation activity. Enzymes involved in cytosine demethylation or synthesis of signaling transmitters, carnitine and collagen have protein domains similar to jumonji dioxygenases and will be responsive to the same metabolic cues. Active KDMs have numerous metabolic enzymes as part of their target gene repertoire shown by their binding and demethylase activity in matched ChIP-Seq assays [25]. Activation of metabolic pathways supporting glycolysis, amino acid, organic acid and lipid synthesis ultimately leads to proliferative advantages of cancer cells (Figure 3B). Further, the ability of KMTs and KDMs to cooperate with the transcriptional machinery can amplify or mute specific transcriptional responses (Figure 3C). In the case of nuclear hormone receptors, targets of transcriptional cooperation include steroid biosynthesis as well as lipogenesis and enforces therapy resistance and steroid-dependent signaling in adenocarcinomas [25, 36]. However, an unambiguous mode of interaction is not identified and ranges from coexpression, protein contacts, to synergy in local chromatin environment, or even posttranslational modification. Indeed, non-histone protein modification by histone demethylases adds complexity to the regulatory ability of demethylases. Protein demethylation of TP53 suppressed proapoptotic functions by inhibiting its transcriptional activity [37–39]. Presence of regulatory POLYCOMB, COMPASS or COREST protein complexes can control, stabilize or shift the epigenetic balance [40]. Interestingly, KDMs cooperate with a network of transcription factors rather than an isolated partner, while maintaining and accomplishing gene target and DNA sequence specificity. Prominent players in cooperation with the epigenomic landscape of histone lysine methylation are MYC oncogene, androgen receptor, estrogen receptor, TP53 tumor suppressor protein, and hypoxia inducible factors, HIFs. HIF-hydroxylases are closely related to KDM dioxygenases and responsive to changes in physiological oxygen concentration. Even though these hydroxylases are cellular oxygen sensors, it is unknown whether 2OG-dependent dioxygenases like KDMs are similarly sensitive. KDMs are operated downstream of HIFs and direct gene targets of the transcription factors [41]. Such a synergistic relationship is counterintuitive, as hypoxia would expect to abolish KDM function. However, whether the KDM dioxygenase cycle is blocked in hypoxia is yet to be shown.
Although cancer and stem cells can be challenged by enzyme inhibition [42–46], mechanistic details of factors and 2-oxoacid metabolites that are relevant under physiological conditions in the development of disease are yet to be defined. With both fields maturing, it is evident that further synergy between epigenetic and metabolomics will deliver new therapeutic agents as well as fundamental insights into how cellular chemistry regulates gene expression essential for tissue homeostasis.

**Key Points**

- Histone methylation is part of the central histone code. Histone remodelers such as lysine methyltransferases (KMTs) and lysine demethylases (KDMs) are complementary but interconnected forces in the network of different histone-modifying enzymes that write, erase and read epigenetic marks. Histone methylation of distinct epigenetic marks leads to condensed, transcriptionally silent heterochromatin, whereas distinct loss of epigenetic marks by histone demethylation causes local formation of transcriptionally active euchromatin.

- Epigenetic master regulators are at the top of cellular hierarchies and control a distinct phenotypic program via chromatin modification without altering the core DNA sequence. Epigenetic oncogenes or tumor suppressors can arise when an epigenetic master regulator is somatically activated or lost and contributes to cancer initiation and progression.

- Deregluation of KMTs and KDMs turns epigenetic master regulators into cancer drivers (Table 1): histone hypomethylation can cause transcriptional activation of oncogenes; histone hypermethylation can cause transcriptional repression of tumor suppressor genes.

- Histone methylation and DNA methylation are tightly linked and rely mechanistically on each other. Lysine methylation initiates, targets or maintains DNA methylation, and vice versa. In addition, there is a strong cooperation of epigenetic factors with the transcriptional complex. Cooperation with transcription factors or other members of the epigenetic machinery can target, amplify or mute specific transcriptional responses.

- High-throughput technology in combination with multiomics systems biology is necessary to decipher the dynamic interplay between epigenomics and functional output in biological and biomedical settings. In particular, a solid bridge between next-generation sequencing platforms such as metabolomics has not yet been established and remains a future challenge.

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Parents rarely let go of their children, so children let go of them. They move on; they move away. The moments that used to define them—a mother’s approval, a father’s nod—are covered by moments of their own accomplishments. It is not until much later, as the skin wrinkles and the bones weaken, that children understand; their stories, and all their accomplishments, sit atop the stories of their parents, stones upon stones, beneath the waters of their lives. Dedicated to Leland Volker, Franziska Violet, Lennart Curt, Frederic Carl Wolf, and Annabelle Clara Nia, beloved grandchildren of Ingrid Liselotte and Volker Eduard Filipp.

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