Published in final edited form as:

Curr Mol Med. 2008 December ; 8(8): 805–815.

Menin, Histone H3 Methyltransferases, and Regulation of Cell Proliferation: Current Knowledge and Perspective

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Abstract

Menin is a tumor suppressor encoded by the *MEN1* gene that is mutated in patients with an inherited syndrome, multiple endocrine neoplasia type 1 (MEN1). Loss of menin has potent impact on proliferation of endocrine and non-endocrine cells. However, until recently little has been known as to how menin regulates cell proliferation. Rapid research progress in the past several years suggests that menin represses proliferation of endocrine cells yet promotes proliferation in certain types of leukemia cells via interacting with various transcriptional regulators. Menin interacts with histone H3 methyltransferases such as MLL (mixed lineage leukemia) protein. Increasing evidence has linked the biological function of menin to epigenetic histone modifications, control of the pattern of gene expression, and regulation of cell proliferation in a cell type-specific manner. In light of these recent findings, an emerging model suggests that menin is a crucial regulator of histone modifiers by acting as a scaffold protein to coordinate gene transcription and cell proliferation in a cell context-dependent manner. This recent progress unravels the coordinating role of menin in epigenetics and regulation of cell cycle, providing novel insights into understanding regulation of beta cell functions and diabetes, as well as the development and therapy of endocrine tumors and leukemogenesis.

Keywords

Menin; MEN1; beta cells; endocrine tumors; histone methyltransferase; transcriptional factor; mixed lineage leukemia (MLL)

1. Introduction

The tumor suppressor gene *MEN1* is mutated in patients with a dominantly inherited tumor syndrome, multiple endocrine neoplasia type 1 (MEN1) [1,2]. The *MEN1* gene encodes a protein of 610 amino acid residues, menin, which is located predominantly in the nucleus [3]. The *MEN1* patients develop a variety of endocrine neoplasms, including parathyroid hyperplasia and adenomas, pituitary adenomas and pancreatic islet cell tumors [1,2]. Menin does not reveal homology to any other known proteins. However, menin has orthologs in organisms ranging from *Drosophila* to zebrafish and mice (47%, 97%, and 98%, homology, respectively), while no ortholog has been identified in the yeast *Saccharomyces cerevisiae* and *C. elegans* [4–6]. This suggests that MEN1 was formed only in arthropods or higher metazoan species, relatively late during evolution.

Genetic studies in mice indicate that menin is a tissue specific tumor suppressor. Homozygous deletion of *Men1* (the murine equivalent of human *MEN1*) is lethal, and the

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embryos die at the mid-gestation with defects in the neural tube, the liver and the heart [4,7]. Heterozygous *Men1* knockout mice exhibit a phenotype similar to that of the human MEN1 disorder and develop endocrine tumors in pancreatic islets, the anterior pituitary, parathyroids, adrenal cortex and adrenal medulla later in life [8,9]. It is well known that menin is widely expressed in both nonendocrine and endocrine tissues [4,10]. However, recent studies suggest that menin is expressed in endocrine organs such as pancreatic islets at a relatively high level [11,12]. Consistent with this observation, homozygous loss of *MEN1* in tissues that are not normally predisposed to developing MEN1 tumors does not lead to development of MEN1 tumors. For instance, mice with tissue-specific and homozygous loss of *Men1* in the liver remains tumor free in the liver throughout their life [13], highlighting the tissue specific effect of menin in tumor suppression.

Mutation of menin in endocrine cancers from patients demonstrates that it is a bona fide tumor suppressor. Both alleles of a tumor suppressor gene in tumor cells are often mutated during a process called “two hits” [14]. A common example is that a patient inherits a mutant form of a tumor suppressor gene, e.g., *MEN1*, and after birth, the remaining normal allele of the *MEN1* gene is often lost due to missing a whole or part of the chromosome that carries the normal allele of *MEN1* [15]. This phenomenon is called loss of heterozygosity (LOH). A classic definition for a tumor suppressor gene is that the tumor suppressor represses tumorigenesis, and both alleles in tumor cells are disrupted through “two hits” involving LOH. Parathyroid tumors and insulinomas from MEN1 patients often lose the remaining normal allele of the *MEN1* gene, resulting in LOH at the *MEN1* locus [16]. As *MEN1* fits with this classic definition, *MEN1* is a bona fide tumor suppressor in endocrine cells.

Menin has been shown to suppress proliferation of several types of cells. For instance, overexpression of menin represses proliferation of Ras-transformed NIH 3T3 cells, and their clonogenicity in soft agar and inhibits tumor growth in nude mice [17]. In addition, reduction of menin expression by antisense cDNA increases proliferation of rat duodenal crypt-like cells [18]. In agreement with this, menin expression is modestly decreased in mid-G1 phase, suggesting that menin expression may be cell cycle-regulated in rat pituitary GH4 and Chinese hamster ovary CHO cells [10,19]. On the other hand, menin plays a crucial role in promoting proliferation of certain types of leukemia cells [20]. Thus, menin regulates cell proliferation in a cell type-dependent manner.

To explore the molecular circuitry underlying menin-dependent regulation of cell proliferation, intensive efforts have been made to identify menin-interacting proteins by many groups, in hopes of uncovering clues to menin-mediated regulation of cell cycle as reviewed by Balogh et al. [21]. Several transcriptional factors, such as Jun D, NF- κ B and Smad3, have been found to interact with menin [22]. However, it remains unclear how menin's interaction with these proteins leads to controlling cell proliferation. One important clue on the role of menin in controlling gene transcription and cell proliferation came from identifying menin's interaction with MLL (mixed lineage leukemia) and MLL2, two large proteins that harbor a SET domain (suppressor of variegation [Su(var)3–9] [23], enhancer of zeste [E(z)] [24] and trithorax [25]). The SET domain is conserved among many chromatin-associated proteins and displays histone lysine methyltransferase activity as reviewed [26,27]. Indeed MLL and MLL2 possess intrinsic histone H3 lysine 4 (H3K4) methyltransferase activity [28,29].

These findings have provided novel insights into the role of menin in regulating cell proliferation by acting as a crucial epigenetic regulator and chromatin modifier [28,29]. Recently, we and others uncovered a novel role of menin as an essential co-factor in proliferation and differentiation of MLL-fusion protein (FP) transformed cells [20,30]. These data link menin with the histone methyltransferase machinery of the MLL family, and

point to a role of menin-MLL interaction in regulating endocrine tumorigenesis and leukemogenesis. Hence, this review will focus on the recent progress in identifying menin as a regulator of MLLs and histone H3K4 methylation and gene transcription, menin's crucial role in MLL fusion protein-mediated leukemogenesis, and the role of menin in regulating histone H3 methylation and proliferation of endocrine cells. Moreover, histone H3K4 methylation-mediated recruitment of histone binding proteins to gene loci and removal of H3K4 methylation marks by specific demethylases will also be reviewed. Further, the link between menin and nuclear receptor signaling has been uncovered, coupled with discovery of the menin-MLL cooperation in controlling the global pattern of gene expression and chromatin-based memory of gene expression pattern. Overall, these recent findings have pushed the field to the frontier of epigenetics, cell memory, cell cycle, and controlling tumorigenesis.

2. Menin and MLL complex

Menin was first found as a component of a multi-protein complex containing a trithorax group protein MLL2 (mixed lineage leukemia 2), which displays a histone H3 lysine 4 methyltransferase activity conferred by the conserved SET domain [28]. Interestingly, several tumor-derived menin point mutants failed to associate with MLL2 [28]. H3K4 trimethylation is the symbol of active chromatin (for reviews, see [31,32]). Menin also interacts with RNA Polymerase II, a large subunit that was phosphorylated at Ser 5 of the Carboxyl-Terminal Domain (S5P-CTD). These results suggest that the menin/histone methyltransferase (HMTase) complex is associated with transcriptional machinery [28]. Another independent group identified menin as a component of a MLL-containing histone H3 methyltransferase complex [29]. They mapped the very N-terminal part of MLL as a ~10 amino acid residue sequence that mediates interaction with menin [29]. A recent study showed that MLL sequence required for interaction with menin is longer, extending from residue 5 to 44 [33].

Consistent with physical interaction between menin and MLL, menin is essential for maintenance of expression of homeotic box genes such as *Hoxa9*, *Hoxc6*, *Cyt19*, and *Hoxc8* and H3K4 trimethylation at the *Hoxc8* and *Hoxa9* gene loci, underscoring the functional significance of MLL and menin interaction in regulation of gene expression [28,29]. Moreover, Chromatin immunoprecipitation (ChIP) has also shown that menin and MLL bind to *Hox* gene loci and are required for H3K4 trimethylation [28]. These studies have shed light on menin's biological function by linking menin to MLL and chromatin modifications. The physical interaction between menin and MLL-N terminus provides a fresh clue as to whether menin plays a crucial role in MLL or MLL2-mediated H3K4 methylation and gene transcription. Further, these findings raise a crucial question about whether menin regulates cell proliferation via controlling transcription of cell cycle regulators in concert with histone H3K4 methyltransferase activity of the MLL complex.

3. MLL protein, SET domain, Histone H3K4 methylation/demethylation and the function of wild type MLL

MLL is a homologue of *trithorax group* (*trx*) genes in *Drosophila melanogaster* and consists of 3969 amino acid residues [34]. Members of the Trithorax and Polycomb group (PcG) gene families collectively maintain a cellular memory system through a respectively positive and negative regulation of *Hox* gene expression throughout embryonic and adult life [35]. Three regions of significant homology shared by *trx* and MLL include subnuclear localization signals, central PHD finger domains, and the SET domains as shown in Fig. 1a. Among them, SET domain, a highly conserved motif, confers an intrinsic H3K4 methyltransferase (HMT) activity to MLL protein [36].

Methylation of lysines in histones, with the exception of histone H3K79, is catalyzed exclusively by the conserved SET domain family proteins originally identified in *Drosophila* (suppressor of variegation [Su(var)3-9] [23], enhancer of zeste [E(z)] [24] and trithorax [25]). The modified lysine can exist in a mono-, di-, or tri-methylated form. Set1, a yeast homologue of MLL, is required for all H3K4 methylation in yeast [37]. Set1 is predominantly associated with the coding regions of genes transcribed by RNA polymerase II (Pol II) in yeast, and the presence of trimethylated H3K4 closely correlates with Set1 occupancy on the gene [38]. MLL specifically methylates H3K4, resulting in mono-, di-, and tri-methylated H3K4 [39]. Trimethylated H3K4 is enriched in the promoter region in mammalian cells [40]. Three structural components in MLL complex, RbBP5, ASH2L and WDR5 are also required for H3K4 trimethylation by MLL in vitro [41]. Trimethylated H3K4 represents an epigenetic mark typically associated with transcriptionally active chromatin [28,39]. Though recent genome-wide analysis shows H3K4me3 Is Enriched at Active and Inactive Genes throughout the Genome, The signals observed for histone H3K4me3 were typically lower (about 3-fold) at the inactive genes than at active genes [42].

MLL is required for H3K4 trimethylation at its target genes, transcription of certain *Hox* genes, and normal hematopoiesis. MLL is proteolytically cleaved at two conserved sites in the middle part of the protein to generate an N-terminal 320-kD fragment (N320) and a C-terminal 180-kD fragment (C180) by Taspase 1, as shown in Fig. 1a, which heterodimerize to stabilize the complex and confer its subnuclear localization [43]. Like *trx* in *Drosophila*, MLL functions as a regulator to maintain the expression of multiple *Hox* genes during development. MLL mutants exhibit hematopoietic abnormalities [44]. For instance, yolk sac progenitors from MLL null embryos at E10.5 generated definitive CFU-M and CFU-GEMM colonies in hematopoietic assays, however, the colonies were consistently smaller, fewer in number, and exhibited a slower growth rate compared to colonies generated from wild type littermates [45]. In vitro, Milne et al. showed that MLL regulates *Hox* gene expression through direct binding to promoter sequences. They determined that the MLL SET domain is a H3K4-specific methyltransferase whose activity is stimulated with acetylated H3 peptides [36]. This methyltransferase activity is correlated with *Hox* gene activation and H3K4 methylation at the cis-regulatory sequences in vivo [36]. MLL2 is another member of MLL family and also contains a SET domain conferring histone methyltransferase activity [28]. These results indicate that this complex is similar to the SET-1 complex (COMPASS) in yeast [46].

Identification of specific H3K4 demethylases adds another layer of complexity to address menin's role in histone H3 modifications [47–50]. For example, JARID1 protein, or retinoblastoma binding protein 2 (RBP2) is a mammalian enzyme capable of erasing methyl groups from trimethylated H3K4 [47,50]. Moreover, RBP2 is displaced from *Hox* genes during embryonic stem (ES) cell differentiation, correlating with an increase of H3K4 trimethylation (H3K4me3) and target gene expression [47]. Interestingly, RBP2 knockout mice display decreased apoptosis and increased entry into G1 phase of cell cycle in hematopoietic stem cells (HSC) and myeloid progenitors [50], consistent with increased transcription of *Hox* genes in HSC and progenitor cells. Also, loss of menin arrests the MLL-AF9 and MLL-ENL transformed bone marrow cells at G1 phase or promotes differentiation [20,30]. These results raise the possibility that menin may functionally interact the demethylase activity of RBP2 or other H3K4 demethylase activity in controlling G1/S transition and H3K4 methylation. Though it remains unclear if RBP2 plays a role in regulation of *Hox* genes in pathological conditions such as leukemic transformation, it is conceivable that the H3K4 methylation level, either globally or locally at a particular locus, is a result dictated by the combined effect of menin, histone H3K4 methyltransferase such as MLL and histone H3K4 demethylases including RBP2.

4. MLL chromosomal translocation and the development of mixed lineage leukemia

The *MLL* gene was originally identified as a common target of chromosomal translocations that take place in ~ 5% of acute leukemia patients. Chromosomal translocations involving *MLL* are predominant in infant leukemia and leukemia occurring in patients previously treated with DNA topoisomerase II inhibitors [51,52]. *MLL* has been shown to fuse with over 40 different partners, resulting in formation of a diverse collection of MLL fusion proteins (MLL-FPs) as reviewed by Mitterbauer-Hohendanner and Mannhalter [51]. MLL partner proteins can be classified into two functional categories: signaling molecules that normally localize to the cytoplasm/cell junctions and nuclear factors implicated in various aspects of transcriptional regulation, as reviewed by Ayton and Cleary [53]. Both wild-type MLL and oncogenic MLL-FPs transcriptionally regulate *Hox* genes, which are spatially and temporally regulated during embryonic development and have been shown to play a critical role, respectively, in hematopoiesis and leukemogenesis [54–56].

Both wild type and oncogenic MLL proteins retain the amino terminal AT hook motif of MLL that directly binds DNA [34], suggesting that the *MLL* fusion gene may recognize putative *MLL* response elements of target genes. However, it is not known whether loss or gain of function of MLL-FPs is crucial for transformation by MLL fusion genes. Many MLL partners may cause the fusion proteins to gain function and cause abnormal regulation on target genes that are responsible for leukemogenesis. However, MLL fusion protein MLL-AF9 strongly activates expression of endogenous *Hoxc8* in mouse embryonic fibroblasts (MEFs) through a mechanism that does not involve H3K4 methylation nor requires wild-type MLL, suggesting that a distinct mechanism of gene regulation by MLL fusion proteins in a wild type MLL-independent manner in MLL^{-/-} MEFs cells [36].

5. Menin, H3K4 trimethylation, and MLL-FP-mediated transformation of hematopoietic cells

Enlightened by the functional interaction between menin and MLL, our group and Cleary's group explored the role of menin in regulating *Hox* gene transcription, H3K4 methylation, and MLL-FP-mediated leukemic transformation. Results from these studies have uncovered an essential role for menin in MLL-FP-mediated leukemogenesis and also hematopoiesis [20,30]. The striking conclusion is supported by multiple lines of evidence: 1) Acute ablation of *Men1* reduces aberrant *Hox* gene expression mediated by MLL-menin associated complexes [20]; 2) Menin is essential for oncogenic MLL-FP-mediated transformation of bone marrow cells, as evidenced by bone marrow colony formation, methylcellulose serial replating assays and the ability to induce leukemia in syngeneic recipient mice. However non-MLL oncoprotein-mediated transformation is entirely independent of menin [30]; 3) Loss of menin relieves the differentiation block of MLL-ENL-transformed leukemic blasts [30], but only arrests MLL-AF9-transformed cells at G1 phase without blocking differentiation [20] (unpublished data, Chen, Y.); 4) *Men1* excision suppresses *Hoxa9* expression, and ChIP assay suggests that both menin and MLL may bind to promoters *Hoxa7*, *Hoxa9* and *Hoxa10* genes [20,30].

Importantly, for the first time, menin was found to be essential for histone H3K4 trimethylation at the *Hoxa9* locus [20], which is the first piece of evidence to link menin's biological function with histone H3 modifications in hematopoietic cells. As *Hox* genes, especially *Hoxa9*, are important target genes of MLL oncoproteins during leukemogenesis, these results suggest that menin recruits histone modifiers to the menin target genes and

enhances gene transcription and subsequent leukemic transformation. Moreover, conditional menin knockdown markedly reduces MLL binding to the *Hoxa9* locus [20].

MLL-FPs lead to enhanced *Hoxa9* expression, which correlates with H3K4 methylation at the *Hoxa9* locus and leukemogenesis. However, all of the MLL-FPs lack the SET domain, which confers specific H3K4 methylation, but retain the DNA binding and menin-interacting domains [30]. Moreover, it is impossible that menin-dependent and MLL-FP-mediated transformation is through MLL-FP-mediated H3K4 methylation, because MLL-FPs are deprived of their SET domain and thus the ability to methylate H3K4. On the other hand, the wild type MLL has the H3K4-methylating SET domain but MLL per se is not oncogenic. Further, menin is only essential for MLL-FP-mediated, but not non-MLL-FP-mediated, transformation [30]. Therefore, current knowledge strongly suggests that a gain of function by MLL-FPs leads to upregulation of *Hoxa9*, H3K4 methylation, and leukemogenesis in a menin-dependent fashion. How does menin positively regulate Hox genes in MLL-FP-mediated transformation? As menin specifically interacts with the very N-terminal part of MLL [29], menin may not only recruit MLL-FPs but also wild type MLL to the *Hoxa9* and other loci to facilitate cell proliferation and leukemogenesis [20,30].

6. Histone H3 lysine 79 methylation mediated by MLL fusion proteins and Dot1L

Histone H3 lysine 79 (H3K79) can be specifically methylated by a human methyltransferase enzyme, hDOT1L [57,58]. A recent report shows that hDot1L is essential for MLL-AF10-mediated bone marrow transformation [59]. Interestingly, fusion of MLL with hDOT1L is sufficient for hematopoietic transformation and upregulation of *Hoxa9* gene, concomitant with the enhanced local H3K79 methylation. Since it has been shown that the four most frequently occurring MLL translocation partners (AF4, AF9, ENL and AF10) are part of a protein network involved in histone H3K79 methylation [60], it is likely that hDOT1L may be recruited to the loci of Hox genes to modify H3K79 when hematopoietic stem cell/progenitors are transformed by other ML-FPs (such as MLL-AF9, MLL-ENL) as well. Menin may also be essential for recruitment of hDOT1L. In this regard, gain of H3K79 methyltransferase activity to “write” H3K79 marker at the loci of the MLL-FP target genes may explain that loss of SET1 domain does not block the Hox gene expression in MLL oncoprotein-induced leukemic transformation. Conversely, H3K4 methylation is reduced at *Hoxa9* locus in MLL-hDOT1L and MLL-AF10 transformed cells compared to normal cells infected with vector only [59]. The author concludes that MLL-hDOT1L or MLL-AF10 may compete with endogenous MLL for binding to their target genes. Therefore, an attractive question is that among H3K4 methylation or H3K79 methylation, which one is more important for MLL oncoprotein-mediated leukemic transformation? Since it is still hard to manipulate these specific histone H3 marks, addressing these questions still need further technical advancement.

7. “Readers” of methylated histone H3K4: specific methylated H3K4 binding proteins and their functions

How does H3K4 methylation affect gene transcription? Several models are proposed to explain the effect of H3K4 methylation on gene transcription, including the change of chromatin conformation and the recruitment of methylated H3K4-specific binding proteins to the nucleosomes harboring the methylated H3K4 [61]. Definitive results on the recruitment of specific methylated H3K4 binding proteins have been recently reported. For instance, CHD1 has been shown to bind specifically to trimethylated H3K4 by several groups [62–64]. CHD1 is a component of the nucleosome remodeling complex that

possesses ATPase and DNA helicase activity [65]. Hence, guiding CHD1 by methylated H3K4 to nucleosomes may lead to the opening of local chromatin and thus activation of gene transcription.

Recently four independent groups have demonstrated how trimethylated H3K4 (H3K4me3) “mark” is “read” by two distinct proteins with a well conserved PHD domain, relaying the H3K4me3 signal to these proteins [66–69]. One of these proteins is BPTF, a large subunit of nucleosome remodeling factor (NURF), which is recruited to the H3K4me3-rich chromatin region through a PHD domain [69]. Importantly, recruitment of BPTF through the H3K4me3 mark is crucial for activation of developmental genes [69]. In contrast to BPTF, another H3K4me3 “reader,” ING2, is recruited to H3K4me3-rich nucleosomes through its PHD domain while carrying a histone deacetylase (HDAC), leading to repression of target genes such as cyclin D1 [68]. Thus, in most cases H3K4me3 leads to activation of gene transcription, but it appears that the histone mark can also cause specific repression of gene transcription through HDACs. However, it remains unclear how H3K4me3 is differentially recognized by either activating BPTF or repressing ING2. Moreover, these studies point out a crucial function of the well-conserved PHD domain in binding H3K4me3 mark, but it is not yet clear how many proteins with a PHD domain can bind the methylation mark specifically. For example, MLL protein harbors PHD domains that may help the protein “memorize” its target genes or chromatin domain after DNA replication, if its PHD domain specifically binds the H3K4me3 mark. This mode of action conceivably allows a cell to “memorize” its identity and pattern of gene expression during multiple rounds of DNA replication. It is conceivable that menin may also recruit specific chromatin remodeling factors through H3K4me3 to open the local chromatin and facilitate gene transcription. Indeed, we have demonstrated that menin is essential for recruitment of CHD1 to the *Hoxa9* locus in MLL-AF9 transformed bone marrow cells, raising the possibility that CHD1 and other H3K4me3 binding proteins may also play a role in hematopoiesis and leukemogenesis [20].

Adding a further layer of complexity to how histone H3K4 methylation regulates gene transcription, several groups have identified proteins that bind either unmethylated or dimethylated H3K4 (H3K4me2) specifically. WDR5, a WD domain-containing protein, binds di-methylated H3K4 preferentially and recruits MLL to convert H3K4me2 to H3K4me3 by adding the third methyl group [70]. On the other hand, BHC80, another PHD-domain containing protein, specifically binds unmethylated H3K4 and recruits histone H3 demethylase LSD1 to erase H3K4 methylation [71]. Linking unmethylated H3K4 to methylation of genomic DNA, DNMT3L specifically binds the unmethylated H3K4 with its N-terminus and recruits DNA methyltransferase DNMT3 to facilitate de novo methylation of DNA [72]. Thus, differential methylations of H3K4 may relay distinct signals to regulate gene transcription. Currently, little is known as to whether menin plays a role in directly regulating the association of these various H3K4 binding proteins with chromatin.

8. Menin as a regulator of histone modifier in MLL-FP-mediated transformation

To summarize our current understanding of menin’s role as a regulator of histone modifiers in MLL-FP-mediated transformation, we use menin-dependent regulation of *Hoxa9* transcription as an example to propose a model to illustrate how menin recruits histone methyltransferases to regulate *Hoxa9* gene expression in normal and transformed hematopoietic progenitor cells. In normal myeloid cells as shown in Fig. 1b, menin recruits MLL complex to the *Hoxa9* locus and promotes transcription of MLL target genes, via MLL’s H3K4 methyltransferase activity to methylate H3K4 at an appropriate level. However, when MLL fuses through chromosomal translocations with one of its fusion

partners (FPs), such as AF9, AF10 and ENL, it loses its C-terminus harboring the SET1 domain. Since MLL -FPs retain the N-terminal menin binding domain, menin should still be able to recruit MLL -FPs to its target gene loci. Theoretically, MLL fusion with its FPs must lead to loss as well as gain of function for MLL. Loss of HMTase activity of MLL occurs by truncation of the SET1 domain in the C-terminus. Likely, it will lead to reduction of H3K4 methylation at the Hoxa9 locus, as reported by Okada et al in MLL-hDOT1L and MLL-AF10-transformed cells [59]. However, the fact that loss of menin substantially reduces H3K4 trimethylation at the Hoxa9 locus in MLL-AF9 transformed cells suggests that H3K4 seems still important for Hoxa9 expression [20]. How does menin still maintain H3K4 methylation in MLL-FP-transformed cells is an unanswered question. Further, it remains unclear whether MLL or its SET domain is crucial for the MLL-FP-transformed leukemic cells. Further studies on the role of H3K4 methylation and unknown components of the MLL complex in transformed cells will shed light on these questions.

9. Menin in endocrine cells: histone H3K4 methylation and regulation of CDK inhibitors

p18^{INK4c} (p18) and p27^{CIP/KIP} (p27) belong to two distinct families of cyclin-dependent kinase (CDK) inhibitors that inhibits cell cycle progression [73]. p27 is a member of the CIP/KIP family, encodes a protein that binds to and inhibits the activation of cyclin E/A-CDK2 or cyclin D-CDK4 complexes, and thus hinders cell cycle progression from G1 to S transition. On the other hand, p18 is from the INK4 family that specifically inhibits CDK4 and CDK6 kinases at late G₁ phase. Previous studies suggest that loss of both p18 and p27 function result in hyperplasia and/or tumors in the pituitary, adrenals, thyroid, parathyroids, testes, pancreas, duodenum, and stomach [74]. Most of these hyperplastic tissues are from endocrine organs. Therefore, it is tempting to hypothesize that menin may exert its antiproliferative effects via regulation of p18 and p27. Given that p27 and p18 are established cell cycle regulators by inhibiting various CDK complexes, it is conceivable that transcriptional activation of these CDK inhibitors by menin may confer inhibitory effects on endocrine cell proliferation. Indeed, menin has been shown to be essential for p18 and p27 expression in mouse embryonic fibroblasts (MEFs) [75].

Recently, Karnik et al reported that menin may inhibit proliferation of islet cells by promoting histone H3K4 methylation and transcription of p27 and p18 [11]. In vivo study revealed that islet enlargement is correlated with an increase in the number of insulin+ β cells in menin heterozygous mice [11]. Ectopic menin expression in cultured β cells reduces & [A-Za-z]{1,10}; proliferation of MIN6, an insulinoma-derived cell line, whereas MEN1 specific siRNA restored MIN6 cell growth [11]. Furthermore, p27 and p18 are regulated by menin, and the regulation is essential for the inhibitory effects of menin in islet cells [11]. ChIP assay revealed that menin directly binds to the promoters of p27 and p18 and increases H3K4 methylation at these promoters. Moreover, H3K4 methylation at the p18 and p27 loci is reduced in islet tumors from *Men1* mutant mice [11]. In particular, H3K4 trimethylation at the p27 and p18 locus is reduced in islet tumors from MEN1 mutant mice. Methylation of histone tails has been implicated in long-term epigenetic memory, and H3K4 tri-methylation is a generally conserved mark associated with transcriptionally active chromatin [39,76]. Thus, menin-dependent trimethylation of H3K4 may facilitate transcription of p27 and p18. In agreement with this study, our recent data show that excision of the floxed *Men1* in MEFs accelerates G0/G1 to S phase entry [12]. The accelerated S-phase entry is accompanied by increased CDK2 activity as well as decreased expression of CDK inhibitors p18 and p27 [12]. Furthermore, we extended the present results to in vivo condition and observed obvious pancreatic islets enlargement within 7 days after *Men1* excision [12], indicating an acute and direct effect of menin in suppressing β cell proliferation.

Our previous studies demonstrate that only growth of islets cells but not adjacent exocrine cells was significantly enhanced, even though *Men1* was excised in both islet cells and non-islet cells [12]. Moreover, β cell proliferation is observed as early as seven days after *Men1* excision from β cells, highlighting an acute and direct role of menin in regulating β cell proliferation [12]. The specific and direct role of menin in repressing islet cell proliferation has important implications because it may prompt us to develop a novel therapeutic approach by inhibiting menin or menin pathway to treat type I and type II diabetes, diseases in which the mass of β cells are eventually compromised [77]. This idea is further supported by the recent findings that the level of menin expression is normally repressed by prolactin during pregnancy in mice, indicating a physiological downregulation of menin to facilitate islet cell proliferation [78]. Importantly, substantial downregulation of menin for several months in mice leads to enlargement of islets and resistance of gestational diabetes yet fails to lead to tumorigenesis in islets.

As MLL methylates H3K4 and menin interacts with MLL [29], it is conceivable that Menin transcriptionally activates p27 and p18 through controlling MLL-mediated H3K4 modification. p27 expression is reduced in human insulinomas [75] and insulinomas from *Men1* mutant mice [79]. In agreement with this, germline mutations in the p27 gene in humans and rats also lead to a syndrome with endocrine neoplasia [80]. Further, p18 and MEN1 cooperate in repressing the development of insulinoma as shown in *p18* and *Men1* double knock-out mice [81]. Consistent with the role of *p18* in suppressing endocrine hyperplasia, *p18* and *Men1* also cooperate in suppressing the development of non-small cell lung cancer [82]. These results indicate that menin and p18 cooperate, and p18 may be one of major menin effectors in suppressing MEN1 tumorigenesis and development of tumors in other organs, such as the lung.

Milne et al also demonstrated that menin and MLL cooperatively regulate p27 and p18 in MEFs [75]. ChIP assay indicates that both MLL and menin bind to the promoter and the coding region of the p27 and p18 genes [75]. Consistent with these results, MLL-null cells and menin-null cells express a much lower level of p27 and p18 compared to wild-type MEFs [75]. Thus, both MLL and menin appear to be essential for the optimal expression of p27 and p18. Further, menin-null cells proliferate faster than the control wild type cells [75]. However, the study did not address whether menin facilitates MLL binding to the p27 and p18 loci and methylation of the local H3K4.

Approximately 23% of mice with insulinomas from the *Men1*^{+/-} mice do not show a reduction of p27 expression [79]. In addition, mice with the *Men1*^{+/-} and *p27*^{+/-} genotype do not reveal an synergistic nor additive effect in endocrine hyperplasia [81]. These results implicate that the role of p27 in suppressing endocrine hyperplasia or MEN1 tumorigenesis is not absolute, and other molecular events may also be involved in menin-mediated suppression of MEN1 tumorigenesis [79]. With regard to the essential role of menin in MLL-FP-mediated leukemogenesis, an outstanding question is whether menin negatively regulates CDK inhibitors in hematopoietic cells with a manner distinct from its role in endocrine tissues. According to our unpublished data and report from Yokoyama et al. [30], loss of menin did not affect p27 and p18 expression in MLL-AF9-transformed myeloid cells. Therefore, it is most likely that p18 and p27 are not target genes of menin in hematopoietic cells. These results also highlight that the function of menin, or the menin-dependent histone methylation, may modulate gene transcription in a cell type specific manner.

In endocrine cells, *Men1* mutations accelerate cell proliferation as shown in Fig. 1c. Conceivably, menin targets different genes in endocrine cells from those in myeloid cells. For instance, in endocrine cells, menin controls cell proliferation by recruiting MLL to the

p27 and p18 loci and positively regulates their expression. Loss of menin or mutated menin reduces H3K4 methylation level and p27/p18 expression, resulting in increased cell proliferation [11]. Thus, it is likely that menin interacts with the histone modifier MLL in both endocrine and non-endocrine cells, but targets different genes in a tissue-specific manner and leads to opposite phenotypes in regulating cell proliferation. Alternatively, it is also likely that menin targets similar genes to regulate H3K4 methylation at the gene loci, but activating or repressing H3K4me3-binding proteins are recruited to the loci in a tissue specific manner. This possibility is consistent with the opposing role of BPTF and ING2 in transcription, two specific H3K4me3 binding proteins [68,69]. In particular, ING2 recognizes H3K4me3 and represses cyclin D1 expression [68]. Also, it was reported that reduced menin expression leads to overexpression of cyclin D1 and inhibits cell proliferation [18]. In this regard, it is possible that menin may regulate cyclin D1 expression via h3k4me3 binding protein ING2 as shown in Fig. 1c. It remains unclear what mechanism underlies menin's tissue specific actions. Due to the complex and uncertain components of the MLL complex, it is unclear how menin and other components functionally interact and whether there are any additional unidentified components of MLL or menin complex that affect menin's tissue specific actions.

10. Role of menin in nuclear receptor pathway

Another recent report extends the role of menin in regulating steroid hormone receptors in the context of H3K4 methylation [83]. Menin acts as a transcriptional coactivator of the nuclear receptors for estrogen and vitamin D [83]. Activation of the endogenous estrogen-responsive gene TFF1 (pS2) results in recruitment of menin to the TFF1 promoter and elevates trimethylation of H3K4 at the promoter. Menin directly interacts with the estrogen receptor- α (ER α) in a hormone-dependent manner in vitro [83]. Moreover, H3K4 methylation plays a pivotal role in defining the nuclear receptor ligand-mediated induction or regulation of gene expression [84]. These findings are consistent with the role of menin in regulating histone modification, epigenetics, gene transcription, and cell proliferation. Interestingly, another study has also shown that MLL2 associates with estrogen receptor (ER) α and is recruited to promoters of estrogen receptor target genes [85]. Inhibition of MLL2 also inhibit ER target gene expression [85]. However, they did not show if the local H3K4 modification is affected by MLL2 at ER target gene loci. Given that menin is a component of MLL and MLL2 complexes, it is conceivable that menin may serve as a co-activator in estrogen receptor-mediated gene transcription through histone methylation by MLL and/or MLL2.

Given that so many members of the nuclear receptor superfamily may respond to common ligands, these results have multiple implications: 1) Does menin act as a co-activator of estrogen receptor and play a role in ER mediated biological function, i.e. to control cell growth? For example, it is established that estrogen receptor β inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest [86]; 2) Does the interaction of menin with the nuclear receptor contribute to MEN1-related tumorigenesis? Which target genes are involved? 3) Does menin interact with other nuclear receptor members? For instance, Dreijerink et al. has reported a ligand-dependent interaction between menin and retinoid X receptor [83]. Does this interaction take place in vivo and have functional effects on cell proliferation? Does menin also act as a co-activator in RAR or RXR mediated transcriptional gene regulation? Given fusion proteins of PML, PLZF with RAR are key players in acute promyelocytic leukemia (APL), it is unclear whether co-activator menin (if it is) is essential for regulating target genes of PML-RAR and PLZF-RAR fusion protein mediated leukemogenesis.

11. Global role of menin and MLL in regulating H3K4 methylation in genome

A recent study mapped genomic binding sites of menin using ChIP assays coupled with microarray analysis [87]. Menin has been shown to localize to the promoters of thousands of human genes in HeLa S3, HepG2 and pancreatic islet cells, suggesting a global role for menin in gene transcription in both endocrine and non-endocrine-derived cells [87]. Notably, menin and MLL also co-localize to the promoters of many human genes. For instance, menin sites were also bound by MLL1 and Rbbp5: 49.3% in HeLa S3 cells; 56.7% in HepG2 cells, and 46.1% in pancreatic islets [87]. Although menin does not always bind to promoters together with MLL, menin binds to nearly half of MLL target genes and appears to be essential for MLL-mediated H3K4 methylation at these loci. In this study, menin has also been shown to enhance expression of p27 and p18 in pancreatic islets. However, the expression of p27 and p18 in the liver where both the *Men1* alleles were ablated was also significantly reduced [87]. This study has also demonstrated that expression of 184 genes whose promoters were bound by menin in wild-type human islets was altered in *Men1*-null mouse islets. Of these 184 genes, only about a third were downregulated in their expression by *Men1* excision [87]. Thus, although menin binds a large group of gene loci, it is absolutely required for regulation of transcription of only a relatively small group of genes, suggesting a redundancy in its function. In addition to p18, whose regulation by menin was reported in menin-null cells, some other highly cell proliferation-related genes were also listed [87]. Such as proliferating cell nuclear antigen (PCNA), cyclin C, cyclin I and ataxia telangiectasia-mutated gene (ATM) [87]. Whether these genes are involved in menin-mediated inhibition of cell growth warrants further investigation. In another gene expression profiling attempt in insulinomas of β cell-specific *Men1* mutant mice, multiple cell cycle related genes (including cyclin A2, B2 and D2) and epigenetic changes were also identified, suggesting that epigenetic mechanisms and cell cycle regulation are involved in tumorigenesis of islet insulinoma after loss of menin [88].

12. Perspective

Accumulating evidence in recent years suggests that menin is not only a tumor suppressor gene in multiple endocrine organs but also an oncogenic cofactor promoting proliferation of hematopoietic cells and development of mixed lineage leukemia. The essential role of menin in MLL-FP-mediated leukemogenesis revealed the “evil face” of the tumor suppressor gene menin in contrast to its benign role in suppressing endocrine cell proliferation as shown in Fig. 1b.

Many outstanding questions remain to be addressed: 1) What mechanisms account for the cell or tissue specific roles of menin in non-endocrine tissues? Based on the current knowledge, menin acts as an essential co-factor in MLL-FP-mediated leukemogenesis. Since MLL fusion with other partner proteins normally only occurs in hematopoietic cells, it is conceivable that menin interacts with MLL, MLL-FPs and other cofactors in hematopoietic cells and promotes cell proliferation and differentiation arrest. 2) In endocrine tissues, menin exerts an antiproliferative role, at least in part through transcriptional regulation of p18 and p27, while *Men1* excision in liver cells does not affect cell proliferation and tumorigenesis in the liver [13]. It remains unclear whether menin-dependent H3K4 methylation plays a crucial role in menin-dependent repression of proliferation of endocrine cells, nor is it understood how menin represses proliferation of endocrine cells yet stimulates proliferation of MLL cells. 3) We recently showed that ablation of menin almost abolished both active and repressive histone modifications in the *Hoxa9* locus, suggesting an even more broad role of menin in histone modification [89]. However, how does menin regulate repressive histone markers, such as Histone 3 lysine 9

di-methylation and histone 3 lysine 27 di-methylation, remains an open question. 3) It usually takes 6–9 months for mice to develop overt endocrine tumors after homozygous *Men1* excision, suggesting that secondary events are necessary for formation of MEN1 associated tumors. However, it is elusive as to what the secondary events are, e.g., mutations in other tumor suppressors and oncogenes in MEN1 tumorigenesis. 4) It was recently reported that MEN1 was predicted by TargetScan as a target of microRNA mir-24 and expression of most microRNAs is highly tissue specific [90,91]. This raises an interesting question as to whether this microRNA or other hitherto unidentified miRNAs also regulate menin expression in a tissue-specific manner. 5) Menin is phosphorylated at multiple sites [92], and also its own transcription is regulated by prolactin during mouse pregnancy [78]. Interesting questions arise as to how various extracellular signals regulate menin expression and posttranslational modifications to control proliferation of endocrine and non-endocrine cells. Answers to these questions will certainly further our understanding of how menin regulates cell proliferation, development of endocrine tumors and leukemia, function of beta cells, and diabetes. Insights from these studies will likely shed new light on treating endocrine tumors, leukemia, and diabetes.

Acknowledgments

We thank Jizhou Yan and Austin Thiel for critical reading of the manuscript and discussion. This work is in part supported by NIH grants (R01 CA113962 and R01 CA100912), a Leukemia and Lymphoma SCOR grant, and a grant from American Diabetes Association. We appreciate the valuable comments from other members of our laboratories.

Abbreviations

CDK	cyclin-dependent kinase
ChiP	Chromatin immunoprecipitation
FPs	Fusion proteins
H3K4	histone H3 lysine 4
H3K4me3	H3K4 trimethylation
H3K79	Histone H3 lysine 79
HDAC	histone deacetylase
LOH	loss of heterozygosity
MEF	mouse embryonic fibroblasts
MEN1	Multiple endocrine neoplasia type 1
MLL	<u>m</u> ixed <u>l</u> ineage <u>l</u> eukemia
NURF	nucleosome remodeling factor
PcG	Polycomb
Pol II	RNA polymerase II
trx	<i>trithorax</i>

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Fig. 1.

(a) A schema of wild type MLL and MLL fusion-proteins resulted from chromosomal translocation. The MLL protein functional domains, menin binding domain, breaking point and cleavage point are denoted. Abbreviations: PHD, plant homeo domain; SNL, subnuclear localization. (b) A Model to illustrate the role of menin in transcriptional regulation on Hoxa9 expression in concert with MLL in MLL-FPs transformed hematopoietic cells. Abbreviations: BPTF, bromodomain PHD finger transcription factor; CHD1, chromodomain helicase DNA binding protein 1; TFX, transcription factor X. (c) A model that illustrates the role of menin in transcriptional regulation of p27, p18 and cyclin D in endocrine tissues.

Simultaneous association of CHD1/BPTF, which promotes transcription, and ING2, which represses transcription, is hypothetical.