Review

Menin: a scaffold protein that controls gene expression and cell signaling

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The protein menin is encoded by the *MEN1* gene, which is mutated in patients with multiple endocrine neoplasia type 1 (MEN1) syndrome. Although menin acts as a tumor suppressor in endocrine organs, it is required for leukemic transformation in mouse models. Menin possesses these dichotomous functions probably because it can both positively and negatively regulate gene expression, as well as interact with a multitude of proteins with diverse functions. Here, we review the recent progress in understanding the molecular mechanisms by which menin functions. The crystal structures of menin with different binding partners reveal that menin is a key scaffold protein that functionally crosstalks with various partners to regulate gene transcription and interplay with multiple signaling pathways.

Menin: an orphan protein mutated in an inherited tumor syndrome

The menin protein is encoded by the *MEN1* gene, which is mutated in patients with MEN1 syndrome [1,2]. MEN1 syndrome is a dominantly inherited disease that is characterized by tumor formation in endocrine organs including the pituitary gland, parathyroid gland, and pancreatic islets [3–7]. The majority of patients with the inherited form of this disease have one germline mutation in the *MEN1* gene, with loss of heterozygosity (LOH) at the *MEN1* alleles in the endocrine tumor, highlighting menin as a bona fide tumor suppressor in endocrine organs. These mutations spread throughout the coding region of the gene, with no obvious mutation hotspots [5,8]. *MEN1* is also frequently mutated in patients with sporadic parathyroid [9] and pancreatic endocrine tumors [8,10]. Consistently, mice with heterozygous *Men1* mutation phenocopy the human MEN1 syndrome [11–13]. Complete *Men1* ablation in the mouse is embryonic lethal at E11.5–13.5, with defects in multiple organs [11].

Menin consists of 610 residues and is conserved from *Drosophila* to humans [14], but is not found in yeast or *Caenorhabditis elegans*, indicating that it is evolutionarily a relatively new gene. Although menin is ubiquitously expressed in various organs during mouse embryonic development [15,16], its function is tissue-specific; sometimes displaying opposing roles between different organs. For instance, it suppresses tumorigenesis in endocrine organs, yet is essential for leukemogenesis [17,18].

It has also been reported that menin plays a role in suppressing hyperplasia or tumors in several other organs, such as the lung [19,20], prostate [21], and breast [22], and it exacerbates diabetes in mouse models [23–25]. Menin also influences the function of other organs such as bone [26–31] and liver [32,33]. Detailed mechanisms for how menin affects these organs are less clear, but it likely functions via regulating various distinct signaling pathways. Menin itself is also regulated by multiple signaling proteins such as prolactin [23] and transforming growth factor (TGF)-β [34], and by post-translational modifications such as phosphorylation and SUMOylation. Additionally, MEN1 disease-associated single amino acid substitutions can lead to enhanced polyubiquitylation and degradation via proteasomal pathways [35–38].

Menin lacks domains that are homologous to other proteins, therefore, it is challenging to elucidate its biochemical function. As such, intensive efforts have been made by many groups to identify menin-interacting proteins, in the hopes of finding clues about how menin biochemically suppresses tumorigenesis, as previously reviewed [39,40]. These menin partners provide valuable information regarding the biochemical function of menin. However, the detailed underlying structural mechanism has been elusive. Recent progress in elucidating the crystal structure of menin, coupled with the revelation that menin crosstalks with various signaling pathways, provide new insights into how menin controls gene expression and cell signaling [41,42]. This review focuses on the latest progress in understanding how menin, as a scaffold protein, controls gene expression and interplays with multiple signaling pathways to control cell behavior. Certain studies were performed in one cell line or one model system, and in some situations only a limited number of studies were performed, therefore, caution is to be exercised regarding generalizing the role of menin and underlying mechanisms in these situations.

Menin interacts with proteins involved in regulating gene transcription and cell signaling

Although menin is ubiquitously expressed, its expression levels vary from tissue to tissue [15,23,43]. Menin is primarily a nuclear protein, but lesser amounts are also detectable in the cytoplasm and even the cell membrane. Its nuclear localization sequences (NLSs), located in its C-terminal region, can directly interact with DNA in a se-

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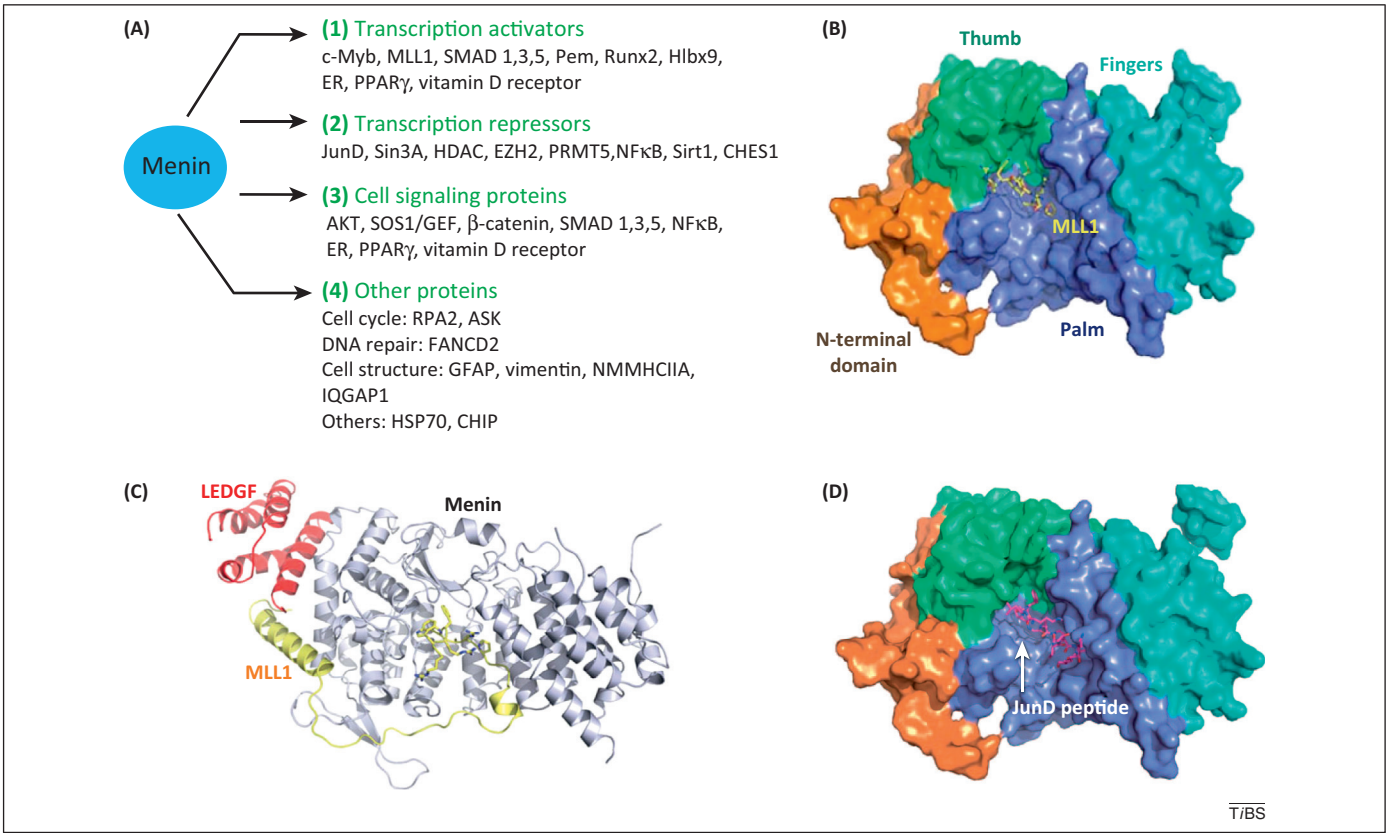

Figure 1. Menin-interacting proteins and structures of menin with selected interacting proteins. **(A)** Four classes of menin-interacting proteins based on their cellular functions in context of their interaction with menin: transcription activators; transcription repressors; cell signaling proteins; and other proteins. Some menin-interacting proteins can have dual roles as transcription factors and signaling molecules. **(B)** The surface representation of menin complexed with menin binding motif of mixed lineage leukemia protein (MLL)1 ($MLL1_{MBM}$). **(C)** Overall ternary structure of menin complexed with $MLL1_{MBM-LBM}$ [MLL1 menin binding motif–lens epithelium-derived growth factor (LEDGF) binding motif] and $LEDGF_{IBM}$ (LEDGF integrase binding motif). **(D)** Crystal structure of menin in complex with transcription factor, JunD peptide. Menin interacts directly with JunD. Menin binding motif of JunD consists of residues 27–47. $JunD_{MBM}$ is shown as purple stick model. (B–D) Reproduced, with permission, from [41]. ASK, activator of S phase kinase; CHES1, checkpoint suppressor; CHIP, COOH terminus of Hsp70-interacting protein; ER, estrogen receptor; EZH2, enhancer of zeste homolog 2; FANCD2, Fanconi anemia complementation group; GEF, guanine nucleotide exchange factor; GFAP, glial fibrillary acidic protein; HDAC, histone deacetylase; Hlxb9, homeobox HB9; IQGAP, IQ motif containing GTPase activating protein; HSP, heat shock protein; NFκB, nuclear factor κB; NMMHC, non-muscle myosin heavy chain; Pem, protein-energy malnutrition; PPAR, peroxisome proliferator-activated receptor; PRMT, protein arginine methyltransferase; RPA, replication protein A; Runx, runt-related transcription factor; Sin3A, Sin3 transcription regulator homolog A; Sirt, a member of sirtuin family of proteins; SOS, Son of Sevenless.

quence-independent manner [44,45]. Menin-interacting proteins can be classified into four main categories based on their cellular role in context to their association with menin: transcription activators, transcription repressors, and cell signaling proteins, and the other remaining interacting partners, which have diverse functions ranging from regulation of DNA repair to the structural integrity of the cell (Figure 1A). Menin-interacting transcriptional activators include transcription factors c-Myb, protein-energy malnutrition (Pem) and runt-related transcription factor (Runx)2 [29,31,46,47], homeobox protein, Hlxb9 [48] and histone modifiers such as mixed lineage leukemia proteins (MLL)-1 and -2 and histone H3 lysine 4 (H3K4) methyltransferases [49,50]. The transcriptional repressors that directly or indirectly interact with menin include transcription factor JunD, nuclear factor (NF)κB [51,52], histone deacetylase (HDAC)1/2, histone deacetylase Sirt1 (a member of sirtuin family of proteins), the histone H3 lysine 27 methyltransferase EZH2, and protein arginine methyltransferase (PRMT)5 [20,53–55]. Notably, menin also interacts with multiple proteins that mediate several signaling pathways, such as the SMAD proteins, which transduce TGFβ signaling [29,56]; β-catenin, a member of the Wnt signaling pathway [57–60]; nuclear receptors such as estrogen receptor and peroxisome proliferator-activator receptor (PPAR)γ; and Ras-activating protein Son of Sevenless (SOS)1 [19,61,62]. The physiological and genetic impact of the interaction between menin and many of its interacting proteins in model organisms remains to be explored. It is noteworthy that these signaling proteins often can also act as either transcription activators or repressors, however, for the purposes of this discussion we classify them based on the their activity in the context of their interaction with menin.

Our group and others have proposed that menin might act as a scaffold or adaptor protein to interact with other proteins in controlling gene transcription, based on cellular and biochemical studies [45,63]. This concept is consistent with the observation that menin distributes in a broad spectrum in gel filtration chromatography [54], and that menin binds to thousands of gene loci in various cell lines [64,65]. However, until recently the crystal structure of menin was not known, hindering the understanding of how menin interacts with its partners to exert its functions.

Crystal structure of menin and its interacting partners

Great efforts by many groups have been made to solve the crystal structure of menin. It has been challenging to

obtain high quality menin protein crystals to decipher its structure. However, Huang *et al*. and Murai *et al*. have recently succeeded in resolving the structure of menin alone [41,42], in complex with an MLL1 peptide (Figure 1B), in a ternary complex with MLL1 and lens epithelium-derived growth factor (LEDGF) (Figure 1C), or in complex with a peptide from JunD (Figure 1D) [41].

The crystal structure of menin looks like a curved left hand, in which the N-terminal domain is represented by a long β-hairpin, transglutaminase-like domain that resembles a thumb; the middle region adopts the shape of the palm; and the C terminus resembles curved fingers (Figure 1B). Notably, the palm forms a deep pocket that is occupied by the conserved MLL1 peptide (Figure 1B). Mutagenesis studies show that the residues in either MLL1 or the menin pocket that mediate the menin–MLL1 interaction are crucial for binding each other, as well as for the menin-mediated upregulation of Hox genes [41].

Biochemical studies show that menin not only interacts with MLL1, but also interacts simultaneously with LEDGF, a protein important for MLL-AF9-induced leukemia [63]. The crystal structure of menin–MLL1–LEDGF clearly shows that the N-terminal region of MLL1 (yellow, Figure 1C) binds the deep central pocket of menin, and the further downstream sequence of MLL1 loops around the N-terminal part of menin, forming an α helix that directly contacts the menin N terminus. On the top of the V shape structure that is co-formed by the α helix of MLL1 and the surface of the N-terminal part of menin, LEDGF (red, Figure 1C) directly interacts with both MLL1 and menin. This is strong evidence demonstrating that menin acts as a scaffold protein to interact with multiple proteins. Consistent with this notion, a mutation in menin at the LEDGF-interaction site diminishes the ability of menin to promote expression of Hoxc8. Further work is needed to determine how menin structurally interacts with other partners.

Similar to its interaction with MLL1, menin also binds JunD via its central pocket. The region of JunD that binds menin spans residues 27–47, and is known as the menin-binding motif ($JunD_{MBM}$) (Figure 1D) [41]. Comparison of the $JunD_{MBM}$ with the MLL1 peptide that binds menin ($MLL1_{MBM}$) reveals a conserved sequence (FPXXP). Indeed, the co-crystal of menin and the JunD peptide shows that $JunD_{MBM}$ and $MLL1_{MBM}$ bind the menin pocket with the same orientation (Figure 1B,D). $MLL1_{MBM}$ effectively competes out JunD binding to menin [41], demonstrating that menin can use the same pocket to bind either MLL1 or JunD. MLL2 also binds to menin via a similar conserved sequence. It remains to be determined whether menin can also interact with other proteins containing similar sequences. Binding of menin to MLL1 serves to recruit these proteins to promoters of the genes, leading to an increase in gene transcription [41].

Menin activates gene transcription

Menin upregulates the expression of cyclin-dependent kinase inhibitors (CDKIs) p18 and p27 [43,66,67], thereby reducing β cell proliferation. It activates transcription of the CDKIs at least partly via MLL1, which adds trimethylation to histone H3 at lysine 4 (H3K4me3); a chromatin modification associated with transcriptional activation [48,66,67] (Figure 2A). Consistent with these findings, genetic ablation of Retinoblastoma binding protein 2 *Rbp2*, a histone H3 lysine 4 demethylase, reduced the development of insulinoma in a β cell-specific *Men1* knockout mouse model [68]. Whether a DNA sequence-specific factor recruits menin–MLL1 to the loci, and which factor that might be, remains unclear.

The *MLL1* gene can undergo chromosomal translocations with one of various partner genes, resulting in the expression of MLL1 fusion proteins (MLL-FPs) that can induce leukemia. In these circumstances leukemogenesis is driven by c-Myb, a transcription factor that directly

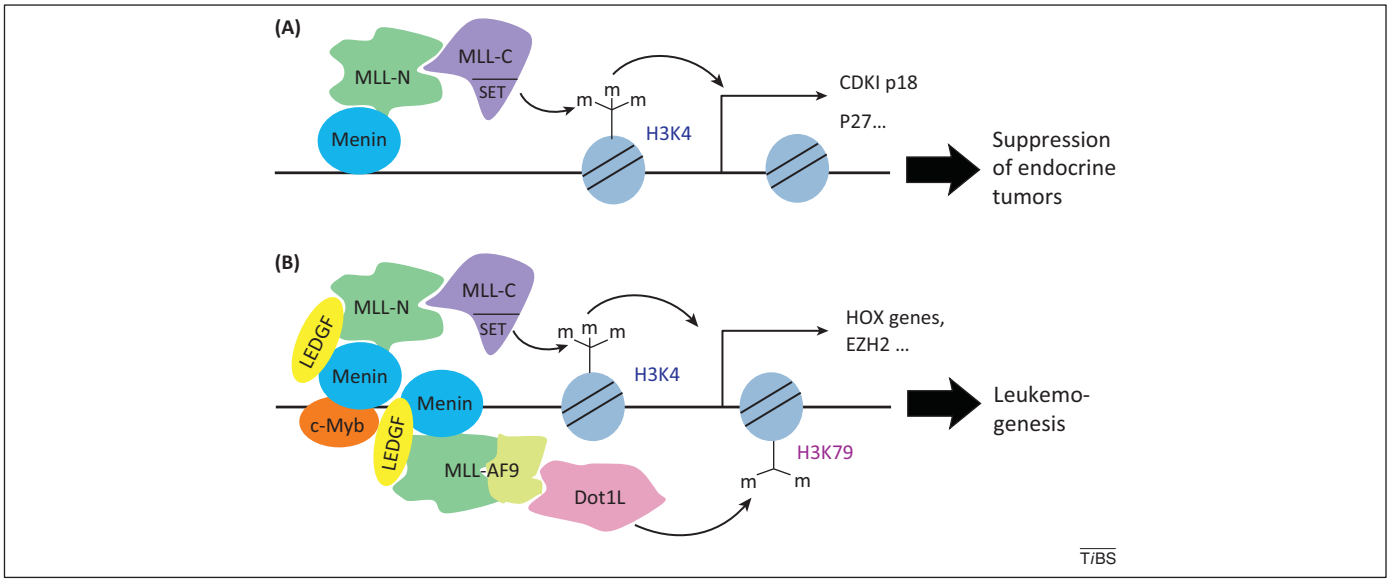

Figure 2. Menin acts as a scaffold protein to activate gene transcription tissue-specifically. **(A)** In endocrine cells, menin recruits mixed lineage leukemia protein (MLL)1 to the promoters of cyclin-dependent kinase inhibitor (CDKI) p18 and p27. **(B)** In MLL-fusion protein (FP)-induced leukemia cells, c-Myb binds and recruits menin. Menin further recruits, via its central pocket, either wild type MLL1 (green and violet) or MLL1-FPs, recruitment of MLL (wild type containing MLL-N and MLL-C or MLL1-FP) can help recruit lens epithelium-derived growth factor (LEDGF) and DOT1 like histone H3 methyltransferase (Dot1L). Menin can directly deposit histone H3 at lysine 4 with trimethylation (H3K4me3), whereas Dot1L deposits histone 3 lysine 79 (H3K79) methylation, both leading to increased transcription of *Hox* genes, *Meis1*, and *Ezh2*, and blockade of myeloid differentiation. Depiction of c-Myb is not stoichiometric in this figure.

binds menin and likely recruits MLL1-FP, wild type (WT) MLL1, and LEDGF to Hoxa9 and Meis1 gene loci to promote their expression (Figure 2B) [41,46,63,69]. Deletion of menin in these leukemia cells abolishes recruitment of WT MLL1 and MLL1-FPs and decreases H3K4me3 at these gene loci, demonstrating that menin is an essential cofactor for MLL1 function (Figure 2B) [70]. Consistent with their role in promoting Hoxa9 and Meis1 expression, MLL1-FPs are found in complexes associated with enhancing transcriptional activation, including the histone methyltransferase, Dot1L containing complex, which methylates histone 3 lysine 79 (H3K79), and the positive transcription elongation factor (pTEFb) complex, which mediates transcriptional elongation [71,72].

As menin plays an important role in regulating gene expression through interaction with various partners such as MLL1 and MLL1-FP, small molecule inhibitors that block menin–MLL1 interaction were developed. These inhibitors suppress menin–MLL1-dependent expression of Hox genes and inhibit proliferation of MLL1 fusion-transformed leukemia cells [73]. These findings support a new therapeutic strategy for aggressive leukemias with MLL1 rearrangements. Further optimization of these menin inhibitors yielded another compound (MI-2-2) that binds to menin with low nanomolar affinity [K(d) = 22 nM] and effectively disrupts the interaction between menin and MLL1 [74]. Moreover, co-crystallization of the human menin protein with MI-2-2 gives a high resolution (1.6 Å) structure that displays a close interaction between menin and MI-2-2. MI-2-2 has increased efficacy in blocking the menin–MLL1 interaction and expression of Hox genes compared to the earlier compounds [74]. These findings provide a structural basis to design better inhibitors to inhibit effectively the menin–MLL1 interaction.

Consistent with these findings, recently reported structure-based design of cyclic peptidomimetics, based on the co-crystal structure of menin–MLL1 peptide [75], also generated a potent macrocyclic peptidomimetic compound that binds to menin with a Ki value of 4.7 nM; >600 times more potent than the corresponding acyclic MLL1 peptide [75]. Collectively, this fast progress in designing small molecule compounds to inhibit menin–MLL1 interaction, and expression of menin–MLL1 targets such as the Hox genes in leukemia, pave the way to develop better compounds with ideal pharmacokinetics, and might eventually lead to effective drugs to treat aggressive acute myeloid leukemia (AML).

Menin suppresses gene transcription

Menin has also been shown to repress gene transcription directly or indirectly. Recent studies have identified more factors that repress gene transcription through association with menin, such as various chromatin-modifying enzymes. Menin also regulates the expression of miRNAs and indirectly controls post-transcriptional expression. Moreover, menin directly interacts with JunD (as discussed previously), and represses JunD-induced transcription through its interaction with the co-repressor mammalian sin3 protein (mSin3A) and its associated HDAC (Figure 3A) [50,55]. It also represses the G2–M phase transition by repressing cyclin B2 expression, partly via recruiting HDAC3 to its promoter [76].

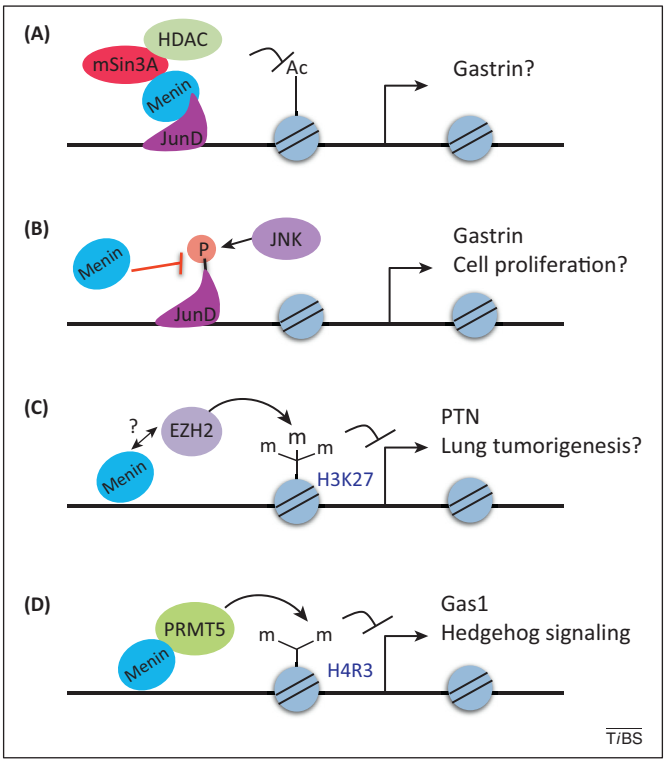

Figure 3. Menin represses gene expression via multiple mechanisms. **(A)** Menin interacts with transcription factor JunD and recruits the Sin3 transcription regulator homolog (Sin3A)/histone deacetylase (HDAC) complex to reduce histone acetylation and suppress the expression of JunD targets such as gastrin. The endogenous targets of JunD in this setting are unclear. **(B)** Menin also blocks C-jun N-terminal kinase (JNK)-mediated phosphorylation of JunD, leading to repression of JunD transcriptional targets such as gastrin. **(C)** In lung cancer cells, menin recruits enhancer of zeste homolog 2 (EZH2) to the promoter of pleiotrophin (PTN) to increase histone 3 lysine 27 (H3K27) trimethylation, a repressive mark, to downregulate the transcription of PTN. Whether menin directly interacts with EZH2 is yet to be determined. **(D)** Menin can inhibit Hedgehog signaling by interacting with protein arginine methyltransferase (PRMT)5 at the growth arrest-specific1 (Gas1) promoter in pancreatic islets to increase PRMT5-mediated symmetric histone 4 arginine 3 (H4R3) dimethylation, which inhibits the expression of Gas1 and therefore Hedgehog signaling.

Menin inhibits JunD-mediated gene transcription through an additional mechanism involving competition for a binding partner [41]. c-Jun N-terminal kinase (JNK) normally phosphorylates JunD, activating JunD-induced gene expression. The consensus JNK-docking domain (D domain) in JunD contains a cluster of basic amino acids preceding two leucine residues. The $JunD_{MBM}$ sequence partially overlaps with a putative D domain of JunD (JunDD) (Figure 3B) [77]. Both the basic residues and the leucine residues in JunDD are essential for JNK to dock on (or bind to) JunD and phosphorylate it, as well as for binding by menin (Figure 3B). As such, menin binding to JunD blocks JNK-mediated phosphorylation and activation of JunD. Menin and JunD both bind to the promoter of the endogenous *Gastrin* gene and suppress its expression [41]. These findings unravel a new means for menin-mediated suppression of JunD activity and provide a structural basis for the mechanism of suppression (Figure 3B). These findings also help explain why JunD activates proliferation of mouse embryonic fibroblasts (MEFs) in the absence of menin but suppresses proliferation in its presence. Presumably, JunD binds promoters of pro-proliferative genes, and the presence or absence of menin controls whether JunD inhibits or activates expression of these genes, respectively [78].

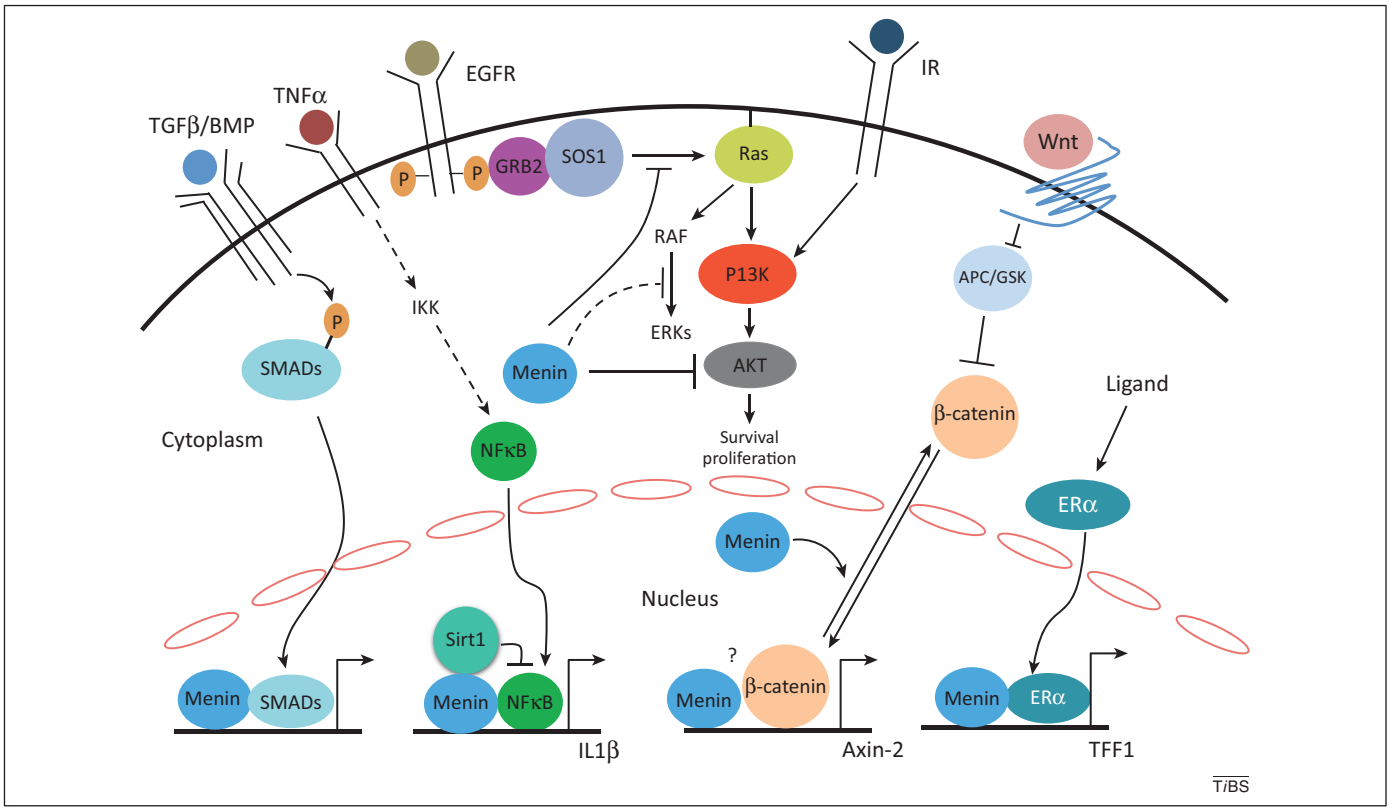

Figure 4. Menin regulates multiple signaling pathways. Menin interacts with SMAD3 or SMAD1/5 to enhance transforming growth factor (TGF)β or bone morphogenetic protein (BMP) signaling, respectively, in various types of cells. Menin also interacts with nuclear factor (NF)κB and recruits Sirtuin, Sirt1 to deacetylate p65 to suppress NFκB-induced gene expression. β-Catenin is located in the cell membrane, and in presence of Wnt signaling it is translocated to the nucleus. In insulinoma cells, menin interacts with β-catenin to upregulate gene transcription, but whether they associate with each other at the promoter of Axin 2 is not known. By contrast, in mouse embryonic fibroblasts (MEFs), menin promotes nuclear export of β-catenin to suppress its transcriptional activity. Menin also interacts with nuclear receptors such as estrogen receptor (ER)α to promote expression of their target genes. In the cytoplasm, menin inhibits receptor tyrosine kinase signaling through multiple mechanisms: inhibition of AKT, inhibition of Son of Sevenless (SOS)1-dependent activation of Ras, and suppression of extracellular signal-regulated kinase (ERK) activation. APC, adenomatous polyposis coli; EGFR, epidermal growth factor receptor; GRB, growth factor receptor-bound; GSK3b, glycogen synthase kinase; IKK, inhibitor of nuclear factor κ-B kinase; IL, interleukin; PI3K, phosphoinositide 3-kinase; TFF, trefoil factor; TNF, tumor necrosis factor.

Menin also directly interacts with the p65 subunit of NF-κB and represses NF-κB-dependent transcription, as shown by luciferase reporter assays in cultured cells [51]. It has recently been reported that menin inhibits NF-κB-mediated transactivation via recruiting Sirt1, a histone deacetylase, to deacetylate lysine 310 (K310) of p65 in hepatocellular carcinoma cells (Figure 4) [53]. It remains unclear whether menin-induced and Sirt1-mediated deacetylation of p65 represses expression of the endogenous targets of NF-κB.

Menin is also reported to repress expression of the *PTN* gene, which encodes a pro-proliferative receptor, pleotrophin, in lung cancer cells [20]. Both menin and polycomb repressive complex (PRC)2 bind to the PTN gene promoter and increase the repressive chromatin mark H3K27me3 at the locus (Figure 3C). A direct interaction between menin and EZH2, the PRC2 enzyme that catalyzes H3K27 trimethylation, has not been demonstrated.

Menin also directly interacts with PRMT5, a negative regulator of gene transcription [54], and recruits it to the promoter of the *Gas1* gene; a crucial factor for binding of Sonic hedgehog (Shh) ligand to its receptor [79]. Thus, menin antagonizes Shh signaling, partly via increasing PRMT5-mediated repressive histone arginine dimethylation (H4R3me2) at the *Gas1* promoter (Figure 3D).

Menin is reported to repress gene expression post-transcriptionally via upregulation of miRNA expression, which can reduce protein translation or the stability of the target mRNA. Specifically, menin induces expression of miRNA-26a (miR-26a) by binding to the promoter of the miR-26a gene [80]. miR-26a targets and reduces expression of the bone morphogenetic protein (BMP) signaling effector SMAD1, antagonizing the osteoblastic differentiation of human adipose-tissue-derived stem cells by BMP. Interestingly, menin also promotes BMP signaling by binding SMADs at target loci and promoting expression of SMAD-target genes. Thus, induction of miR-26a by menin is likely a dampening or desensitizing mechanism for menin-mediated BMP signaling (Figure 4). It remains unclear whether or how these various modes operate in different types of cells or for different target genes.

Menin regulates multiple signaling pathways

TGFβ signaling pathway

Menin directly interacts with the TGFβ downstream signaling molecule SMAD3 in COS cells [56]. Menin knockdown (KD) reduces SMAD3 binding to DNA in GH4C1 cells, a pituitary endocrine tumor cell line, suggesting a role for menin in recruiting SMAD3 to target genes to regulate their expression. Consistently, menin KD reduces the effect of TGFβ-induced inhibition of proliferation [56].

BMP signaling

Menin also regulates BMP signaling, which is critical for bone development [29]. Consistent with this role, *Men1*-null

embryos display defects in cranial and facial development [29]. Menin is required for BMP-dependent multipotent mesenchymal stem cell commitment to the osteoblast lineage, and it facilitates this commitment through its interaction with the BMP effectors SMAD1/5 and their binding partner Runx2 [31]. At later stages of differentiation, menin inhibits differentiation, likely through inhibiting JunD function [30]. These results indicate that menin-mediated regulation of various signaling pathways may be not only tissue specific, but also specific to a certain stage of differentiation.

Wnt Signaling

Wnt signaling stimulates pancreatic islet β cell proliferation, possibly by increasing expression of paired-like homeodomain (Pitx2) [81]. At least some of the effects of Wnt on β cells are mediated by the canonical Wnt effector and transcription factor β-catenin. Overexpression of menin reduces nuclear accumulation of β-catenin and therefore its transcriptional activity in MEFs; in part, by directly interacting with β-catenin and excluding it from the nucleus (Figure 4) [58]. By contrast, menin is crucial for canonical Wnt/β-catenin signaling in cultured rodent islet tumor cells via interaction with β-catenin [59]. It is possible that menin promotes Wnt signaling in certain stages of islet tumor development or inhibits Wnt signaling to prevent β cells from tumorigenesis (Figure 4). Further detailed biochemical and genetic mechanisms remain to be explored.

Nuclear receptor signaling

Menin interacts with several nuclear receptor transcription factors including estrogen receptor (ER)α and PPARγ [61,62]. Menin directly interacts with ERα in a hormone-dependent manner and is recruited to the ERα target gene, trefoil factor (TFF)1. There, it increases the active histone mark H3K4me3, probably via recruitment of MLL, and activates target gene expression (Figure 4). Menin also binds to ERα to enhance its activity in MCF7 breast cancer cells [82], and menin expression correlates with a poorer prognosis in ER-positive breast cancer patients treated with tamoxifen. It is not yet clear whether menin can regulate endogenous ER targets to promote proliferation or survival of normal or transformed breast epithelial cells.

Ras signaling

Consistent with a role for menin in suppressing proliferation, menin overexpression slows the proliferation of Ras-transformed NIH-3T3 cells [83]. Menin inhibits extracellular signal-regulated kinase (ERK)-dependent phosphorylation, a downstream target in Ras pathway, and activation of JunD [84]. Consistently, menin also inhibits ERK activation and lung cancer cell migration [85]. Upstream of ERK, menin antagonizes Ras/ERK signaling by reducing the level of active Ras-GTP. This is achieved at least partly by preventing the Ras guanine nucleotide exchange factor SOS1, and its adaptor, growth factor receptor-bound protein (GRB)2, from binding to Ras in lung cancer cells (Figure 4) [19]. It remains to be determined how menin blocks SOS1 from binding to Ras and precisely how menin inhibits ERKs.

Akt and FOXO signaling

Menin has been reported to interact with the protein kinase Akt1 in cultured cells and mouse pancreata and reduce the level of active Akt1; at least in part by reducing its kinase activity [86]. Menin suppresses both Akt1-dependent proliferation and antiapoptotic activity in nonendocrine and endocrine cells; partly by reducing the translocation of Akt1 from the cytoplasm to the plasma membrane (Figure 4). It is unclear how menin prevents translocation of Akt1 to the cell membrane. Further work will be needed to determine the role of Akt1 in menin-mediated suppression of endocrine tumors, which can be done using tissue-specific double knockout of Men1 and Akt1. Menin is also reported to interact with transcription factor FOXO1 (Forkhead box protein O1) in the cytoplasm of hepatocytes [87], but it is unclear whether menin directly or indirectly interacts with FOXO1 and what the biological consequence of this interaction is. The Akt/mTOR pathway is upregulated in pancreatic neuroendocrine tumors, therefore, it is important to investigate precisely how menin suppresses this pathway.

Hedgehog signaling

Menin directly interacts with PRMT5, a negative regulator of gene transcription [54], and recruits PRMT5 to the promoter of the *Gas1* gene, a crucial factor for binding of Shh ligand, to activate the Hedgehog signaling, a pro-proliferative pathway (Figure 3D).

Overall, it is proposed that menin plays an important and tissue-specific role in various signaling pathways, ranging from Ras to Akt to Hedgehog signaling. However, more experimental evidence is needed to understand completely the function of menin with reference to these signaling pathways.

Regulation of menin by various proteins and signaling pathways

Prolactin pathway

It is reported that menin influences islet growth in pregnant mice [23]. Prolactin, a hormonal regulator of pregnancy, represses islet menin expression via the signal transducers and activators of transcription 5, Stat5 /B cell lymphoma 6 (Bcl6) axis, leading to enhanced β cell proliferation during mouse pregnancy. Consistent with these findings, another recent report also shows that prolactin signaling and menin regulate pregnancy-induced β cell proliferation [88].

TGFβ pathway

TGFβ stimulation leads to the upregulation of menin expression in cultured parathyroid cells, suggesting crosstalk between menin and TGFβ signaling [89]. Consistently, menin expression is also upregulated by TGFβ in MLL–AF9-transformed leukemia cells and mouse hepatocytes [34]. As menin also interacts with SMAD3, a TGFβ signal transducer, to increase gene expression (Figure 4), these studies suggest a potential positive feedback loop. It is unclear how TGFβ upregulates menin expression and whether the crosstalk between menin and TGFβ signaling operates in animal models.

Somatostatin pathway

Gastrin is a peptide hormone that stimulates gastric acid secretion from parietal cells of the stomach, whereas

somatostatin is a peptide hormone and inhibitor of gastrin secretion as well as expression. It is proposed that menin inhibits the expression of gastrin. Menin protein expression is reduced in the gastrointestinal system of somatostatin-null mice [90]. Mice treated with the somatostatin analog octreotide show an increased number of menin-expressing cells, menin mRNA, and menin protein expression predominantly in the duodenum. Menin induction appears to depend on suppression of protein kinaseA (PKA). Whether and how the somatostatin pathway regulates PKA to induce the expression of menin remains to be elucidated.

Glucose and the phosphoinositide 3-kinase (PI3K)/Akt pathway

Menin acts as a negative regulator of β-cell proliferation, especially during pregnancy [23]. Also, acute ablation of menin prevents the development of streptozotocin-induced diabetes and reverses pre-existing diabetes in several mouse diabetes models [24,25]. Short-term glucose stimulation reduced menin levels and increased proliferation in INS1 insulinoma cells and primary rat islets in a PI3K/Akt signaling-dependent manner [91]. It is reported that PI3K/Akt reduces expression of menin by phosphorylating and inhibiting the transcription factor FOXO1, which binds the promoter and stimulates transcription of the *Men1* gene [91]. It remains to be explored whether this mechanism operates in primary islets and mouse diabetes models.

Silencing of MEN1 expression by K-Ras-induced DNA methylation or miRNA

K-Ras-activation inhibits menin expression via DNA methyltransferase 1 (DNMT1)-dependent DNA demethylation of the promoter of the *MEN1* gene in human lung cancer cells [19]. Moreover, Luzi *et al*. discovered that miR-24-1 binds to the 3′ untranslated region (UTR) of *MEN1* mRNA, and reduces *MEN1* expression post-transcriptionally, unraveling another means of suppression of the *MEN1* gene [92].

Post-translational modifications of menin

Menin is post-translationally modified by SUMOylation, phosphorylation and ubiquitylation. Menin is SUMOylated by small ubiquitin like modifier protein, (SUMO1) at lysine 591 [36]. Point mutations associated with the MEN1 disease lead to rapid degradation of menin by the ubiquitin–proteosome pathway in 293T cells [35]. Menin is specifically phosphorylated at serine 394 on treatment with γ-IR and UV. Menin is also phosphorylated at Ser487 dynamically and at Ser543 constitutively, but the impact of phosphorylation on menin function remains unclear [93].

Menin-mediated regulation of genome integrity

Menin interacts with various proteins involved in DNA replication and repair, such as checkpoint suppressor (CHES)1 [94], and menin is localized to telomeres during meiosis [95], suggesting a role for menin in telomere maintenance. Recently, mutations in chromatin modifier Daxx/ATRX genes as well as the *MEN1* gene were identified in pancreatic neuroendocrine tumors (PNETs) [10]. Daxx is localized at the telomere in PNETs, and it is not yet clear whether there is any functional crosstalk between menin and Daxx/ATRX in regulating the telomere. Whether and how these interactions of menin with DNA modifiers influence the biochemical function of menin in normal cells or disease states is not yet clear.

Box 1. Outstanding questions

- How does menin choose its partner among a diverse group of interacting proteins? Does menin choose certain partners in distinct types of cells or in distinct settings of biological conditions?
- How does menin functionally interact with its partners *in vivo* in model organisms such as mice?
- How is menin regulated and how do various post-translational modifications affect its function?
- How does menin exert its crucial functions in various organ systems in development, homeostasis, and disease?
- Can *MEN1* mutation-induced dysregulation of tumorigenic pathways, such as the Hedgehog signaling pathway, be targeted to improve therapy of MEN1 syndrome?
- Can the structure of menin be used to develop better inhibitors to treat relevant diseases such as MLL1-FP-induced leukemia, perhaps by blocking menin–MLL1/MLL1-FP interactions?

Concluding remarks

Recently, rapid progress has been made in understanding the molecular and biochemical functions of menin. In particular, as the crystal structures of menin with several of its binding partners have been resolved, these new findings have established menin as a key scaffold protein that can functionally crosstalk with various partners in controlling a diverse range of biological functions. Menin positively or negatively controls gene transcription by interacting with distinct proteins. Menin may also exert its functions via either its central pocket or other parts of its surface. Moreover, menin not only regulates multiple signaling pathways, but itself is also regulated by multiple proteins, signaling pathways, and numerous types of post-translational modifications. It is noteworthy that these various mechanisms could be highly tissue specific, or cell type or context dependent, and more genetic and biochemical work remains to be done to expand these observations. It is noteworthy that certain important findings were only observed in a limited number of studies or conditions; caution need to be exercised in generalizing such functions of menin and further detailed studies are expected to resolve these issues. Nevertheless, this progress has provided novel insights into how menin controls gene transcription and interplays with various signaling pathways. With the accumulation of this novel knowledge regarding the molecular mechanisms underlying the function of menin, many exciting questions (Box 1) can now be raised and addressed to improve the therapy of MEN1 disease and other related diseases such as leukemia. In particular, how does menin choose its partners in different contexts? For example, in MLL1 fusion protein (MLL1-FP)-induced leukemia, the interaction between menin and MLL1/MLL1-FP dimers is particularly important, as a menin/MLL1/MLL1-FP are required to maintain high level expression of HOX genes, which are crucial for MLL1-FP-induced leukemia. By contrast, menin interaction with MLL1 may be important

for upregulation of CDKI, p18, and p27 in endocrine cells, and menin interaction with JunD may be important to inhibit endocrine cells, but not necessary for MLL1-FP-induced leukemia. As such, further studies are necessary to explore these potential mechanisms.

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