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Epigenetic Mechanisms Regulate Mallory Denk Body Formation in the Livers of Drug-primed Mice

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Abstract

The mechanism of Mallory Denk body formation is still not fully understood, but growing evidence implicates epigenetic mechanisms in MDB formation. In a previous study the epigenetic memory of MDB formation remained intact for at least four months after withdrawal from the DDC diet. In the present study, mice were fed a diet containing DDC or a diet containing DDC and S-adenosylmethionine (SAME) to investigate the epigenetic memory of MDB formation. DDC feeding caused an increase in histone 3 acetylation, a decrease in histone 3 trimethylation, and an increase in histone ubiquitination. The addition of SAME to the DDC diet prevented the DDC induced decrease of H3K4 and H3K9 trimethylation and the increase in histone ubiquitinylation. Changes in histone modifying enzymes, (HATs and HDACs) were also found in the liver nuclear extracts of the DDC/SAME fed mice. Data mining of microarray analysis confirmed that gene expression changed with DDC refeeding, particularly the SAME-metabolizing enzymes, *Mat2a*, *AMD*, *AHCY* and *Mthfr*. SAME supplementation prevented the decrease of *AHCY* and *GNMT*, and prevented the increase in *Mthfr*, which provide a mechanism to explain how DDC inhibits methylation of histones. The results indicate that SAME prevented the epigenetic cellular memory involved in the MDB formation

INTRODUCTION

Mallory Denk bodies (MDBs) are cytokeratin-rich inclusion bodies that form in human liver cells, mainly in chronic liver disease and in drug-primed mouse livers (Kachi 1993, Yuan 1996). The diethyl-1, 4-dihydro-2, 4, 6-trimethyl-3, 5-pyridinedicarboxylate (DDC)-drug-primed mouse model was used to study the phenomenon of MDB formation (Yuan 1996).

DDC was fed to mice for 10 weeks, at which time MDBs were formed. When these mice were withdrawn from DDC feeding for 4 weeks, most MDBs disappeared. However MDBs reformed when the mice were refed DDC for one week (Fickert 2002). The phenomenon of MDB formation suggested the existence of a heritable cellular memory in the liver cells of the primed mice. This enabled them to reform MDBs in a short interval (7 days) when the drug was refed (drug primed livers). Epigenetic mechanisms were studied as an approach to understanding the mechanism of MDB formation

Histone H3 is one of the five main histone proteins involved in the structure of chromatin in eukaryotic cells. The N-terminal tail of histone H3 protrudes from the globular nucleosome

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core. It can undergo several different types of epigenetic modifications that modify gene expression. Post-translational addition of methyl groups to the amino-terminal tails of histone proteins has biological importance. Lysine and arginine methylation of histone tails influences changes in gene expression (Barski 2007). Trimethylation of H3K9 is associated with gene silencing and the formation of relatively inactive regions of DNA known as heterochromatin. Trimethylation of H3K4 is associated with increased gene expression (Vakoc 2006). LSD1 can demethylate mono- and di-methylated lysines, specifically histone 3, lysine 4 and 9 (H3K4 and H3K9). Jumonji domain-containing (JmjC) histone demethylases were discovered, which are able to demethylate mono-, di-, or tri-methylated lysines (Lin 2006).

Acetylation of histone H3 occurs at several different lysine positions in the histone tails. This acetylation is controlled by a family of enzymes known as histone acetyl transferases (HATs). Deacetylation was effected by a family of histone deacetylase enzymes (HDACs). Very recently two families of histone demethylating enzymes were discovered.

These studies indicate that specific histone modifications and modifying enzymes that control levels and patterns of histone acetylation and methylation, play essential roles in both global and tissue-specific chromatin organization (Lin 2006). It is the change in the balance of the effects of these histone modifications that accounts for epigenetic changes in gene expression.

In the present study, histone 3 modifications were investigated with respect to MDB formation. The data showed that MDB formation was associated with hypomethylation of histone 3K9 and K4. A change in histone 3 acetylation and H2A ubiquitination was also found. Histone 3 modifying enzymes were also screened to further establish an epigenetic basis for MDB formation. SAME, the most effective methyl group donor, prevented histone modifications that occurred with DDC refeeding, correlating with the prevention of MDB formation by SAME. This further strengthened the hypothesis that MDB formation is an epigenetic phenomenon.

MATERIALS AND METHODS

Animals

One-month-old C3H male mice (Harlan Sprague-Dawley, San Diego, CA) were fed DDC (0.1% diethyl 1,4-dihydro-2, 4, 6-trimethyl-3, 5-pyridinedicarboxylate (Aldrich, St Louis, MD) for 10 weeks to induced MDB formation *in vivo*. The mice were then withdrawn from the drug for 1 month (n = 4) and re-fed DDC with or without S-adenosylmethionine (SAME) (4 g/kg body weight/day) by gavage for 7 days (Li 2006). All mice were treated in a humane manner as approved by the Animal Care Committee at Harbor-UCLA Laboratory BioMedical Research Institute according to the Guidelines of the National Academy of Science. The control mice were fed control diet (Yuan 1996).

Nuclei Isolation

The isolation of nuclei was carried out according to the method of Umlauf *et al.* (Umlauf 2004). Liver tissue, frozen in isopentane immersed in liquid nitrogen was homogenized in a Dounce homogenizer with 10 strokes in 1 ml of buffer-I. The homogenates were centrifuged for 10 min at 6000×g. The Pellets were then resuspended in buffer-II, placed on ice for 10 min and then centrifuged 20 min at 9000g on a sucrose cushion (buffer III). All buffers used contained protease inhibitors: 10 mM benzamidine, 0.7 µg/ml leupeptin, 50 µg/ml soy bean trypsin inhibitor, 0.2 µg/ml aprotinin, 2 µg/ml antipain, 0.7 µg/ml pepstatin, 0.5 mM PMSF, and 0.5 mM AEBSF (Calbiochem, La Jolla, CA), sodium butyrate 5 mM and DTT 1 mM. Protein concentrations were measured using the Bradford method (Bardford 1976). Bovine serum albumin was used as the protein standard

Histone Isolation

The protocol for histone isolation was the Shechter *et al.* method (Shechter 2007). Briefly, isolated nuclei were mixed with 0.4 N H₂SO₄ and incubated on a rotator for 30 min. at 4°C. Samples were spun in a microcentrifuge at 16,000 g, 10 min. Dissolved histones in the supernatant were then precipitated with 33% TCA. After the acetone wash the histones were dissolved in an appropriate buffer and proceeded to further analysis.

Western Blot Analysis

Proteins (50 µg) from liquid nitrogen frozen stored livers nuclear extracts and isolated histones were separated by SDS-PAGE gels and transferred to a PVDF membrane (Bio-Rad, Hercules, CA) for 1 h in 25 mM Tris-HCl (pH 8.3), 192 mM glycine and 20% methanol. The membranes were stained using primary antibodies to the antigens (Table 1). Appropriate species polyclonal and monoclonal HRP-conjugated antibodies were used as the secondary antibodies. The membranes were subjected to chemiluminescence detection using luminal, according to the manufacturer's instructions (Amersham Pharmacia Biotech, Piscataway, NJ).

Microarray Analysis

Total liver RNAs were extracted with Ultraspec™ RNA Isolation Systemic (Biotex Laboratories, Houston, TX) and cleaned with Rneasy columns (Qiagen, Valencia, CA). Five micrograms of total RNA were used for preparing biotin-labeled cRNA. Labeled and fragmented cRNA was subsequently hybridized to Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA). Labeling, hybridization, image scanning and initial data analysis were performed by the Microarray Core at Los Angeles Biomedical Research Institute. Sample preparation and loading, hybridization, staining and microarray data analysis were performed (Bardag-Gorce 2007).

Quantitative Real-time RT-PCR Assay

Total liver RNAs were extracted with Trizol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA) as described previously (Li 2007). PCR primers for histone demethylase LSD1 was (FmLSD1; CAAAGATCCAGCTGACGTTTGA, and RmLSD1 GGACAAGCACAGTATCGCTGTT). The methionine adenosyltransferase II-alpha Mat2a sequence for FmMAT2a is; GTGGGCCTCAGGGTGATG, and for RmMAT2a is; TCCCCAACCGCCATAAGTATC. And the primer for GNMT was: FmGNMT TGCTGAAATATGCGCTTAAGGA, and RmGNMT TTGGCTTCTTCAATGACCCAAT

Immunohistochemistry

Liver tissue was fixed in 10% buffered zinc formalin. Liver sections were double stained using a mouse monoclonal antibody to CK-8 (Fitzgerald, RDI, Concord, MA) and a rabbit polyclonal antibody to ubiquitin (Dako, Carpinteria, CA). Texas-red and FITC-conjugated second antibodies were used. DAPI was used as the nuclear stain.

Statistical Analysis

P values were determined by ANOVA and student-Newman-Keuls for multiple group comparisons (Sigma-Stat software, San Francisco, CA).

RESULTS

To induce MDB formation, mice were fed the DDC drug for 10 weeks, then withdrawn from DDC for 4 weeks (drug primed mice). One group of the drug primed mice was refed DDC for one week. A second group was refed DDC + SAME. At the end of each treatment, the mice were sacrificed and the liver cells were examined for MDB formation. Figure 1 hematoxylin

and eosin stain showed MDB forming cells in the DDC refed group, very few small MDBs in the DDC + SAME refed group.

Several epigenetic mechanisms contributed to the phenomenon of MDB formation. For example, histone modifications are known to be involved in the regulation of several critical cellular processes. This may explain the mechanism of an epigenetic cellular memory (Li 2007), seen when MDBs form. How does this link to critical cellular processes? Histone modifications that could be linked to MDB formation were investigated.

Nuclei were isolated from control, DDC and DDC+SAME mouse livers to evaluate the epigenetic changes present, particularly in MDB forming livers. These nuclei were also used to isolate the histones (Figure 2A) in order to investigate histone modifications. Figures 2B and 2C show a significant change in histone 3 acetylation at lysine 9 (H3K9Ac) which was increased in the DDC refed groups compared to the controls. SAME supplementation produced a further increase of this modification. When the acetylation of H3K18 was analyzed, the results showed a decrease due to refeeding and a slight but significant increase with SAME feeding. These results indicated that each of the two H3 lysine residues had undergone changes in different directions. Since SAME did not prevent the changes induced in DDC refed mice, these changes observed did not correlate with MDB formation.

The histone acetyltransferases involved in histone 3 acetylation are summarized in Table 2. These HATs acetylate histone 3 tails from K9 to K27 and therefore are involved in a variety of epigenetic mechanism.

Figure 3A showed that GCN5 was induced by DDC feeding, which substantiates the increased acetylation of H3K9. However, the decrease of H3K18 acetylation may require the involvement of another epigenetic mechanism, such as the decrease of specific acetyltransferase activity. These results demonstrated the complexity of the epigenetic mechanisms involved in histone modifications. One of the major functions of histone acetylation is to open up the chromatin allowing transcription factors to gain access to regulatory elements in the DNA (Fischle 2003). This leads to an increase in gene expression. The activity of histone deacetylase enzymes (HDACs) reverses this process. Sirt1 is an HDAC class III that requires nicotinamide adenine dinucleotide (NAD⁺) as a cofactor (De Gennaro 2004). It is involved in histone 3 deacetylation of K56 in yeast (Dali-Youcef 2007). Sirt1 was significantly reduced in both DDC R and DDC R SAME (Figure 3B). Also, HDAC2 which is an H3K9 deacetylase was previously shown to be significantly down regulated by DDC refeeding (Bardag-Gorce 2007). This indicated that there is a tendency for HDAC activity to be decreased when MDB are forming in the liver cells. The GCN5 change was prevented by SAME, thus correlating with MDB formation. Changes in SIRT1 did not correlate with MDB formation.

Histone methylation is even more complex than acetylation. Arginine and lysine are residues that can be mono di or trimethylated. The complexities of the types and levels of methylation provide increased regulatory potential. Each event may have specific effects on the chromatin structure and on the interactions of regulatory proteins with chromatin (Zhang 2005). Dimethylation of Histone 3 lysine 9 (H3K9me₂) and lysine 4 (H3K4me₂) was analyzed and shown to have no differences between controls and experimental samples (data not shown). Trimethylation of H3k9me₃ and H3k4me₃, which are more stable, is involved in chromatin stability. They were found to be significantly reduced in the DDC R samples and significantly increased up to the control level by SAME supplementation (Figures 4A and 4B). Thus, SAME prevented the decreased trimethylation of H3K4 and K9, which correlated with the prevention of MDB formation.

The most common histone methyltransferases that may be responsible for the methylation of H3K9me3 and H3H4me3 are the SET domains containing lysine methyltransferases, which include the SUV39 family (Dodge 2005, Tachibana 2001) (see Table 3).

The SET7/9 methyltransferase that regulates the methylation of H3K4me3, and the SUV39-H1 methyltransferase that regulates the methylation of H3K9me3 were studied. Western blots showed that SUV39H1 was significantly reduced in the liver of mice refed DDC. Its level was brought to the control level by SAME supplementation (Figure 5A). SET 7/9 was up regulated by DDC refeeding. DDC refeeding plus SAME prevented the up regulation (Figure 5B). SAME, therefore, prevented both the decrease in H3K9me3 and SUV39H1 levels, which correlated with MDB formation.

Peters *et al.* (Peters 2001) showed that a loss of H3K9 methylation was directly linked to SUV39H1 mutation and was associated with dramatic genome instability. The results presented here showed a significant decrease of histone 3 methylation and a significant decrease of SUV39H1. This reflects a high level of gene expression, because H3K9 methylation is usually associated with gene repression (Lachner 2003).

LSD1, the enzyme responsible for H3K4 demethylation, functions as a transcriptional repressor because it demethylates H3K4 directly and reverses H3K4 methylation (Shi 2004). Both the measurement of LSD1 protein and its gene expression showed an increase of LSD1 when the mice were fed DDC. SAME supplementation did not affect LSD1 levels.

Histone ubiquitination has been reported to open up the condensed chromatin for subsequent methylation by the HMTs (Rathke 2007). The level of histone ubiquitinylation on isolated histones was studied here. Figure 6 shows a significant increase of histone ubiquitinylation in the DDC refed samples. This increase was prevented by SAME feeding. Ubiquitinylation and methylation are linked as indicated by the increase of ubiquitinylation when methylation is decreased. SAME prevented the increase in ubiquitinylation of histones, which correlated with MDB formation.

SAME supplementation produced significant changes in the liver of DDC refed mice. It played a significant role in epigenetic modifications and in reducing MDB formation, SAME metabolizing enzymes were therefore studied. Data mining of microarray analysis performed on the livers of mice from the drug primed mouse model showed a change in gene expression of several enzymes involved in S-adenosylmethionine metabolism.

MAT2a (methionine adenosyltransferase II- α), and S-adenosylmethionine decarboxylase (Amd) were up regulated by DDC refeeding (Figure 7A) and SAME prevented Amd increase but did not affect Mat2a significantly (Figure 7B). In contrast, Ahcy (S-adenosylhomocysteine hydrolase) and GNMT, the glycine N-methyltransferase which demethylates SAME to form adenosylhomocystein (SAH), were down regulated by DDC refeeding. DDC fed with SAME up regulated Ahcy and GNMT (Figure 7C, 7D).

DISCUSSION

Mallory Denk body (MDB) formation is observed in the liver cells of alcoholic patients, as well as in other non-alcoholic chronic liver diseases. To mimic MDB formation that occurs in these liver diseases, the Denk mouse model was used. Previous studies have consistently shown that mice need to be fed DDC for 10 weeks before MDBs appear (Rathke 2007). When DDC is withdrawn, a few residual small MDBs are seen, and when DDC is refed, numerous MDBs form after 7 days of refeeding. This indicated that liver cells have a memory of the previous treatment. The memory was expressed when the liver was rechallenged with the drug. Evidence supporting an epigenetic mechanism explaining this phenomenon has been reported (Umlauf

2004). The present paper provides additional support for the epigenetic hypothesis. It is reported here that changes in methylation, acetylation and ubiquitinylation of histones are part of memory of liver cells during MDB formation. The expression of this memory was prevented by feeding SAME with DDC. For instance, SAME prevented the decrease in trimethylation of H3K4 and K9.

Histone lysine methylation plays an important role in the regulation of gene expression, which impacts on many fundamental biological processes. In contrast to other histone modifications, histone methylation can exist in a mono-, di- or trimethylated state, which influences both transcription activation or repression, depending on the particular site and degree of methylation involved. Trimethylation of histones is regarded as a more robust signal for the establishment of long term epigenetic memory (Jenuwein 2006). Trimethylation of H3K9 is stable and can persist through mitoses without demethylation over several cell generations (Schotta 2004). H3K9me3 sets the molecular stage for DNA methylation (Völkel 2007). In the present study, data showed that DDC refeeding decreased significantly both H3K9 and H3K4 trimethylation and this was prevented by SAME supplementation with DDC refeeding. The low level of histone 3 trimethylation in the livers of refeed mice could explain, in part, the large number of changes in gene expression seen in the DDC refeed mice (Bardag-Gorce 2007).

H3K9me3 and H3K4me3 were changed by DDC refeeding, with or without SAME treatment. In both instances, trimethylation was significantly decreased by DDC refeeding and increased when SAME was added to the DDC refeeding. Histone methyl transferases (HMTases) require the cofactor SAME to methylate histones (Jenuwein 2006). SAME metabolizing enzymes were changed significantly by DDC refeeding, particularly decreased Mat1a, increased Mat2a, decreased AHCY, GNMT, and increased AMD. Since SAME supplementation prevented the DDC induced changes, it is likely that SAME metabolism was shifted away from the methylation pathway. Feeding SAME is a promising approach that prevented the epigenetic modifications occurring when MDBs formed (Li 2007). It is hypothesized that SAME prevented the changes in the SAME methylation pathway which DDC inhibited. DDC increased AMD gene expression which led to the increase in methylthioadenosine, an inhibitor of methylation (Mato 2007). MTHFr is a potent inhibitor of GNMT activity (Mato 2007). MTHFr reduces the methyltransferase methylation activity and conversion of SAME to S-adenosylhomocysteine.

Six lysine residues of histones H3 and H4 and one lysine residue of histone H1 have been identified as target sites of methylation: lysine 4, 9, 27, and 79 of H3, lysine 20 of H4, and lysine 26 of histone H1b (Magueron 2005). This is a very complex molecular machinery that controls gene expression and therefore it is not possible to suggest that only hypomethylation of H3K4 and H3K9 explain all the changes in gene expression observed. It is a balance between all the modifications, which accounts for the gene expression establishing a change in the histone code.

Histone lysine methylation does not change the overall charge density between nucleosomal DNA and histones. Histone acetylation does do this. This change in histone methylation cannot directly alter chromatin structure. A model was proposed that explains how histone methylation changes provide a recognition surface for highly specific chromatin bindings. Multiple protein modules of histone modifying enzymes, which, in turn, mediate changes in chromatin structure, change biological functions downstream of the binding (Strahl 2000). LSD1, responsible for histone demethylation, functions as a transcriptional repressor because it demethylates H3K4 directly (Lachner 2003). The data reported here showed no change in LSD1 in the nuclear extracts of the DDC refeed mice, with or without SAME feeding.

Histone residue modifications are tightly linked. The modification of one residue depends on the modification of another residue. For instance methylation of histone H3K4 or H3K79 is dependent on ubiquitinylation of H2B (Jason 2002, Osley 2004). In addition both histone 3 and histone 4 have been reported to be ubiquitinylated. This ubiquitinylation was required to weaken the interaction between DNA and chromatin, as does acetylation, i.e., to facilitate the recruitment of proteins involved in DNA repair (Wang 2006). The data reported here showed an increase of histone ubiquitinylation when methylation was decreased, which reflected again a tendency for up regulation of gene expression in the DDC fed mice.

In conclusion, the data reported here on histone methylation and ubiquitinylation changes constitute the beginning of understanding the role played by an epigenetic mechanism in the regulation and heritability of global gene changes of expression involved in MDB formation. It would be naïve, at this point, to think that the data reported here is complete. It is just the “tip of the iceberg” of a complex regulatory system, which includes the effects of histone modification, DNA methylation, DNA damage and repair and noncoding RNAs.

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A

B

C

D

Figure 1.

Mouse liver sections showing MDB formation due to DDC feeding and a significant decrease of MDB formation when SAME was added to the diet. (**A**): H&E liver section staining, $\times 400$; (**B**, **C**, and **D**) Double stained for ubiquitin and cytokeratin 8 which colocalized in the MDBs (yellow) (arrows). The DDC Refed + SAME showed only a few small MDBs (arrow) $\times 800$

Figure 2.

Histone 3 Acetylation analysis. Histones were isolated using acid extraction and then analyzed by SDS-Page electrophoresis and Coomassie Blue staining (A). The same amount of protein was used for Western blot to assess histone modifications (loading control). (B) Immunostaining for acetylated histone 3 at lysine 9, and (C) acetylated histone 3 at lysine 18.

Figure 3.

(A) Histone acetyltransferase GCN5 levels in the liver of mice refed DDC (DDC R) and the liver of mice refed DDC with SAMe (DDC R SAMe). (B) Histone Deacetylase Sirtuin 1 (Sirt1) levels in the liver of mice DDC R and the liver of mice DDC R with SAMe. (Mean $\pm$ SE, $n = 3$)

Figure 4. H3K9me3 and K9me3 trimethylation. The histones were isolated from the nuclear extracts of mouse livers. Western blots were performed using antibodies against H3K9me3 (A) and H3K4me3 (B). (Mean $\pm$ SEM, $n = 3$).

Figure 5. (A) Histone methyltransferase (HAT) SUV39H1; (B) SET 7/9; (C) demethylase LSD1. Western blot; and (D) LSD1 Gene expression in the liver of DDC refed mice and the liver of DDC refed +SAME mice. (Mean $\pm$ SE, $n = 3$).

Figure 6.

Histone ubiquitinylation. Histones were isolated from the nuclear extracts of livers. Western blots were performed using antibodies against ubiquitin. DDC increased H2A (arrow) and this was prevented by SAmE. (Mean $\pm$ SEM, $n = 3$).

Figure 7.

(A) Microarray analysis of SAME metabolizing enzymes comparing DDC refed mice to the controls; (B) Mat2a increase in the liver of mice fed DDC; and (C) AHYC, S-adenosylhomocysteine hydrolase, decrease were confirmed by PCR and Western blot analysis. (D) The methyltransferase GNMT was significantly decreased in the liver of mice fed DDC and this decrease was restored by SAME supplementation.

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Table 1

Used Antibodies

Antigen	Location
Histone 3	Upstate, Lake Placid, NY
Histone 3 Acetylated lysine 9 (AcH3K9)	Cell Signaling Technology, Danvers, MA
Histone 3 Acetylated lysine 18 (AcH3K18)	Cell Signaling Technology, Danvers, MA
Histone 3 Methylated lysine 4 (H3K4me3)	Upstate, Lake Placid, NY
Histone 3 Methylated lysine 9 (H3K9me3)	Upstate, Lake Placid, NY
Sirtuin	Abcam, Inc Cambridge MA
GCN5	Abcam, Inc Cambridge MA
SET 7/9	Cell Signaling Technology, Danvers, MA
Ubiquitin	CALBIOCHEM Inc, La Jolla, CA
LSD1	Cell Signaling Technology, Danvers, MA
Cytokeratin 8	RDI, Concord MA
SUV39H1	Cell Signaling Technology, Danvers, MA
AHCY	ABR–Affinity BioReagents Golden, CO

Table 2

Histone 2 Acetylation

Site	Enzyme	Role
K9	GCN5, SRC1, PCAF	Activation
	Unknown	Histone deposit
K14	GCN5, PCAF, SRC1, p300/CBP	Activation
	Tip60 (MYST4)	DNA repair
	hTFIIIC90	RNA polIII
	TAF1	RNA polIII
	sas3	Elongation
	sas2	Euchromatin
K18	GCN5, p300/CBP	Activation
	Gcn5	DNA repair
	p300/CBP	DNA replication
K23	GCN5 p300 CBP	Activation
	p300/CBP ?	Elongation
K27	GCN5	Activation
K56	RTT109	?
K115		?
K122		?

Table 3**Histone 3 Methylation**

Site	Enzyme	Role
R2	CARM1	Activation
K4	mixed-lineage leukemia: MLL1/2/3/4/5, SET1A/B, ASH1 SET9, SET1P	Activation Act elongation memory
R8	PRMT5	Repression
K9	G9a, SETDB1, EuHMTase, CLL8 ? Suv39H1/2 Suv39H1/2 G9a RIZ1	Repression Silencing DNA Meth (TriMeth) Imprinting Activation Tumor Suppressor
K14		Unknown
R17	CARM1	Activation
K23		Unknown
R26	CARM1	Activation
K27	Ezh2	Silencing
K36	? Set2, NSD1, SYMD2	Repression Elongation
K79	Dot1p Dot1p	Elongation Transcription/elongation