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Chapter Title	Metabolic Deregulations Affecting Chromatin Architecture: One-Carbon Metabolism and Krebs Cycle Impact Histone Methylation	
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Abstract	The methylation status of a particular amino acid results from the interplay of two enzymes: "Writers" (methyltransferases) and "Erasers" (demethylases). Methylation of histones in chromatin can be recognized by "Readers" which induce changes in chromatin organization and gene expression, directed by the methylation status. Importantly, the reactions of methylation and demethylation involve several metabolites. Some, such as folate and *S*-adenosyl-L-methionine, act as cofactors for methyltransferases while flavin adenine dinucleotide and α-ketoglutarate act as cofactors for demethylases. Other metabolites, such as succinate and fumarate, function as enzyme inhibitors. Factors that modulate the levels of these metabolites in the cell therefore affect the dynamics of protein methylation. These factors can include diet, as well as altered expression of enzymes involved in cofactor synthesis through mutations and/or post-translational modifications. For example, methionine is a substrate for *S*-adenosyl-L-methionine formation, and reduction in its abundance ultimately induces a global reduction in histone methylation in vitro, affecting gene expression. Changes in the metabolic states of cells in diseases such as cancer, and regulation of metabolites required for histone methylation and demethylation, have thus been highlighted as avenues for therapeutic development. In this review, we evaluate the current knowledge concerning methylation of histones, and also of other protein substrates. We document how this is linked to metabolites such as *S*-adenosyl-L-methionine and other intermediates in the Krebs cycle. Finally, we discuss the implications of deregulation at this level in cancer.

Keywords (separated by '-')	Histone methylation - One carbon metabolism - *S*-adenosyl-L-methionine - Methyltransferases - Demethylases

Metabolic Deregulations Affecting Chromatin Architecture: One-Carbon Metabolism and Krebs Cycle Impact Histone Methylation

Francisco Saavedra, Ekaterina Boyarchuk, Francisca Alvarez, Geneviève Almouzni, and Alejandra Loyola

Contents

Abstract The methylation status of a particular amino acid results from the interplay of two enzymes: "Writers" (methyltransferases) and "Erasers" (demethylases). Methylation of histones in chromatin can be recognized by "Readers" which induce

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Keywords Histone methylation · One carbon metabolism · *S*-adenosyl-L-methionine · Methyltransferases · Demethylases

1 Chromatin Methylation: Function and Regulation

1.1 Basic Concepts of Chromatin Structure

The genetic instructions of cells are carried on DNA molecules which encode information relating to the basic processes required for normal cellular function, such as replication, transcription, and DNA repair. In every human cell, a nucleus of around 2 μm diameter contains around 2 m of DNA packaged in a structure called chromatin—a nucleoprotein complex comprising DNA, RNA, and proteins, organized in several hierarchical levels. Correct and dynamic organization of chromatin is necessary for accurate genome functioning. The basic unit of chromatin is the nucleosome, which comprises an octamer with two copies each of the core histones H2A, H2B, H3, and H4, around which is wrapped 147 bp of DNA and a variable linker DNA segment associated with the H1 linker histone (Luger et al. 1997). Additional chromatin-binding proteins including transcription factors and structural RNAs cause the chromatin filaments to fold further, resulting in highly compact DNA. Modulation at each level of chromatin organization ensures that adaptation to environmental cues can occur (Sitbon et al. 2017; Hug and Vaquerizas 2018; Luo et al. 2018; Yadav et al. 2018). Chemical modifications of

the histones, termed post-translational modifications (PTM), or onto DNA are major mechanisms of chromatin alteration (Gurard-Levin and Almouzni 2014; Jones 2012). In addition, the properties of nucleosomes can be further modulated by the inclusion of histone variants, which can confer particular properties to chromatin (Sitbon et al. 2017). The expression of these variants differs depending on the cell cycle phase, tissue in which they are expressed, and the mode of their incorporation into the chromatin (Mendiratta et al. 2018). Together, these features are critical for proper chromatin functioning in various processes such as development, aging, or tumorigenesis.

1.1.1 Post-Translational Modifications, the Histone Code, and the "Writer-Eraser-Reader" Model

Post-translational modifications can occur throughout the entire lifespan of a protein, from synthesis to degradation (Loyola and Almouzni 2007; Alvarez et al. 2011; Rivera et al. 2015). Methylation of lysine residues in calf thymus was the first histone PTM to be identified (Murray 1964), reported before the discovery of histone acetylation (Allfrey et al. 1964) or phosphorylation (Gutierrez and Hnilica 1967). Since then, over 15 different types of PTMs have been identified on histones (Zhao and Garcia 2015). These modifications provide a stable but reversible system with which the cell can react to external stimuli (Gurard-Levin and Almouzni 2014). Particular PTMs, such as phosphorylation or acetylation, can alter the physical properties of the nucleosome including charge, thereby affecting histone-DNA interactions (Bowman and Poirier 2015).

The most common mechanism of action of histone PTMs is the modulation of protein binding through the recruitment of non-histone proteins, which can consequently modify the chromatin state. The density of a particular PTM at a given chromatin locus can be critical, because a single mark on one histone is unlikely to have significant effects. Rather, it is likely that a certain level of modified histones exists, above which significant effects will be observed with regards to chromatin.

Multiple types of modification can occur at particular residues. For instance, lysines can be methylated, sumoylated, ubiquitinated, or acetylated in an exclusive manner. The large number of possible combinations gave rise to the hypothesis of the "histone code", whereby histone modifications work sequentially or in combination to affect gene regulation (Jenuwein and Allis 2001). Many enzymes have been identified to be involved in catalyzing the chemical modification of histones ("Writers") or removing such modifications ("Erasers") (Kouzarides 2007). Effector proteins ("Readers") recognize and bind to histones or DNA that carry certain chemical modifications, in order to achieve a specific chromatin state at a given locus (Nicholson et al. 2015). Whilst PTMs are generally considered important for the recruitment of proteins, they can also inhibit histone-protein interactions (Wen et al. 2014).

1.1.2 Histone Methylation: Effects, Localization, and "Readers"

Of all histone PTMs, methylation has been identified as one of the key modifications in the regulation of gene expression. Methylation predominantly occurs on lysine and arginine residues, but has also be detected on histidine, aspartic, and glutamic acid residues (Zhao and Garcia 2015).

Specific methylation of histone lysine residues has enabled the correlation of methylation at a given locus with its transcriptional activity to be analyzed. Depending on the particular lysine residue, its degree of methylation (mono- [Kme1], di- [Kme2], or trimethylation [Kme3]), and the position of the methylated nucleosome within the gene and genome, this modification can be associated with transcriptionally active or inactive chromatin (Table 1). In general, methylation of the histone H3 lysine 4 (H3K4), H3K36, and H3K79 have been linked to activation of gene expression; whereas di- and trimethylation of H3K9, H3K27, and H4K20 have been associated with gene silencing and/or heterochromatin formation (Mozzetta et al. 2015). In addition, methylation of histone lysine residues has been associated with the regulation of splicing (Luco et al. 2010). For instance, H3K36me3 is present on highly transcribed exons, and is more enriched on constitutive exons compared with alternatively spliced ones (Kolasinska-Zwierz et al. 2009). Moreover, local increases in H3K9me2 and H3K9me3 enhance exon inclusion, whereas H3K9 demethylation is associated with exon skipping (Bieberstein et al. 2016).

These diverse effects require a series of "Readers" that possess methyl-lysine recognition domains. Methylation does not significantly affect the charge of the histone; instead it frequently functions to provide a docking site for Reader proteins. The Reader can then serve as a platform to recruit other effector proteins and form multiprotein complexes to direct either transcriptional activation or repression. Methyl-lysine recognition domains can be divided into four classes: ankyrin repeats, tryptophan-aspartic acid (WD40) repeat domains, plant homeodomain (PHD) fingers, and Royal family proteins. Royal family proteins are classified based on the presence of the conserved barrel-like protein fold called the "Tudor barrel". This superfamily includes the Tudor domain, chromodomain, malignant brain tumor (MBT) domain, chromo barrel domain, and proline-tryptophan-tryptophan-proline (PWWP) domain families (Teske and Hadden 2017). Each of them exhibit specific binding features which are related to the methylation status of the residue, and whether the modification occurs cis or trans (Teske and Hadden 2017).

The principle of the mechanism behind the modulation of chromatin structure and regulation of transcription by PTMs and associated Readers can be illustrated by H3K9 methylation in heterochromatin. Heterochromatin-meditated gene silencing is thought to result from changes in the packing of nucleosomes to create a dense, compact structure, which prevents transcriptional machinery from accessing the DNA or establishing the modifications that recruit transcriptional activators. At heterochromatin sites that are enriched for H3K9me2/3, direct binding of HP1 via its chromodomain can promote chromatin compaction or phase transition by dimerization or oligomerization of HP1 (Canzio et al. 2011; Machida et al. 2018). This

Table 1 Histone lysine methylation in mammals

Histone	Lysine and degree of methylation	Writer	Eraser	Function
H3	K4me1	KMT7 (SET7)	KDM1A (LSD1)	Transcription activation
		KMT2A (MLL2)	KDM1B (LSD2)	Enhancer function
		KMT2B (MLL3)	KDM5B (JARID1B)	
		KMT2C (MLL4)		
		KMT2D (MLL5)		
		KMT2F (SET1A)		
		KMT2G (SET1B)		
	K4me2	KMT2A (MLL2)	KDM1A (LSD1)	Transcription activation
		KMT2B (MLL3)	KDM1B (LSD2)	Enhancer function
		KMT2C (MLL4)	KDM5A (JARID1A)	
		KMT2D (MLL5)	KDM5B (JARID1B)	
		KMT2F (SET1A)	KDM5C (JARID1C)	
		KMT2G (SET1B)	KDM5D (JARID1D)	
		KMT3E (SMYD3)	ROIX1 (NO66)	
	K4me3	KMT2A (MLL2)	KDM2B (JHDM1B)	Transcription activation
		KMT2B (MLL3)	KDM5B (JARID1B)	Enhancer function
		KMT2C (MLL4)	KDM5C (JARID1C)	
		KMT2D (MLL5)	KDM5D (JARID1D)	
		KMT2F (SET1A)	ROIX1 (NO66)	
		KMT2G (SET1B)		
		KMT3E (SMYD3)		
	K9me1	KMT1E (SETDB1)	KDM1A (LSD1)	Transcription repression
		KMT1C (G9A)	KMT3A (JLHDM2A)	

(continued)

t1.27 **Table 1** (continued)

t1.28 Histone	Lysine and degree of methylation	Writer	Eraser	Function
t1.26		KMT1D (GLP)	KMT3B (JLHDM2B)	
t1.27		KMT8E (PRDM3)	KDM3C (JMJD1C)	
t1.28		KMT8D (PRDM8)	KDM7A (JHDM1D)	
t1.29		KMT8F (PRDM16)	KDM7B (JHDM1F)	
t1.30		KMT2H (ASH1)	KDM7C (JHDM1E)	
t1.31		KMT3F (NSD3)	HR (HAIR, hairless)	
t1.32	K9me2	KMT1A/B (SUV39H1/2)	KDM1A (LSD1)	Transcriptional repression
t1.33		KMT1E (SETDB1)	KMT3A (JLHDM2a)	Heterochromatin formation
t1.34		KMT1C (G9A)	KMD4A (JMJD2A)	
t1.35		KMT1D (GLP)	KMD4B (JMJD2B)	
t1.36		KMT8A (PRDM2)	KMD4C (JMJD2C)	
t1.37		KMT8D (PRDM8)	KMD4D (JMJD2D)	
t1.38		KMT2H (ASH1L)	KDM7A (JHDM1D)	
t1.39		KMT3F (NSD3)	KDM7B (JHDM1F)	
t1.40			KDM7C (JHDM1E)	
t1.41			HR (HAIR, hairless)	
t1.42	K9me3	KMT1A/B (SUV39H1/2)	KMD4A (JMJD2A)	Transcriptional repression
t1.43		KMT1E (SETDB1)	KMD4B (JMJD2B)	Constitutive heterochromatin formation
t1.44		KMT1F (SETDB2)	KMD4C (JMJD2C)	X-chromosome inactivation
t1.45		KMT8A (PRDM2)	KMD4D (JMJD2D)	
t1.46		KMT2H (ASH1L)	RIOX2 (MINA)	
t1.47		KMT3F (NSD3)		
t1.48	K27me1	KMT6B (EZH1)	KDM7A (JHDM1D)	Transcriptional repression
t1.49		KMT1C (G9A)	KDM7C (JHDM1E)	

(continued)

Table 1 (continued)

Histone	Lysine and degree of methylation	Writer	Eraser	Function
		KMT1D (GLP)		
		KMT2H (ASH1L)		
		KMT3G (NSD2)		
		KMT3F (NSD3)		
	K27me2	KMT6A (EZH2)	KDM6A (UTX)	Transcriptional repression
		KMT6B (EZH1)	KDM6B (JMJD3)	Facultative heterochromatin formation
		KMT2H (ASH1L)	KDM7A (JHDM1D)	X-chromosome inactivation
		KMT3G (NSD2)	KDM7C (JHDM1E)	
		KMT3F (NSD3)		
	K27me3	KMT6A (EZH2)	KDM6A (UTX)	Transcriptional repression
		KMT6B (EZH1)	KDM6B (JMJD3)	Facultative heterochromatin formation
		KMT2H (ASH1L)		X-chromosome inactivation
		KMT3G (NSD2)		
		KMT3F (NSD3)		
	K36me1	KMT3A (SET2)	KDM2A (JHDM1a)	Transcription activation
		KMT3B (NSD1)	KDM2B (JHDM1b)	
		KMT3G (NSD2)		
		KMT3F (NSD3)		
		KMT2H (ASH1)		
	K36me2	KMT3A (SETD2)	KDM2A (JHDM1a)	Transcriptional activation
		KMT3B (NSD1)	KDM2B (JHDM1b)	Transcription elongation
		KMT3G (NSD2)	KMD4A (JMJD2A)	
		KMT3F (NSD3)	KMD4B (JMJD2B)	
		KMT2H (ASH1L)	KMD4C (JMJD2C)	
			KMD4E (JMJD2E)	
			KMD8 (JMJD5)	
			RIOX1 (NO66)	

(continued)

t1.78 **Table 1** (continued)

t1.79 Histone	Lysine and degree of methylation	Writer	Eraser	Function
	K36me3	KMT3A (SETD2)	KMD4A (JMJD2A)	Transcriptional activation
t1.77				
t1.78		KMT2H (ASH1L)	KMD4B (JMJD2B)	Transcription elongation
t1.79		KMT3C (SMYD2)	KMD4C (JMJD2C)	
t1.80			KMD4E (JMJD2E)	
t1.81			RIOX1 (NO66)	
t1.82	K56me1	KMT1C (G9A)	KMD4B (JMJD2B)	DNA replication
			KMD4E (JMJD2E)	Heterochromatin formation
t1.83				
t1.84	K56me3	KMT1A/B (SUV39H1/2)	KMD4B (JMJD2B)	DNA replication
			KMD4E (JMJD2E)	Heterochromatin formation
t1.85				
	K64me	Unknown	Unknown	Heterochromatin formation
t1.86				
	K79me1	KMT4 (DOT1L)	KDM2B (JHDM1b)	Transcriptional activation
t1.87				
t1.88				Telomeric silencing
t1.89				DNA damage response
	K79me2	KMT4 (DOT1L)	KDM2B (JHDM1b)	Transcriptional activation
t1.90				
t1.91				Telomeric silencing
t1.92				DNA damage response
	K79me3	KMT4 (DOT1L)	KDM2B (JHDM1b)	Transcriptional activation
t1.93				
t1.94				Telomeric silencing
t1.95				DNA damage response
H4	K5me1	KMT3E (SMYD3)	Unknown	Contributes to cancer phenotype
t1.96				
	K20me1	KMT5A (PR-SET7)	KDM7A (JHDM1D)	Transcriptional silencing
t1.97				
t1.98		KMT3B (NSD1)	KDM7B (JHDM1F)	Mitotic condensation
t1.99		KMT3G (NSD2)		
t1.100	K20me2	KMT5B/C (SUV4-20H1/2)	KDM7C (JHDM1E)	Transcription repression
		KMT3B (NSD1)		Heterochromatin formation/silencing
t1.101				
t1.102		KMT3G (NSD2)		DNA damage responses

(continued)

Table 1 (continued) t1.104

Histone	Lysine and degree of methylation	Writer	Eraser	Function	
	K20me3	KMT5B/C (SUV4-20H1/2)	KDM7C (JHDM1E)	Transcription repression	t1.103
		SMYD5		Heterochromatin formation/silencing	t1.104
				DNA damage response	t1.105
H1	K26me*2/3*	KMT1C (G9A)	KMD4A (JMJD2A)	Heterochromatin formation/silencing	t1.106
		KMT1D (GLP)	KMD4B (JMJD2B)		t1.107
		KMT6A (EZH2)	KMD4C (JMJD2C)		t1.108
H2A.Z	K7me1	SETD6	Unknown	Transcription repression	t1.109

In the table, only methylations with a documented biological outcome and/or modifier are listed. t1.110
Modified from Alli et al. (2007), Greer and Shi (2012), Wagner and Carpenter (2012), Mozzetta et al. (2015), Zhao and Garcia (2015), Park et al. (2016) AU4

dimerization/oligomerization bridges neighboring nucleosomes that carry H3K9me2/3. In regions of constitutive heterochromatin, HP1 recruits diverse sets of regulators including chromatin modifiers, DNA replication and repair factors, and nuclear structural proteins as well as RNA (Kwon and Workman 2008). These regulators act in combination to mediate the establishment and maintenance of heterochromatin (Probst and Almouzni 2011; Rivera et al. 2014).

Arginine methylation can occur in three different forms: modification of one of the ω-nitrogens to produce monomethyl arginine (MMA, Rme), addition of two methyl groups onto the same ω-nitrogen to produce asymmetric dimethyl arginine (ADMA, Rme2a); or addition of one methyl group to each ω-nitrogens to produce symmetric dimethyl arginine (SDMA, Rme2s). Such modifications do not change the positive charge of arginine, but can affect its involvement in protein-protein interactions. As is the case for lysine methylation, the outcome of arginine methylation depends on the particular residue, the degree of methylation, and the symmetry of the modification. The most well-characterized methylated arginine residues include R2, R8, R17, and R26 of histone H3; and R3 of histones H4 and H2A. Key transcriptional activation marks involving arginine methylation include H4R3me2a, H3R2me2s, H3R17me2a, and H3R26me2a; while H3R2me2a, H3R8me2a, H3R8me2s, and H4R3me2s are associated with transcriptional repression (Blanc and Richard 2017) (Table 2).

Arginine methylation affects protein function via at least two different mechanisms. First, methylation can directly alter the ability of arginine to form bonds with hydrogen-bond acceptors by introducing steric constraints. It is noteworthy that unmodified arginine has five potential hydrogen-bond donors. The modification H4R3me2a, for example, prevents recruitment of lysine methyltransferase MLL4, and therefore impairs H3K4 methylation and transcriptional activation (Dhar et al.

t2.1 **Table 2** Histone arginine methylation in mammals

t2.2 Histone	Arginine and degree of methylation	Writer	Function
t2.3 H3	R2me2a	PRMT6	Transcription repression
t2.4	R2me2s	PRMT5 PRMT7	Transcription activation
t2.5	R8me2a	PRMT2	Transcription repression
t2.6	R8me2s	PRMT5	Transcription repression
t2.7	R17me2a	PRMT4 (CARM1)	Transcription activation
t2.8	R26me2a	PRMT4 (CARM1)	Transcription activation
t2.9	R43me2a	PRMT4 (CARM1) PRMT6	Transcription activation
t2.10 H4	R3me2a	PRMT1 PRMT6	Transcription activation
t2.11	R3me2s	PRMT5 PRMT7	Transcription repression
t2.12	R17me1	PRMT7	In vitro substrate
t2.13	R19me1	PRMT7	In vitro substrate
t2.14 H2A	R3me2a	PRMT1 PRMT6	Transcription activation
t2.15	R3me2s	PRMT5 PRMT7	Transcription repression
t2.16	R29me2a	PRMT6	Transcription repression
t2.17 H2B	R29me1	PRMT7	In vitro substrate
t2.18	R31me1	PRMT7	In vitro substrate
t2.19	R33me1	PRMT7	In vitro substrate

t2.20 Modified from Di Lorenzo and Bedford (2011), Greer and Shi (2012), Alam et al. (2015), Jahan and Davie (2015), Zhao and Garcia (2015)

170 2012). This is the mechanism behind H3R2me2a-dependent transcriptional repres-
171 sion, which counteracts H3K4 methylation by inhibiting the binding of the H3K4
172 methyltransferase MLL1 and several other H3K4me3 effectors (Hyllus et al. 2007).
173 Interestingly, the opposite is true for symmetrically methylated H3R2, which
174 enhances the binding of H3K4me3 Readers. For example, the RAG2 PHD domain
175 preferentially binds to the H3R2me2sK4me3 modifications, with a 20-fold increased
176 affinity compared to H3K4me3 (Yuan et al. 2012). The second mechanism of action
177 relies on the ability of Tudor domain family proteins to "read" methylated arginine
178 residues and subsequently recruit chromatin modifiers to these residues. Individual
179 PHD and WD40 domains are also able to bind methylated arginines (Gayatri and
180 Bedford 2014).
181 Interestingly, the majority of methylarginine Readers that have been characterized
182 to date recognize the methylation of non-histone proteins (see below). One of the
183 factors that is recruited by methylated H4R3 is Staphylococcal nuclease domain-
184 containing protein 1 (SND1), also known as Tudor domain-containing protein
185 11 (TDRD11), which acts as a transcriptional coactivator by recruiting histone
186 acetyltransferases, thereby promoting histone acetylation (Gayatri and Bedford 2014).

1.1.3 Histone Methyltransferases: Classification and Recruitment of "Writers"

The human genome encodes around 60 methyltransferases, comprising both SET-domain lysine methyltransferases (KMTs) and seven-beta-strand enzymes that methylate different residues (Clarke 2013).

In mammals, all of the KMTs identified to date are highly specific toward a particular lysine residue within a histone, but also toward a number of non-histone substrates (see below). All KMTs apart from DOT1L belong to a large protein family characterized by the presence of the conserved SET domain, whose name was coined based on the three *Drosophila melanogaster* proteins that were first identified: *S*uppressor of variegation 3–9 (Su(var)3–9), *E*nhancer of zeste (E(z)), and the homeobox gene regulator *T*rithorax (Trx) (Jenuwein et al. 1998). The SET domain catalyzes the transfer of a methyl group to the ε-amino groups of lysine residues using *S*-adenosyl-L-methionine (SAM) as the methyl group donor. Based on the sequence homology within and around the catalytic SET domain, SET-containing KMTs can be divided into six sub-families: SET1, SET2, SUV39, EZH, SMYD, and PRDM (Volkel and Angrand 2007). The features of the SET domain of a protein often reflect its substrate specificity (Herz et al. 2013). The majority of SET-containing KMTs have at least one additional module, which confers the ability to recognize various PTMs, usually including the modification that they catalyze. The coupling of "writing" and "reading" properties provides a mechanism for the nucleation and spreading of lysine methylation along the chromatin. In contrast to SET domain-containing methyltransferases, disruptor of telomeric silencing-like protein (DOT1L, also known as KMT4) contains a domain similar to that of glycine *N*-methylase (Nguyen and Zhang 2011), and mono-, di-, or trimethylates H3K79 in a non-processive manner (Frederiks et al. 2008).

One of the most critical—and debatable—aspects of KMTs functions as regulators is their capacity to target a particular genomic locus. No KMT aside from PRDM family members possess DNA-binding properties, and so they rely on protein-binding partners and other mechanisms to target chromatin. Generally, methyltransferases are recruited to their genomic target loci through interaction with sequence-specific transcription factors, other chromatin-binding proteins, and non-coding RNAs, and thus methylate nucleosomal histones (Mozzetta et al. 2015). However, some methyltransferases are involved in the methylation of non-nucleosomal histones, such as SETDB1 (a member of SUV39 family), which binds ribosomes and monomethylates H3K9 co-translationally (Rivera et al. 2015).

Arginine methylation is catalyzed by a family of enzymes called protein arginine methyltransferases (PRMTs) that belong to the seven-beta-strand group of methyltransferases. PRMTs are generally classified by activity as type I, II, or III. Types I and II catalyze the formation of a mono-methylarginine intermediate, which then gives rise to an asymmetric dimethylarginine in the case of type I PRMTs (PRMT1, 2, 3, 4, 6, and 8), or to a symmetric dimethylarginine in the case of type II PRMTs (PRMT5 and 9). The only known type III PRMT is PRMT7, which exclusively generates mono-methylarginine residues (Morales et al. 2016). A fourth group of arginine methyltransferases, type IV, catalyze the monomethylation of the

internal guanidino nitrogen (δ-MMA) of arginine residues. These enzymes have been identified in yeast, but no mammalian homologs have been identified. Nevertheless, such modifications have been recently described in humans (Martens-Lobenhoffer et al. 2016).

1.1.4 Histone Demethylases: Classification and Activities of "Erasers"

For about 40 years, histone lysine methylation was considered to be a modification that could not be actively removed, until the discovery of the first histone lysine demethylase (KDM), denoted lysine-specific demethylase 1 (LSD1) (Shi et al. 2004). Other lysine demethylases have since been identified, and there are only a few lysine residues that are not associated with a demethylase (Black et al. 2012). Demethylases can be grouped in two families: LSDs and Jumonji C (JmjC) domain-containing families.

The LSD family consists of two members, LSD1/KDM1A and LSD2/KDM1B, each characterized by the presence of a C-terminal amine oxidase domain (AOD). This domain confers demethylase activity through a flavin adenine dinucleotide (FAD)-dependent amine oxidation mechanism, and a substrate specificity that is limited to mono- and dimethylated lysines (Shi et al. 2007). Via this domain, LSD1 can demethylate mono- and dimethylated H3K4 and H3K9 residues and is thus considered a corepressor or coactivator, respectively (Shi et al. 2004; Metzger et al. 2005). On the other hand, LSD2 can only demethylate mono- and dimethyl marks on H3K4, and is therefore considered a transcriptional corepressor (Fang et al. 2010).

The JmjC domain-containing family includes more than 30 proteins with different substrate specificities and distinct catalytic mechanisms, which are further divided into several subfamilies (KDM2, KDM3, KDM4, KDM5, KDM6, KDM7, and KDM8) (Allis et al. 2007). The JmjC KDMs are dioxygenases that use iron (Fe (II)) and α-ketoglutarate (2-oxoglutarate or 2-OG) as cofactors (Klose et al. 2006). These enzymes can demethylate all three methylation states of lysine on a range of substrates (Table 1). Currently, the KDM for H3K79me remains enigmatic, but a recent report suggests that KDM2B is capable of catalyzing H3K79me2/me3 demethylation (Kang et al. 2018). As for KMTs, the targeting of JmjC KDMs to their loci relies on two features of the enzymes. First, they are associated with large multimeric complexes, which may guide them to the histones surrounding specific target genes. Second, other conserved domains such as PHD, Tudor, zinc finger (zf-C2HC4), F-box, and AT-rich interactive (ARID) domains, as well as leucine-rich regions (LRR), participate in the targeting of JmjC KDMs to specific regions (Klose et al. 2006).

The reversibility of arginine methylation is unclear. Several studies have reported the modulation of methylation of particular arginine residues in a window of minutes following induction of transcription, or within one cell cycle, which strongly supports the existence of an active mechanism for arginine demethylation (Metivier et al. 2003; Le Romancer et al. 2008). To date, only a few proteins with potential arginine-demethylating activity have been identified. These include the JmjC protein

6 (JMJD6) (Chang et al. 2007), peptidylarginine deiminase 4 (PAD4) (Wang et al., 2004) and the JmjC protein 1B (JMJD1B) (Li et al. 2018). Notably, JMJD6 also possesses lysine hydroxylase activity, and PAD4 cannot be considered a classical demethylase because it cannot demethylate dimethylated arginines. Notably, although a subset of JmjC KDMs (KDM3A, KDM4E, and KDM5C) are able to demethylate arginine residues in vitro, their in vivo activity is yet to be proved (Walport et al. 2016).

1.2 Non-Histone Substrates for HMTs

Methylation is not restricted to histones. Methylated lysine and arginine residues are found in many cellular proteins including those involved in transcription, RNA processing, DNA repair, cell signaling, and translation. The processes involved in the regulation of methylation should therefore be considered beyond the histones. In recent years, advances in liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and the generation of a set of specific antibodies have enabled comprehensive large-scale proteomic analyses of arginine methylation in different organisms (Wesche et al. 2017). Although a lack of good lysine methylation-specific antibodies has limited the proteome-wide analysis of this PTM, promising strategies have been developed for the identification of this modification which involve enrichment of methylated peptides using native methyl-lysine recognition domains (Moore et al. 2013).

Histone methylation modifiers control the methylation state of non-histone substrates, in order to regulate their activities or stabilities. Key components of several signaling pathways are classified as methylated non-histone substrates, including nuclear factor-kappa B (NFκB), estrogen receptor (ERα), β-catenin, and p53 (Alam et al. 2015; Biggar and Li 2015; Mozzetta et al. 2015) (Table 3). Importantly, the impact of non-histone protein methylation depends on the exact residue that is modified and its degree of methylation, similarly to histone methylation. A striking example is the case of p53. For instance, the monomethylation of K372 on p53 (p53K372me1) by SET7/SET9 results in increased stability of the protein, enhanced expression of the p53 target gene *p21*, and increased p53-induced apoptosis (Chuikov et al. 2004); while the SMYD2-mediated monomethylation of p53 at K370 (p53K370me1) functions as an inactivating modification, repressing its activity as a transcriptional regulator (Huang et al. 2006). On the other hand, p53K370me2 enhances the transcriptional activity of p53 by promoting its interaction with p53-binding protein 1 (53BP1), which is a p53 coactivator and a regulator of the DNA-damage response (Tong et al. 2015). The p53K382me1 modification (mediated by SET8) and p53K373me2 (mediated by G9a/GLP) both inhibit p53 function (Shi and Whetstine 2007; Huang et al. 2010). Arginine methylation also has a role in the regulation of p53; PRMT5 methylates R333, R335, and R337 in a DNA-damage dependent manner. These residues are located within the oligomerization

t3.1 **Table 3** Non-histone substrates of Lys- and Arg- methyltransferases categorized by their biological functions

t3.2 Function	Substrate	KMT	Biological outcome
t3.3 Transcription factors	C/EBPb (K39)	KMT1C (G9a)	Inhibits transactivation activity
t3.4	MyoD (K104me1/2)	KMT1C (G9a)	Inhibits transcriptional activity
t3.5	MEF2D (K267 me1/2)	KMT1C (G9a)	Inhibits transcriptional activity
t3.6	p53 (K373me2)	KMT1D (GLP)	Inhibits transcriptional activity and p53-dependent apoptosis
t3.7		KMT1C (G9a)	
t3.8	p53 (K382)	KMT5A (PR-SET7)	Represses transcriptional activity
t3.9	p53 (K370)	KMT3C (SMYD2)	Reduces DNA-binding ability and apoptosis
t3.10	p53 (K372)	KMT7 (SET7/9)	Increases p53 stability and p53-dependent apoptosis
t3.11	p53 (R333/335/337)	PRMT5	Alters recruitment to target genes;
t3.12			Inhibits p53 oligomerization
t3.13	GATA4 (K299me1)	KMT6 (EZH2)	Inhibits transcriptional activity
t3.14	RORα (K38me1)	KMT6 (EZH2)	Enhances proteasomal degradation
t3.15	UBF (K232/K254me3)	KMT1E (SETDB1)	Increases nucleolar chromatin condensation; decreases rDNA transcription
t3.16	TAF10 (K189me1)	KMT7 (SET7/9)	Enhances binding to pol II
t3.17	ERα (K302me1)	KMT7 (SET7/9)	Stabilizes ERα
t3.18			Promotes ERα recruitment and ER-dependent gene activation
t3.19	ERα (K266)	KMT3C (SMYD2)	Inhibits ERα activity
t3.20	ERα (R260)	PRMT1	Promotes interactions with PI3K and Src
t3.21	FOXO3 (K270/271me1)	KMT7 (SET7/9)	Decreases protein stability;
t3.22			Inhibits DNA-binding activity and FOXO3-dependent transcription
t3.23	RUNX1 (R206/210)	PRMT1	Abrogates association with co-repressor SIN3A
t3.24	RB (K810me1, K873me1)	KMT7 (SET7/9)	Promotes interaction with HP1;
t3.25			Promotes Rb-dependent cell cycle arrest and transcriptional repression

(continued)

AU5

Table 3 (continued)

Function	Substrate	KMT	Biological outcome
	RB (K810me1, K860me1)	KMT3C (SMYD2)	Promotes interaction with transcriptional repressor L3MBTL1
	E2F1 (K185me1)	KMT7 (SET7/9)	Stimulates ubiquitination and protein degradation
	E2F1 (R111/R113 m2s)	PRMT5	Promotes protein degradation
			Favours cell proliferation
	E2F1 (R109m2a)	PRMT1	Promotes E2F1-dependen expression of genes connected with apoptosis
Chromatin-modifiers and chromatin-binding proteins	P300 (R2142)	PRMT4	Inhibits interaction with glucocorticoid receptor interacting protein (GRIP1)
	DNMT1 (K142me1)	KMT7 (SET7/9)	Promotes proteasome-mediated degradation
	DNMT3 (K44me2)	KMT1D (GLP)	Stimulates binding of MPP8
		KMT1C (G9a)	
	KMT1D (GLP) (K174)	KMT1D (GLP)	Stimulates binding of MPP8
	KMT1C (G9a) (K165me2/3, K239me3)	KMT1C (G9a)	Stimulates binding of HP1 and CDYL
	KMT1A (SUV39H1) (K105/ K123 me1)	KMT7 (SET7/9)	Inhibits methyltransferase activity
	CBX4/PC2	KMT1A (SUV39H1)	Promotes TUG1 ncRNA-dependent recruitment to Polycomb bodies
	SMARCC1 (R1064m2a)	PRMT4	Modulates targeting to subset of genes of c-Myc pathways
	CDYL (K135me3)	KMT1C (G9a)	Decreases interaction with H3K9me3
	RUVBL2 (K67me1)		Negative regulates hypoxia-inducible genes
		KMT1C (G9a)	
	PCNA (K248me1)	KMT5A (PR-SET7)	Stabilizes PCNA
	PARP1 (K508me1)	KMT7 (SET7/9)	Stimulates PARP activity and its recruitment to sites of DNA damage
Signaling pathway	STAT3 (K180)	KMT6 (EZH2)	Increases STAT3 phosphorylation
			Enhances STAT3 activity
	STAT3 (K140me2)	KMT7 (SET7/9)	Inhibits STAT3 activity

(continued)

t3.49 **Table 3** (continued)

t3.50 Function	Substrate	KMT	Biological outcome
	p65 (K218, K221)	KMT3B (NSD1)	Activates NF-kB signaling pathway
	p65 (K37)	KMT7 (SET7/9)	Activates NF-kB signaling pathway
	p65 (K314, K315)	KMT7 (SET7/9)	Reduces of p65 stability
	p65 (K310)	SETD6	Inhibits p65-driven transcription
	p65 (R30)	PRMT5	Activates NF-kB signaling pathway
	MAP3K2 (K260)	KMT3E (SMYD3)	Activates MAP3K2
	EGFR (R1175)	PRMT5	Negative regulates EGFR signaling
	Axin (R378)	PRMT1	Negative regulates Wnt signaling
RNA binding and processing	SPT5 (R681/696/698)	PRMT1	Inhibits basal transcription;
		PRMT5	Decreases interaction with RNA polymerase II
	LSM4 (80–139, me2s)	PRMT5	Stimulates binding to SMN; promotes formation of spliceosome
	SNRPD1 (90–119, me2s)	PRMT5	Stimulates binding to SMN; promotes formation of spliceosome
	SNRPD3 (110–126, me2s)	PRMT5	Stimulates binding to SMN; promotes formation of spliceosome
	SNRPB (107–210, me2s)	PRMT5	Stimulates binding to SMN; promotes formation of spliceosome
	SNRPB (PGM motifs, me2a)	PRMT4 (CARM1)	Stimulates binding to SMN; splicing regulation
	SNRPC (PGM motifs, me2a)	PRMT4 (CARM1)	Stimulates binding to SMN; splicing regulation
	SF3B4 (190–424, PGM motifs, me2a)	PRMT4 (CARM1)	Stimulates binding to SMN; splicing regulation
	TAF2S (CA150), (1–136, me2a)	PRMT4 (CARM1)	Stimulates binding to SMN; splicing regulation
Other	HSP90 (K615 me1)	KMT3C (SMYD2)	Promotes interaction with titin and its stabilization in myofibers
	HSP70 (K561me2)	KMT2F (SETD1A)	Promotes interaction with Aurora kinase B
			Stimulates kinase activity
	Tat (K50/51)	KMT1E (SETDB1)	Inhibits HIV transcription
	Tat (R52/53 me2a)	PRMT6	Inhibits transactivation activity
			Inhibits HIV replication

t3.73 Modified from Alam et al. (2015), Biggar and Li (2015), Mozzetta et al. (2015)

domain and affect p53 function by interfering with the promoter-binding specificity (Jansson et al. 2008).

The wide variety of cellular processes that are regulated by methyltransferases and demethylases have made these enzymes attractive targets for medical research and therapeutic development. Many of them are altered in several tumor types; for example, KMT2C/MLL3, KMT2D/MLL2, Ezh2, and SETD2 (Lawrence et al. 2014). Therefore, targeting these epigenetic factors presents an opportunity for the development of therapeutics. Although clinical evaluation of drugs that target histone methylation is still in its infancy, promising targets among the KMTs, PRMTs, and KDMs have already been identified (Song et al. 2016; McCabe et al. 2017). However, the modulation of enzymatic activity of methyltransferases and demethylases might be achieved via different mechanisms, and this must be considered for successful drug development.

In the following sections, we review how modulation of the availability of enzymatic cofactors of methyltransferases and demethylases can impact the methylation landscape of chromatin. We discuss examples of pathologies in which such cofactors are deregulated, and demonstrate how this knowledge has been exploited to generate potential therapies.

2 Metabolites Involved in the Regulation of Methyltransferases: *S*-Adenosylmethionine and *S*-Adenosylhomocysteine

2.1 *Regulation of* S-*Adenosylmethionine and* S-*Adenosylhomocysteine Levels: One-Carbon Metabolism*

The production of SAM—the primary methyl group donor for reactions catalyzed by methyltransferases—relies on the use of methionine as a substrate. While plants and bacteria synthesize methionine from aspartate, animals cannot synthesize this amino acid and must acquire it from their diet. Despite this, mammals can regenerate methionine via the one-carbon metabolic pathway which takes carbon groups from nutrient and mediate its incorporation into different outputs, such as nucleotides, glutathione SAM, and others, occurring mainly in the liver (Suganuma and Workman 2018). This pathway includes two different cycles, the methionine and folate cycle, as illustrated in Fig. 1.

The methionine cycle produces SAH as a byproduct, which is a potent pan-inhibitor of methyltransferases. Thus, the SAM/SAH ratio is an indicator of the "methylation potential" of a cell, and determines the activity of methyltransferases (Caudil and Wang 2001). Hydrolysis of SAH to homocysteine (HCY) is important in maintaining the SAM/SAH ratio. Although the reaction is reversible, the equilibrium is shifted toward SAH hydrolysis by the constant removal of HCY via three different mechanisms: (1) methylation of HCY, mediated by methionine synthase (MS) or

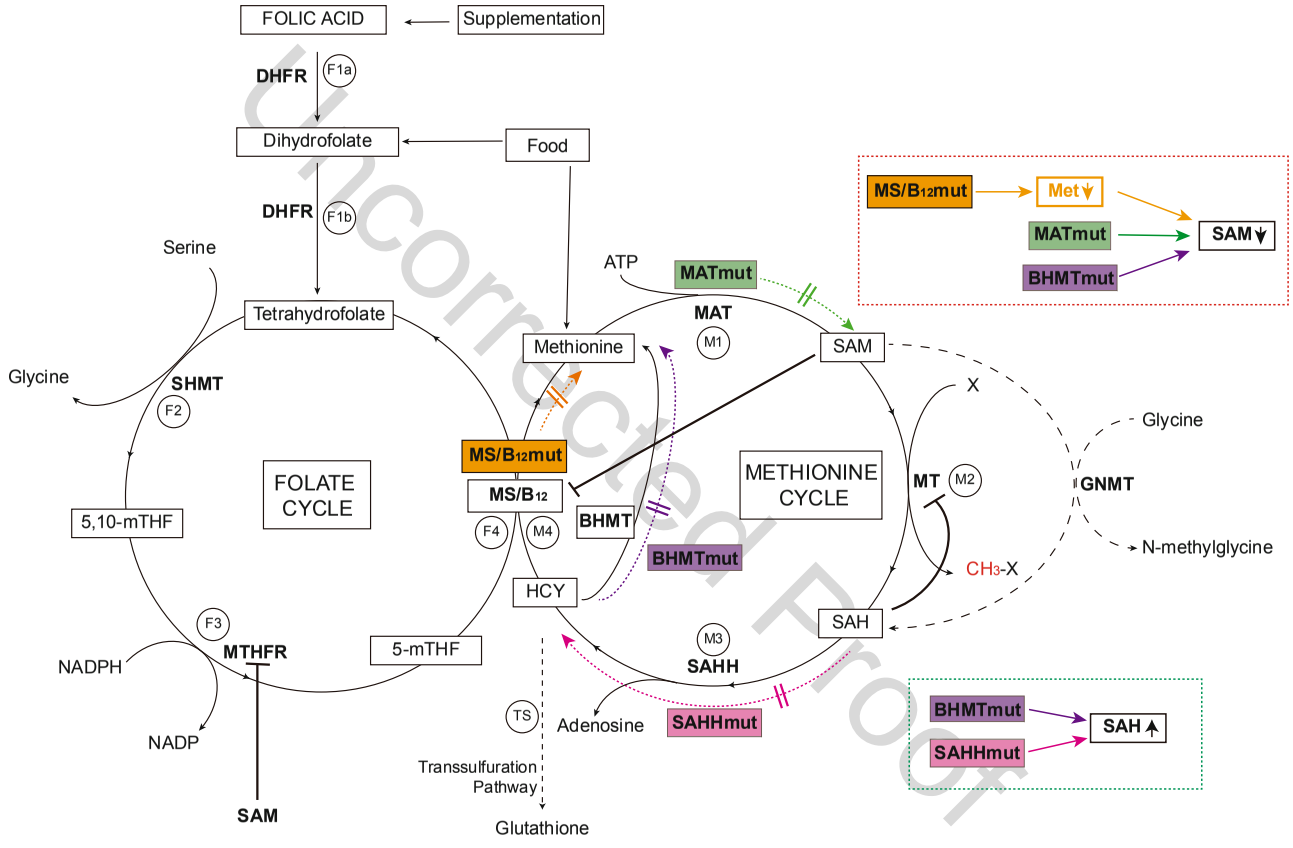

Fig. 1 One-carbon metabolic pathway. Representative scheme showing the association of the methionine and the folate cycles to synthesize SAM. We numbered the steps of each cycle respectively M1 to M4 for methionine and F1a to F4 for folate. The common point between the two cycles is the re-methylation

Fig. 1 (continued) of homocysteine (Step F4/M4). Abbreviations: *SAM* *S*-adenosylmethionine, *SAH* *S*-adenosylhomocysteine, *HCY* homocysteine, *MAT* methionine adenosyltransferase, *SAHH* SAH-hydrolase, *MS* methionine synthase, *B12* vitamin B12, *5,10-mTHF* 5,10-methylene tetrahydrofolate, *5-mTHF* 5-methylene tetrahydrofolate, *DHRF* dihydrofolate reductase, *MTHFR* methylenetetrahydrofolate reductase, *SHMT* serine hydroxymethyltransferase, *ATP* adenosine triphosphate, *NADPH* nicotinamide adenine dinucleotide phosphate. The suffix "mut" inside boxes indicates a mutated version of the corresponding enzyme

betaine-homocysteine *S*-methyltransferase (BHMT); (2) the use of HCY in the transsulfuration pathway for glutathione synthesis; or (3) release of HCY to the extracellular space (Grillo and Colombatto 2008). Deregulation of the pathways involved in regulating the SAM/SAH ratio—either by increasing or decreasing the ratio—affects the chromatin methylation landscape and may therefore contribute to the development of diseases, especially cancer (Shlomi and Rabinowitz 2013). The deregulation of enzymes involved in one-carbon metabolism also affects histone methylation. This mechanism and its important role in carcinogenesis are discussed below.

2.2 *Deregulation of One-Carbon Metabolism, Its Impact on Histone Methylation, and Its Association with Diseases*

As Fig. 1 illustrates, the synthesis of SAM from methionine is catalyzed by methionine adenosyl transferases (MATs). In humans, three MAT isoforms exist: MATI, MATII, and MATIII. The isoforms MATI and MATIII are liver-specific isoforms, while MATII is expressed in various tissues (Murin et al. 2017). Due to its structure and composition, MATII is the only isoform that is susceptible to inhibition by SAM (Halim et al. 1999). Deregulation of MAT expression has been reported in different types of cancers. For example, an isozyme switch from MATI/III to MATII occurs in hepatocellular carcinomas and bile duct cancer (cholangiocarcinoma), and contributes to the depletion of SAM which results in genome-wide histone hypomethylation, with subsequent activation of oncogenic pathways (Murin et al. 2017). It is hypothesized that this isozyme switch is induced by a reduction in SAM levels. Because of this reduction, the normally hypermethylated *mat2a* promoter, which encodes the catalytic subunit of MATII, becomes hypomethylated during the development of hepatocellular carcinoma, occasioning its upregulation and a further decrease in the SAM levels (Yang et al. 2001).

Under normal conditions, MATII participates in the methylation of specific genes through its SAM-integrating transcription (SAMIT) regulatory module. Thus, MATII physically interacts with methyltransferases and transcription factors at specific chromatin loci, providing a direct supply of SAM for histone methylation (Igarashi and Katoh 2013). For example, the repressive mark H3K9me3 at the COX-2 locus is mediated by SetDB1, and requires expression of the catalytic subunit of MATII. When MATII is silenced, the repressive methylation on COX-2 is absent, the oncogene is upregulated, and carcinogenesis is promoted (Kera et al. 2013).

Another important enzyme of one-carbon metabolism is SAH-hydrolase (SAHH), which catalyzes the hydrolysis of SAH to give HCY and adenosine. In nonalcoholic steatohepatitis (NASH) and nonalcoholic fatty liver disease (NAFLD), the SAHH gene is silenced by DNA hypermethylation (H3K27me3) and/or deacetylation of H4K16. This leads to an accumulation of SAH that not only induces global chromatin hypomethylation, but also deregulates processes such as the transsulfuration and transmethylation pathways, thus affecting the redox state of the cell

and favoring the development of disease (Pogribny et al. 2018). Despite this, the accumulation of SAH due to the action of SAHH inhibitor, such as neplanocin and 3-deazaneplanocin in mammary adenocarcinoma had resulted in a global decrease in levels of H3K79me2 that are established by the SAM-dependent methyltransferase DOT1; and, ultimately, in a reduction in cancer cell proliferation (Zhang et al. 2014).

Other types of cancer such as adenocarcinoma and squamous cell carcinoma present increased SAM availability through increased one-carbon metabolism. This phenomenon occurs via upregulation of the methionine transporters LAT1 and LAT4, and by redirection of some of the glycolytic intermediates to the serine-glycine biosynthesis pathway. This pathway supports the folate cycle, which in turn leads to aberrant histone methylation (Wong et al. 2017).

Importantly, as well as deregulation of enzymes involved in one-carbon metabolism, environmental factors can also affect the levels of available SAM and, therefore, histone methylation. For example, mice who were fed a diet deficient in choline-methyl showed reduced hepatic H3K9me3 and H4K20me3 due to impairment of the folate and methionine cycles which decreases the SAM/SAH ratio (Pogribny et al. 2012). Similarly, chronic alcohol consumption also leads to SAM depletion, mainly because the metabolism of ethanol induces high oxidative stress in the cells due to increased levels of ROS (Albano 2006). Constant consumption of ethanol results depletion of glutathione (GSH) which is one of the main systems of ROS detoxification, especially in the brain (Mytilineou et al. 2002). Since GSH is synthesized through the trans-sulfuration pathway using HCY as substrate, depletion leads to concomitant depletion of HCY, methionine, and SAM (Fig. 2). Indeed, the amount of SAM is reduced in alcoholic liver disease models, while the amount of SAH is increased and the GSH/GSH disulfide ratio is reduced (Halsted et al. 2002). This ROS-mediated depletion of SAM ultimately leads to global DNA and histone hypomethylation, as well as deregulation of other histone PTMs, including histone acetylation and ubiquitination (Jangra et al. 2016). Importantly, chronic alcohol consumption also affects folate metabolism, reducing uptake and favoring excretion (Medici and Halsted 2013). Through these mechanisms, alcohol induces epigenetic changes that are important for the progression of various cancers including esophageal, hepatic, and colorectal cancers (Dumitrescu 2018).

2.3 *Deregulation of Enzymes Outside of One-Carbon Metabolism that Affect the SAM/SAH Ratio and Histone Methylation*

Other deregulations that affect the SAM/SAH ratio and are observed in cancers include those involving nicotinamide *N*-methyltransferase (NNMT). This enzyme catalyzes the methylation of nicotinamide, consuming the cellular pool of SAM, and has been seen to cause a decrease of up to 50% in the SAM/SAH ratio in certain types of cancers such as liver, kidney, colon, lung, and bladder cancer. This is associated with a significant, genome-wide decrease in histone methylation at H3K4, H3K9,

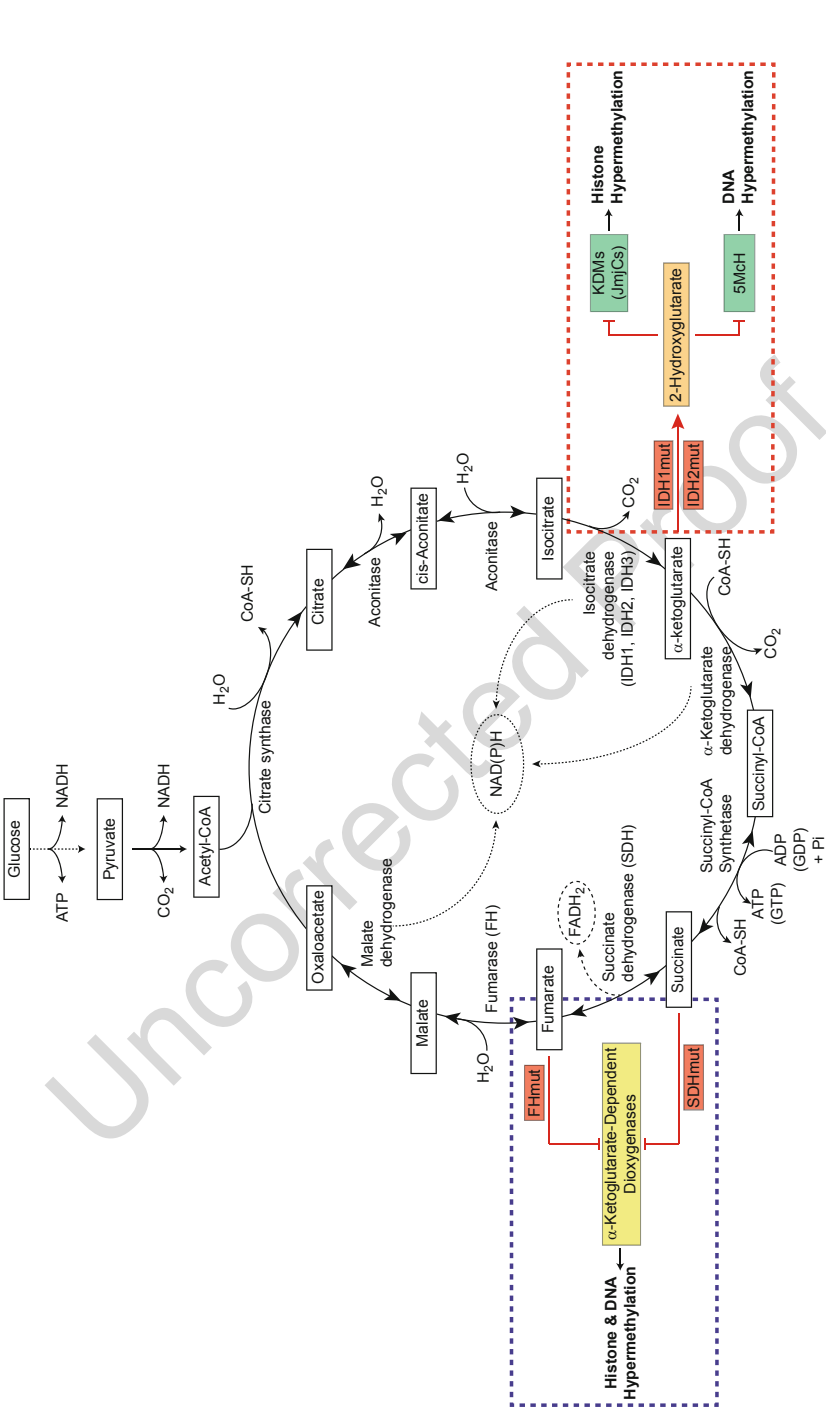

Fig. 2 Krebs Cycle. Representative scheme of the reactions involved including the steps with two arrowheads for reversible reactions, while those with a single arrowhead indicate irreversibility. Mutations in the enzymes isocitrate dehydrogenase (IDH), fumarase (FH), and succinate dehydrogenase (SDH) affect

Fig. 2 (continued) processes highlighted by dotted boxes. Abbreviations: *CoA* co-enzyme A, *ATP* adenosine triphosphate, *ADP* adenosine diphosphate, *GTP* guanosine triphosphate, *GDP* guanosine diphosphate, *NADPH* nicotinamide adenine dinucleotide phosphate, *NADH* nicotinamide adenine dinucleotide, *FADH2* flavin adenine dinucleotide, *IDH* isocitrate dehydrogenase, *FH* fumarase, *SDH* succinate dehydrogenase, *KDMs* histone lysine demethylases, *5McH* 5-hydroxymethylcytosine. Adapted from Nelson et al. (2017)

H3K27, and H4K20, resulting in a phenotype which is considered more pluripotent and can, therefore, increase cancer aggressiveness (Ulanovskaya et al. 2013).

As mentioned previously, an abnormal increase in the SAM/SAH ratio can promote carcinogenesis. Glycine *N*-methyltransferase (GNMT) catalyzes glycine methylation using SAM, and it has been suggested that the only purpose of this enzyme is to maintain SAM levels in normal conditions (Martínez-Chantar et al. 2008). Inactivating mutations of this enzyme have been demonstrated to induce a 40-fold increase in SAM levels, leading to enrichment of H3K27me3 on the promoters of tumor suppressor genes such as RASSF1 and SOCS2, causing transcriptional silencing and subsequent activation of oncogenic pathways. This mechanism is particularly common in cells of steatosis and hepatocellular carcinoma (Martínez-Chantar et al. 2008; Luka et al. 2009).

3 Metabolites Involved in the Activity of Demethylases: Flavin Adenine Dinucleotide, α-Ketoglutarate, Succinate, and Fumarate

Sugars, fatty acids, and most amino acids are oxidized to CO_2 and H_2O via the respiratory chain and the Krebs cycle, also known as acid citric cycle or tricarboxylic acid (TCA) cycle (Fig. 3). Interestingly, except for Fe(II), all the cofactors required by demethylases have a role in the Krebs cycle as intermediaries or products, linking energy metabolism to gene regulation(Nieborak and Schneider 2018). For example, the histone demethylase LSD1 contains a flavin adenine dinucleotide (FAD)-dependent amine oxidase domain (Black et al. 2012), whose activity is dependent on FAD levels. One family of enzymes that are particularly sensitive to the products of the Krebs cycle are the α-ketoglutarate-dependent dioxygenases, especially the JmjC histone demethylases (Black et al. 2012). These enzymes require α-ketoglutarate, O_2, and Fe (II) to function; and are inhibited by succinate, fumarate, and 2-hydroxyglutarate (Fig. 3, red dotted box). In this section, we will review how mutations of enzymes involved in the Krebs cycle affect α-ketoglutarate-dependent histone demethylases.

3.1 Oncometabolites Arising from Deregulations in the Krebs Cycle

3.1.1 Accumulation of 2-Hydroxyglutarate: The Prominent Case of Isocitrate Dehydrogenase Mutations in Glioblastoma Multiforme

Intracellular accumulation of 2-hydroxyglutarate is a concern for several reasons. Among them, its activity as a competitive inhibitor of α-ketoglutarate-dependent

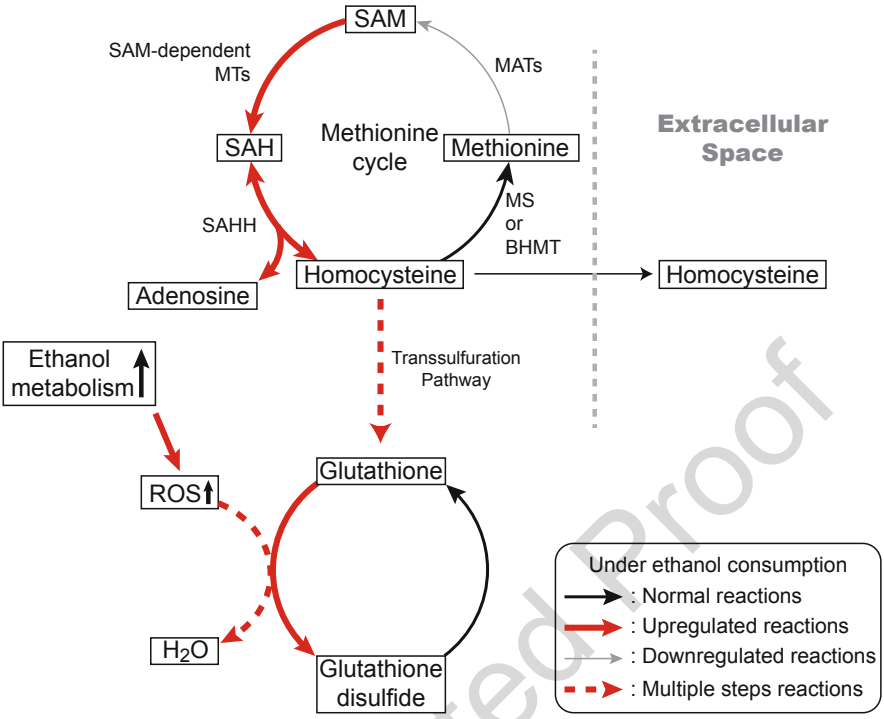

Fig. 3 Constant alcohol consumption leads to an imbalance in the methionine cycle. ROS generation, caused by ethanol metabolism, leads to an increase in the cellular demand in Glutathione to react to the oxidative stress. Depletion of Glutathione leads to an engagement of homocysteine into the transsulfuration pathway, which in turn, diminishes homocysteine re-methylation and promotes depletion of SAM. Red arrows indicate reactions that are stimulated under alcohol consumption

dioxygenases impedes normal histone demethylation and, therefore, induces chromatin hypermethylation (Xu et al. 2011; Yang et al. 2012).

Glioblastoma multiforme (GBM) is one of the most common adult malignant gliomas, accounting for more than 50% of glioma cases (Alifieris and Trafalis 2015), and representing the most aggressive type of primary brain tumor in humans (Stafford et al. 2016). Due to its aggressiveness and its rapid recurrence following treatment (Stafford et al. 2016), patients have a median survival time of 15 months after diagnosis (Lacroix et al. 2001; Martinez et al. 2010) Among the mutations that have been identified in GBM patients, those occurring in isocitrate dehydrogenase (IDH) genes have caught the attention of researchers (Chesnelong 2015). In 2008, a genome wide analysis identified mutations in the active site of IDH1—specifically, at arginine 132 (R132)—in about 12% of the analyzed samples (Parsons et al. 2008). One year later, the same group identified mutations in the IDH2 gene at codon 172, which encodes an arginine residue analogous to R132 (Yan et al. 2009). Mutations in the IDH1 and IDH2 genes are mutually exclusive and heterozygous, with mutations of IDH1 being more commonly observed (Parsons et al. 2008; Yan

et al. 2009). Notably, IDH mutations occur not only in GBM, but also in acute myeloid leukemia, in which they are associated with a worse prognosis (Abbas et al. 2010; Paschka et al. 2010).

The amino acids R132 and R172 in IDH1 and IDH2, respectively, form hydrogen bonds with the isocitrate substrate, suggesting that these mutations affect the catalytic activity of the enzymes (Xu et al. 2004). Indeed, mutation of R132 of IDH1 or R172 of IDH2 result in a loss of the canonical function (Guerra et al. 2009), but confer the ability to reduce α-ketoglutarate to 2-hydroxyglutarate (Dang et al. 2009; Ward et al. 2010). Today, 2-hydroxyglutarate is considered an "oncometabolite", and has been reported to be accumulated in glioma samples that harbor IDH mutations (Xu et al. 2011).

The pathway for intracellular accumulation of 2-hydroxyglutarate is illustrated in Fig. 3 (red dotted box). In normal conditions, isocitrate is converted to α-ketoglutarate by IDHs. This metabolite can then either continue into the Krebs cycle or function as a cofactor for the α-ketoglutarate-dependent dioxygenases of the JmjC family. Glioblastoma cells that have mutations in only one allele of IDH1/2 contain a functional copy of IDH, which acts to maintain the supply of α-ketoglutarate, while the mutated allele converts this continuous supply into 2-hydroxyglutarate (Chesnelong 2015). In this way, IDH mutations lead to major epigenetic deregulations, changing the transcriptional program at a genome-wide scale, with notable effects on tumor suppressors, oncogenes, pro-differentiation genes, DNA repair, and metabolic genes (Chesnelong 2015).

The "hypermethylator" phenotype of IDH-mutant gliomas is associated with genome-wide hypermethylation of CCCTC-binding factor (CTCF)-binding sites, which inhibits the binding of this insulator protein and disrupts the proper establishment of boundary elements that partition topological domains of chromatin. This additional deregulation leads to aberrant upregulation of the canonical glioma oncogene, platelet-derived growth factor receptor A (PDGFRA) (Flavahan et al. 2016).

Knowing that mutated IDHs could be potential targets for the treatment of glioblastomas and other cancers, multiple clinical trials have focused on different IDH inhibitors such as AG-120, AG-221, or AG-881 to inhibit IDH1, IDH2, or both, respectively. Moreover, other clinical trials have been carried out to study molecules that exploit the metabolic sensitivity of IDH mutated gliomas, such as metformin, or molecules that can revert the hypermethylation of transformed cells (Han and Batchelor 2017). Table 4 includes a summary of 20 ongoing clinical trials of different IDHs inhibitors, demethylating agents, and/or metabolic modulators in different types of cancer.

3.1.2 Succinate and Fumarate, Oncometabolites that Promote Histone Hypermethylation

In addition to IDH1 and IDH2, mutations of the *fh, sdha, sdhb, sdhc, sdhd*, and *sdhaf2* genes, which encode subunits of fumarase (FH) and succinate dehydrogenase

Table 4 Clinical trials focused on IDHs inhibitors as therapy for different types of cancer t4.1

Title of the trial	Targeted conditions	Evaluated drugs and therapies	NTC identifier at ClinicalTrails.gov	t4.2
A Study of FT 2102 in Participants with Advanced Solid Tumors and Gliomas with an IDH1 Mutation	Cohort 1a and 1b: Glioma, cohort 1a and 1b: Glioblastoma Multiforme, cohort 2a and 2b: Hepatobiliary tumors (hepatocellular carcinoma, bile duct carcinoma, intrahepatic cholangiocarcinoma, other hepatobiliary carcinomas), cohort 3a and 3b: Chondrosarcoma, cohort 4a and 4b: Intrahepatic cholangiocarcinoma, cohort 5a: Other solid tumors with IDH1 mutations	FT-2102 (IDH1 inhibitor), Azacytidine (DNA demethylating agent), Nivolumab (monoclonal antibody against PD-1), gemcitabine and cisplatin (standard chemotherapy drugs)	NCT03684811	t4.3
Treatment with Azacytidine of recurrent gliomas with IDH1/2 mutation	Recurrent IDH1/2 mutated glioma	Azacytidine (DNA demethylating agent)	NCT03666559	t4.4
IDH1 inhibition using Iopidine as maintenance therapy for IDH1-mutant myeloid neoplasms following allogeneic stem cell transplantation	IDH1 mutation myeloid neoplasms	AG-120 (also known as Ivosidenib, IDH1 inhibitor)	NCT03564821	t4.5
CB-839 with radiation therapy and Temozolomide in treating participants with IDH-mutated diffuse astrocytoma or anaplastic astrocytoma	Anaplastic astrocytoma with mutant IDH, diffuse astrocytoma with mutant IDH	CB-839 hydrochloride (Glutaminase inhibitor), radiation, Temozolomide (alkylating agent, standard chemotherapy drug)	NCT03528642	t4.6
IDH1 (AG 120) inhibitor in patients with IDH1 mutated myelodysplastic syndrome	Myelodysplastic syndromes, acute myeloid leukemia	AG-120 (also known as Ivosidenib, IDH1 inhibitor)	NCT03503409	t4.7
Study of Venetoclax with the mIDH1 inhibitor Ivosidenib (AG120) in IDH1-mutated hematologic malignancies	Other diseases of blood and blood-forming organs, advanced hematologic malignancies, acute myeloid leukemia	AG-120 (also known as Ivosidenib, inhibitor of IDH1), Venetoclax (inhibitor of Bcl-2)	NCT03471260	t4.8

(continued)

t4.9 **Table 4** (continued)

t4.10	Title of the trial	Targeted conditions	Evaluated drugs and therapies	NTC identifier at ClinicalTrails.gov
t4.11	Study of AG-120 and AG-881 in subjects with low grade glioma	Glioma	AG-120 (also known as Ivosidenib, IDH1 inhibitor), AG881 (pan-mutant IDH inhibitor)	NCT03343197
t4.12	Study of AG-120 (Ivosidenib) vs. placebo in combination with Azacytidine in patients with previously untreated acute myeloid leukemia with an IDH1 mutation	Newly diagnosed acute myeloid leukemia, untreated acute myeloid leukemia, acute myeloid leukemia arising from myelodysplastic syndrome	AG-120 (also known as Ivosidenib, IDH1 inhibitor), Azacytidine (DNA demethylating agent)	NCT03173248
t4.13	BAY1436032 in patients with mutant IDH1(mIDH1) advanced acute myeloid leukemia (AML)	Acute myeloid leukemia	BAY1436032 (pan-mutant IDH1 inhibitor)	NCT03127735
t4.14	Study of DS-1001b in patients with gene IDH1-mutated gliomas	Glioma	DS-1001b (inhibitor of certain mutant forms of IDH1)	NCT03030066
t4.15	Study of AG-120 in previously treated advanced cholangiocarcinoma with IDH1 mutations (ClarIDHy)	Advanced cholangiocarcinoma, metastatic cholangiocarcinoma	AG-120 (also known as Ivosidenib, IDH1 inhibitor)	NCT02989857
t4.16	Phase I Study of BAY 1436032 in Patients with IDH1-mutant Solid Tumors	Neoplasms	BAY1436032 (pan-mutant IDH1 inhibitor)	NCT02746081
t4.17	A safety and efficacy study of Oral AG-120 plus subcutaneous Azacytidine and Oral AG-221 plus subcutaneous Azacytidine in subjects with newly diagnosed acute myeloid leukemia (AML)	Acute myeloid leukemia	AG-120 (also known as Ivosidenib, IDH1 inhibitor), Azacytidine (DNA demethylating agent), AG-221 (mutant IDH2 inhibitor)	NCT02677922
t4.18	Safety study of AG-120 or AG-221 in combination with induction and consolidation therapy in patients with newly diagnosed acute	Newly diagnosed acute myeloid leukemia, untreated acute myeloid leukemia, acute myeloid leukemia arising from myelodysplastic	AG-120 (also known as Ivosidenib, IDH1 inhibitor), AG-221 (mutant IDH2 inhibitor), Cytarabine, Daunorubicin, Idarubicin,	NCT02632708

(continued)

Table 4 (continued) t4.19

Title of the trial	Targeted conditions	Evaluated drugs and therapies	NTC identifier at ClinicalTrails.gov t4.20
myeloid leukemia with an IDH1 and/or IDH2 mutation	syndrome, acute myeloid leukemia arising from antecedent hematologic disorder, acute myeloid leukemia arising after exposure to genotoxic injury	Mitoxantrone, etoposide (standard chemotherapy drugs)	
Metformin and chloroquine in IDH1/2-mutated solid tumors	Glioma, cholangiocarcinoma, chondrosarcoma	Metformin (regulator of glucose production in liver and sensitivity to insulin), chloroquine (autophagy inhibitor)	NCT02496741 t4.19
Study of orally administered AG-881 in patients with advanced hematologic malignancies with an IDH1 and/or IDH2 mutation	Acute myeloid leukemia, myelodysplastic syndrome, hematologic malignancies	AG881 (pan-mutant IDH inhibitor)	NCT02492737 t4.20
Study of orally administered AG-881 in patients with advanced solid tumors, including gliomas, with an IDH1 and/or IDH2 mutation	Glioma	AG881 (pan-mutant IDH inhibitor)	NCT02481154 t4.21
Study of orally administered AG-120 in subjects with advanced hematologic malignancies with an IDH1 mutation	Relapsed or refractory acute myeloid leukemia, untreated acute myeloid leukemia, other IDH1-mutated positive hematologic malignancies	AG-120 (also known as Ivosidenib, IDH1 inhibitor)	NCT02074839 t4.22
Study of orally administered AG-120 in subjects with advanced solid tumors, including glioma, with an IDH1 mutation	Cholangiocarcinoma, chondrosarcoma, glioma, other advanced solid tumors	AG-120 (also known as Ivosidenib, IDH1 inhibitor)	NCT02073994 t4.23
Study of the Glutaminase inhibitor CB-839 in solid tumors	Solid tumors, triple-negative breast Cancer, non-small cell lung Cancer, renal cell carcinoma, mesothelioma, fumarate hydratase deficient tumors, succinate dehydrogenase deficient gastrointestinal stromal tumors, succinate	CB-839 (Glutaminase inhibitor), paclitaxel, Everolimus, Erlotinib, docetaxel, Cabozantinib (standard chemotherapy drugs)	NCT02071862 t4.24

(continued)

t4.25 **Table 4** (continued)

t4.26 Title of the trial	Targeted conditions	Evaluated drugs and therapies	NTC identifier at ClinicalTrails.gov
	dehydrogenase deficient non-gastrointestinal stromal tumors, tumors harboring IDH1 and IDH2 mutations, tumors harboring amplifications in the c-Myc gene		

t4.25 In the table, 20 trials retrieved from ClinicalTrials.gov at the moment of writing the manuscript. Each trial may be evaluating the IDH inhibitor in addition to DNA demethylating agents and/or metabolic modulators, plus chemotherapy drugs, as indicated

(SDH) complexes, are also found in some cancers such as paragangliomas, renal cell carcinomas, pheochromocytoma, and gastrointestinal stromal tumors (Toro et al. 2003; Bayley et al. 2008; Hao et al. 2009; Kaelin 2009; Bardella et al. 2011; Gill 2018; Matsumoto et al. 2018). Such mutations correspond to a loss of function, and therefore cause accumulation of fumarate and succinate (Pollard et al. 2005), which disrupts the histone and DNA methylation patterns through inhibition of α-ketoglutarate dependent dioxygenases, in a similar way to 2-hydroxyglutarate (Xiao et al. 2012) (Fig. 3, blue dotted box). Indeed, they are also considered oncometabolites. Mutations of SDHs in samples of paragangliomas and mouse models have been observed to produce a "hypermethylator" phenotype, with global increases in the histone methylation marks H3K9me3, H3K27me2, and H3K27me3. These modifications induce transcriptional changes and cell migration (Letouzé et al. 2013).

In summary, several mutations in various enzymes of the Krebs cycle are involved in cancer development through the accumulation of certain metabolites and intermediaries of the cycle, which in turn promote changes in the epigenetic landscape. This knowledge has informed the design of new strategies to combat these diseases and opened new opportunities which are already being explored.

4 Final Thoughts on the Topic: Modulation of Metabolism as a Tool to Fight Disease

Methionine and folate, unlike α-ketoglutarate, fumarate, and succinate; cannot be synthesized by humans, meaning that appropriate supplementation is important. As we have discussed, the epigenetic information mediated by histone methylation is highly dependent on an appropriate SAM/SAH ratio. This is, in turn, completely dependent on the availability of methionine and folate, as well as the appropriate

functioning of the cycles in which these molecules participate. This is important because epigenetic deregulation can lead to carcinogenesis, but also because epigenetic information is a major influence on embryonic development. Although we did not discuss this aspect here, it is well known that folate is essential for neural tube development and for pregnancy health in general (Greenberg et al. 2011; Viswanathan et al. 2017). Since 2007, the World Health Organization has recommended that pregnant women should take a folic acid supplement of 400 μg daily, from conception until at least 12 weeks of gestation.

Considering the metabolites generated by the Krebs cycle, it is important to study the generation of excess fumarate and succinate due to mutations in the genes *fh*, *sdha*, *sdhb*, *sdhc*, *sdhd,* and *sdhaf2*, and to develop strategies to normalize the levels of these metabolites. Furthermore, the development of drugs that inhibit mutants of IDHs with increased 2-hydroxyglutarate synthesis activity is crucial, as these mutants have key roles in the development of certain types of cancer, particularly those associated with the brain.

Finally, it must be noted that this review focuses on the modulation of histone methylation by specific metabolites, emphasizing the deregulations observed in cancer. However, the contribution of metabolic processes to epigenetic mechanisms and the role of this in human health is beyond the scope of this paper. For example, acetyl-coA metabolism influences histone modifications beyond acetylation. Eight additional types of "acylations" have been recently described, each one with a different effect on gene expression (Sabari et al. 2017). Thus, it is essential that research into diseases in which gene regulation plays a role must also consider the influence of nutrition, gene mutations, and changes in the affinity of metabolic enzymes as well as other potentially related factors such as epigenetic silencing or derepression of genes.

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