The future: genetics advances in MEN1 therapeutic approaches and management strategies

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Abstract

The identification of the multiple endocrine neoplasia type 1 (MEN1) gene in 1997 has shown that germline heterozygous mutations in the MEN1 gene located on chromosome 11q13 predisposes to the development of tumors in the MEN1 syndrome. Tumor development occurs upon loss of the remaining normal copy of the MEN1 gene in MEN1-target tissues. Therefore, MEN1 is a classic tumor suppressor gene in the context of MEN1. This tumor suppressor role of the protein encoded by the MEN1 gene, menin, holds true in mouse models with germline heterozygous Men1 loss, wherein MEN1-associated tumors develop in adult mice after spontaneous loss of the remaining non-targeted copy of the Men1 gene. The availability of genetic testing for mutations in the MEN1 gene has become an essential part of the diagnosis and management of MEN1. Genetic testing is also helping to exclude mutation-negative cases in MEN1 families from the burden of lifelong clinical screening. In the past 20 years, efforts of various groups worldwide have been directed at mutation analysis, molecular genetic studies, mouse models, gene expression studies, epigenetic regulation analysis, biochemical studies and anti-tumor effects of candidate therapies in mouse models. This review will focus on the findings and advances from these studies to identify MEN1 germline and somatic mutations, the genetics of MEN1-related states, several protein partners of menin, the three-dimensional structure of menin and menin-dependent target genes. The ongoing impact of all these studies on disease prediction, management and outcomes will continue in the years to come.

Introduction

Multiple endocrine neoplasia type 1 (MEN1) is a rare tumor syndrome with an autosomal dominant pattern of inheritance with >95% penetrance by age 40–50 years (Online Mendelian Inheritance in Man, OMIM number: 131100). The approximate prevalence of MEN1 has been reported as 1 in 30,000 individuals with no apparent gender bias. The main characteristic clinical features of MEN1 include at least two of the following three endocrine tumors in an individual: multi-gland parathyroid adenomas, anterior pituitary adenomas and entero-pancreatic neuroendocrine tumors (gastrinoma/Zollinger–Ellison syndrome and pancreatic neuroendocrine tumors). These characteristics can present in a non-hereditary form with no family history of
MEN1 (sporadic MEN1) or in several members of a family (familial MEN1) (Marx et al. 1998, Brandi et al. 2001, Thakker et al. 2012). The penetrance of the main MEN1 tumors by age 50 years is as follows: the parathyroids (90–100%), entero-pancreatic neuroendocrine (30–70%) and anterior pituitary (30–40%) (Brandi et al. 2001, Thakker et al. 2012). In addition to these main endocrine tumors causing associated circulating hormone excess, MEN1 patients present with various hormone-secreting, hormone non-secreting and non-endocrine tumors. These include adrenal cortical tumor; foregut carcinoids of the lung, thymus or gastric enterochromaffin-like cells; skin lesions facial angiofibroma, truncal collagenoma and lipoma; central nervous system tumors meningioma and ependymoma and smooth muscle tumors leiomyomas (uterine in females or in the esophagus) (Brandi et al. 2001, Thakker et al. 2012, Agarwal 2013). Malignant gastrinoma or foregut carcinoids are the cause of death in approximately 25% of MEN1 patients (Doherty et al. 1998, Dean et al. 2000, Goudet et al. 2010, Ito et al. 2013). The various individual tumor types observed in the MEN1 syndrome also occur sporadically. The cloning of the gene causative for the MEN1 syndrome in 1997 has provided a unique opportunity to gain insights into the normal physiology and pathophysiology of the wide range of MEN1-associated cell types affected. This review covers the advances in molecular genetic studies and basic findings about the MEN1 syndrome and related states, the MEN1 gene and its protein product menin.

**Positional cloning of the MEN1 gene and identification of inactivating mutations**

Genetic mapping, segregation analysis of candidate markers in MEN1 families and loss of heterozygosity (LOH) studies in MEN1-associated tumors led to the localization of the putative tumor suppressor gene that causes the MEN1 syndrome to a narrow interval on chromosome 11q13 near the PYGM locus (Larsson et al. 1988, Bale et al. 1989, Friedman et al. 1989, Nakamura et al. 1989, Thakker et al. 1989, Sawicki et al. 1992, Debelenko et al. 1997, Emmert-Buck et al. 1997). The polymorphic markers in the 11q13 region were useful in genetic tests to identify disease carriers among family members; however, they could not be used to diagnose MEN1 genetically in index cases.

Two different large collaborative groups used a positional cloning approach to sequence a MEN1-linked minimal interval that is mapped at 11q13 to identify the MEN1 gene (Chandrasekharappa et al. 1997, Guru et al. 1997a, b, Lemmens et al. 1997a, b). They showed that the MEN1 gene spans about 9000 bp of genomic DNA containing 10 exons and transcribed into a 2.8 kb mRNA with the translational start codon (ATG) in exon-2 and the stop codon in exon-10. The protein product of the MEN1 gene consisting of 610 amino acids was named menin (GenBank Accession No.: U93236.1). Germline heterozygous inactivating mutations were found in the coding region of the MEN1 gene in index cases and affected family members, together with LOH for markers at the MEN1 gene locus in their tumors as expected for a causative tumor suppressor gene (Knudson 1971, 1993, Chandrasekharappa et al. 1997, Lemmens et al. 1997b).

It is important to note that there are two versions of menin in the gene databases with 610 or 615 amino acids. The 615 amino acids version has an alternative splice site at the end of exon-2 that adds five amino acids after codon 148. While the mutations in most publications are written as per the 610 amino acids version of menin, most current databases have used the 615 amino acids version of menin to annotate MEN1 mutations. Therefore, the same version of menin must be used for mutation annotation to compare MEN1 mutations after amino acid 148.

**Germline and somatic mutations in the MEN1 gene**

Since 1997, germline DNA samples have been screened for mutations in the MEN1 gene that belong to cases suspected to have familial MEN1 or sporadic MEN1. Germline heterozygous mutations in the MEN1 gene are observed in 70–90% of familial MEN1 cases and the frequency of finding a de novo mutation is significantly lower in sporadic MEN1 cases (Agarwal et al. 1997, Bassett et al. 1998, Giraud et al. 1998, Cebrian et al. 2003, Cardinal et al. 2005, Klein et al. 2005, Tham et al. 2007, Sakurai et al. 2012, de Laat et al. 2016, Giusti et al. 2017). Over 1200 germline mutations in the MEN1 gene have been reported with no obvious genotype–phenotype correlation of specific mutations with the MEN1-associated tumor spectrum even among family members with the exact same mutation. Clustering of mutations is observed in some regions of exon-2 and exon-10 that have been attributed to the nature of the underlying repetitive nucleotides prone to DNA polymerase errors (Agarwal et al. 1998, Bassett et al. 1998, Thakker 2010).
Table 1  

<table>
<thead>
<tr>
<th>Type of mutation</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Protein truncation</td>
<td></td>
</tr>
<tr>
<td>Nonsense</td>
<td>14.0</td>
</tr>
<tr>
<td>Frame-shift</td>
<td>42.0</td>
</tr>
<tr>
<td>Splicing</td>
<td>10.5</td>
</tr>
<tr>
<td>Large deletion</td>
<td>2.5</td>
</tr>
<tr>
<td>Missense</td>
<td>25.5</td>
</tr>
<tr>
<td>In-frame deletion/insertion</td>
<td>5.5</td>
</tr>
</tbody>
</table>


Two comprehensive studies have tabulated and analyzed germline MEN1 mutations published from 1997–2007 to 2007–2015 identifying 459 and 117 (total 576) unique mutations, respectively, in MEN1 patients (Lemos & Thakker 2008, Concolino et al. 2015). These mutations are scattered over the entire coding region of MEN1 with no hot spots. The largest category of MEN1 germline mutations (69%) predict obvious pathologic consequence due to premature truncation of menin from nonsense mutations (14%) and frame-shift mutations (42%) or exon region deletion due to splicing defects (10.5%) or large deletions (2.5%) (Table 1) (Concolino et al. 2015). Missense mutations (25.5%) and single or few amino acids in-frame deletions or insertions (5.5%) do not predict obvious inactivation of menin, and their classification as benign or pathologic needs further investigation (Table 1) (Lemos & Thakker 2008, Concolino et al. 2015). For example, the pathologic nature of many of these missense and in-frame mutations could be verified based on their tracking with the disease in families. Seven common polymorphisms that constitute a synonymous or non-synonymous amino acid change in the meningocoding region have also been reported in the MEN1 gene (Table 2). For accurate genetic diagnosis, it is important to know about common polymorphisms so that they can be excluded as benign variants. Interestingly, the two non-synonymous common variants, p.Arg171Gln and p.Ala541Thr, have been studied for their potential association with the MEN1 phenotype (De Carlo et al. 2008, Nozieres et al. 2014).

The discovery of susceptibility genes for hereditary tumor syndromes can be informative for the identification of the genetic cause of their sporadic counterpart tumors (Marx & Simonds 2005). Indeed, somatic inactivating mutations in the MEN1 gene have been reported in sporadic tumors of the types observed in the MEN1 syndrome. In contrast to MEN1 where the 1st hit to the MEN1 gene is in the germline and the 2nd hit is somatic, in sporadic tumors, the 2 hits to the MEN1 gene for biallelic inactivation are both somatic. Using targeted sequencing of MEN1 exons or whole-exome sequencing approaches, the frequency of somatic MEN1 mutations reported in sporadic tumors is as follows: glucagonoma (60%), VIPoma (57%), non-functioning PNETs (44%), gastrinoma (38%), bronchial carcinoid (35%), parathyroid adenoma (35%), lipoma (28%), insulinoma (2–19%), angiofibroma (10%), anterior pituitary tumor (3.5%) and adrenocortical tumor (2%) (Heppner et al. 1997, Zhuang et al. 1997, Gortz et al. 1999, Jiao et al. 2011, Cromer et al. 2012, Newey et al. 2012, Cao et al. 2013, Thakker 2014, Scarpa et al. 2017). Similar to the distribution of germline mutations, the somatic mutations are also spread over the entire coding region of MEN1 with no hot spots. The percentage of the types of somatic MEN1 mutations (nonsense, frame-shift, splicing, missense and in-frame deletions or insertions) is also similar to the germline MEN1 mutations (http://cancer.sanger.ac.uk/cosmic). Also, no obvious genotype–phenotype correlations have been identified for specific somatic mutations.

Table 2  

<table>
<thead>
<tr>
<th>Genomic Location hg19 (dbSNP)</th>
<th>Codon change</th>
<th>Consequence</th>
<th>Annotation</th>
<th>Minor allele frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:64577552 G/T (rs371192390)</td>
<td>CTG/CTT</td>
<td>p.Leu10Leu</td>
<td>synonymous</td>
<td>0.07</td>
</tr>
<tr>
<td>11:64577147 C/T (rs61736636)</td>
<td>AGC/AGT</td>
<td>p.Ser145Ser</td>
<td>synonymous</td>
<td>2.9</td>
</tr>
<tr>
<td>11:64575505 G/A (rs607969)</td>
<td>CGG/CAG</td>
<td>p.Arg171Gln</td>
<td>non-synonymous</td>
<td>1.2</td>
</tr>
<tr>
<td>11:64572602 C/T (rs2071313)</td>
<td>GAC/GAT</td>
<td>p.Asp418Asp</td>
<td>synonymous</td>
<td>39.3</td>
</tr>
<tr>
<td>11:64572560 G/A(rs138770431)</td>
<td>CTG/CTA</td>
<td>p.Leu432Leu</td>
<td>synonymous</td>
<td>0.1</td>
</tr>
<tr>
<td>11:64572557 C/T (rs540012)</td>
<td>CAC/CAT</td>
<td>p.His433His</td>
<td>synonymous</td>
<td>0.72</td>
</tr>
<tr>
<td>11:64572018 G/A (rs2959656)</td>
<td>GCA/ACA</td>
<td>p.Ala541Thr</td>
<td>non-synonymous</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Minor allele frequency data from the Exome Aggregation Consortium (ExAC) database, which spans 60,706 unrelated individuals sequenced as part of various disease-specific and population genetic studies. Minor allele frequency of the less (or least) frequent allele in a population (>0.05% considered as a common variant).
Genetic testing for mutations in the MEN1 gene

Currently, genetic testing of the MEN1 gene includes PCR-based screening for mutations in the coding region and splice junctions, and if a mutation is not identified, then multiplex ligation probe amplification (MLPA)-based screening is performed for the detection of large deletions of the MEN1 gene. Clinical practice consensus guidelines for MEN1 and related states developed by a panel of experts including physicians, surgeons and geneticists from international centers, have made recommendations for mutational analysis of the MEN1 gene (Brandi et al. 2001, Thakker et al. 2012). Before and after testing, all individuals should be provided genetic counseling. The tests must be performed in a clinical genetics laboratory that is accredited to perform MEN1 mutation analysis. Individuals who are the relatives of a patient with a known MEN1 mutation should be offered genetic testing prior to biochemical and radiological screening for the detection of MEN1-associated tumors, because the chance of inheriting the mutation is 50%, and the germline genetic testing would help to avoid the screening for tumors in mutation-negative cases. Individuals who test positive for the known mutation in their family should be screened on an annual basis for the development of MEN1-associated tumors. For asymptomatic members of a family with a known MEN1 mutation, the guidelines recommend that MEN1 germline testing should be offered at the earliest opportunity, as early as age 5 years, because MEN1 manifestations have been observed in some children at age 5–10 years (Stratakis et al. 2000, Machens et al. 2007, Goudet et al. 2015). MEN1 germline testing may be offered to symptomatic young patients (<40 years) (with no family history) who present with an atypical phenotype such as an MEN1-associated tumor (e.g., multi-gland hyperparathyroidism) and who may have not yet developed the full spectrum of MEN1-associated tumors. Also, the guidelines advise to offer genetic testing to symptomatic family members with a known MEN1 mutation in the family, as well as to index cases with two or more of the MEN1-associated endocrine tumors. This is because of the report of phenocopies wherein symptomatic individuals in MEN1 kindreds have tested negative for their known familial MEN1 mutation (Thakker et al. 2012).

MEN1-like disease from germline mutation in MEN1 or other susceptibility genes

MEN1-like cases and families have been reported who show incomplete clinical manifestations of MEN1 with as few as any one of the three main MEN1-associated endocrine tumors. Germline MEN1 mutations have been found in a few kindreds of the parathyroid-only disorder, familial isolated primary hyperparathyroidism (FiHP) (Simonds et al. 2002, Hannan et al. 2008, Lemos & Thakker 2008, Concolino et al. 2015). Among these germline mutations, a higher frequency of MEN1 missense mutations have been observed but with no hot spots or genotype-phenotype correlations (Lemos & Thakker 2008, Concolino et al. 2015). Also, germline MEN1 mutations have been reported in five cases of apparently sporadic PNETs (with no family history or other tumors of MEN1) (Scarpa et al. 2017). However, MEN1 germline mutations have not been found in the pituitary-only disorder of familial isolated pituitary adenoma (FIPA) (Daly et al. 2006).

Because the involvement of the MEN1 gene was excluded in cases with MEN1-like characteristics, efforts could be directed to identify susceptibility genes for such conditions. Identification of the susceptibility gene for the hyperparathyroidism-jaw tumor syndrome by a positional cloning approach has shown that germline mutations in the CDC73 gene are causative for parathyroid cancer (Carpten et al. 2002, Shattuck et al. 2003). An exome sequencing approach has shown that germline activating mutations in the GCM2 gene occur in 18% of FiHP kindreds (Guan et al. 2016). Germline mutations in the AIP tumor suppressor gene identified by a positional cloning approach, which is also located on 11q13, occur in 20% of FIPA patients and in 30–50% of patients with familial acromegaly and germline GPR101 microduplication or mutation occurs in patients with X-linked acrogigantism (X-LAG) (Vierimaa et al. 2006, Daly et al. 2007, Trivellin et al. 2014). Identification of a homozygous germline mutation in the cyclin-dependent kinase inhibitor (CDKI) gene CDKN1B/p27 in a rat strain with combined MEN1 and MEN2 features (MENX) prompted the germline mutation analysis of this gene in human patients with MEN1-like features, and the identification of the MEN4 disorder (Pellegata et al. 2006). Heterozygous germline mutations in p27 have been identified in 1–2% of MEN1-like cases (MEN4) who present with incomplete features of MEN1 such as primary hyperparathyroidism only, parathyroid and pituitary tumor or MEN1 features (Pellegata 2012). No obvious genotype-phenotype correlation has been reported for MEN1 or MEN1-like features with p27 mutations. The CDKI genes belong to two families, the INK4 family (p15, p16, p18 and p19) and the Cip/Kip family (p21, p27 and p57). They are cell-cycle regulators that inhibit specific cyclin–CDK complexes.
Mutation analysis of the members of the INK4 and Cip/Kip family of CDK1 genes in MEN1-like cases has shown that less than 1% carry probable p15, p18 or p21 heterozygous germline mutations (Agarwal et al. 2009, Thakker 2014).

The MEN1-encoded protein menin and its functional characterization

The amino acid sequence of menin does not show homology to any known proteins. Menin is highly conserved in animal species, and there are no homologs of menin in yeast and nematodes (HomoloGene, Saccharomyces Genome Database (SGD), and Nematode.net).

Menin is ubiquitously expressed and detected at 67 kDa by western blot analysis. Studies of post-translation modifications of menin have shown that phosphorylation of menin could be detected at amino acid residues Ser394, Thr397, Thr399, Ser487, Ser543 and Ser583 (Francis et al. 2011). Menin has also been shown to undergo SUMOylation and palmitoylation (Feng et al. 2013, He et al. 2016). Sub-cellular localization studies have shown that menin is predominantly nuclear, but a very small amount of menin has also been detected in the cytoplasm and at the cell membrane (Guru et al. 1998, Cao et al. 2009, He et al. 2016). The amino acid sequence of menin shows two main nuclear localization signals (NLS), NLS1 (amino acid residues 479–497) and NLS2 (amino acid residues 588–608) and a third accessory NLS, NLSa (amino acid residues 546–572) (Guru et al. 1998, La et al. 2006). Germline and somatic MEN1 mutations that predict premature protein truncation (frame-shift and nonsense) would cause loss of one or both main NLSs leading to abnormal localization of the truncated menin and functional inactivation. Transfection and protein analysis in mammalian cell lines has shown that menin missense mutant proteins undergo degradation by the proteasome pathway that would prevent any functional activity (Canaff et al. 2012).

Menin does not possess any intrinsic enzymatic activity, and its amino acid sequence does not predict any obvious functional domains. To determine the mechanisms by which menin acts as a tumor suppressor (as well as the consequence after menin loss), the elucidation of the physiological functions of menin has mainly relied on protein–protein interaction methods. Efforts from many groups have identified more than 50 different proteins that could partner with menin (Table 3). These interactions predict that menin is a multi-functional protein with functional contributions in transcriptional regulation as a co-repressor or co-activator (through interaction with chromatin modifying proteins, transcription factors, and transcription initiation or elongation proteins), DNA-repair associated with response to DNA damage, cell signaling, cytoskeletal structure, cell division, cell adhesion or cell motility (Hendy et al. 2009, Balogh et al. 2010, Matkar et al. 2013, Thakker 2014). Genetic studies in Drosophila melanogaster have shown that loss of menin may lead to genomic instability (Busygina et al. 2004).

Due to a prominent role of menin in transcriptional regulation, many groups have studied the target genes of menin by analyzing menin-dependent differential mRNA expression, and menin-occupancy or menin-dependent histone modifications at target genes by chromatin immunoprecipitation coupled with next-generation sequencing. However, a consensus signature of genes affected upon menin loss in MEN1 target tissues warrants further investigation. Regarding the regulation of menin, a microRNA (miR24-1 and its mature form miR24) has been shown to regulate the expression of menin with a feedback mechanism (Luzi et al. 2012, Vijayaraghavan et al. 2014). Further studies of this miRNA that targets menin may shed light on yet another aspect of the multi-functional roles of menin and its regulation.

The functional characterization of menin has been advanced by the deciphering of the three-dimensional (3D) structure of Nematostella menin (the starlet sea anemone) and human menin (Protein Data Bank No. 3RE2 and 3U84). The deletion of disordered regions facilitated the generation of menin crystals – deletion of an internal unstructured loop (amino acid residues 426–442) and truncation of the C-terminus (amino acid residues 487–539) in Nematostella menin, and deletion of a single internal unstructured loop (amino acid residues 460–519) in human menin (Murai et al. 2011, Huang et al. 2012). The 3D structure of menin resembles a ‘curved left hand’, with a deep pocket formed by the ‘thumb’ and the ‘palm’. The structure shows 4 domains: a long β-hairpin N-terminal domain, a transglutaminase-like domain that forms the ‘thumb’, a helical ‘palm’ domain that contains three tetratricopeptide motifs, followed by a C-terminal ‘fingers’ domain. The presence of the deep pocket formed by the thumb and the palm has been shown to act as a domain for protein–protein interaction. Co-crystallization efforts with two known menin-interacting proteins has shown that the pocket can bind short peptides of the AP1 transcription factor JUND (amino acid residues 27–47) or the mixed lineage leukemia (MLL) protein MLL1 (amino
acid residues 6–25) (Huang et al. 2012). Interaction analysis of purified menin protein (normal or with missense mutations) and a peptide consisting of the MLL1 menin-binding motif (MBM) or interaction analysis of menin mutants and MLL1 overexpressed in cells, has revealed that menin missense mutations at key residues located at the menin–MLL1 interface completely abolishes the menin–MLL1 interaction (Murai et al. 2011, Huang et al. 2012). Such assays can prove to be useful to decipher the benign or pathologic nature of menin missense variants of unknown significance (VUS).

### An unexpected function of menin as a pro-oncogenic factor in MLL fusion leukemia

The MLL proteins of the lysine methyltransferase (KMT) family, MLL1/KMT2A and MLL2/KMT2B are part of a protein complex that catalyzes a specific histone modification for transcription initiation or elongation factors.
gene activation, histone H3 lysine-4 trimethylation (referred to as the H3K4me3 mark in chromatin) (Shilatifard 2012). Loss of menin has been shown to coincide with the loss of H3K4me3 at specific genes, and at the same genes, gain of H3K27me3 that is an epigenetic mark of gene repression (Saccheri et al. 2006, Agarwal & Jothi 2012, Lin et al. 2015). MLL1 gene (chromosome 11q23) translocation with several different genes that encode transcription factors leads to the formation of MLL1-fusions that activate the expression of genes such as the HOX genes that cause leukemia (Yokoyama 2017). The interaction of menin in the Trithorax-like MLL protein complex has shown that menin is essential in this complex for the oncogenic activity of MLL-fusion proteins to cause leukemia (Hughes et al. 2004, Yokoyama et al. 2005). Evidence from studies conducted by using bone marrow of mouse models has confirmed this pro-oncogenic action of menin (Chen et al. 2006). Therefore, menin–MLL interaction inhibitors have been developed, which has been facilitated by the deciphering of the 3D structure of menin with the MLL1 MBM (Grembecka et al. 2012). These inhibitors named MI (Menin-MLL inhibitor) and further improved versions of these inhibitors (MI-463, MI-503, MI-538 and compound 27) have been shown to block the proliferation of MLL1-fusion leukemia cells from patients in vitro and in mouse xenografts in vivo (Borkin et al. 2016). Such menin–MLL interaction inhibitors hold promise to conduct future clinical trials as a potential treatment for patients with MLL1-fusion leukemia. Menin–MLL interaction inhibitors in mouse xenograft models have been shown to block the growth of specific types of prostate cancer, Ewing sarcoma and childhood gliomas (Funato et al. 2014, Malik et al. 2015, Svoboda et al. 2017).

Genetically engineered mouse models

Over the past two decades, many genetically engineered mouse models of Men1 loss have been generated to examine the causality of Men1 loss as a driver of tumorigenesis. These mouse models can serve as valuable surrogates to study the initiation, maintenance and evolution of MEN1-associated tumors and can be utilized to discover and validate pathways downstream of menin loss and to evaluate the utility of potential drug treatments.

Mice with germline knockout of the Men1 gene have been generated in four different laboratories by deleting genomic regions encompassing different exons of the mouse Men1 gene (U.S.A. (exons 3–8), France (exon 3), Australia (exon 2) and UK (exons 1–2)) (Crabtree et al. 2001, Bertolino et al. 2003a,b, Loffler et al. 2007a, Harding et al. 2009). Collectively, these mouse models have shown that germline homozygous loss of the Men1 gene (Men1<sup>−/−</sup>) leads to death in utero between embryonic days 10.5 and 14.5 due to delayed development with craniofacial abnormalities, defective neural tube closure, heart hypertrophy, abnormal liver organization, hemorrhages and edemas. Mice with germline heterozygous loss of the Men1 gene (Men1<sup>+/−</sup>) gestate normally, but they are predisposed to tumor development in a tissue-restricted manner, which resembles the condition in human MEN1 patients (Table 4). Also, these tumors exhibit loss of the non-targeted Men1 allele (LOH), thus supporting a tumor suppressor function of the Men1 gene. Adult Men1<sup>+/−</sup> mice (age 12–18 months) develop MEN1-associated endocrine tumors of the parathyroid, multiple

### Table 4  Prevalence of MEN1-associated tumors in Men1<sup>+/−</sup> mouse models compared to human MEN1.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Parathyroid</td>
<td>Parathyroid</td>
<td>90%</td>
<td>24%</td>
<td>47%</td>
<td>9%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Pancreas</td>
<td>40%</td>
<td>–</td>
<td>15%</td>
<td>–</td>
</tr>
<tr>
<td>Insulinoma</td>
<td>Insulinoma</td>
<td>10%</td>
<td>83%</td>
<td>88%</td>
<td>82%</td>
</tr>
<tr>
<td>Pituitary</td>
<td>Pituitary</td>
<td>20%</td>
<td>26%</td>
<td>12%</td>
<td>10%</td>
</tr>
<tr>
<td>Prolactinoma</td>
<td>Prolactinoma</td>
<td>5%</td>
<td>5%</td>
<td>–</td>
<td>7%</td>
</tr>
<tr>
<td>GH secreting</td>
<td>GH secreting</td>
<td>2%</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ACTH secreting</td>
<td>ACTH secreting</td>
<td>25%</td>
<td>20%</td>
<td>35%</td>
<td>10%</td>
</tr>
<tr>
<td>Adrenal</td>
<td>Adrenal</td>
<td>2%</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cortex NF</td>
<td>Cortex NF</td>
<td>25%</td>
<td>20%</td>
<td>35%</td>
<td>10%</td>
</tr>
<tr>
<td>Pheochromocytoma</td>
<td>Pheochromocytoma</td>
<td>–</td>
<td>7%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gonads</td>
<td>Gonads</td>
<td>22%</td>
<td>–</td>
<td>47%</td>
<td>–</td>
</tr>
<tr>
<td>Testis (Leydig cell in male)</td>
<td>Testis (Leydig cell in male)</td>
<td>–</td>
<td>8%</td>
<td>–</td>
<td>40%</td>
</tr>
<tr>
<td>Ovary (Sex-cord stromal in female)</td>
<td>Ovary (Sex-cord stromal in female)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

ACTH, adrenocorticotrophic hormone; GH, growth hormone, NF, non-functioning.
pancreatic islets (mainly insulinoma; also, glucagonoma, glucagon + insulin mixed-hormone tumors or gastrinoma), anterior pituitary (mainly prolactinoma in female; also, GH-secreting somatotropinoma), adrenal cortex, thyroid, gonads (Leydig cells in male or ovarian stroma in female) and mammary glands. The delay in tumor development could be due to the time it takes for the spontaneous LOH of the Men1 locus. Among the spectrum of tumors that develop in Men1-/- mice, some are not observed in human MEN1 patients, e.g., bilateral pheochromocytoma and gonadal tumors; and some human MEN1-associated tumors have not been reported in the mouse models, e.g., foregut carcinoids and skin lesions (3% of Men1-/- mice in one mouse model have shown lipoma (Harding et al. 2009)).

Genetically engineered mouse models with homozygous somatic loss of the Men1 gene specifically in MEN1-associated endocrine tissues have been generated by the Cre-Lox system that uses floxed Men1 alleles and Cre-recombinase expression driven by tissue-specific promoters. These mice develop normally and show tumor development at an early age because they do not rely on the spontaneous loss of the non-targeted Men1 allele as in the germline Men1-heterozygous mice. However, despite biallelic Men1 loss during early embryogenesis due to Cre expression from promoters that are active during embryogenesis, tumor development still takes 6–12 months. These observations indicate that loss of menin alone may not be sufficient for tumorigenesis. Parathyroid-specific Men1-knockout mice (using parathyroid hormone promoter/PTH-Cre) develop parathyroid hyperplasia and hypercalcemia (Libutti et al. 2003). Pancreatic islet β-cell-specific Men1-knockout mice (using rat insulin promoter/RIP-Cre) develop insulinomas (adenoma or carcinoma) (Bertolino et al. 2003c, Crabtree et al. 2003, Biendi et al. 2004). Pancreatic α-cell-specific Men1-knockout mice (using glucagon promoter/GLU-Cre) show mostly insulinomas rather than the expected glucagonomas (Lu et al. 2010, Shen et al. 2010). The reason for this phenotype has been attributed to the possible involvement of paracrine signals that induce β-cell proliferation or due to transdifferentiation of α-cells into β-cells indicating a role of menin in maintaining the cellular plasticity of islet cells. Men1 knockout in the whole pancreas (using pancreas/duodenum homeobox protein-1/PDX1-Cre) leads to a single tumor only of the endocrine pancreatic β-cells (insulinomas) while other pancreatic endocrine and non-endocrine cells remain unaffected (Shen et al. 2009). Also, knockout of Men1 in the liver (using albumin promoter/ALB-Cre), a tissue not targeted in MEN1, does not lead to tumors in the liver despite the complete loss of Men1 (Scacheri et al. 2004). Other mouse models with knockout of Men1 in pancreatic endocrine cell lineages, bone cells and intestinal cells have also helped to gain insight into the physiological actions of menin (Veniaminova et al. 2012, Bonnavion et al. 2015, Kanazawa et al. 2015, Sundaresan et al. 2016, Liu et al. 2017). These elegant studies in genetically engineered mouse models show that menin may function as a cell-type-specific tumor suppressor and that its action is only required in MEN1-associated target tissues to prevent tumorigenesis.

A search of mouse models that show MEN1-associated tumors has revealed that mice with knockout of some cell cycle regulators (p18 and p27) develop tumors of the parathyroid, pancreatic islets and anterior pituitary (prolactinoma) (Franklin et al. 1998). Therefore, mice with knockout of genes that are known to regulate the cell cycle (Rb, p53, p18, p27, Cdk2 or Cdk4) have been generated in the Men1-/- background to study the effect of the combined loss on the development of PNETs (insulinomas). Combined loss of Men1 with Rb or p53 has not shown any significant effect (Loffler et al. 2007b, 2012, Matoso et al. 2008). Combined loss of Men1 with p18 but not p27 results in the acceleration of insulinoma formation and increased tumor incidence (Bai et al. 2007). Combined loss of Men1 with Cdk4 but not with Cdk2 blocks the formation of insulinomas (Gillam et al. 2015). These mouse models show that decreased activity of p18 or increased activity of Cdk4 may contribute to the development of PNETs upon menin loss.

Mouse models have also been generated to look at the effect of pancreatic islet β-cell-specific Men1 loss together with other candidate target genes associated with β-cell proliferation and function. These mouse models have shown that the expression of activated K-RAS (p.G12D) enhances rather than inhibits β-cell proliferation, β-catenin loss can suppress insulinoma tumorigenesis, histone demethylase retinoblastoma-binding protein-2 (Rbp2) loss decreases insulinoma formation and prolongs survival, ActivinB loss prolongs survival and MLL1/KMT2A loss leads to earlier onset of tumor formation (4 months vs 6 months) and shortened lifespan by promoting tumor progression and angiogenesis (Lin et al. 2011, 2016, Chamberlain et al. 2014, Jiang et al. 2014, Ripoche et al. 2015). Such studies have dual benefits as they help to understand tumorigenesis and to reveal β-cell proliferation mechanisms for developing β-cell replacement strategies to help diabetic patients who suffer from functional β-cell loss (Garcia-Ocana & Stewart 2014).
The pre-clinical utility of the mouse models of \textit{Men1} loss is highlighted by several studies that have investigated the efficacy of potential treatment options in mouse PNETs (insulinoma) and pituitary tumors (prolactinoma). Some of the potential treatment options were predicted from protein–protein interaction studies where loss of menin in the interaction led to pro-tumorigenic actions of proteins or pathways that could be controlled with drugs (Hedgehog pathway, \(\beta\)-catenin and epigenetic histone modifications). Other potential treatment options targeted a tumor angiogenesis factor (vascular endothelial growth factor, VEGF), receptors associated with tumor angiogenesis (VEGF receptors) or specific somatostatin receptors. These pre-clinical studies include treatment using the following: menin replacement, an apoptosis-promoting factor (tumor necrosis factor, TNF) transgene delivery, an angiogenesis inhibitor (anti-VEGF-A monoclonal antibody, mAb G6-31), a small molecular tyrosine kinase inhibitor of all VEGF receptors (Sunitinitib), a somatostatin analog (Pasireotide/\textit{SOM230}), a Hedgehog pathway inhibitor GDC-0449, a \(\beta\)-catenin inhibitor PKF115-584 and an epigenetic drug (bromodomain and extra-terminal motif (BET) inhibitor JQ1) (Korsisaari \textit{et al.} 2008, Quinn \textit{et al.} 2012, Walls \textit{et al.} 2012, 2016, Gurung \textit{et al.} 2013b, Jiang \textit{et al.} 2014, Smith \textit{et al.} 2016, Lines \textit{et al.} 2017).

The impact of molecular genetic studies on disease prediction and management

The discovery of the causative gene for the MEN1 syndrome has changed the clinical management of MEN1 and related states with a direct impact on reducing disease-related morbidity and mortality. Genetic counseling and germline \textit{MEN1} genetic testing under recommended guidelines has facilitated the accuracy of disease prediction in index cases and their immediate family members and relatives (Brandi \textit{et al.} 2001, Thakker \textit{et al.} 2012). Mutation-negative individuals in families with a known \textit{MEN1} mutation are spared the uncertainty and anxiety of knowing whether they have the disease or not, and asymptomatic mutation-positive individuals can undergo early screening and monitoring for \textit{MEN1}-associated tumors. A positive genetic test also helps to confirm the clinical diagnosis in both familial and sporadic cases of MEN1. Although a positive genetic test for \textit{MEN1} mutation does not lead to any immediate medical or surgical treatment decisions, the negative-genetic test eases the burden of lifelong clinical investigations. A few studies have shown that in family members of mutation-positive index cases who opted for genetic testing and who tested positive for an \textit{MEN1} mutation, early surveillance has helped to detect tumors at an average of 10 years before clinically evident disease, allowing careful monitoring of tumors that have the potential to become malignant. Reports of a few mutation-negative symptomatic individuals in \textit{MEN1} kindreds has suggested the existence of \textit{MEN1} phenocopies, which may occur in 5–10\% of families with \textit{MEN1}, mainly associated with features of parathyroid and pituitary disease (Newey & Thakker 2011). The identification of other susceptibility genes for disease features similar to \textit{MEN1} has prompted several clinical genetic testing laboratories to offer genetic testing of a panel of genes that can be screened by direct sequencing or whole-exome sequencing (WES) to improve early disease prediction and management. For example, a hyperparathyroidism panel (Invitae), and XomeDxSlice - a phenotype-driven specific gene list using WES capture (GeneDx).

Future directions

No mutation in the \textit{MEN1} gene is identified in 10–30\% of cases diagnosed with \textit{MEN1} based on clinical features. Screening of \textit{MEN1} regions not included in the current mutation analysis methods may help to find the missing mutations; for example, mutations in gene regulatory regions that may be located far away from the gene. The identification of such \textit{MEN1} gene regulatory regions may facilitate such studies. It is possible that other causative gene/s may be responsible for the clinical features, such as mutations in genes that encode menin-interacting proteins. The identification of these genes can be attempted through collaborative efforts using WES or whole-genome sequencing of germline and tumor DNA samples of patients (and family members) who present with the various \textit{MEN1}-associated tumors. Such efforts may also help to identify disease-associated potential genetic modifiers.

Recent studies have proposed rare phenotypes among the \textit{MEN1} characteristics such as breast cancer in the Dutch cohort of \textit{MEN1} patients (van Leeuwaarde \textit{et al.} 2017). Follow-up studies could be conducted to verify this observation in other large cohorts of \textit{MEN1} patients such as in the French and Belgian GTE network (Groupe d’étude des Tumeurs Endocrines), the Italian patient database and the Japanese database of \textit{MEN1} (Sakurai \textit{et al.} 2012, Thevenon \textit{et al.} 2015, Giusti \textit{et al.} 2017).
Also, mouse models of Men1 loss could help to elucidate whether menin loss alone or other factors contribute to this phenotype.

The tissue-restricted pattern of the endocrine tumors of MEN1 is also evident among the different mouse models of Men1 loss. The reason for this tissue-selective anti-tumor function of menin remains to be determined. Exploring tissue-specific actions of menin that may result from interactions with the various protein partners that have been identified may help to reveal therapeutic targets to facilitate translational efforts. Stimulating the outcome of these actions of menin may serve as a potential anti-tumor therapeutic option in MEN1-associated endocrine tumors. The development of cell lines derived from mouse or human MEN1-associated endocrine tumors could facilitate such studies. Another area for future investigation is the reason for the delay in tumorigenesis after the loss of both copies of the Men1 gene (as observed in mouse models).

Future studies directed at the identification of the missing genes that contribute to the development of the tumors characteristic of MEN1, and further elucidation of the molecular pathways that are affected upon menin loss hold promise for enhancing management strategies for MEN1 and related states.

Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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