MENIN Loss as a Tissue-Specific Driver of Tumorigenesis

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Abstract

The *MEN1* gene encodes MENIN, a tumor suppressor that plays a role in multiple cellular processes. Germline and somatic mutations in *MEN1* have been identified in hereditary and sporadic tumors of neuroendocrine origins suggesting context-specific functions. In this review, we focus on the development of mutational *Men1 in vivo* models, the known cellular activities of MENIN and efforts to identify vulnerabilities in tumors with MENIN loss.

History of *MEN1* and Discovery of MENIN

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal-dominant syndrome that classically manifests as parathyroid, pituitary and pancreatic neuroendocrine tumors. The first description of a MEN1 patient is attributed to Jakob Erdheim who described a patient with acromegaly, a pituitary adenoma, and four hypertrophic parathyroid glands on postmortem examination (Erdheim 1903; Ballard, Fame, and Hartsock 1964). In 1927, Cushing and Davidoff reported the first patient with classic findings of MEN1: adenomas were identified in the pituitary, the parathyroid glands and within a pancreatic islet (Ballard, Fame, and Hartsock 1964; Cushing 1927). Over the subsequent three decades, multiple investigators suggested the syndrome may be hereditary. In 1954, Wermer proposed that the disease was transmitted by a single autosomal dominant gene with a high degree of penetrance (Wermer 1954). This was further corroborated by Ballard and colleagues' description of a "syndrome of multiple endocrine adenomatosis" that included six generations consisting of 42 family members. They reported endocrine neoplasia involving

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the pituitary (64.9%), pancreas (81.2%), parathyroids (88.2%), adrenals (37.6%) and thyroid (18.8%) (Ballard, Fame, and Hartsock 1964).

Subsequently, linkage analysis mapped *MEN1* in insulinoma specimens to chromosome 11q13 (Larsson et al. 1988). By studying two brothers who inherited MEN1 syndrome from their mother, it was discovered that tumor tissue had loss of one constitutional allele at two loci on chromosome 11 when compared with matched normal tissue. The two loci spanned from band p15 to band q23 (Larsson et al. 1988). To ultimately identify the specific gene, polymorphic markers were mapped to the 11q13 region, identifying 33 candidate genes (Chandrasekharappa et al. 1997). To further narrow down the boundaries of the gene, they examined interstitial deletions and mitotic crossing-over events in patient tumor samples from hereditary MEN syndrome type I and sporadic gastrinomas that had been laser-dissected, identifying the minimal interval bounded by marker PGYM and D11S4936 as the candidate transcript for MEN1. As confirmation, the team designed primers to amplify the exons from the genomic DNA of unrelated MEN1 patients, finding mutations in 14 of the 15 patients in this candidate MEN1 transcript. Of these mutations, five were frameshift mutations, three were nonsense mutations, two were in-frame deletions and two were missense alterations (Chandrasekharappa et al. 1997). The transcript was estimated to be 2.8 kilobases and the 610-amino acid protein was named MENIN, reflecting its causality to MEN1 syndrome.

MENIN was first characterized as a nuclear protein after localization studies were performed by Guru *et al.* (Guru et al. 1998). With immunofluorescence and immunoblotting of subcellular fractions, they detected MENIN as a nuclear protein. Using green fluorescent protein-tagged MENIN deletion constructs transfected into HEK-293T cells, the group discovered that MENIN had at least two nuclear localization signals (NLS) and an accessory NLS (NLSa) in its C-terminal end (La et al. 2006). However, none of the known 22 missense or 3 inframe germline mutations in MEN1 localized to the NLS. Furthermore, of the 43 frameshift/nonsense mutations, 39 were truncated such that the NLS would not have been translated, which would result in a cytosolic protein.

MENIN is a well-established tumor suppressor protein, which was initially inferred from pedigree and sequencing studies (Agarwal et al. 1997; Heppner et al. 1997; Zhuang et al. 1997; Debelenko et al. 1997). By cloning and sequencing tumors from familial MEN1 syndrome, sporadic MEN1 and familial hyperparathyroidism, Agarawal *et al.* found forty different mutations across the *MEN1* gene (Agarwal et al. 1997). The mutations predominately predicted a loss of function in the protein, thus suggesting that *MEN1* is a tumor suppressor gene. Additionally, dideoxy fingerprinting (ddF) and then sequencing of amplified DNA fragments with aberrant ddF patterns on 33 sporadic parathyroid tumors identified 13 (39%) as having loss of heterozygosity (LOH) at 11q13 and seven had *MEN1* mutations (Heppner et al. 1997). These data supported the tumor suppressor function of *MEN1* and the biallelic loss-of-function mechanism.

MEN1 Mutations in Human Tumors

MEN1 mutations were first reported in insulinomas and gastrinomas in 1997. By using fluorescent *in sito* hybridization analysis on tumors from 40 patients, one copy of *MEN1* was found to be deleted in 25 of 27 (93%) sporadic gastrinomas and 6 of 12 (50%) sporadic insulinomas. Of all forty tumors, only one exhibited a germline mutation (Zhuang et al. 1997). Somatic mutations of *MEN1* have also been identified in 2 of 12 (17%) of sporadic insulinomas and 9 of 27 (33%) of sporadic gastrinomas (Zhuang et al. 1997). Subsequently, whole-exome sequencing of 10 non-familial pancreatic neuroendocrine tumors (pNETs), found *MEN1* inactivating/missense mutations in 50% and identified mutations in 44% (30/68) of tumors in a validation set (Jiao et al. 2011). *MEN1, DAXX* (death-domain associated protein), *ATRX* (a thalassemia/mental retardation syndrome X-linked) and mTOR pathway genes (i.e. *PTEN, TSC2, PIK3CA*) were among the most commonly altered genes. This is in stark contrast to the genes mutated in tumors of the exocrine pancreas (e.g. *TP53, KRAS, CDKN2A, TGFBR1, SMAD3, SMAD4*) (Jones et al. 2008).

In non-neuroendocrine solid tumors, *MEN1* mutations are infrequent suggesting a unique dependency of MENIN in neuroendocrine tissues. An aggregation of published analyses of the Cancer Genome Atlas (TCGA) shows that *MEN1* mutations occur 0.3–6.5% of examined solid tumors (Figure 1).

Improved survival has been observed in patients with either *MEN1/DAXX/ATRX* mutations or *MEN1* in combination with *DAXX/ATRX* mutation (Jiao et al. 2011). This genotype-specific survival effect is not observed in all series and may vary depending on disease stage, as loss of *DAXX* and *ATRX* has also been associated with poorer prognosis (Marinoni et al. 2014). In another study of 102 sporadic, resected primary pancreatic neuroendocrine tumors that underwent whole-genome sequencing, somatic *MEN1* mutations were present in 41% of the tumors and germline mutations in 6% (Scarpa et al. 2017). The mutations were distributed across the gene without evidence of clustering. A majority of these tumors also had an inactivating copy number change in *MEN1*.

In an institutional cohort of approximately 17,000 sequenced tumors across cancer types, we observed multiple amino acid positions that have an increased frequency of mutations (Figure 2). The most frequent somatic mutation (found in multiple cancer types) occurred at codon 521 which is in proximity to the NLS although mutations at this site have not been functionally validated. MENIN has at least three NLS (NLS1, NLS2 and an accessory NLSa) and single point mutations in these regions only attenuate but do not completely block nuclear translocation of MENIN (La et al. 2006). Cytosolic localization was only achieved when all three of the NLSs were concurrently mutated. While none of these NLSs is crucial for the recruitment of MENIN to the chromatin, the key positively-charged residues at the NLSs are crucial for MENIN contacting the promoter DNA as shown by chromatin immunoprecipitation (ChIP) assay (La et al. 2006).

Mouse Models of Menin Loss

The sequence of *MEN1* is highly conserved across species, from drosophila to human. In mice, the *Men1* gene is approximately 6.7 kilobases, maps to chromosome 19 and is 611 amino acids long (Stewart et al. 1998). Compared to the human protein, murine Men1 has 97% amino acid sequence homology. Like human *MEN1*, murine *Men1* has ten exons of which the first exon is not translated. Several constitutively deleted (Crabtree et al. 2001; Bertolino, Radovanovic, et al. 2003; Bertolino, Tong, Galendo, et al. 2003; Loffler et al. 2007; Harding et al. 2009; Wiedemann and Pellegata 2016) and conditionally inactivated (Bertolino, Tong, Herrera, et al. 2003) *Men1* alleles have been used to study its effect *in vivo* (Table 1).

Mouse Models of *Men1* Conventional Knockout

The earliest murine model of *Men1* knockouts was developed at the National Human Genome Research Institute by Crabtree *et al* (Crabtree et al. 2001). The group created a targeted *Men1* gene by inserting a floxed PGK-neomycin cassette into intron 2 and a third loxP site in intron 8 (Men1 $^{3-8/+}$). The homozygous phenotype was found to be embryonic lethal, with developmental delay and exhibited cranio-facial defects by E11.5–12.5. Heterozygous mice developed islet cell hyperplasia and pancreatic islet tumors at 9 months. By 16 months, larger and multi-focal islet tumors were observed. 20/71 mice (28%) developed a pancreatic islet tumor by the age of 22 months and disease progression from hyperplastic islet cells tumor formation correlated with worsening of insulin elevation (2.4–15 ng/ml versus 15–39 ng/ml) and loss of heterozygosity at the *Men1* locus. Comparable to human MEN1 syndrome, these animals also had tumors in the parathyroid, pituitary and adrenal glands (carcinoma and pheochromocytoma) in 7–26% of the animals.

In 2003, Bertolino *et al.* created a mouse model of constitutive *Men1* deletion spanning exon 3 (Men1$^{1/}$) (Bertolino, Radovanovic, et al. 2003). Once again, the Men1 $^{/}$ genotype were embryonic lethal with defects found in organogenesis of the neural tube, heart and liver. The heterozygous animals exhibited multiple endocrine neoplasms around one year of age mimicking the human condition, with pancreatic islet cell tumors, gastrinomas, parathyroid adenomas, pituitary adenomas, and thyroid hyperplasia and tumors (Bertolino, Tong, Galendo, et al. 2003). However, islet cell tumors were found to progress to carcinoma and then metastasis in aged mice.

Loffler *et al.* developed the third conventional mouse model of *Men1* knockout (Loffler et al. 2007). Exon 2, including the translation start site, was deleted from *Men1.* By 270 days, 33% of the heterozygous animals had pancreatic islet hyperplasia and 58% had an adenoma. Pancreatic islet adenomas were observed in 72% by 360 days, 83% by 450 days, and >90% by 540 days. Other organs that developed a phenotype include pituitary gland, parathyroid, thyroid, testes, ovaries and adrenal glands.

Harding *et al.* followed in 2009 with a conventional mouse model knocking out exons 1 and 2 of *Men1* (Harding et al. 2009). Heterozygous animals had a worse survival compared to wildtype animals and developed a broad spectrum of endocrine tumors. *Men1*$^{+/-}$ animals

developed pancreatic tumors (9 months), pituitary tumors (12 months) and parathyroid adenomas and adrenal cortical tumors (15 months).

Mouse Models of *Men1* Conditional Inactivation

As homozygous knockout of the *Men1* gene led to embryonic lethality, it was desirable to develop conditional targeting of the *Men1* gene to investigate the function of menin in various organs in temporally controllable manner. Using their *Men1* floxed exon 3 to 8 transgene, Crabtree *et al.* crossed mice with floxed *Men1* loci with mice expressing a Cre-recombinase transgene under the rat insulin promoter (RIP) to specifically delete *Men1* in β-cells (Crabtree et al. 2003). These animals developed pancreatic neuroendocrine tumors at 6 months of age.

Bertolino *et al.* also created a model of *Men1* deletion (Men1$^{+/}$) that was specific to β-cells (RIP-Cre) (Bertolino, Tong, Herrera, et al. 2003). They observed islet hyperplasia around two months with progression to dysplasia and insulinoma by six months of age. By ten months, 100% penetrance in tumor formation was observed. Like the previous murine models, insulin levels were correlated with the degree of malignant transformation in the β-cells. Similarly, Biondi *et al.* induced conditional deletion of exon 2 of *Men1* with RIP-Cre ($_{Men1}$$^{loxP/loxP}$-*RipCre*$^+$$^)$ (Biondi et al. 2004) By. four months, β-cells hyperplasia was observed and by 9 months, the animals developed insulinomas (33.3%) and prolactinomas (71.4%).

Another conditionally inactivated *Men1* murine model was created by Shen *et al.* and relies on a Cre recombinase specifically driven by a pancreatic progenitor promoter, PDX1 (pancreatic and duodenal homeobox 1) (Shen et al. 2009). The *Men1* allele is floxed from exon 3 to 8 (*Men1*$^{f/f}$). Although the Pdx1 promoter expresses Cre recombinase in both endocrine and exocrine pancreatic cells, only endocrine tumors manifested, indicating the specific impact of the *Men1* gene deletion on certain endocrine tissues. These mice have elevated serum insulin levels by 5 months and then develop an insulinoma by 10–12 months.

Recently, Lines et al. described a temporally controlled model of MEN1 pancreatic neuroendocrine tumor using tamoxifen-inducible Cre recombinase under the control of rat insulin promoter (Lines et al. 2017). Exons 3 and 8 of *Men1* were floxed by loxP sites. The mice were aged to three months and then fed a tamoxifen diet for 5 days. They were then subsequently aged until at least 5 months and necropsies were performed. 100% of animals fed tamoxifen had pancreatic NETs and significantly larger non-malignant islet cells.

Interest in knocking out *Men1* in the endocrine islets of Langerhans has been not limited to β-cells. Ablation of exons of *Men1* alleles in α-cells has been achieved by two groups with the use of a Cre recombinase under control of a rat glucagon promoter (Shen et al. 2010) or a proglucagon promoter (Lu et al. 2010). Although the absence of *Men1* was proven to be limited to α-cells, Shen et al. observed only β-cell insulinomas where as the animals developed by Lu et al. exhibited α-cell glucagonomas, β-cell insulinomas and tumors that consisted of both α-cells and β-cells. To elucidate why insulinomas develop in light of ablating menin in α-cells only, Lu et al. performed cell-lineage analysis by staining for a reporter fluorescent protein marker and concluded that α-cells trans-differentiate into β-

cells. On the contrary, Shen et al. performed fluorescence-activated cell sorting to isolate α-cells or β-cells and detected *Men1* deletion only α-cells.

Double Knockout Mouse Models of *Men1* Conditional Inactivation

With the discovery of additional interacting partners of menin, several mouse models knocking out both menin and the interacting partner have been developed. As discussed in the following sections, Kmt2a (MLL1) is a histone H3 lysine 4 specific methyltransferase that associates with menin and promotes leukemogenesis. However, the role of Kmt2a is largely not understood in the pathogenesis of pancreatic neuroendocrine tumor. As such, Lin et al. developed a *Kmt2a$^{f/f}$;Men1$^{f/f}$;RIP-Cre* animal (Lin et al. 2016). *Kmt2a$^{f/f}$;RIP-Cre* animals exhibited islet hyperplasia at 10 months but even with later time points, no frank tumors developed. With the addition of *Men1$^{f/f}$*, *Kmt2a$^{f/f}$;Men1$^{f/f}$;RIP-Cre* animals had a significantly worsened survival compared to *Men1$^{f/f}$;RIP-Cre* animals (334 days vs. 274 days). Hyperinsulinemia was used to approximate time of tumor formation and higher levels of insulin were achieved in the animals deficient of Kmt2a at 6 months than *Men1$^{f/f}$;RIP-Cre* animals. Islet cell tumors were also more numerous, larger and higher grade in *Kmt2a$^{f/f}$;Men1$^{f/f}$;RIP-Cre* animals as compared to *Men1$^{f/f}$;RIP-Cre* animals.

When Gillam et al. noted hypoplasia in pituitary and pancreatic islet cells with the ablation of *Cdk4*, they became interested in studying this in the context of neuroendocrine tumorigenesis. As such, they made animals that were heterozygous in *Men1* with the addition of homozygous knockout of either *Cdk4* or *Cdk2*, a CDK which did not exhibit any neuroendocrine effects. Compared to the *Men1$^{+/-}$* animals which developed pituitary (45%) and islet cell tumors (62%), *Men1$^{+/-}$;Cdk4$^{-/-}$* animals did not develop neuroendocrine tumors at 15 months. In addition, *Men1$^{+/-}$;Cdk2$^{-/-}$* animals developed both types of tumors, suggesting that *Cdk4* is required for neuroendocrine tumorigenesis secondary to *Men1* loss (Gillam et al. 2015).

MENIN Function

Across species, MENIN is highly conserved and does not share homology with any other family of proteins. The delineated activities of MENIN are diverse and often context specific. MENIN is thought to play a role in GTPase activity, transcriptional regulation and cell cycle regulation, among others.

GTPase Activity and Transcriptional Regulation

Within its N-terminus, MENIN contains five consensus sequence motifs characteristic of GTPases. In the presence of nm23, a tumor metastasis suppressor, MENIN acts as a GTPase (Yaguchi et al. 2002). Individually, MENIN and nm23 both have very low affinity to GTP and GDP and no GTPase activity. As a corollary, MENIN does not influence the functions of nm23 (i.e. NDP kinase, protein kinase, or Rad-GAP activity). Moreover, the crystal structure of MENIN and its interacting proteins have been solved using crystallography but no GTPase-like folding or domain has been identified (Huang et al. 2012; Murai et al. 2011). Thus, the significance of the GTPase activity for MENIN remains undefined.

The discovery of the interaction of MENIN and activator protein 1 factor (AP1) JunD were the first hints suggesting that MENIN played a role in cell cycle regulation. Unique among the AP1 proteins, JunD is an inhibitor of cell growth (Shaulian and Karin 2001). Transcription factor JunD was identified as an interacting partner with the N-terminus of MENIN via a yeast two-hybrid screen (Gobl et al. 1999; Agarwal et al. 1999). MENIN does not interact with any other member of the AP1 family. In the presence of mutant MENIN, JunD-mediated transcription was not repressed and with the addition of a histone deacetylase inhibitor, MENIN lost its ability to repress JunD-mediated transcription. This paradoxical repression of JunD, a suppressor of growth, by MENIN, remained without biologic significance until it was discovered that JunD had growth promoting activity in the absence of MENIN (Agarwal et al. 2003). *In vitro* cell growth assays in *JunD*-null, *Men1 WT* immortalized fibroblasts showed a rescue of the growth inhibition phenotype with overexpression of wildtype *JunD* whereas overexpression of mutant *JunD,* which could not bind to Men1, exhibited growth promotion. In immortalized fibroblasts that were *JunD* WT, *Men1* null, overexpression of wildtype *JunD* exhibited a more transformed-like morphology but no change in growth index. Furthermore, it was further corroborated that the mechanism through which MENIN represses JUND is through recruitment of histone deacetylase (Kim et al. 2003).

Coimmunoprecipitation experiments have shown that MENIN also interacts with the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) family of transcriptional factors, which induce a cellular stress response (e.g. to ionizing radiation, mitogens, growth factors and cytokines) (Heppner et al. 2001). MENIN was shown to repress NF-kB transactivation in a dose-dependent manner.

Menin is implicated in histone modification through its interactions with mixed lineage leukemia protein 1 (MLL1, also called KMT2A) (Huang et al. 2012), mixed lineage leukemia protein 2 (MLL2, also called MLL4, KMT2B, or KMT2D) and trithorax protein absent, small homeotic 2 (Ash2L) (Hughes et al. 2004). Menin was shown to physically associate with Set-domain containing histone methyltransferase (HMTase) complexes MLL2 and Ash2L by nondenaturing immunoprecipitation and mass spectrometry (Hughes et al. 2004). The menin-associated HMTase specifically methylates histone H3 on lysine 4 (H3K4). In cells retrovirally infected with mutant *Men1*, HMTase activity significantly decreases. Since menin also interacts with Rpb2, the menin-HMTase complex likely associates with transcriptional activation complexes. Using microarray hybridization, gene expression of *Men1* wildtype and null embryos revealed null embryos had a significant decrease in expression of *Men1* and developmental genes *Hoxc6, Cyt19* and *Hoxc8* with menin binding specifically to the *Hoxc8* locus. Huang et al. discovered that menin binds to MLL1, a H3K4 methyltransferase, and JunD in the same pocket (Huang et al. 2012). However, contrary to the transcription repressing activity of the menin-JunD complex, menin-MLL1 complexes associate with the HOXA9 promoter which consequently upregulates transcription and promotes leukemic transformation (Yokoyama et al. 2005).

Menin's transcription and growth-suppressing role in pancreatic beta cells has been shown to rely on its interaction with protein arginine methyltransferase 5 (PRMT5) to downregulate the Hedgehog signaling pathway (Gurung et al. 2013). PRMT5, which suppresses

transcription through its histone arginine methyltransferase activity, was shown to coelute with menin by chromatography, followed by mass spectroscopic analysis. PRMT5 is recruited by menin to the promoter of *Gas1*, a crucial factor for Sonic hedgehog (Shh) ligand to bind to its receptor patched homolog 1 (PTCH1). By increasing the histone H4 arginine 3 dimethylation at the Gas1 promoter, PRMT5 suppresses *Gas1* expression and subsequently, Hedgehog-signaling mediated growth. Menin-mediated recruitment of PRMT5 has also been described to the *GLI1* promoter (Gurung, Feng, and Hua 2013), which is downstream of *GAS1* and smoothened (*SMO*). These findings raise the interesting possibility that pNETs with mutated *MEN1* and high Hedgehog signaling could be treated with a Hedgehog inhibitor.

In neuroendocrine cell lines, Menin interacts with death-domain-associated protein (DAXX) to enhance histone H3 lysine9 trimethylation (H3K9me3) resulting in suppression of the membrane metallo-endopeptidase Mme promoter (Feng et al. 2017). This interaction has clinical implications as *DAXX* is mutated in 40% of human neuroendocrine tumors (Jiao et al. 2011). By cDNA microarray analysis, *Mme*, *DCN* and *Gria3* were identified to be upregulated in *Men1*- or *Daxx*-null mouse embryonic fibroblasts. Increased *Mme* expression has been associated with larger tumor size, higher ki-67 index and metastasis (Deschamps et al. 2006). Menin binds to Daxx in its C-terminal region with one mutation associated with MEN1 tumor syndrome resulting in the failure of menin to bind Daxx. Menin and Daxx enhance histone 3 lysine 9 trimethylation. Without this suppression of *Mme*, cell proliferation of neuroendocrine tumors goes unchecked. Either with shRNAs or drug (Thiorphan), decreasing levels of Mme significantly decreases tumors size.

Cell Cycle Regulation

Inspired by the differential expression of other tumor suppressors in accordance with the cell cycle (i.e. BRCA1 (Gudas et al. 1996) and BRCA1 (Bertwistle et al. 1997)), Kaji et al. investigated the levels and localization of menin in conjunction with JunD at various points of the cell cycle in rat pituitary GH4C1 cells (Kaji et al. 1999). Menin was mostly localized to the nucleus. Higher levels of menin were present during quiescence but levels drop after entry of the cell cycle. As the cell enters S phase, levels of menin increase again. JunD levels corresponded with levels of menin.

Later in 2005, after menin was found to interact with MLL2 through regulation of *Hox* expression, Milne et al. hypothesized that menin may additionally regulate cell growth by interfacing with cyclin dependent kinase inhibitors, specifically $p18^{Ink4c}$ and $p27^{Kip1}$ (Milne et al. 2005). Chromatin immunoprecipitation revealed that menin binds directly to the coding sequences of $p18^{Ink4c}$ and $p27^{Kip1}$. In fibroblasts deficient in menin or MLL2, expression of $p18^{Ink4c}$ and $p27^{Kip1}$ decreased and cell growth increased. Although MLL2 is dependent on menin to bind to $p18^{Ink4c}$ and $p27^{Kip1}$ loci, menin seems to recruit MLL2 to particular loci in order to regulate transcription (Milne et al. 2005). In a separate study by Schnepp et al., cells deficient of menin were found to decrease the levels of protein and RNA of $p18^{Ink4c}$ and $p27^{Kip1}$, increase CDK2 activity and accelerate G_0/G_1 to S phase entry (Schnepp et al. 2006). With the restoration of menin by retrovirus, expression of $p18^{Ink4c}$ and $p27^{Kip1}$ and cell cycle progression both normalized.

As a tumor repressor, MENIN was hypothesized to play a role in the TGF-ß pathway, which is crucial in cell growth inhibition (Kaji et al. 2001). Using antisense RNA to inactivate menin, TGF-ß was found to less efficiently inhibit rat anterior pituitary cell growth through reduced transcriptional activity. Smad2, Smad3 and Smad4 are the canonical downstream effectors of the TGF-ß pathway, translocating into the nucleus upon phosphorylation to act as transcriptional activators. By coimmunoprecipation, menin was found to specifically interact with Smad3. Inactivation of menin interrupts Smad3 binding with DNA, thus preventing transcriptional activation. The loss of *Men1* in neuroendocrine tumors may prevent TGF-ß from halting the cell cycle when exposed to stress and thus contribute to tumor formation (Kaji et al. 2001). Of note, interaction with JunD and NF-kB suggests that menin acts as a transcriptional repressor but its interaction with Smad3 indicates that it can also act as a transcriptional activator.

MENIN in Leukemia

As opposed to its tumor repressing functions in endocrine organs, MENIN is critical for the leukemogenic activity of mixed lineage leukemia (MLL) gene in acute human leukemia. MLL chromosomal translocations form a diverse cohort of over 40 MLL-fusion proteins that constitutively activate MLL's transcriptional effector properties (Mitterbauer-Hohendanner and Mannhalter 2004). MLL is a histone methyltransferase that maintains embryonic *Hox* gene expression (Yu et al. 1998). However, *MLL* fusions lose histone methyltransferase activity of MLL but gain other histone modification functions, resulting in oncogenicity. Interestingly, MENIN is the only interacting partner of MLL to maintain its interaction to the MLL-fusion protein (Yokoyama et al. 2004). Moreover, MENIN and wildtype MLL are also crucial for maintenance of MLL fusion protein-induced acute myeloid leukemia (Thiel et al. 2010; Thiel et al. 2013).

MENIN was found to be an interacting partner with MLL by immunopurification (Hughes et al. 2004). It associates with MLL oncoproteins on *HOX* gene promoters via a conserved, high-affinity MENIN binding motif (hMBM) (Yokoyama et al. 2005). Without a functioning hMBM, cells were incapable of clonogenic activity and oncogenic transformation (Yokoyama et al. 2005; Caslini et al. 2007). As such, the interaction between MENIN and MLL served to be a druggable target.

High-throughput screening identified a small-molecule MI-1 (with MI-2 and MI-3, as analogs), which could reversibly inhibit the MENIN-MLL binding motif (Grembecka et al. 2012). Treatment with MI-2 and MI-3 showed significant growth arrest, inhibition of transformation and induced hematopoietic differentiation *in vitro*. Further modifications of this MI small molecule yielded MI-463 and MI-503 which showed growth inhibition of patient-derived leukemia xenografts (Borkin et al. 2015).

Targeting MENIN Loss

Because MENIN is multifunctional and is context specific in its actions, there are considerable challenges to developing a robust therapeutic strategy. In order to target tumors with MENIN loss, focus has turned to orthogonal pathways that contain druggable targets.

Activating mutations in *Kras* are frequently observed in pancreatic ductal adenocarcinoma and not pNET, suggesting *Kras* and its effectors are suppressed in these tissues (Chamberlain et al. 2014). Mice with islet-specific expression of the gain of function mutant, *Kras*^{G12D}, exhibited slower proliferation of ß-cells as compared to control animals. However, in animals with overexpressed *Kras*^{G12D} but decreased *Men1*, there was an increase in proliferation of ß-cells suggesting that the Kras-induced growth suppression in endocrine ß-cells is dependent on Men1. Since Kras both activates MAPK pathway-mediated cell proliferation and RASSF1A-mediated cell growth suppression, MEN1-mediated suppression of the MAPK pathway is crucial to maintain physiologic balance in ß-cells. Currently, the exact mechanism of MEN1-mediated suppression of the MAPK pathway is unknown. However, pharmacologic targeting to re-instate MAPK pathway suppression in the context of a *Men1* mutation has potential clinical relevance. Recently, it has also been reported that menin suppresses glucagon-like peptide 1 (GLP-1)/PKA-induced phosphorylation of FOXO1 and CREB, two transcription factors, to suppress ß-cells proliferation (Muhammad et al. 2017). It is not yet known whether this pathway is relevant to ß-cell proliferation induced by menin deletion or only to ß-cell regulation in diabetic stress conditions.

The Wnt/ß-catenin pathway has been thought to play a role in pancreatic development and cell proliferation (Rulifson et al. 2007; Murtaugh et al. 2005). Moreover, pNETs with mutations in *MEN1* exhibit increased active ß-catenin and decreased phosphorylated ß-catenin (Jiang et al. 2014). This finding was reproduced in animals that were *Men1*-deficient. In addition, in *Men1*-deficient animals, concurrent knockout of *ß-catenin* resulted in smaller insulinoma size and reduced rates of tumorigenesis. Treatment with a small-molecule antagonist of the TCF/ß-catenin complex, PKF115–584, in *Men1*-deficient animals resulted in reduced hyperinsulinemia and Ki67 staining. *Men1*-deficient ß-cells treated with PKF115–584 showed significantly suppressed replication *in vitro*.

As previously discussed, the discovery of MENIN and DAXX interaction resulting in suppressed *Mme* (Feng et al. 2017) yields a potentially targetable interaction for patients with neuroendocrine tumors. Similarly, the physiologic interaction between MENIN and PRMT5 represses GLI1 and GAS1 (mediators of the Hedgehog pathway), which may serve as additional pharmacologic targets. A Hedgehog signaling inhibitor, GDC-0449, was used in MEN1$^{f/f}$ - *Ripcre* mice which develop insulinomas (Gurung et al. 2013) and found to decrease serum insulin levels. Treatment with GANT-61, a small molecule inhibitor of GLI1, resulted in a decrease in cell proliferation in MEN1 null cells as well as a reduction in C-MYC protein levels (Gurung, Feng, and Hua 2013).

Conclusion

MENIN is a multi-functional protein with pivotal roles in physiologic endocrine organ maintenance and neuroendocrine tumorigenesis. With diverse functions including transcription regulation, histone modification, and cell cycle regulation, MENIN holds immense promise in increasing understanding of pathogenesis and its interacting partners may provide new therapeutic targets for patients with somatic or germline MEN1 mutations.

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Highlights for review

- *MEN1* encodes MENIN, a tumor suppressor that is altered in select cancers.

- Pancreatic neuroendocrine and parathyroid tumors are enriched with *MEN1* mutations.

- Conventional and conditional mouse models of *Men1* loss produce PanNETs.

- MENIN has GTPase activity and regulates transcription and the cell cycle.
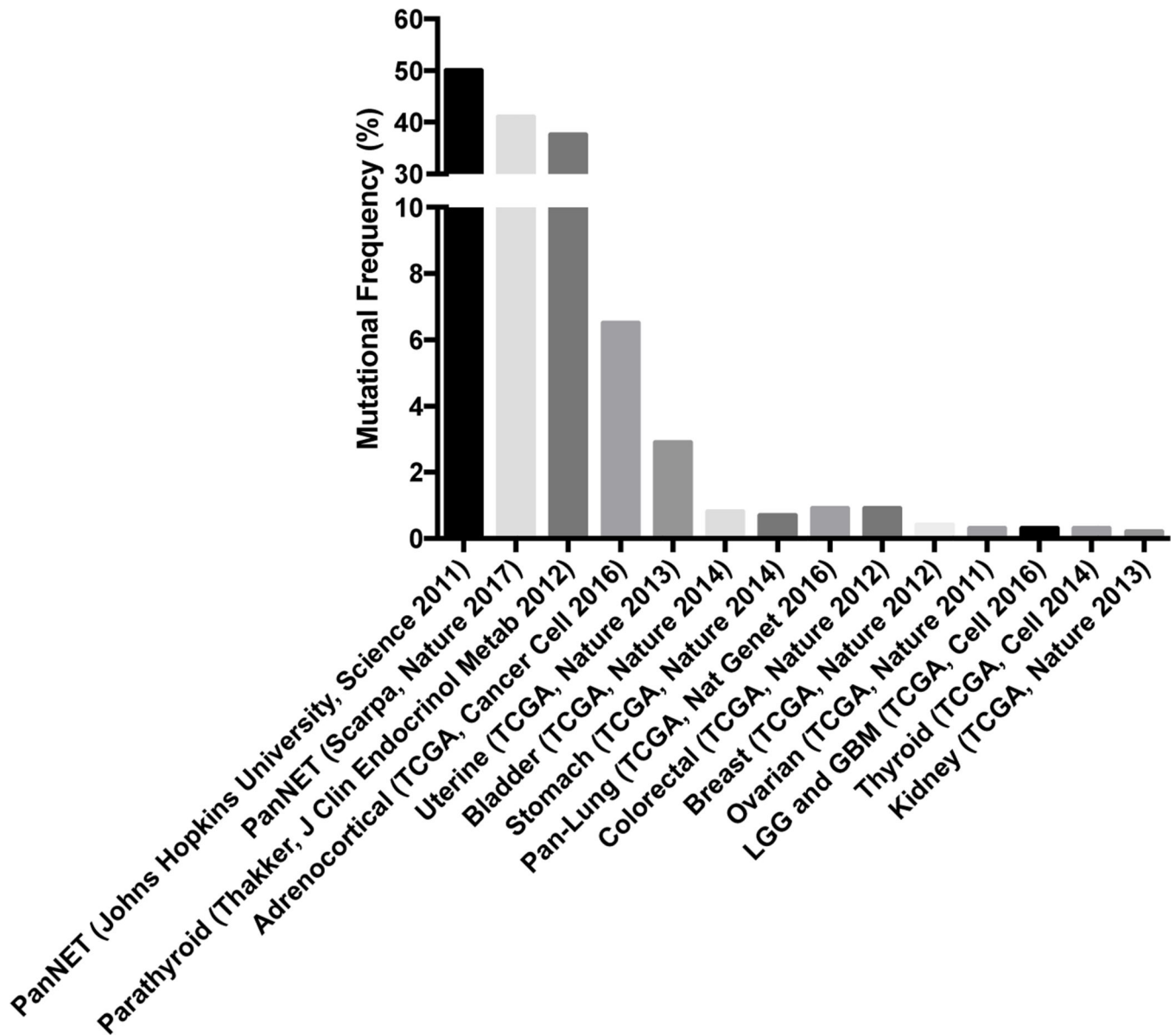
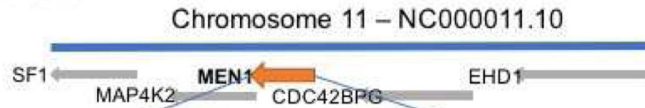## Cross-Cancer Mutation Summary for MEN1
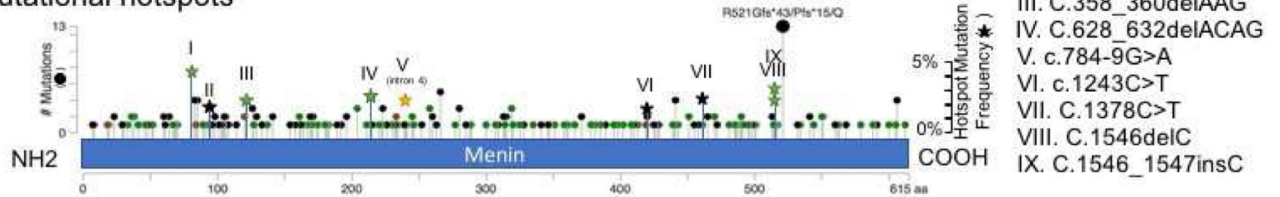

Figure 1:

MEN1 mutations in cancers

Aggregation of singular studies and TCGA analyses demonstrates that *MEN1* mutations are relatively infrequent in cancers with the exception of pancreatic neuroendocrine tumors and parathyroid adenomas (cbiportal.org).

Figure 2.

A. *MEN1* is on chromosome 11q13.1. It spans 2.8kb and is 610 amino acids long.

B. *MEN1* mutations are spread diffusely across the entire gene with certain mutations occurring with slightly higher frequency than others (cbioportal.org). (Green dot: missense mutation, black dot: truncating mutation)

Table 1.

Select mouse models of *Men1* loss

Reference	Genetic Modification	Background Strain	Phenotype	Time to pNET	Hormonal Aberrations	LOH present?
Crabtree et al. 2001	Conditional Deletion of exon 3–8					
	Men1$^{tsm/tsm}$	NIH Black Swiss	Embryonic lethal (E11.5–12.5)			
	Men1$^{tsm/+}$	129Sv/EvTacFBR	- Pancreatic islet tumors (28%) - Parathyroid adenomas (24%) - Pituitary adenomas (26%) - Adrenocortical carcinomas (20%), pheochromocytoma (74%) - Lung adenocarcinomas (22%) - Gastric neuroendocrine tumors (4%) - Prostate carcinoma (4%) - Ovarian adenoma (4%) - Thyroid follicular adenomas	9 months	Insulin	No
	Men1$^{\Delta 3-8/\Delta 3-8}$ (Men1$^{tsm/tsm}$ x EIIa-cre)	129Sv/EvTacFBR	Embryonic lethal (E10.5-E11.5)			
Bertolino, Radovanovic, et al. 2003	Deletion of exon 3					
	Men1$^{T/T}$	129/Ola/Sv	Embryonic lethal (E11.5–13.5)			
	Men1$^{+/T}$	129/Ola/Sv	-Testis Leydig carcinoma (88%) - Pancreatic islet cell tumor adenoma 19.7%), carcinoma (60.6%) - Parathyroid adenoma (63.6%), carcinoma (3%) - Adrenal glands adenoma (27.8%), carcinoma (18%) - Extrapancreatic gastrinoma adenoma (17.6%), carcinoma (5.9%) - Pituitary gland adenoma (16.6%), - carcinoma (20%) - Mammary carcinoma (8.3%) - Thyroid tumor (6.5%)	12–13 months	Insulin, PTH, glucagon	Yes
Loffler et al. 2007	Deletion of exon 2					
	Men1$^{+/-}$	C57/129	-Pancreatic islet cell adenoma (79.2%) - Pituitary adenomas (61.1%) -Thyroid neoplasm (15.2%) - Testis (34.4%)/ovaries (15.9%) - Parathyroid adenoma (6.9%) - Adrenal adenomas (0.8%)			Yes
Harding et al. 2007	Deletion of exon 1 and 2					

Reference	Genetic Modification	Background Strain	Phenotype	Time to pNET	Hormonal Aberrations	LOH present?
	Men1$^{+/-}$	C57BL/6	- Pancreatic islet cell adenoma (83.6%) - Pituitary adenomas (31.4%) - Parathyroid adenoma (41.8%) - Adrenal adenomas (11.6%)	9–12 months		Yes
Crabtree et al. 2003	Conditional deletion of exon 3 – 8					
	Men1$^{N/N}$- RIP2-cre	B6;FVB;129 Sv	- Pancreatic islet cell tumor (100%) - Pituitary adenomas (12%)	6 months	Insulin	
Bertolino, Tong, Galendo, et al. 2003	Conditional deletion of exon 3					
	Men1$^{F/F}$-RipCre$^+$	129/Ola/Sv	- Pancreatic islet cell tumors (100%) - Pituitary adenomas - Parathyroid adenomas	6 months	Insulin	
Biondi et al. 2004	Conditional deletion of exon 2					
	Men1$^{loxP/loxP}$-RipCre$^+$	C57BL/6J	- Pituitary adenomas (71.4%) - Pancreatic islet cell tumors (33.3%)	9 months		
Shen et al. 2009	Conditional deletion of exons 3 to 8					
	Men1$^{F/F}$-PDX1Cre$^+$	FVB;129Sv	- Pancreatic islet cell tumor			
Lines et al. 2017	Temporal/ conditional deletion of exons 3 to 8					
	Men1$^{L/L}$/RIP 2-CreER (tamoxifen-induced)	Tg(Ins2-cre/ERT)1D am/J;129S(FVB)Men1tm 1.1 Ctre/J	- Pancreatic islet cell tumor (100%)	5 months		
Shen et al. 2010	Conditional deletion of exons 3 to 8					
	Glu-Cre;Men1 $^{f/f}$ (α−cell specific)	FVB;129Sv	- Pancreatic islet cell tumor	13 months		
Lu et al. 2010	Conditional deletion of exon 3					
	Men1$^{+/-}$, Men1$^{F/F}$, Men1$^{F/F}$-RipCre$^+$, Glu Cre$^+$ (α−cell specific)	129/Ola/Sv	- Pancreatic islet cell tumor (insulinoma)(15%) - Pancreatic a-cell tumor (glucagonoma)(30%) - Mixed insulin-glucagonoma (55%)	8 months	Glucagon, insulin	
Lin et al. 2016	Conditional deletion of exon 3–8 of MEN1 and insertion of *lacZ* reporter in-frame of Kmt2a					
	Kmt2a$^{f/f}$;Men1$^{f/f}$;RIP-Cre	129s6, FVB/N, and C57BL/6 background	- Pancreatic islet cell tumor	6 months	Insulin	
Gillam et al. 2015	Conditional eletion of exon 3–8 of MEN1 and deletion of exon 1–2 of CDK4 and three point mutations					
	Men1$^{+/-}$; Cdk4$^{-/-}$	FVB/N, 129S6 and *C57BL/6; 129sv*	- No neuroendocrine tumors	15 months		

Reference	Genetic Modification	Background Strain	Phenotype	Time to pNET	Hormonal Aberrations	LOH present?
	Men1$^{+/-}$; Cdk2$^{-/-}$	FVB/N, 129S6 and *C57BL/6; 129sv*	- Pituitary tumor (52%) - Pancreatic islet cell tumor (65%)	15 months		