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Epigenetic regulation by the menin pathway

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Abstract

There is a trend of increasing prevalence of neuroendocrine tumors (NETs), and the inherited multiple endocrine neoplasia type 1 (MEN1) syndrome serves as a genetic model to investigate how NETs develop and the underlying mechanisms. Menin, encoded by the *MEN1* gene, at least partly acts as a scaffold protein by interacting with multiple partners to regulate cellular homeostasis of various endocrine organs. Menin has multiple functions including regulating several important signaling pathways by controlling gene transcription. Here, we focus on reviewing the recent progress in elucidating the key biochemical role of menin in epigenetic regulation of gene transcription and cell signaling, as well as posttranslational regulation of menin itself. In particular, we will review the progress in studying structural and functional interactions of menin with various histone modifiers and transcription factors such as MLL, PRMT5, SUV39H1 and other transcription factors including c-Myb and JunD. Moreover, the role of menin in regulating cell signaling pathways such as TGF-beta, Wnt, and Hedgehog, as well as miRNA biogenesis and processing will be described. Further, the regulation of the *MEN1* gene transcription, posttranslational modifications and stability of menin protein will be reviewed. These various modes of regulation by menin as well as regulation of menin by various biological factors broaden the view regarding how menin controls various biological processes in neuroendocrine organ homeostasis.

Keywords

MEN1; menin; neuroendocrine tumor; epigenetic regulation

Introduction

In recent years, prevalence of neuroendocrine tumors is increasing (Jiao, et al. 2011), and the multiple endocrine neoplasia type 1 (MEN1) gene, which encodes protein menin, is

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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genetically well characterized for its function in regulating cell homeostasis in various endocrine organs including the pituitary, parathyroid glands, and pancreatic islets. Various excellent reviews on the structure and functions of menin in regulating cell proliferation and tumor suppressing have been reported (Agarwal, et al. 2005; Balogh, et al. 2010; Balogh, et al. 2006; Matkar, et al. 2013; Wu and Hua 2008; Yang and Hua 2007). Here, we mainly focus on reviewing the recent progress in the key biochemical role and the underlying mechanisms of menin in epigenetic regulation, as well as posttranslational regulation of menin itself.

Regulation of histone modifying enzymes by menin

Menin regulates MLL—It has been reported that menin interacts with mixed lineage leukemia 1 (MLL1) (Canaff, et al. 2012a) and MLL2 (Hughes, et al. 2004), both harbor a SET-domain that possess histone 3 lysine 4 (H3K4) methyltransferase. MLL1 was originally identified as a gene interrupted in chromosomal translocations responsible for the development of acute leukemia in adults (Marschalek 2011), with an even higher incidence in infant leukemia (Tomizawa, et al. 2007). The *MLL* gene can fuse with one of over 60 partner genes via chromosomal translocation, leading to formation of chimeric MLL fusion proteins. These fusion proteins retain an approximately 1,400 amino acid N-terminal fragment. Expression of MLL fusion proteins enhances proliferation and blocks differentiation of hematopoietic cells, ultimately leading to acute leukemia (Slany 2005).

Crystallographic studies indicate that menin adopts a rectangular-shaped conformation that resembles a curved left hand, with a deep pocket formed by the thumb and the palm (Huang, et al. 2012). Menin binds a short peptide from the N-terminus of MLL1 with a pocket structure. The part of MLL binding to menin is the N-terminus retained in all MLL fusions (Yokoyama, et al. 2005; Yokoyama, et al. 2004). Menin forms a complex with MLL and several other cofactors including WDR5 and ASH2L, leading to histone 3 lysine 4 trimethylation (H3K4me3) at the promoter of the target genes (Caslini, et al. 2007; Chen, et al. 2006; Hughes et al. 2004; Milne, et al. 2005; Onodera, et al. 2010; Thiel, et al. 2010; Yokoyama et al. 2005). For instance, in MLL fusion protein-induced leukemia cells, menin is required for expression of certain homeotic genes, such as *Hoxa9*, *Hoxc6*, and *Hoxc8* in concert with MLL1 (Hughes et al. 2004; Yokoyama et al. 2004). Targeted deletion of the *Men1* gene reduces the binding of MLL1 to the *Hoxa9* locus (Chen et al. 2006; Tomizawa et al. 2007). On the other hand, in endocrine cells or mouse embryonic fibroblasts (MEFs), menin is required for MLL1 binding to the cyclin-dependent kinase (CDK) inhibitors, $p27^{Kip1}$ and $p18^{Ink4c}$ loci to increase H3K4me3 and induce their expression (Milne et al. 2005). Menin is also required for recruiting MLL1 to the GATA3 locus to regulate GATA3 expression and Th2 cytokine production in T helper type 2 (Th2) cells (Onodera et al. 2010). These studies all strongly suggested that menin plays an important role in recruiting MLL methyltransferase complex to the promoters of the target genes and increase their transcription.

Besides directly binding to the N-terminus of MLL1 via the central pocket, menin also directly binds chromatin associating protein LEDGF (lens epithelium-derived growth factor) (van Nuland, et al. 2013; Yokoyama and Cleary 2008). LEDGF is crucial for co-localization

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of menin and wild type MLL1 or MLL fusions to the loci of menin/MLL target genes such as *Hox* and *CDKIs* (Yokoyama and Cleary 2008). Importantly, crystallographic studies indicate that menin, the N-terminal part of MLL, and LEDGF form a ternary complex in which a helical structure from LEDGF sits on a “V” shape structure formed from a MLL helix and menin. This structure clearly shows that menin acts as a scaffold to recruit both MLL and LEDGF (Huang et al. 2012). Moreover, although menin has no DNA binding domains, menin can associate with chromatin (Jin, et al. 2003), and directly binds to genomic DNA via the positively charged amino acid residues in the nuclear localization signals (NLSs) of menin (La, et al. 2006). These findings indicate that menin acts as a scaffold to recruit the MLL complex to its target genes partly via its binding to genomic DNA via the NLSs.

To broaden the view of menin binding to genomic DNA in an unbiased manner, genome-wide analysis shows that menin and MLL1 co-localize to the promoters of thousands of human genes, but they do not always bind together (Scacheri, et al. 2006). In addition, menin and MLL could regulate distinct pathways separately in hematopoietic stem cells and developing B cells (Li, et al. 2013). Nevertheless, targeted deletion or knockdown of the *Men1* gene in various types of cells only affects expression of several dozens of genes (Li et al. 2013), indicating that menin is not required for regulating expression of all the genes it binds as shown in the chromatin immunoprecipitation (ChIP) assay. Consistent with this, menin is not absolutely required for MLL1-mediated normal hematopoiesis (Li et al. 2013), but is required for maintenance of MLL fusion protein-induced leukemia cells (Bouffioux and Dusart 1974; Grembecka, et al. 2012).

Menin regulates PRMT5—Menin also regulates protein arginine methyltransferase 5 (PRMT5) function (Gurung, et al. 2013b), a member of PRMT family (Bedford and Clarke 2009; Stopa, et al. 2015). Based on distinct catalytic effect on forming either asymmetric or symmetric dimethylation at arginine, PRMT family members are classified into either type 1 or type 2, accordingly. PRMT5 belongs to type 2 enzyme and works together with its cofactor MEP50 to mediate the methylation of histones H2A and H4 at arginine 3 and histone H3 at arginine 8 (Karkhanis, et al. 2011). PRMT5 also has multiple non-chromatin protein substrates such as p53, p65 and HoxA9 (Bandyopadhyay, et al. 2012; Jansson, et al. 2008; Le Guezennec, et al. 2006; Wei, et al. 2013; Yang, et al. 2014). In addition, PRMT5 can repress globin gene expression through recruitment of DNA methyltransferase 3A (DNMT3A), indicating a potential cross talk between histone arginine methylation and DNA methylation (Girardot, et al. 2014; Rank, et al. 2010; Zhao, et al. 2009). Posttranslational histone modifications catalyzed by PRMT5 significantly affect gene expression and regulate cell growth and proliferation (Scoumanne, et al. 2009). Our previous data shows that menin directly binds to PRMT5 and target histone 4 arginine 3 symmetric dimethylation (H4R3me2s) at promoters of *Gas1* and *Gli1* to suppress Hedgehog (HH) signaling and proliferation of neuroendocrine cells (Gurung, et al. 2013a; Gurung et al. 2013b).

Menin regulates PRMT5’s function by several modes. First, menin can suppress Glucagon-like peptide-1 (GLP1) induced and PKA-mediated phosphorylation of both FOXO1 and cyclic adenosine monophosphate (cAMP) response element binding protein (CREB), likely through PRMT5 or PRMT5-like enzyme-mediated methylation of FOXO1 and CREB

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(Muhammad, et al. 2017). Second, menin recruits PRMT5 to the promoter of the *Gas1* gene, increases repressive H4R3me2s and suppresses Gas1 expression. Third, menin also can recruit PRMT5 to the promoter of *Gli1* and subsequently repress Gli1 expression and thus Hedgehog signaling (Gurung et al. 2013a). However, the further molecular details remain to be investigated.

Menin regulates SUV39H1 and Daxx—Menin has been reported to silence transcription of target genes via interacting with the suppressor of variegation 3–9homolog protein 1 (SUV39H1) and increasing the histone 3 lysine 9 trimethylation (H3K9me3) at the promoter of the target genes (Feng, et al. 2017; Song, et al. 2014; Yang, et al. 2013). SUV39H1 is a histone 3 lysine 9 (H3K9) methyltransferase (Rea, et al. 2000). Menin interacts with SUV39H1 through 360–445 amino acid region of menin and recruits SUV39H1 to the promoters of *GBX2* and *IL6* and represses their expression via enhancing H3K9me3 (Song et al. 2014; Yang et al. 2013). Recently, we found that menin directly binds to Daxx/ATRX complex and further recruits SUV39H1 to the promoter of membrane metallo-endopeptidase (Mme, or CD10), and represses Mme expression by enhancing H3K9me3 at the Mme promoter (Feng et al. 2017). Daxx is a H3.3-specific histone chaperone and deposits H3.3 at specific chromatin regions in cooperation with ATRX (Drane, et al. 2010; Lewis, et al. 2010) and frequently mutated in neuroendocrine tumors (Jiao et al. 2011). Menin and Daxx are required for each other to recruit SUV39H1 to the Mme promoter, and menin directly binds to Daxx through 396–450 amino acid region (Feng et al. 2017). Daxx also directly binds to ATRX which may also bind SUV39H1 binding domain (Feng et al. 2017; Tang, et al. 2004; Yang et al. 2013). These findings suggest that menin and Daxx/ATRX firstly form a complex and then recruit SUV39H1 to the Mme promoter and increase H3K9me3, leading to suppression of Mme expression.

Crosstalk between menin and transcription factors

Several transcription factors have been reported to interact with menin. Some of them have been shown to be functionally important in their interaction.

JunD—Menin interacts with JunD and represses the JunD activity (Agarwal, et al. 1999; Huang et al. 2012). JunD is a transcription factor belonging to the AP1 family and is involved in negative control of cell proliferation (Hernandez, et al. 2008). The crystal structural study showed that menin binds JunD via its central pocket which can also bind to the MLL1 peptide (Huang et al. 2012). In other words, menin uses the same pocket to bind either MLL1 or JunD. A conserved sequence (FPXXP) is found in the menin binding domain (MBD) of both JunD and MLL (Huang et al. 2012). The region of JunD that binds menin spans 27–47 amino acid residues (Agarwal et al. 1999; Huang et al. 2012). Earlier biochemical studies have suggested that menin binds to JunD to repress JunD-induced transcription through interaction with co-repressors mSin3A and HDAC 1 or 2 in cultured cells (Agarwal et al. 1999; Gobl, et al. 1999; Kim, et al. 2003). Recently, we unraveled a new mechanism whereby menin binds JunD and thus block c-Jun N-terminal kinase (JNK)-mediated phosphorylation of JunD at residues S90, S100, and T117, an event crucial for activating JunD function in transcriptional regulation (Huang et al. 2012). Coincidently, both the basic residues and the leucine residues in JunD are bound by JNK to phosphorylate

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JunD, and these residues are also bound by menin. As such, menin binding to JunD effectively blocks JNK's interaction with JunD and thus inhibits JNK-mediated phosphorylation and activation of JunD. This finding well explains why JunD activates proliferation of MEFs in the absence of menin, but suppresses proliferation in the presence of menin (Agarwal, et al. 2003). In addition, JunD has two isoforms: full-length and truncated isoforms, and menin only can bind to full-length isoform of JunD (Yazgan and Pfarr 2001).

c-Myb and c-Myc—c-Myb binds and recruits menin to the promoters of *Hox* genes (Jin, et al. 2010). c-Myb, GATA-3, and menin form a core transcription complex that promotes expression of *GATA-3* and *Hox* genes in leukemia cells. This complex also recruits MLL to promote Th2 cell maturation (Nakata, et al. 2010). However, unlike regulation by menin/MLL-mediated H3K4me3 at the promoter (Jin et al. 2010) of the target genes, a recent report shows that menin can directly interact with the transactivation domain (TAD) of Myc and then bind to E-boxes to enhance transcription of Myc target genes (Wu, et al. 2017). This enhanced transcription of MYC target genes depends on P-TEFb, a key factor to facilitate transcription regulation by MYC (Wu et al. 2017). The above findings indicate that, by transcriptionally promoting the expression of MYC target genes, menin can stimulate cell proliferation, cellular metabolism and cancer progression in certain types of cancers including MLL fusion protein-induced leukemia cells.

NF-κB—The NF-κB has several components including p50, p52 and p65, and found to interact specifically with menin *in vitro* and *in vivo* (Heppner, et al. 2001). The binding region of NF-κB proteins with menin is at their N-terminal part. The interaction between menin and NF-κB represses PMA (phorbol 12-myristate 13-acetate)-stimulated or TNF-α-stimulated NF-κB activation in HeLa, Cos7, and NTERA-2 cells. p65 phosphorylation was dramatically increased by RET constitutive activation and was negatively correlated with menin expression in parathyroid neoplasia (Corbetta, et al. 2005). In menin knockdown IEC-17 cells, a non-transformed crypt-like cell line, cyclin D1 expression is increased and the increased expression is partly due to the up-regulation of NF-κB-mediated transcription (Theillaumas, et al. 2008). These findings demonstrate a correlation among menin, NF-κB, and cyclin D1 in regulating proliferation of the intestinal epithelial cells. However, it remains to be investigated whether menin and NF-κB are co-localized to the loci of their target genes to regulate gene expression or not. One study in hepatocellular carcinoma showed that menin could repress p65 acetylation through recruitment of Sirt1, an enzyme that deacetylases p65 in lysine 310 (K310) (Gang, et al. 2013). In RPA2 high expression-dependent breast cancer cells, RPA2 decreases the binding level between menin and NF-*k*B in a competitive way and relieves the antagonistic function of menin on NF-κB-regulated transcription (Chen, et al. 2017). These studies suggest that menin regulates gene transcription via directly modifying NF-κB pathway in certain types of cancer cells.

Menin regulates various signaling pathways

TGF-beta signaling pathway—Transforming growth factor beta (TGF-beta) superfamily proteins have many important biological functions, including regulation of tissue differentiation, cell proliferation, and migration in both normal and cancer cells (Yokobori

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and Nishiyama 2017). Menin regulates TGF-beta signaling and TGF-beta-induced gene transcription by interacting with Smad3, a TGF-beta downstream effector (Kaji, et al. 2001). Menin directly interacts with Smad3 and inhibit Smad3/4-DNA binding at specific transcriptional regulatory sites to regulate their expression (Kaji et al. 2001). When the *Men1* gene is deleted in leydig cells, the effect of TGF-beta-induced inhibition of proliferation was reduced (Hussein, et al. 2008). Consistently, Canaff et al showed that menin mutation compromises menin's function in promoting TGF-beta-induced Smad3 transcriptional activity (Canaff et al. 2012a). Thus, menin mutation deprives its function in promoting TGF-beta signaling-induced repression of cell proliferation, likely contributing to the development of MEN1. On the other hand, it is also reported that TGF-beta up-regulates menin expression in MLL-AF9 transformed mouse bone marrow cells (Zhang, et al. 2011). Moreover, menin expression was down-regulated in MLL-AF9 transformed mouse bone marrow cells when TβRII, a vital component in TGF-beta signaling pathway, was deleted. Menin expression was also decreased in liver samples from the conditional TβRII knockout mice after TβRII excision (Zhang et al. 2011).

Wnt signaling—Menin was shown to be crucial for regulating canonical Wnt/β-catenin signaling in cultured rodent islet tumor cells via interaction with β-catenin (Chen, et al. 2008). In *Men1*-null MEFs and insulinomas from β-cell-specific *Men1* knockout mice, β-catenin accumulates in the nucleus, but overexpression of menin reduces nuclear accumulation of β-catenin and suppresses its transcriptional activity (Cao, et al. 2009). Wnt signaling stimulates pancreatic islet β cell proliferation, possibly by increasing expression of paired-like homeodomain 2 (Pitx2) (Rulifson, et al. 2007). When menin and activated β-catenin are overexpressed in islet tumor cells, the Wnt/β-catenin downstream target gene, *Axin2*, is significantly enhanced, correlating with increased H3K4me3 at the promoter of the *Axin2* gene (Chen et al. 2008). It is possible that menin promotes Wnt signaling in late stage of islet tumor development or inhibits Wnt signaling to prevent β cells from tumorigenesis at the early stage. These opposite results for the role of menin on the Wnt signaling pathway and cell proliferation may depend on distinct context of cells.

Conditional knockout of β-catenin in *Men1*-deficient mice leads to suppression of tumorigenesis and significantly improved hypoglycemia and the survival rate of the mice (Jiang, et al. 2014). Antagonizing β-catenin signaling by the small molecule inhibitor PKF115–584 in *Men1*-deficient mice also suppresses tumor cell proliferation *in vitro* and *in vivo* (Jiang et al. 2014). These findings suggest that suppression of β-catenin signaling inhibits the expression of pro-proliferative genes in *Men1*-null islets and improves hyperinsulinemia and hypoglycemia in the mice.

Hedgehog signaling—In pancreatic ductal adenocarcinoma, menin expression was suppressed by DNA methyltransferase 1 (DNMT1) downstream of the Hedgehog signaling pathway, and menin overexpression strongly antagonized the promotion effect of Hedgehog signaling on pancreatic cancer cell proliferation (Cheng, et al. 2016). Aberrant activity of the Hedgehog signaling pathway has been reported in many types of cancers including basal cell carcinoma (BCC) (Xie, et al. 1998) and medulloblastoma (Thompson, et al. 2006). Moreover, the expression of Hedgehog ligand is enhanced in human gastrointestinal

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neuroendocrine tumors and in mouse small cell lung cancer (Park, et al. 2011; Shida, et al. 2006). In genetically engineered mouse models of pancreatic ductal adenocarcinoma (PDAC), inhibition of Hedgehog signaling results in depletion of the stroma surrounding the tumor, stimulating angiogenesis and enhancing delivery of chemotherapeutic drugs to inhibit the cancer cells (Olive, et al. 2009).

It is suggested that the Hedgehog/DNMT1/menin axis is a potential molecular target for pancreatic cancer therapy (Cheng et al. 2016). Our previous study indicated that menin antagonizes Hedgehog signaling, partly via increasing PRMT5-mediated repressive H4R3me2s at the *Gas1* promoter in neuroendocrine cells (Gurung et al. 2013b). *Men1*-null cells complemented with menin mutants fail to repress Gas1 mRNA levels, but the wild type did so (Gurung et al. 2013b). Moreover, menin and PRMT5 also suppress Hedgehog signaling at a second step besides Gas1, by inhibiting the expression of Hedgehog downstream effector Gli1. These findings indicate that loss of menin-mediated repressive histone methylation, H4R3me2s, at the *Gas1* and *Gli1* promoters and resulted up-regulated Hedgehog signaling play a role, at least in part, towards pathogenesis of the MEN1 syndrome.

Menin and PI3K/AKT signaling—Heterozygous *Men1* knockout mice developed insulinoma (Bertolino, et al. 2003) and *Men1* excision increased proliferation of β cells and islet size (Yang, et al. 2010a; Yang, et al. 2010b), indicating that menin is crucial for regulating β cells. Menin expression was reduced in high glucose stimulated INS1 insulinoma cells and primary rat islets (Zhang, et al. 2012). PI3K/AKT inhibitors suppress glucose induced repression of menin expression. (Zhang et al. 2012). A major downstream target of the PI3K/AKT pathway, FOXO1, promotes menin expression in INS1 cells by binding to the promoter of menin (Wang, et al. 2011; Zhang et al. 2012). Activated AKT can phosphorylate FOXO1 and decline its transcription activity (Martinez, et al. 2006), further to down-regulate menin expression.

Menin regulates pancreatic islet alpha cell to beta cell differentiation—Menin is also a crucial factor inhibiting pancreatic islet alpha cell trans-differentiation into beta cell lineage. Mice with alpha cell-specific knockout of the *Men1* gene developed insulin-secreting beta cell tumors or insulinomas 6 months following the *Men1* deletion (Lu, et al. 2010). Genetic cell lineage tracing analysis showed that insulinoma cells were directly derived from transdifferentiating glucagon-expressing cells (Lu et al. 2010). It remains to be investigated as to what pathway(s) is perturbed or the epigenetic reprogramming altered in the *Men1* deleted alpha cells, resulting in alpha cell to beta cell trans-differentiation.

Regulation of GI-NETs by menin—As a tumor suppressor, menin epigenetically inhibits proliferation of neuroendocrine tumors via several different pathways, such as menin binds to PRMT5 and enhances H4R3me2s at promoters of *Gas1* and *Gli1* to suppress Hedgehog signaling (Gurung et al. 2013a; Gurung et al. 2013b) and menin also recruits Daxx/ATRX and SUV39H1 to Mme promoter and represses Mme expression through increasing H3K9me3 level (Feng et al. 2017). Neuroendocrine tumors have several different types, such as gastrointestinal neuroendocrine tumors (GI-NETs), which are the most frequent type due to about 75% of these tumors have stimulation of gastrin. GI tract NETs

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are usually associated with MEN1 or Zollinger-Ellison syndrome (ZES), especially in the duodenum or stomach. 23% of the GI-NETs are gastric carcinoids (Burkitt and Pritchard 2006), the incidence of which has been increased recent years. Type 2 gastric carcinoids comprise 5–8% of all the gastric carcinoids. 16% duodenal gastrinomas have the mutations in the *MEN1* gene and these tumors developed type 2 gastric carcinoids (Anlauf, et al. 2005). Merchant group reported that *Men1* deletion in the gastrointestinal mucosa induces hypergastrinemia and epithelial hyperplasia, but no tumors developed (Veniaminova, et al. 2012). However, their further study showed that mice with double conditional knockout of *Men1* and somatostatin genes developed gastric carcinoids (Sundaresan, et al. 2017). They also found that the developed gastric carcinoids are correlated with nuclear export of p27 and the human gastric carcinoids have the p27 protein loss. The loss of p27 protein is possible due to the phosphorylation induced degradation. On the other hand, menin recruits MLL to the promoters of $p27^{Kip1}$ and $p18^{Ink4c}$ and increases their expression by enhancing the H3K4me3 (Milne et al. 2005). Therefore, the reduced p27 protein level may be also partly due to the inactivating mutations of *MEN1* gene.

Menin and K-Ras signaling regulate each other—Menin suppresses both proliferation and migration of lung adenocarcinoma cells partly via inhibiting PTN and its receptor RPTP β/ζ signaling (Feng, et al. 2010; Gao, et al. 2009). However, menin also is repressed by K-Ras through DNMT1 dependent DNA demethylation of the promoter of the *MEN1* gene in lung adenocarcinoma cells and inversely menin reduces the level of active Ras-GTP at least partly by preventing GRB2 and SOS1 from binding to Ras (Wu, et al. 2012), these studies suggest a potential negative feedback loop between menin and K-Ras, and menin plays a crucial role in K-Ras induced lung cancer development.

Menin and miRNAs

miRNA biogenesis—We previously found that menin interacts with arsenite-resistant protein 2 (ARS2), a component of the nuclear RNA CAP-binding complex that is crucial for biogenesis of certain miRNAs including let-7a (Gurung, et al. 2014). Menin does not affect levels of primary-let-7a (pri-let-7a), but increases the levels of mature let-7a (Gurung et al. 2014). Let-7a targets, including insulin receptor (INSR) and insulin receptor substrate 2 (IRS2), pro-proliferative genes that are crucial for insulin-mediated signaling, are up-regulated in *Men1*-excised cells (Gurung et al. 2014). Inhibition of let-7a using anti-miRNA in wild type cells is sufficient to enhance the expression of IRS2. Depletion of menin inhibits conversion of pri-miRNA to pre-miRNA. Knockdown of ARS2 in menin-expressing cells repressed let-7a processing. However, ARS2 knockdown had little impact on let-7a expression in menin-deleted cells. These findings unravel a mechanism whereby menin suppresses cell proliferation, at least partly by promoting the biogenesis and processing of certain miRNAs, including let-7a, to insulin signaling and likely endocrine cell proliferation.

Other miRNA processing—MiR-24-1 directly binds to the highly conserved 3'UTR region of *MEN1* mRNA, and represses menin expression, and the negative feedback loop between miR-24-1 and menin protein is essential for MEN1 tumorigenesis (Luzi, et al. 2012). Vijayaraghavan et al. also found that miR-24 directly decreases menin expression and impacts downstream cell cycle inhibitors in neuroendocrine tumors (Vijayaraghavan, et al.

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2014). Moreover, miR-24 inhibition increases menin expression and decreases cholangiocarcinoma cell proliferation (Ehrlich, et al. 2017). Except for miR-24, several other miRNAs have been found to have the ability to repress menin expression in various tissues. Overexpression of miR-421 represses menin expression and enhanced cell proliferation and invasion of neuroblastoma cells (Li, et al. 2014). Ectopic expression of MiR-29b precursor reduced, but inhibition of miR-29b increased the mRNA and protein levels of menin (Ouyang, et al. 2015). MiR-17 induced by glucose inhibits menin expression via targeting its 3'-untranslated region and promotes pancreatic β cell proliferation (Lu, et al. 2015). More recent findings showed that expression levels of miR-762 and menin in ovarian cancer tissues are correlated, and miR-762 down-regulates menin expression through a binding site in the 3'UTR region, and further increases the Wnt signaling pathway to promote ovarian cancer (Hou, et al. 2017). Taken together, menin expression can be repressed by multiple distinct miRNAs at posttranscriptional level, while menin also can regulate expression of some miRNAs.

Regulation of menin transcriptional expression

Regulation of menin expression by prolactin signaling—As menin plays an important and pleiotropic role in regulating multiple functions of islets, it is conceivable that its expression is controlled by numerous signals. During late stage of pregnancy of mice, prolactin is produced, and prolactin binds its cell surface receptor, and phosphorylates and activate STAT1 (Jabbour, et al. 1998; Karnik, et al. 2007; Tourkine, et al. 1995). The activated STAT1 translocates into the nuclear in beta cells, and then binds to the promoter of the *Men1* gene, to suppress transcription of the *Men1* gene (Karnik et al. 2007). Notably, controlled expression of the *Men1* transgene in the pancreatic islets attenuates the prolactin-induced repression of the *Men1* expression in the pancreatic islets, leading to gestational diabetes in the mice (Karnik et al. 2007). These findings indicate that menin is physiologically regulated to adapt the pancreatic islets to counteract the development of gestational diabetes. More work remains to investigate whether this molecular circuitry is also conserved in human pancreatic islets.

Regulation by FOXO1—While menin is repressed by the prolactin signaling pathway (Karnik et al. 2007), menin expression is activated by FOXO1 (Zhang et al. 2012), which is a member of Forkhead box containing transcription factor family (Anderson, et al. 1998). A key feature of this family of transcription factors is that many of them can be phosphorylated by receptor tyrosine kinases (RTK) via activating PI3K/AKT pathway (Brunet, et al. 1999). AKT can phosphorylate FOXO1 at three distinct sites, Thr24, Ser256 and Ser319. For instance, insulin induces FOXO1 phosphorylation by activating PI3K and AKT axis, and the phosphorylated FOXO1 binds to 14-3-3 protein, and is sequestered in the cytoplasm (Tzivion, et al. 2011). Moreover, the phosphorylated FOXO1 in the nucleus can also be exported into the cytoplasm. Thus, the net outcome of phosphorylation of FOXO1 is to reduce its nuclear localization and thus its activity in transcriptional activation (Vogt, et al. 2005). Further, the phosphorylated FOXO1 is also channeled for ubiquitin-dependent protein degradation (Aoki, et al. 2004; Matsuzaki, et al. 2003). It is clear that FOXO1 is crucial for regulating insulin signaling and also cell metabolism (Kitamura 2013). As such, it is quite interesting to note that glucose induces activation of AKT (Zhang et al. 2012), and

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suppresses FOXO1 activity. Activation of FOXO1 upon reducing phosphorylation leads to increased menin expression. Therefore, menin regulation is linked to an important signaling pathway regulating beta cell, metabolism and diabetes.

Somatostatin increases menin expression—Somatostatin is a peptide hormone and inhibitor of gastrin expression and secretion. Menin also inhibits the expression of gastrin. Somatostatin analog octreotide increases the mRNA and protein levels of menin in the duodenum of mice. While octreotide inhibited PKA enzyme activity and forskolin treatment, which increases cellular cAMP and PKA activity, suppressed menin protein level (Mensah-Osman, et al. 2008). However, whether and how the somatostatin pathway regulates PKA to induce the expression of menin remains to be elucidated.

Regulation of menin by posttranslational modifications

Posttranslational modifications (PTMs), also known as protein level regulation, play a crucial role for regulating protein functions and thus cellular processes and various biological activities such as transcriptional regulation and cell signaling (Orford, et al. 1997; Waby, et al. 2008). Currently, multiple PTMs have been found for various proteins, including phosphorylation, methylation, acetylation, ubiquitination, myristylation, nitration, glycosylation and SUMOylation (Ribet and Cossart 2010). For instance, tumor suppressor and transcription factor p53 undergoes extensive posttranslational modifications, such as ubiquitination, methylation, SUMOylation, and phosphorylation to regulate it's a variety of biological activities (Brooks and Gu 2011; Meek 2015; Stehmeier and Muller 2009). Recently, menin has been found to be regulated by various PTMs including ubiquitination, SUMOylation, phosphorylation, and palmitoylation (He, et al. 2016). However, the biological and functional impact of these PTMs of menin remains to be further investigated.

Ubiquitination—The first posttranslational modification of menin was found to be ubiquitination in 2004 by Naganari Ohkura group (Yaguchi, et al. 2004). They found that MEN1-derived missense mutant menin protein has much short half-life as compared to wild type menin protein in transfected 293 cells. Further studies reveal that the reduced levels of the mutant proteins were a result of rapid menin degradation through ubiquitin-mediated degradation. The unstable menin mutants, but not wild type menin, were associated with a heat shock protein Hsp70 and its co-chaperone CHIP, which was shown to function as a ubiquitin E3 ligase towards several substrates presented by Hsp70 and Hsp90 (Connell, et al. 2001; Meacham, et al. 2001). Ubiquitination of menin missense mutants were also reported by Hendy group, and they showed that reduction of either Hsp70 or CHIP expression by siRNA-mediated knockdown stabilizes the menin missense mutants, indicating that CHIP is the E3 ubiquitin ligase of menin mutants presented by Hsp70 for proteasome-mediated degradation (Canaff, et al. 2012b). These findings are consistent with the observation that menin protein level is reduced in *MEN1*-mutated neuroendocrine tumors. While a much longer period (12 h) of treatment with MG132, a proteasome inhibitor, prevented reduction of the amount of wild type menin protein (Yaguchi et al. 2004), whether the wild type menin is subjected to ubiquitin-mediated degradation in normal cells, or in what type of cells, remains unclear.

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SUMOylation—Another type of PTMs involves attachment of the small ubiquitin-related modifier (SUMO), originally reported as a protein related to Ubiquitin, to protein substrates, and this process is referred to as SUMOylation (Bayer, et al. 1998; Geiss-Friedlander and Melchior 2007). SUMOylation has been shown to be important in regulating many cell processes including transcription, replication, chromosome segregation and DNA repair (Geiss-Friedlander and Melchior 2007; Johnson 2004). We found that menin undergoes SUMO modification and Lys591 is one of the SUMOylation sites (Feng, et al. 2013). Full length menin with L591R mutation can still be SUMOylated, suggesting that multiple SUMOylation sites exist and the menin SUMOylation site (QKVS) is not consistent with the consensus SUMO interacted motif, which implies the possible existence of a menin-specific E3 ligase (Melchior, et al. 2003). However, thus far, no functional effect of SUMOylation on menin has been observed.

Phosphorylation—The first phosphorylation modification of menin was found in 2006 by Matthew Meyerson group (MacConaill, et al. 2006). Protein phosphorylation is considered to be the most abundant posttranslational modification in eukaryotes. They initially found that menin migrated as a doublet on SDS-PAGE gels, indicating a possible posttranslational modification (Hughes et al. 2004). Further study showed that menin is phosphorylated on two serine residues, Ser543 and Ser583 (MacConaill et al. 2006). However, the functional study of the phosphorylation by mutating both serine residues had no impact on menin's ability to recruit trithorax family complex proteins Ash2L, Rbbp5, and MLL2, nor on cell proliferation (MacConaill et al. 2006). While these two phosphorylation sites are situated between the two NLSs located at the C-terminal part of menin, mutations at both of the serine residues do not affect menin localization into the nucleus (MacConaill et al. 2006). Additional phosphorylation sites of menin, Ser394 and Ser487, were also identified by mass spectrometry analysis (Francis, et al. 2011). Phosphorylation of menin at Ser394 was induced in response to irradiation (IR) or UV treatment (Francis et al. 2011), while Ser487 was phosphorylated under normal cell culture conditions. Nonetheless, HMT activity assay showed both Ser394 and Ser487 mutated menin mutant was still able to immunoprecipitate methylated histone 3. Several phosphorylation sites of menin have been found, but the overall functional significance of menin phosphorylation is still unclear. Phosphorylation can be mediated by a plethora of protein kinases, and one possible way to elucidate the function of menin phosphorylation is to identify the cognate protein kinases and then evaluate the role of menin phosphorylation under relevant conditions.

Conclusions

Physiological or pathological regulation of menin modifications and functions

Thus far, while multiple posttranslational modifications of menin have been reported, no clear biological functions attributed to the modifications have been unequivocally demonstrated. It is well documented that menin is phosphorylated in several residues, including the phosphorylation at Ser394 in response to several forms of DNA damage (Francis et al. 2011). However, so far it is unclear whether this phosphorylation has any consequence on menin function. Mutation of serine to alanine at the phosphorylation site did not affect cell cycle and cell proliferation (Francis et al. 2011). Moreover, it is also not clear

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whether SUMOylation of the menin affects its function in regulating homeostasis of the endocrine organs. Going forward, it is important to investigate the potential role of menin modifications on its biological functions and also crucial to uncover new layers of regulation of normal menin and its functional consequences.

Crosstalk between menin and GLP-1 pathway

It has been recently reported that menin represses expression of GLP-1 receptor (GLP1R), likely via interacting with protein arginine methyltransferases including PRMT5 (Muhammad et al. 2017). The precise mechanisms remain to be further investigated, and it is also crucial to understand whether GLP-1 pathway has any interplay with the menin function.

Interplay between the menin pathway and FOXO1 or CREB?

GLP-1 signaling and PKA induce phosphorylation of FOXO1 at the site which is also phosphorylated by insulin pathway-activated AKT (Muhammad et al. 2017). Notably, menin suppresses GLP-1-mediated phosphorylation of FOXO1. It is tantalizing to postulate that menin-PRMT5 can methylate the arginine residues close to the PKA phosphorylation site of FOXO1, but thus far, a direct PRMT5 mediated arginine methylation of the site was not demonstrated. Thus, it is important to find out whether/what arginine methyltransferase(s) may be responsible for the menin-mediated suppression of the FOXO1 phosphorylation. On the other hand, menin also inhibits GLP-1-mediated, PKA-dependent phosphorylation and thus activation of CREB, and it is important to elucidate how menin does so. It is also important to understand how menin coordinately suppresses a pro-beta cell factor CREB yet suppresses a negative beta cell factor FOXO1, both via phosphorylation, as these studies will likely unravel the key role and underlying mechanism of menin in regulating islet homeostasis and likely diabetes, by uncovering the underlying mechanism.

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