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Histone H3 lysine 4 methyltransferase KMT2D

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Abstract

Histone-lysine *N*-methyltransferase 2D (KMT2D), also known as MLL4 and MLL2 in humans and Mll4 in mice, belongs to a family of mammalian histone H3 lysine 4 (H3K4) methyltransferases. It is a large protein over 5,500 amino acids in size and is partially functionally redundant with KMT2C. KMT2D is widely expressed in adult tissues and is essential for early embryonic development. The C-terminal SET domain is responsible for its H3K4 methyltransferase activity and is necessary for maintaining KMT2D protein stability in cells. KMT2D associates with WRAD (WDR5, RbBP5, ASH2L, and DPY30), NCOA6, PTIP, PA1, and H3K27 demethylase UTX in one protein complex. It acts as a scaffold protein within the complex and is responsible for maintaining the stability of UTX. KMT2D is a major mammalian H3K4 mono-methyltransferase and co-localizes with lineage determining transcription factors on transcriptional enhancers. It is required for the binding of histone H3K27 acetyltransferases CBP and p300 on enhancers, enhancer activation and cell-type specific gene expression during differentiation. KMT2D plays critical roles in regulating development, differentiation, metabolism, and tumor suppression. It is frequently mutated in developmental diseases, such as Kabuki syndrome and congenital heart disease, and various forms of cancer. Further understanding of the mechanism through which KMT2D regulates gene expression will reveal why KMT2D mutations are so harmful and may help generate novel therapeutic approaches.

Keywords

KMT2D; MLL4; H3K4 methyltransferase; H3K4 methylation; epigenetic regulation; enhancer; gene expression

1. Introduction

Epigenetic mechanisms play critical roles in regulating gene expression. Methylation of histone H3 lysine 4 (H3K4) is one method that cells use to mark promoters and enhancers. Active promoters are marked with tri-methylation of H3K4 (H3K4me3) while enhancers are marked with mono- and di-methylation of H3K4 (H3K4me1 and H3K4me2, respectively)

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(Calo and Wysocka, 2013; Heintzman et al., 2007). In yeast, the SET1 enzymatic subunit of the Set1 complex accounts for all mono-, di- and tri-methylations on histone H3K4 (Roguev et al., 2001; Ruthenburg et al., 2007). In Drosophila, there are three Set1-like H3K4 methyltransferase complexes with three different enzymatic subunits responsible for their H3K4 methyltransferase activity: dSet1, Trithorax (Trx), and Trithorax-related (Trr). dSet1 is responsible for the bulk of the H3K4me3 while Trr was shown to regulate H3K4me1 (Ardehali et al., 2011; Mohan et al., 2011). As shown in Fig. 1, mammals have six Set1-like H3K4 methyltransferases: KMT2A (or MLL1), KMT2B (or MLL2), KMT2C (or MLL3), KMT2D (or MLL4, ALR, and sometimes MLL2), KMT2F (or SET1A), and KMT2G (or SET1B). Based on the sequence homology of the SET-containing enzymatic subunits, it was determined that KMT2A and KMT2B are homologous to Trx, KMT2C and KMT2D are homologous to Trr, and KMT2F and KMT2G are homologous to dSet1 (Mohan et al., 2011). Consistent with dSet1's role in Drosophila, it was shown that depletion of KMT2F/G's unique CFP1 subunit in mammalian cells decreases global H3K4me3 levels, indicating that KMT2F/G are major H3K4 tri-methyltransferases in mammals (Clouaire et al., 2012). In addition to the KMT2 family, there are several other H3K4 methyltransferases, including SET7, SET9, SMYD3 and Meisetz (PRDM9) (Ruthenburg et al., 2007).

In this review, we will focus on KMT2D, the major mammalian histone H3K4 mono-methyltransferase. Its established importance in gene regulation and frequency of mutation in developmental diseases and cancers warrants an exploration of the literature to inspire further study and development of novel therapies. The review will mainly focus on KMT2D's effects as a H3K4 methyltransferase because non-histone substrates of KMT2D have not been identified yet. We begin with a discussion of the protein's structure and the composition of the KMT2D-associated protein complex. Then we discuss its important role as an enhancer regulator and its various functions in development, differentiation, metabolism, and tumor suppression. We conclude with a discussion of the developmental diseases and cancers that have been associated with mutations in KMT2D.

2. The KMT2D Protein

In mice, the *Kmt2d* gene is located on chromosome 15F1. Its human ortholog is located on chromosome 12q13.12. The mouse and human transcripts are 19,823 and 19,419 base pairs long and contain 55 and 54 exons, respectively (Table 1). Sequence alignment between the cDNA of the mouse and human genes reveals that the two genes are 88% identical. The human *KMT2D* transcript is widely expressed in adult tissues (Prasad et al., 1997). The mouse and human KMT2D proteins are 5,588 and 5,537 amino acids in length, respectively, and both weigh about 600 kDa. Alignment of the mouse and human KMT2D protein sequences reveals that the two proteins are 90% identical.

The KMT2D protein contains two clusters of plant homeotic domains (PHDs) in the N-terminus region (three PHDs per cluster) and an enzymatically active C-terminal SET domain (Ruthenburg et al., 2007). The PHDs in the second cluster (PHD4-6) recognize H4 tails on nucleosomes *in vitro* and could be critical for KMT2D-catalyzed nucleosome methylation (Dhar et al., 2012). Extensive mutation or deletion of the SET domain destabilizes KMT2D, indicating that the SET domain is responsible not only for the H3K4

methyltransferase activity but also for maintaining protein stability of KMT2D in cells (Dorighi et al., 2017; Jang et al., 2016). Near the SET domain are a PHD and FY-rich N/C-terminal (FYRN and FYRC) domains. In addition, a high mobility group (HMG-I) and nine nuclear receptor interacting motifs (LXXLLs) are found within the protein (Rao and Dou, 2015). The amino acids Y5426 and Y5512 are critical for human KMT2D enzymatic activity *in vitro* (Jang et al., 2016). Mutation of Y5477 in mouse KMT2D, which corresponds to Y5426 in human KMT2D, inactivates KMT2D enzymatic activity but does not affect KMT2D protein stability in embryonic stem cells (ESCs) (Dorighi et al., 2017).

3. The KMT2D Protein Complex

KMT2D (ALR in the cited paper) was first purified from HeLa cell nuclear extracts as a component of a protein complex (ASCOM) that also included NCOA6 (ASC-2 in the cited paper), KMT2C (HALR), ASH2L and RbBP5 (Goo et al., 2003). In a later study, affinity purification of Pax transactivation domain-interacting protein (PTIP)-associated proteins surprisingly revealed that in addition to proteins that are involved in DNA damage response, PTIP also associates with ASH2L, RbBP5, WDR5, DPY30, NCOA6, UTX (also known as KDM6A), PA1, KMT2C and KMT2D (Cho et al., 2007). Further purification using FLAG-tagged PA1 revealed that PTIP and PA1 associate with ASH2L, RbBP5, WDR5, DPY30, NCOA6, UTX, and KMT2C or KMT2D in a single protein complex (Fig. 2). The purified KMT2C and KMT2D complexes display strong H3K4 methyltransferase activity (Cho et al., 2007). Three other studies around the same time revealed similar results as part of their findings (Issaeva et al., 2007; Lee et al., 2007; Patel et al., 2007).

WDR5, RbBP5, ASH2L, and DPY30 are homologs of yeast SET1-complex subunits Bre2, Swd1, Swd3, and Sdc1, respectively, and form the four subunit sub-complex WRAD, which is critical for H3K4 methyltransferase activity in all mammalian Set1-like histone methyltransferase complexes (Ernst and Vakoc, 2012). Among WRAD's four subunits, WDR5 binds directly with the FYRN/FYRC domains of C-terminal SET domain-containing fragments of human KMT2C and KMT2D (Cho et al., 2007). Both PTIP and PA1 are subunits unique to the KMT2C and KMT2D H3K4 methyltransferase complexes, which also contain UTX, a histone H3K27 demethylase (Cho et al., 2007; Cho et al., 2012; Hong et al., 2007). Absence of the KMT2D protein results in the collapse of the KMT2D complex and the destabilization of UTX in cells (Jang et al., 2016; Lee et al., 2013).

4. KMT2D is a prominent enhancer H3K4 mono-methyltransferase

Enhancers are genomic regulatory elements, often bound by cell-type specific transcription factors (TFs), that are essential for cell-type specific gene expression in eukaryotic cells (Bulger and Groudine, 2011). An in depth study using adipogenesis and myogenesis as model systems firmly established that KMT2D is a prominent mammalian H3K4 mono-methyltransferase on enhancer regions and has partial functional redundancy with KMT2C (Lee et al., 2013). Studies using the *KMT2D* KO HCT116 human colon cancer cell line and mouse ESCs also provide strong evidence of KMT2D's role in binding enhancer regions and maintaining global H3K4me1 levels (Guo et al., 2013; Hu et al., 2013; Lee et al., 2013; Wang et al., 2016).

Using ChIP-Seq analysis, Lee et al. showed that KMT2D selectively binds to specific enhancer regions depending on cell type and stage of differentiation. Lineage determining TFs recruit KMT2D to establish cell-type specific enhancers during differentiation. During adipogenesis, the early adipogenic TF CCAAT/enhancer-binding protein β (C/EBPβ) recruits and requires KMT2D to establish a subset of adipogenic enhancers. Deletion of *KMT2D* in *KMT2C* KO cells before differentiation prevents the accumulation of H3K4me1/2, H3K27 acetylation (H3K27ac), the transcription coactivator complex Mediator, and RNA Polymerase II on enhancers and results in severe defects in gene expression and cell differentiation (Lee et al., 2013). Furthermore, KMT2D identifies super-enhancers. KMT2C and KMT2D are required for super-enhancer formation in cell differentiation (Lai et al., 2017).

KMT2C and KMT2D are required for the binding of H3K27 acetyltransferases CREB-binding protein (CBP) and/or p300 on enhancers, enhancer activation, and for enhancer-promoter looping (Lai et al., 2017; Wang et al., 2016). Interestingly, the KMT2C and KMT2D proteins, rather than the KMT2C and KMT2D-mediated H3K4me1, control p300 recruitment to enhancers, enhancer activation, and transcription from promoters in ESCs (Wang et al., 2016). Consistently, a recent paper reported that the H3K4 methyltransferase activities of KMT2C and KMT2D are dispensable for enhancer RNA synthesis and transcription from promoters in ESCs (Dorighi et al., 2017). These data support the idea that KMT2C and KMT2D act as coactivators at enhancers and prime enhancers for activation through CBP and p300.

Since acetylation and methylation are mutually exclusive, one could logically hypothesize that the UTX associated with the KMT2C and KMT2D complexes demethylates a given H3K27 site, followed by recruitment of CBP and/or p300 for acetylation of H3K27. However, multiple studies reported that UTX controls cell differentiation and animal development independently from its H3K27 demethylase activity (Faralli et al., 2016; Shpargel et al., 2014; Vandamme et al., 2012; Wang et al., 2012; Yoo et al., 2016). These studies indicate that UTX enzymatic activity is dispensable for enhancer activation and cell-type specific gene expression and that UTX likely functions on enhancers through association with KMT2C and/or KMT2D.

Based on the evidence described in the literature and the fact that the active enhancer mark H3K27ac is often preceded by H3K4me1 (Calo and Wysocka, 2013), a three-step process could be proposed through which KMT2D acts on developmental enhancers, as seen in Fig. 3's model of *Peroxisome proliferator-activated receptor-γ* (*Ppar-γ*) enhancer activation. 1) Pioneer TFs, such as C/EBPβ, bind a region with enhancer like elements. 2) C/EBPβ and lineage-determining TFs, such as PPARγ and C/EBPα, cooperatively recruit KMT2D to prime the enhancer regions and mark them with H3K4me1. 3) KMT2D facilitates the binding of CBP and/or p300, which activate the enhancer regions and mark them with H3K27ac. The associated TFs and the H3K27ac marks recruit bromodomain-containing protein 4 (BRD4), which in turn recruits Mediator and RNA polymerase II and activates cell-type specific gene expression.

5. KMT2D functions in development, differentiation, metabolism, and tumor suppression

KMT2D's function as a major enhancer regulator in mammalian cells translates to various biological processes, including regulation of development, differentiation, metabolism, and tumor suppression.

5.1. Development and differentiation

Kmt2d whole-body knockout (KO) mice exhibit embryonic lethality around E9.5 (Lee et al., 2013). Furthermore, targeted KO of *Kmt2d* using *Myf5* promoter-driven Cre, which is active in muscle and brown adipocyte precursor cells, results in dramatic decreases in brown adipose tissue and muscle mass in mice, demonstrating that KMT2D is required for muscle and adipose tissue development (Lee et al., 2013). In addition, targeted KO of *Kmt2d* in cardiac precursors and myocardium leads to severe cardiac defects and embryonic lethality (Ang et al., 2016). KMT2D mediated H3K4me1/2 appear to be required for maintaining specific gene expression programs during heart development (Ang et al., 2016). Gene KO in mice shows that KMT2D is also required for B cell development (Ortega-Molina et al., 2015; Zhang et al., 2015).

KMT2D is partially functionally redundant with KMT2C and is required for cell differentiation in culture (Lee et al., 2013; Wang et al., 2016). Lee et al. show that *Kmt2c/Kmt2d* double KO brown preadipocytes display severe defects in adipogenesis and myogenesis. RNA-Seq analysis suggests that KMT2D selectively regulates the induction of adipogenic or myogenic genes and is required for cell-type specific gene expression during differentiation (Lee et al., 2013). KMT2D is also required for expression of cell-type specific genes during neuronal and osteoblast differentiation (Dhar et al., 2012; Munehira et al., 2016). Using ESC differentiation into somatic cells and reprogramming of somatic cells into ESC-like induced pluripotent stem cells as model systems, it was shown that KMT2D is required for cell fate transition. KMT2D facilitates cell fate transition by priming enhancers for p300-mediated activation, as mentioned earlier. However, KMT2D is dispensable for maintaining ESC and somatic cell identity (Wang et al., 2016).

5.2. Metabolism

KMT2D, which is functionally redundant with KMT2C in the liver as well, has been shown to have prominent roles in various metabolic processes. Heterozygous $Kmt2d^{+/-}$ mice demonstrate enhanced glucose tolerance and insulin sensitivity and increased serum bile acid (Kim et al., 2015). Using RNA-Seq analysis, Kim et al. revealed that KMT2C and KMT2D are key epigenetic regulators of the hepatic circadian clock and function as transcriptional coactivators of the circadian TFs retinoid-related orphan receptor (ROR)-α and -γ (Kim et al., 2015). The same group also showed that $Kmt2d^{+/-}$ mice exhibit resistance to high fat diet-induced hepatic steatosis (Kim et al., 2016). Consistent with the reported physical interactions between PPARγ and the KMT2D complex (Lee et al., 2008; Lee et al., 2013), KMT2D acts as a coactivator of PPARγ to direct over-nutrition induced steatosis in the mouse liver (Kim et al., 2016).

5.3. Tumor suppression

Several studies have underscored KMT2C and KMT2D's role as important tumor suppressors of various cancers. Cell based assays revealed that NCOA6 and KMT2C or KMT2D act as coactivators of the tumor suppressor and TF p53 and are required for expression of endogenous p53 target genes in response to doxorubicin, a DNA damaging agent (Lee et al., 2009). Three studies in mice demonstrated a tumor suppressor role of KMT2C and KMT2D in acute myeloid leukemia, follicular lymphoma, and diffuse large B cell lymphoma (Chen et al., 2014; Ortega-Molina et al., 2015; Zhang et al., 2015). Using RNAi and CRISPR/Cas9 approaches, Chen et al. showed that ~50% reduction in gene dosage of KMT2C promotes leukemogenesis (Chen et al., 2014). Zhang et al. showed that targeted KO of *Kmt2d* in mice over-expressing the oncogene Bcl2 leads to increased incidence of germinal center-derived B cell lymphoma (Zhang et al., 2015). Ortega-Molina et al. showed that ablation of *Kmt2d* in B-cells promotes lymphoma development in mice and that *Kmt2d* KO affects the expression of tumor suppressor genes *TNFAIP3*, *SOCS3*, *and TNFRSF14* (Ortega-Molina et al., 2015).

On the other hand, KMT2D deficiency in several breast and colon cancer cell lines results in reduced proliferation (Guo et al., 2013; Kim et al., 2014; Mo et al., 2006). In addition, increased KMT2D activity facilitates chromatin opening and recruitment of TFs, including estrogen receptor (ER), in ER-positive breast cancer cells. In MCF7 cells, AKT binds and phosphorylates KMT2D, which attenuates methyltransferase activity and ERα function (Toska et al., 2017). The resulting inactivation of AKT and upregulation of ER target genes upon treatment with PI3K inhibitor may limit therapeutic efficacy of breast cancer drugs (Toska et al., 2017). It seems that KMT2D may have diverse effects in different cell types, which could be dependent on the specific TFs that recruit KMT2D to target genomic locations for gene activation.

6. KMT2D mutations in developmental diseases and cancers

Loss of function mutations in *KMT2D* have been identified in Kabuki syndrome (Ng et al., 2010). Subsequent studies have confirmed *KMT2D* as a major causative gene in Kabuki syndrome with mutational occurrence rates between 56% and 75% (Bogershausen and Wollnik, 2013; Li et al., 2011; Paulussen et al., 2011). Congenital heart disease shows an excess of protein-altering mutations in genes involved in the regulation of H3K4 methylation, including *KMT2D* (Zaidi et al., 2013). This is consistent with the findings in mice in which KO of *Kmt2d* leads to severe cardiac defects (Ang et al., 2016).

What's more striking is the frequent mutation of *KMT2D* in various types of cancer (Lawrence et al., 2014). Frameshift and nonsense mutations in the SET and PHD domains affect 37% and 60%, respectively, of the total *KMT2D* mutations in malignancies (Rao and Dou, 2015). Cancers with somatic mutations in *KMT2D* occur most commonly in the brain, lymph nodes, blood, lungs, large intestine, and endometrium (Rao and Dou, 2015). These cancers include medulloblastoma (Jones et al., 2012; Parsons et al., 2011; Pugh et al., 2012), pheochromocytoma (Juhlin et al., 2015), non-Hodgkin lymphoma (Morin et al., 2011), diffuse large B-cell lymphoma (Lohr et al., 2012; Pasqualucci et al., 2011), cutaneous T-cell lymphoma, Sézary syndrome (da Silva Almeida et al., 2015), bladder, lung, and endometrial

carcinomas (Kandoth et al., 2013), esophageal squamous cell carcinoma (Gao et al., 2014; Lin et al., 2014; Song et al., 2014), pancreatic cancer (Sausen et al., 2015), and prostate cancer (Grasso et al., 2012).

Why *KMT2D* mutations are common in these cancers is still unknown. One possibility could be that the mutations result in defective enhancer regulation and altered cell-type specific gene expression. An example of this was seen when variant enhancers identified by thousands of H3K4me1 sites were differentially enriched in colon cancer compared to normal colon crypts (Akhtar-Zaidi et al., 2012). Another possibility could be due to errors caused by genomic instability during DNA replication and/or transcription. It was shown that cells with *KMT2D* deficiency displayed transcriptional stress correlated with RNA polymerase II pausing, stalling, and backtracking, resulting in abnormalities in early replicating fragile sites within the chromosome (Kantidakis et al., 2016). This kind of stress early in the replication cycle could cause major transcriptional alterations and DNA breaks and lead to formation of cancerous tumors (Tubbs and Nussenzweig, 2017).

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The corresponding Gene Wiki entry for this review can be found here: https://en.wikipedia.org/wiki/KMT2D

Abbreviations

KMT2D	histone-lysine *N*-methyltransferase 2D
H3K4	histone H3 lysine 4
H3K27	histone H3 lysine 27
H3K4me1/2/3	H3K4 mono-, di- and tri-methylation
H3K27ac	H3K27 acetylation
WRAD	WDR5, RbBP5 ASH2L, and DPY30
KO	knockout
KI	knock-in
TF	transcription factor
Trx	Trithorax
Trr	Trithorax-related
C/EBPβ	CCAAT/enhancer-binding protein β
PPARγ	peroxisome proliferator-activated receptor-γ

CBP	CREB-binding protein
BRD4	bromodomain-containing protein 4
ESCs	embryonic stem cells

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Highlights

- KMT2D is a major enhancer H3K4 mono-methyltransferase.
- KMT2D is required for enhancer activation and cell-type-specific gene expression.
- KMT2D regulates development, differentiation, metabolism, and tumor suppression.
- *KMT2D* mutations are associated with developmental diseases and various cancers.

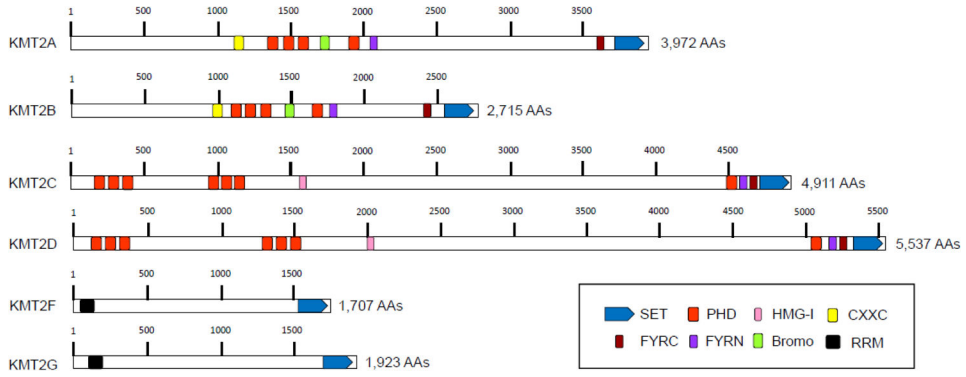

Fig. 1.

The human KMT2 family of H3K4 Methyltransferases. The enzymatic SET domains are shown in blue. PHD: plant homeotic domain, HMG-I: high mobility group I, FYRC/N: FY-rich C/N-terminal, Bromo: bromodomain, RRM: RNA recognition motif, CXXC: nonmethylated-CpG DNA binding domain.

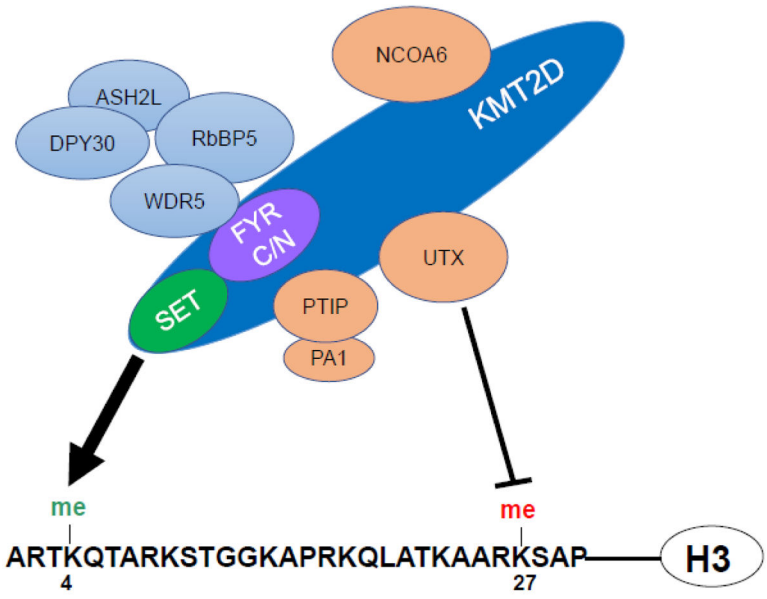

Fig. 2.

The KMT2D Protein Complex. KMT2D associates with WRAD (WDR5, RbBP5, ASH2L, DPY30), UTX, NCOA6, PA1, and PTIP in one complex. The C-terminal SET domain is the source of KMT2D's H3K4 methyltransferase activity. UTX is the complex's H3K27 demethylase. H3K4 methylation is associated with gene activation, whereas H3K27 methylation is associated with gene repression.

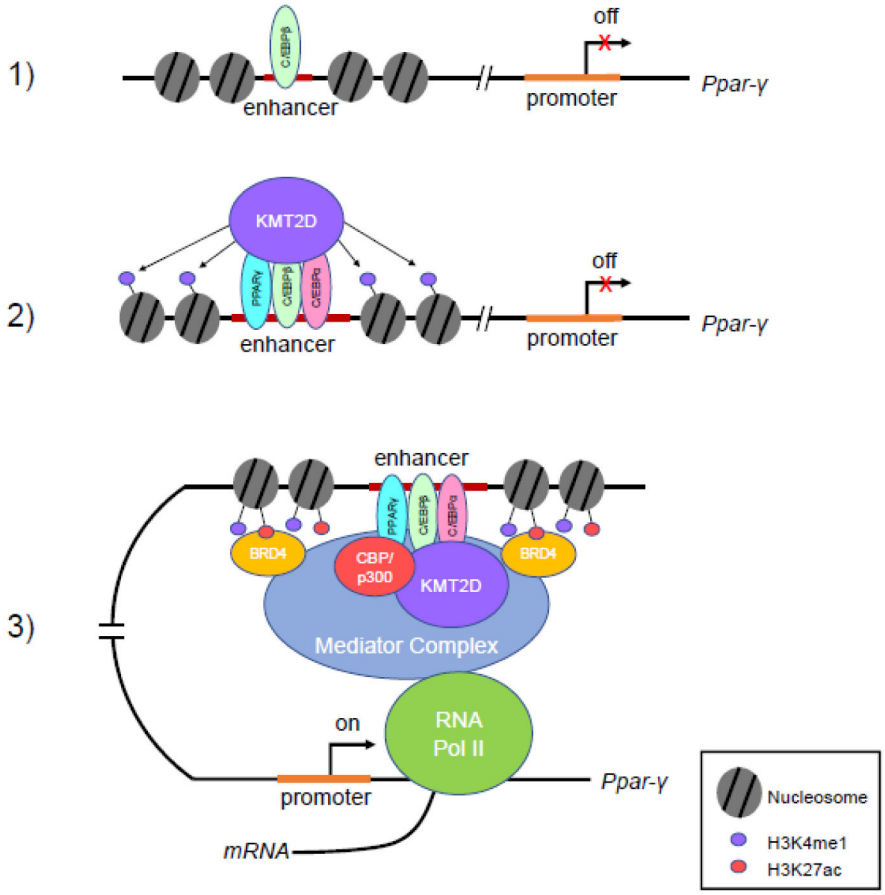

Fig. 3.

Enhancer Regulation via KMT2D Priming. The proposed 3-step model through which KMT2D primes a *Ppar-γ* enhancer and activates cell-type specific gene expression. See text for details.

Table 1

Basic properties of the KMT2D gene/protein

	Human	Mouse
Gene	KMT2D, MLL4, MLL2	Kmt2d, Mll4
Chromosome	12q13.12	15F1
Ensembl ID	ENSG00000167548	ENSMUSG00000048154
Transcript Length (bps)	19,419	19,823
Exons/Introns	54/53	55/54
Uniport ID	O14686	Q6PDK2
Protein Length (amino acids)	5,537	5,588
Protein Size (Da)	593,389	600,303