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Review

A Review of the Scaffold Protein Menin and its Role in Hepatobiliary Pathology

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Multiple endocrine neoplasia type 1 (MEN1) is a familial cancer syndrome with neuroendocrine tumorigenesis of the parathyroid glands, pituitary gland, and pancreatic islet cells. The *MEN1* gene codes for the canonical tumor suppressor protein, menin. Its protein structure has recently been crystallized, and it has been investigated in a multitude of other tissues. In this review, we summarize recent advancements in understanding the structure of the menin protein and its function as a scaffold protein in histone modification and epigenetic gene regulation. Furthermore, we explore its role in hepatobiliary autoimmune diseases, cancers, and metabolic diseases. In particular, we discuss how menin expression and function are regulated by extracellular signaling factors and nuclear receptor activation in various hepatic cell types. How the many signaling pathways and tissue types affect menin's diverse functions is not fully understood. We show that small-molecule inhibitors affecting menin function can shed light on menin's broad role in pathophysiology and elucidate distinct menin-dependent processes. This review reveals menin's often dichotomous function through analysis of its role in multiple disease processes and could potentially lead to novel small-molecule therapies in the treatment of cholangiocarcinoma or biliary autoimmune diseases.

Key words: Biliary epithelium; Fibrosis; Transforming growth factor-β (TGF-β); Mixed lineage leukemia (MLL); Histone deacetylase; Cholangiocarcinoma (CCA); Hepatocellular carcinoma (HCC); JunD; Pancreas; Immunology; Metabolic disease

INTRODUCTION

The protein menin was originally studied within the context of multiple endocrine neoplasia type 1 (MEN1), a rare hereditary autosomal dominant tumor syndrome[1]. MEN1 syndrome penetrance and expressivity are variable, even among patients from the same family tree. The most common manifestation (~100% penetrance by age 50) is primary hyperparathyroidism (HPT), followed by the development of anterior pituitary adenomas (~10%–60% penetrance)[2]. Pancreatic neuroendocrine tumors (PNETs), thymic or bronchial carcinoid tumors, and adrenocortical tumors account for a significant remainder of the morbidity and mortality seen in MEN1 syndrome[3]. Additionally, nonendocrine tumors such as angiofibromas, collagenomas, lipomas, and melanomas are common manifestations[2]. Emerging evidence points to more clinically subtle complications of MEN1 syndrome, possibly due to hormonal imbalances as a result of neuroendocrine growths. Patients often suffer from kidney and bone disease, premature cardiovascular disease, insulin resistance, and glucose intolerance[4].

MENIN STRUCTURE AND FUNCTION

Menin is a 67-kDa nuclear protein (Fig. 1) coded by 10 exons spanning 9 kb of genomic sequence (Fig. 2) and expressed ubiquitously. Patients with MEN1 syndrome inherit a mutated *MEN1* allele (11q13) from one of their parents. Tissue-specific somatic mutation in the remaining functional allele, or "second hit," results in complete loss of menin expression and organ tumorigenesis. Diagnosis of MEN1 syndrome is validated by the identification of a mutation in the exon-coding region or intron–exon junctions of the *MEN1* gene locus[3]. One genomic analysis of >1,300 MEN1 patients revealed that over 70%

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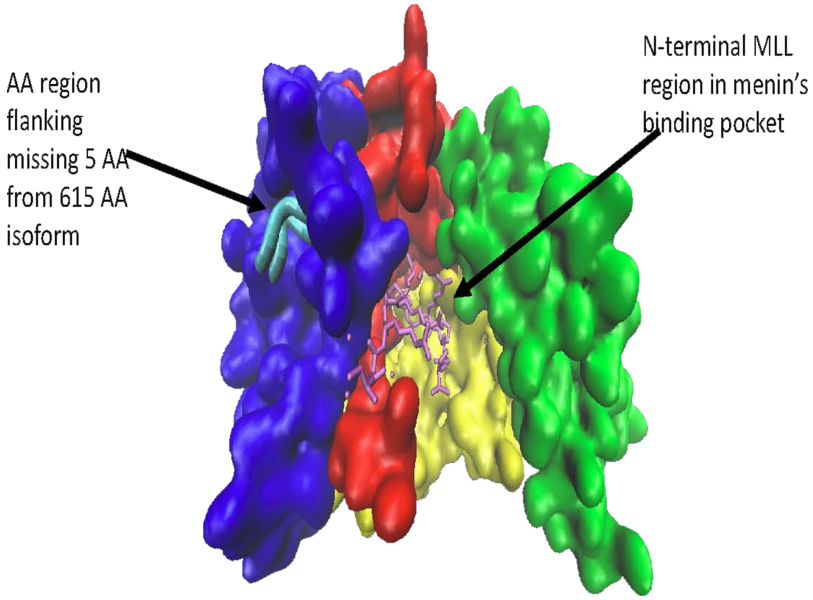

Figure 1. A 610-amino acid menin isoform (type 2). Note how overall binding groove appears intact and the missing five amino acids are located on the surface away from menin's binding pocket. This image was rendered from the online RCSB Protein Data Bank code 4GQ6 using VMD 1.9.3 Graphics (University of Illinois at Urbana-Champaign).

of mutations lead to truncated or nonexistent forms of menin[5]. Inhibition of the proteasome–ubiquitin pathway can restore protein expression in some cases[6]. It should be noted that not all MEN1 families have a mutation in the *MEN1* coding region, indicating a need to understand the regulatory elements surrounding menin expression and function. Thus, it appears that other genetic and environmental factors play a role in the MEN1 syndrome disease process.

Phylogenetically, menin is evolutionarily conserved among vertebrates and *Drosophila*; however, its amino acid sequence does not share homology with other known

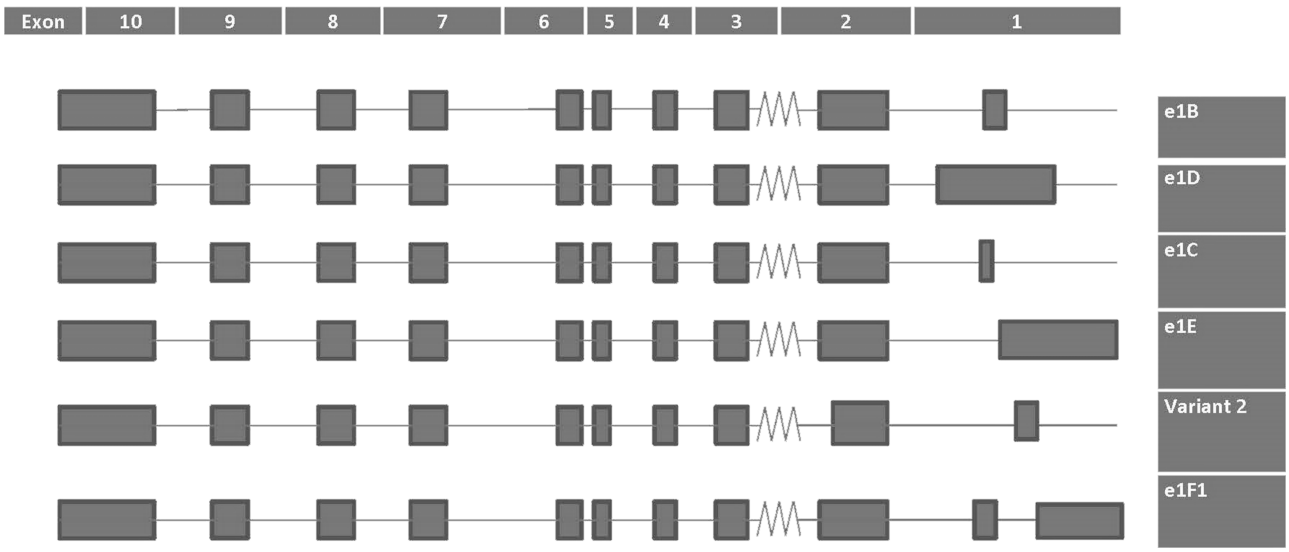

Figure 2. Graphical depiction of multiple endocrine neoplasia type 1 (MEN1) transcript variants taken from UCSC Genome Browser (1/25/17). Variant 2 codes for the shortened menin isoform 2 (610 amino acids). Note how the five amino acids appear to be lost from the C-terminal end of exon 2.

proteins[7]. Mounting evidence points to its role as a scaffold protein with many direct and indirect binding partners[7]. For example, menin associates with many hundreds of loci on the mammalian genome despite its lack of a known DNA-binding sequence[8]. It is generally agreed that menin's primary role is to regulate chromatin architecture through histone modifications resulting in altered gene transcription or even DNA repair[7]. Menin has been shown to directly bind to JunD, an AP-1 transcription factor, to facilitate histone deacetylation and turn off transcription of JunD targets. It has also been shown to enable transcription of mixed-lineage leukemia (MLL) protein target genes by modifying histones with activating trimethylation marks[9,10].

While absolute loss of menin expression is required for MEN1 syndrome tumorigenesis, relative menin expression has since been implicated in cellular proliferation of the lung, stomach, liver, blood, breast, and prostate[11–14]. However, its function is tissue and pathway specific. For instance, it is oncogenic in certain leukemias and prostate cancers, but it acts as a tumor suppressor in canonical MEN1 syndrome organ sites. Furthermore, menin can act through distinct signaling pathways, such as nuclear factor-κB (NF-κB) and transforming growth factor-β (TGF-β), and affect diverse functional outcomes such as apoptosis, cell cycle, gene transcription, and DNA repair[7,13,15]. It remains unclear why tumors arise only in neuroendocrine organs and not in other tissue types in patients with MEN1 syndrome. Furthermore, the surrounding regulatory elements that allow variable expressivity and penetrance seen in MEN1 syndrome are not yet understood.

MEN1 ISOFORMS

One explanation for menin's broad and variable role is the 5′ heterogeneity of the MEN1 mRNA transcript[10]. Northern blot, 5′ RACE, and positional deletion studies revealed that the MEN1 transcripts come in two sizes: a ubiquitously expressed 2.8-kb transcript and a larger 4.2-kb transcript with expression restricted to the pancreas and thymus[16]. The 2.8-kb transcript codes for two isoforms of 615- and 610-amino acid length (NP_000235.2 and NP_570711.1, respectively). There are additional transcript variants based on alternative splicing of exon 1 (UCSC genome browser, 1/25/17) (Fig. 2). *MEN1*'s promoter is located upstream of exon 2 with multiple transcriptional start sites (TSSs) located along with corresponding initiator elements (Inr) directing transcription of individualized exon 1 splice variants. These matching TSS/Inr sequences and splice variants, along with specific upstream and downstream *cis*-regulatory sequences, regulate menin expression on a cell-by-cell basis, suggesting a cell-specific regulation of *MEN1* transcription[17].

Four MEN1 transcript variants were detected using RNA-Sequence data obtained from human cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC) from The Cancer Genome Atlas (http://cancergenome.nih.gov) using Python version 2.7 (http://www.python.org). Example coding can be found at https://github.com/ehrl1ch/RNA-Seq. There is a small shift in menin variant expression between CCA and HCC tumor samples and matched normal tissues (Fig. 3). As noted in Figure 2, menin's various isoforms are quite similar and vary only in their TSS and exon splicing. The difference between the primary 615-amino acid isoform and the 610-amino acid isoform is the loss of Trp–Ser–Pro–Val–Gly at the 149 position (see Fig. 1 for details). While no functional differences between MEN1's various isoforms have been characterized to date, it is possible that the expression of two different isoforms in the same tissue represents distinct pathways or at least an artifact of functional dysregulation. The cause and effect of such dysregulation are unknown.

FIBROSIS

TGF-β signaling is well characterized in the liver as well[18]. For instance, TGF-β inhibits activation of quiescent hepatic stellate cells (HSCs) via Smad7 activity but stimulates fibrotic reaction in activated HSCs or differentiated myofibroblasts via Smad3 activity[18]. TGF-β is antiproliferative and proapoptotic in hepatocytes[18]. Loss of hepatic TGF-β signaling in dominant negative TGF-β receptor type II (dnTGFβrII) mice is a model for primary biliary cirrhosis (PBC) featuring anti-mitochondrial antibodies, lymphocytic infiltration, periportal fibrosis, and increased incidence of HCC[19]. One study showed that dnTGFβrII mice displayed increased proinflammatory Tregs compared to wild type (WT)[20], indicating that TGF-β can affect nonparenchymal cell types.

The relationship between menin and TGF-β in HSC-driven fibrosis was further explored using a bioinformatics approach[21]. This study examined HCC arising in patients with cirrhosis and discovered upregulated *MEN1* gene expression correlated with an increase in TGF-β pathway signaling. Menin expression was associated with HSC activation and positively correlated with profibrotic collagen and metallopeptidase inhibitor gene expression, both TGF-β/Smad transcription targets. Furthermore, TGF-β increases menin expression in both hepatocytes and HSCs[21,22]. Interestingly, one study showed that menin drives HCC formation through epigenetic upregulation of Yes-associated protein (YAP), which itself has a diverse array of functions including antagonism of Smad-associated TGF-β pathway[23]. However, this contradicts previous studies confirming menin's tumor suppressor role in HCC[13]. Further studies may shed light on menin's

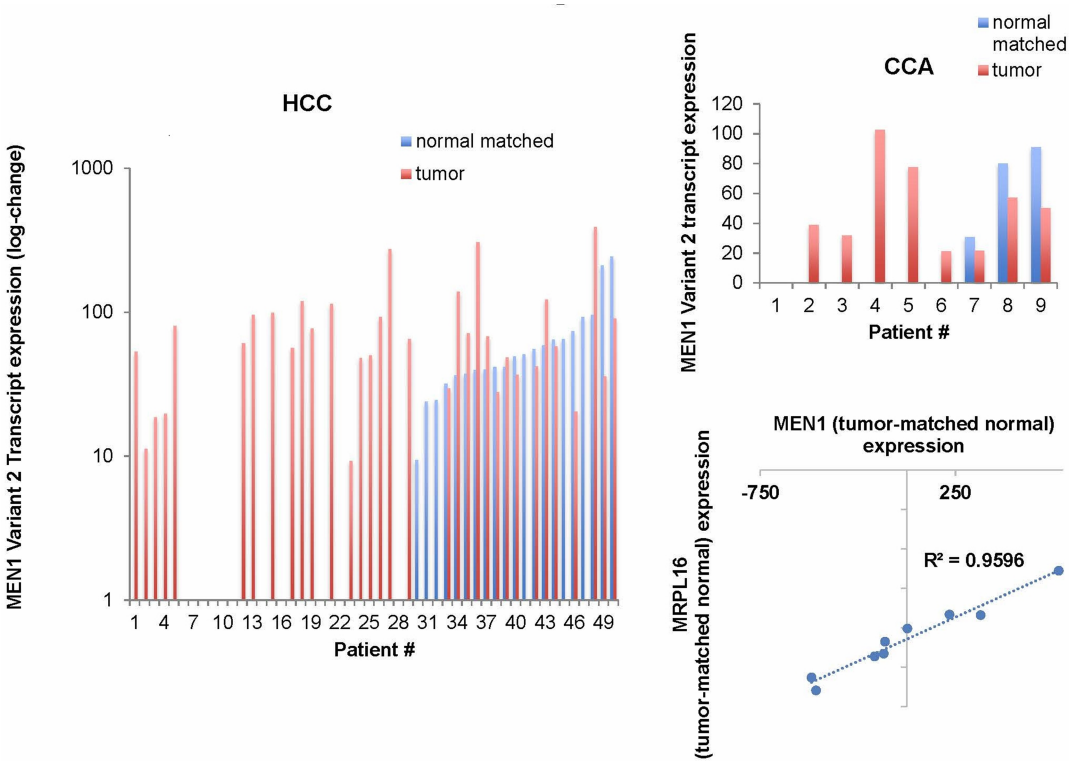

Figure 3. MEN1 transcript variant 2 is aberrantly expressed in human hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) tissues compared to matched normal tissue. *MRPL16*, a nuclear-coded mitochondrial ribosome, shows significant positive correlation with *MEN1* expression in CCA using the Pearson correlation test. *MRPL16* is located on chromosome 11 only a few hundred thousand base pairs away from *MEN1*. It is of potential interest given the amount of mutation in mitochondrial pathways in CCA. $p<0.1$.

role in balancing proliferation and fibrosis during HCC development and progression.

IMMUNOLOGY

As a ubiquitously expressed protein, menin plays a role in immune cells. Recently, menin has been implicated in $CD4^+$ T-cell immunosenescence and senescence-associated secretory phenotype[24]. Senescent $CD4^+$ T cells exhibit irregular homeostasis and cytokine production that can contribute to cancer, infectious disease, and autoimmune disease[24]. Menin binds to the *BACH2* locus and suppresses its expression through histone deacetylation. Decreased BACH2 expression increases cellular senescence. The role of menin in immune cells and hepatobiliary diseases such as primary sclerosing cholangitis (PSC), PBC, and CCA has not been explored.

$Th17^+$ T-cell differentiation is menin dependent as well. In naive $CD4^+$ T cells, menin is recruited to the *IL17* locus to drive its expression. Furthermore, menin binds the *RORC* locus to drive the expression of RORγδ in order to maintain $Th17^+$ T-cell differentiation and function[25]. IL-17 secretion by $Th17^+$ T cells can trigger HSC activation, collagen deposition, and subsequent hepatic fibrosis[26]. Peripheral blood mononuclear cells from patients with PSC showed significantly higher proportion of $Th17^+$ cells compared to healthy patients[27]. In $MDR2^{-/-}$ mice, the prevalence of IL-17^+CD4^+ T cells is thought to come at the expense of anti-inflammatory $Foxp3^+$ regulatory T cells[28–30]. Interestingly, Foxp3 interacts with EZH2, a Polycomb Group protein and menin-binding partner involved in chromatin silencing to suppress gene expression through DNA and histone methylation. $Ezh2^{-/-}$ T cells failed to adequately differentiate into $Foxp3^+$ regulatory T cells nor function properly in vivo[31]. Thus, targeting menin expression in $Th17^+$ or naive $CD4^+$ T cells could ameliorate fibrotic reaction in cholestatic liver disease.

Inhibiting menin expression also seems to decrease effector antigen-specific $CD8^+$ T cells, which were shown to be profibrotic in a bile duct ligated mouse model of cholestasis[29,32]. Yamada et al. infected $CD8^+$ T-cell-specific menin knockout mice with *Listeria monocytogenes* ovalbumin-expressing bacteria in order to assess menin's role in antigen-specific $CD8^+$ T-cell response. $CD8^+$ T cell's lack of menin expression reduced its own proliferation and survival upon infection via induction of cell cycle inhibitors, proapoptotic genes, and transcription factors

associated with effector $CD8^+$ T-cell death[32]. Thus, theoretically overexpressing menin could drive proliferation and survival of effector $CD8^+$ T cells necessary to clear infection but provoke hepatic fibrosis as a consequence.

While menin expression is directly implicated in $CD4^+$ T-cell senescence, $Th17^+$ T-cell differentiation and maintenance, and $CD8^+$ effector T-cell survival, it is indirectly implicated in $CD4^+$ Th2 cell development through the MLL complex[33]. $MLL^{+/-}$ cells were able to differentiate into Th2 memory cells but were not able to maintain H3K4 methylation and H3K9 acetylation at the *Th2* and *Gata* loci nor the expression of Th2 cytokines IL-4, IL-5, and IL-13[33]. Later studies coimmunoprecipitated menin and MLL from the c-Myb/GATA-2 complex necessary for Th2 memory cell formation[34]. Interestingly, menin was more prevalent at the *GATA-3* promoter site under Th2-promoting conditions, while MLL was more prevalent among $CD4^+$ effector/memory cells. Enhanced Th2 activity has been implicated in IG4-related cholangitis[35]. However, overall measurements of Th1/Th2 ratios in PSC/PBC populations have been inconsistent[36].

Chromatin-modifying factors are implicated in the macrophage differentiation between the proinflammatory M1 phenotype and the alternative M2 phenotype[37]. MLL-dependent H3K4me3 and H3K27 demethylation characterize M1 polarization, while M2 polarization is characterized by DNA methyltransferases and histone deacetylases (HDACs)[37]. M1 macrophages treated with a menin–MLL inhibitor exhibited decreased expression of proinflammatory cytokine CXCL10[37]. Interestingly, the M2 macrophage phenotype is more prevalent in CCA tissue and is associated with secretion of factors that suppress immunity and facilitate CCA angiogenesis and epithelial-to-mesenchymal transition (EMT) activity[38]. However, miR-24 activity was shown to suppress cytokine production and M1-type proinflammatory differentiation from macrophage cells[39]. In our CCA xenograft models, miR-24 inhibition increases menin protein expression in CCA tumor cells and leads to increased mononuclear cell tumor infiltration, increased fibrosis, and decreased angiogenesis and proliferation[40]. The role of menin and miR-24 in macrophage polarization remains to be fully understood.

CANCERS

MLL

Of great interest to menin-related pathologies is the potential use of the menin–MLL inhibitor. Originally, it was investigated in the context of a type of leukemia called "mixed-lineage leukemia," from which the MLL protein is named. In MLL, chromosomal translocations at the *MLL* locus yield a myriad of MLL fusion proteins that result in either constitutive activity and/or alternate gene targets for histone methyltransferase (HMT) activity. MLL fusion proteins still require the menin protein in order to traffic to specific genomic locations and trimethylate histone tails. Thus, blocking the menin–MLL interaction with a specific small-molecule inhibitor has the potential to decrease hematopoietic proliferation in MLLs[41].

MLL proteins play a role in bile acid physiology through interaction with nuclear receptors. MLL3 interacts with Farnesoid X receptor (FXR) and retinoic acid receptor (RAR) to positively regulate expression of genes (BSEP, MRP2, and NTCP) involved in hepatic bile acid export[42]. Genes involved in FXR/RAR activation and hepatic cholestasis (ABCC2, ABCC3, FABP6, ASB2, and IL-18) and subunits of cAMP-mediated signaling were enriched following gene expression analysis of WT versus $MLL2^{-/-}$ mice[43]. It is important to note that MLL1/2 interacts with menin, whereas MLL3/4 does not[44]. Recent exome sequencing of CCA tumors revealed mutations in genes coding for chromatin-modifying complexes, notably *ARID1A* and *BAP1*. Mutations in genes encoding MLL family proteins have also been identified[45]. It is not yet known if MLL fusion proteins exist in nonleukemic cancers. Our lab is currently investigating the effects of menin–MLL inhibitor on proliferation, angiogenesis, and fibrosis in cholestatic and CCA models.

Another possible link between menin, MLL, and nuclear receptors involves the vitamin D receptor (VDR). Treatment with vitamin D, and unsurprisingly retinoic acid, was able to force differentiation of an MLL cell line in vitro[46]. Menin was shown to drive expression of VDR transcription targets; however, overall levels of H3K4me3 (an MLL-dependent molecular signature) were unchanged[44]. Hepatic VDR expression is mostly localized to cholangiocytes, Kupffer cells, and HSCs and is negatively associated with cholestasis and liver damage[47,48]. Vitamin D treatment decreased HSC activation in vitro and decreased liver damage in $MDR2^{-/-}$ mice through reduced TNF-R1 expression and inflammatory macrophage cells[48].

TGF-β

TGF-β signaling and menin are well characterized in MEN1 syndrome. TGF-β binds TGF-β receptors to recruit SMAD proteins that translocate to the nucleus and activate transcription. It is shown in both the pituitary and parathyroid glands that menin physically interacts with Smad3 and drives expression of target genes[15]. Loss of menin expression leads to uninhibited proliferation and, ultimately, carcinogenesis as seen in MEN1 syndrome. TGF-β signaling plays a prominent role in pancreatic physiology, albeit its connection with menin is less understood. TGF-β signaling decreases proliferation in a number of pancreatic cell types: β-cells[49], pancreatic stellate cells[50], and pancreatic ductal adenocarcinoma (PDAC) cells[51]. Interestingly, a pancreatic-specific dnTGFβrII

transgenic mouse exhibited less pancreatic fibrosis in response to injury[52]. Thus, exploring the relationship between TGF-β and menin may help elucidate nuances between cellular proliferation and organ fibrosis.

JunD

The Jun family transcription factors (JunD, c-Jun, and JunB) form both hetero- and homodimers to regulate transcription. Menin binds the N terminus of JunD, but not c-Jun or JunB, in an mSin3A binding-specific manner and recruits HDAC complexes[53,54]. The complex includes Sap18, Sap30, SDS3, and RbAP48[55]. The menin–JunD traffics the mSin3A–HDAC complex to JunD target promoters to effectively turn off transcription. Additionally, menin inhibits phosphorylation of JunD, ELK-1, and c-Jun by both the c-Jun N-terminal kinase (JNK) and ERK–MEK pathways[54,56]. Loss of menin abolishes JunD repression of RAS-mediated proliferation and of transcription of JunD target genes[57,58]. Agarwal et al. showed that JunD, in the presence of menin, decreases cyclin D1 expression in order to halt cell growth[57]. Alternative splicing of the JunD mRNA transcript can yield a truncated isoform lacking several crucial amino acids at its N terminus necessary to bind menin. The truncated JunD isoform facilitates transcription at JunD target sites even in the presence of menin[59]. Many of the menin–JunD effects can also be reversed by HDAC inhibitor treatments such as trichostatin A[9] (Fig. 4).

JunD's effects are paradoxical: it halts RAS-mediated cellular transformation but can also prevent premature senescence and apoptosis. JunD$^{-/-}$ cells showed nuclear accumulation of p53 and p27 that resulted in early cell death. JunD responds to TNF-α signaling to drive anti-apoptotic cell survival. TNF-α increased JunD and NF-κB binding to target gene promoter sites, and JunD overexpression drastically increased NF-κB-driven gene expression in rat hepatocytes[58] (Fig. 4). JunD$^{-/-}$ mice were protected from carbon tetrachloride-induced liver damage and fibrosis. JunD was shown to drive expression of TIMP-1, IL-6, and MMP-9 in both hepatocytes and activated HSCs in a JNK-independent fashion[60]. JunD, like menin, appears to serve dichotomous functions in order to maintain the cell in a quiescent state.

Both JunD and menin appear to play roles in hepatic oxidative stress; however, the physical link between the two has yet to be established. Gerald et al. showed that JunD antagonizes RAS-mediated transformation by upregulating antioxidant genes and downregulating ROS-producing

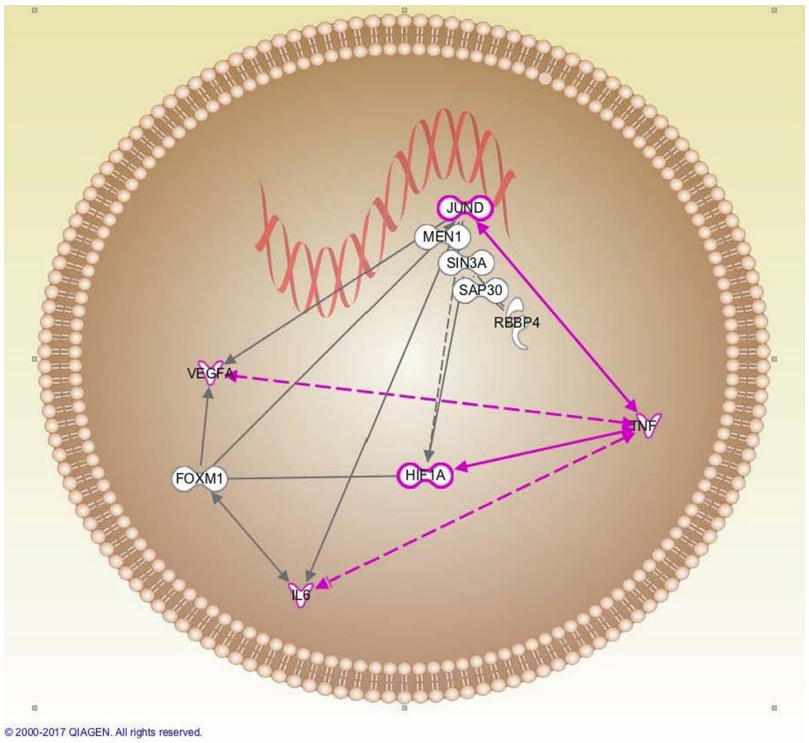

Figure 4. Network analysis depicting menin in complex with JunD, Sin3A, Sap30, and RBBP4 mediating histone deacetylase 1/2 (HDAC1/2)-dependent histone deacetylation and suppression of angiogenesis (VEGF-A) and inflammation (IL-6). Jun–TNF-α drives survival in a HDAC1/2-independent manner by antagonizing menin–JunD–HDAC1/2 activity. QIAGEN's Ingenuity® Pathway Analysis (IPA®; www.qiagen.com/ingenuity; QIAGEN, Redwood City, CA, USA).

genes. Consequently, decreased ROS results in a reduction of HIF-1α levels and represses vascular endothelial growth factor (VEGF-A) transcription and tumor angiogenesis. JunD$^{-/-}$ cells exhibited increased ROS damage, VEGF-A expression, and tumor angiogenesis[61]. In an ischemia/reperfusion (I/R) injury model, characterized by an abnormal level of ROS production, JunD was found to play a protective role by antagonizing expression of other AP-1 transcription factors. JunD reduced expression of cyclin D1 and decreased early phase proliferation that ultimately decreased ROS production and liver damage. Loss of JunD enhances proliferation and caspase-mediated liver damage following I/R injury[62].

One group found that menin expression is required for proper functioning of heat shock proteins in *Drosophila melanogaster*. Loss of menin expression leads to increased hypoxia and oxidative stress[63]. Loss of menin expression was shown to increase vascularity and VEGF-A expression in PNETs[64]. We have previously shown that menin overexpression decreases VEGF-A expression in CCA cell lines, potentially through an upregulation of the JunD–Menin interaction[40].

miR-24

Recently, menin was shown to exist in a negative regulatory feedback loop with miR-24[65]. miR-24 binds to the 3′-untranslated region of the MEN1 mRNA transcript to negatively regulate its protein expression. Menin, in turn, can positively regulate expression of miR-24 via MLL-driven histone trimethylation at both of the miR-24 chromosomal locations, chromosomes 9 and 19[10]. Overexpression of menin represses *MEN1* promoter activity, indicating negative self-regulation[17]. Increase in miR-24 expression is thought to be a possible mechanism driving MEN1 syndrome, and thus targeting miR-24 expression is an attractive therapeutic possibility.

miR-24 activity is documented to drive both pancreatic and liver cancers, although its role in other tissues remains controversial[66–68]. miR-24 is well documented to negatively regulate menin expression in the endocrine pancreas and drive tumorigenesis[65]. There is strong evidence for the oncogenic role of miR-24 in PDAC as well. miR-24 overexpression was shown to decrease FZD5 (WNT receptor), HNF1B (HOX transcription factor), and TMEM92 to drive PDAC EMT. A separate study showed that miR-24 regulated the BIM pathway to drive PDAC angiogenesis[67,68]. Melatonin negatively regulates miR-24 expression in several cancers to inhibit tumor proliferation and migration, and lower melatonin signaling in cholangiocytes leads to increased VEGF expression[69]. miR-24 expression is increased in HCC, and its inhibition reduces proliferation, migration, and invasion[70].

We have shown that miR-24 possesses oncogenic properties in the setting of CCA. Inhibiting its expression increases menin protein expression and decreases CCA proliferation and angiogenesis as demonstrated by both in vitro and mouse xenograft models[40]. miR-24 inhibition in MDR2$^{-/-}$ mice, a model for PSC, actually increased hepatic fibrosis and proliferation while increasing menin expression (unpublished). miR-24 has been shown to negatively regulate SMAD protein expression in vascular endothelial cells[71]. However, its role in TGF-β signaling and hepatic fibrosis has not yet been explored.

Pancreas

While it is well established that patients with MEN1 syndrome develop PNET, the molecular mechanisms underpinning this phenomenon have recently been elucidated[72–76]. The menin–MLL interaction, primarily known for its oncogenic effect in MLL[77], plays a critical tumor suppressor role in islet cells by driving expression of cell cycle inhibitors p27 and p18 with chromatin-activing histone H3 lysine 4 trimethylation (H3K4me3) marks[72]. Agarwal and Jothi further demonstrated menin-dependent H3K4me3 marks within the imprinted Dlk1–Meg3 locus and within all four HOX loci[73], which in turn suppresses islet proliferation[74]. It is important to note that the genetic landscape of HOX loci is functionally diverse, and only certain homeobox genes contained within these clusters may be activated by menin–MLL-driven H3K4me3. Furthermore, epigenetic regulation is a dynamic process where loss of H3K4me3 can functionally enrich H3K27me3 chromatin-silencing marks[78].

Menin is necessary for the antiproliferative effects of Wnt signaling in pancreatic islet cells as well[75]. Menin physically interacts with Wnt pathway proteins such as β-catenin and TCFs, and menin expression positively correlates with downstream target AXIN2 expression and H3K4me3 marks on the *AXIN2* promoter site. Wnt-dependent H3K4me3 is mediated through MLL activity in other tissues[79], but this connection has not yet been established in pancreatic islets. We described above how miR-24 expression decreases FZD5 expression (a WNT signaling receptor) to help drive PDAC growth, potentially through a downregulation of menin[67].

Dreijerink et al. postulate that menin–MLL activity is necessary for nuclear receptor-mediated transcription in MEN1 syndrome[80]. Specifically, they show that menin and estrogen receptors promote H3K4me3 and expression of the TFF1 gene[80], a canonical tumor suppressor critical for the maintenance of epithelial barrier and mucosal protection in both pancreatic and biliary ducts[81,82]. Interestingly, estrogen signaling downregulates TNF by decreasing JNK activity and subsequent phosphorylation of JunD and c-Jun, which is itself a menin-dependent process[83]. This kind of regulation would imply that menin operates under distinct pathways that are often overlapping and either redundant and/or synergistic.

Menin can signal through other pathways in the islet pancreas as well. It binds with protein arginine methyltransferase 5 (PRMT5) to promote H4R3 dimethylation and suppress *GAS1* gene expression and proproliferative hedgehog signaling[84]. HOX gene HOXHB9, also known as HLXB9, is downregulated upon menin overexpression[85]. Menin can participate in posttranslational modifications within the β-cell cytoplasm as well. Menin was shown to bind to IQGAP1, a cytoplasmic scaffold protein, and aggregate E-cadherin and β-catenin at cell-to-cell contact sites to increase adhesion and mitigate Rac1 signaling to decrease migration[86]. Wang et al. documented that menin physically interacts with AKT in the cytoplasm and suppresses its downstream phosphorylation activity[87].

Menin's role in PDAC is less characterized. Recessive homozygosity and promoter methylation at the *MEN1* locus were observed in a small subset of PDAC[88]. Cheng et al. demonstrated that menin interacts with DNA methyltransferase (DNMT1) and observed menin downregulation associated with PDAC development[89]. They showed that menin regulates p27 and p18 expression through DNMT1-dependent promoter methylation. Interestingly, in vitro modulation of menin positively regulated p16, HOXA9, HDAC5, and CBX4 while downregulating CDK2, CDK4, ESM1, IGFBP7, and GAS1 (a member of the Hedgehog signaling family), indicating other molecular mechanisms of action distinct from CpG island methylation. This study reveals how menin can act through different mechanisms in a tissue- and pathway-specific manner.

Liver

Literature regarding menin's role in HCC is controversial. Gang et al. reported a decrease in menin expression in primary human HCC tumor tissues and cell lines and were able to regulate cell proliferation through the modulation of menin expression[13]. They demonstrated that menin physically interacts with Sirt1 to deacetylate p65 and repress NF-κβ-mediated transcription. However, Zindy et al. reported an increase in menin expression in human HCC samples from patients with underlying cirrhosis[21]. In cirrhotic patients, menin expression positively correlated with tumor size and changed uniformly among tumor, matched normal, parenchymal, and nonparenchymal cells. Furthermore, menin expression positively correlated with levels of hepatic fibrosis and expression of fibrotic markers, likely through the association of HSC activation.

We have demonstrated in our lab that menin acts as a tumor suppressor in biliary epithelium and is negatively regulated by miR-24[40]. In vitro modulation of menin inversely regulates proliferation, angiogenesis, migration, and invasion. Inhibition of miR-24 in xenograft CCA models increases menin expression and decreases proliferation and angiogenesis.

METABOLIC DISEASE

Pancreas

Menin plays a role in certain metabolic diseases in addition to cancer. For example, Karnik et al. demonstrated decreased menin expression levels following pregnancy-induced expansion of pancreatic islets in mice[90]. β-Cell-specific menin overexpression reduced islet hyperplasia, induced hyperglycemia, and impaired glucose tolerance, features similar to gestational diabetes. Steroids, such as progesterone, increased menin expression, while prolactin decreased menin expression. It should be noted that insulin levels and insulin gene-related expression were unchanged in this model. Recent evidence suggests that, in addition to menin repression, FOXM1 expression and serotonin signaling are important to β-cell expansion in response to prolactin/lactogen signaling[91]. How menin interacts with these other players remains to be elucidated. It is interesting to note that loss of histone acetylation and trimethylation are known epigenetic features of type 2 diabetes[92].

Neurohormonal stimulation (i.e., prolactin or progesterone stimulation) is one of many mechanisms maintaining islet cell homeostasis. Zhang et al. demonstrated that glucose stimulates islet cell proliferation through decreased menin expression levels via PI3K/Akt/Foxo1 signaling[93]. High glucose stimulation-dependent islet cell hyperplasia both in vitro and in vivo also correlated with reduced menin expression[93]. Growth was reversed with menin overexpression or PI3K/Akt pathway inhibitors.

Duodenum

The duodenum is an important regulatory source for the hepatobiliary system. S cells of duodenum release secretin, which works in conjugation with somatostatin to regulate the bile and pancreatic duct physiology[94]. Somatostatin signaling increases menin expression in the duodenum[7], and our lab has preliminary data showing that secretin decreases menin expression in cholangiocytes (unpublished). Furthermore, menin expression negatively regulates gastric inhibitory polypeptide via PI3K/AKT signaling, which counteracts the effects of secretin[95].

Liver

Wuescher et al. studied a liver-specific hemizygous deletion of menin mouse model. Consistent with other data, these mice showed decreased tolerance to high-fat diet with increased weight gain; decreased glucose tolerance; increased serum insulin, glucose, and glucagon; and increased liver triglycerides[95]. Menin expression itself was tied to fasting, refeeding, and insulin levels. Wuescher et al. demonstrated that insulin signaling via the PKB/AKT pathway downregulated menin protein

levels but not mRNA levels and that menin is required to suppress FOXO1 target gene transcription[96].

Menin interacts with nuclear receptors in the liver as well. Cheng et al. showed that menin expression binds with PPARα to activate transcription of genes involved in fatty acid oxidation. Loss of hepatic menin expression leads to liver steatosis, hepatic triglyceride accumulation, and increased serum levels of ketone bodies[97]. miR-24 expression, a negative regulator of menin expression, was significantly increased in high-fat diet-fed mice. Inhibiting miR-24 leads to upregulation of insulin-induced gene 1 and decreased hepatic fat accumulation[39]. It is interesting to note that MRPL16, a nuclear-coded mitochondrial ribosomal protein located adjacent to *MEN1* loci, significantly correlates with MEN1 expression in CCA, indicating menin may regulate promoter activity on the nearby loci (TCGA, 4/7/16) (Fig. 3).

Hepatic menin expression is decreased in aging and diabetic mice, and further downregulation leads to liver steatosis[98]. While Cheng et al. tied hepatic menin expression to activation of genes involved in fatty acid oxidation, Cao et al. associated loss of menin expression to activation of genes involved in fat deposition[97,98]. Menin and SIRT1, a nuclear NAD^+-dependent HDAC, bind at the promoter sites of several genes involved in fat deposition, including CD36, facilitating histone deacetylation and loss of expression[98]. Sirtuin1 is a regulator of FXR-dependent bile acid signaling[99]. We described above how MLL facilitates transcription of FXR bile acid export target genes in HMT-dependent mechanism[42], and how menin and Sirt1 act together to antagonize p65-mediated transcription of NF-κβ target genes[13]. It is unclear which signaling pathway is at work here.

CONCLUSION/FUTURE PERSPECTIVES

This review focuses on the emerging role of menin in noncanonical MEN1 disease states (Table 1). Since the discovery of its crystal structure in 2011, more detail has emerged surrounding its mechanism[56]. It binds directly with JunD and MLL and indirectly with a host of other binding partners, presumably based on the protein complex it recruits. This review highlights the dichotomous function menin can play in disease. Loss of menin appears detrimental in both hepatobiliary cancers and metabolic disease, whereas menin overexpression appears detrimental in fibrotic autoimmune diseases. Specifically, it appears that loss of menin in parenchymal cells is harmful, whereas menin overexpression in stromal cells (stellate and immune cells) is also harmful. How signaling pathways such as TGF-β, NF-κβ, or nuclear receptor activation affect menin's role as a histone writer remains to be fully elucidated. Of particular interest is understanding how small-molecule inhibitors of menin–MLL, HDAC, or miR-24 activity may be used to further understand

Table 1. Summary of the Various Signaling Pathways Involving Menin From Upstream Extracellular Signals to Menin-Binding Partners, Mechanism of Action, and Functional Outcome as a Result

Organ	Disease	Signaling	Menin Binding Partners	Mechanism	Outcome
Parathyroid/pituitary	Normal	TGF-β	Smad3	?	Inhibit proliferation
Liver	HCC/cirrhosis	TGF-β	Smad3	?	COL1α2 expression, fibrosis, activated HSCs
Liver	HCC	?	?	H3K4me3	YAP transcription
Liver/fibroblast	Normal	?	JunD	HDAC	Cyclin D1 repression
Liver	Normal	TNF-α	JunD/NF-κb subunits	HDAC	Cell survival: increased TIMP, IL-6, and MMP-9 expression. Decreased p53/p27 expression
Liver/stellate cells	$MDR2^{-/-}$	Vitamin D	VDR	?	Decreased fibrosis/HSC activation
Liver	HCC	NF-κβ	P65	?	Decreased transcription/growth
Liver	Steatosis	?	PPARα	?	Fatty acid oxidation
Liver	Steatosis	?	Sirt1	HDAC	Loss of CD36 (fatty acid oxidation)
Pancreas islets	Diabetes	Progesterone/prolactin	?	?	Decreased growth/increased growth
Pancreas	PNET	?	MLL	H3K4	p27/18, HOX loci expression. Decreased islet proliferation
Pancreas	Normal	Wnt	β-Catenin/TCF	H3K4me3	AXIN2 expression/inhibit proliferation
Pancreas	MEN1 syndrome	Estrogen	ER/menin	H3K4me3	TFF1 expression/survival

The left two columns specify the organ and disease state. ? indicates that particular signaling aspect in relation to menin pathways remains to be elucidated.

menin's regulation and function and to combat menin-related pathologies.

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