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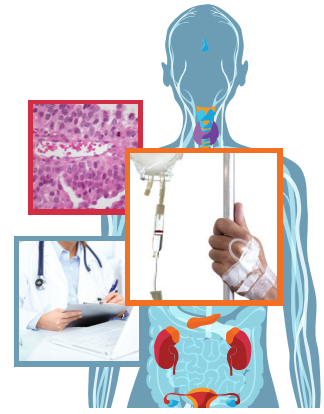

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Exploring the tumors of multiple endocrine neoplasia type 1 in mouse models for basic and preclinical studies

Sunita K Agarwal
National Institutes of Health, NIDDK, Metabolic Diseases Branch, Bldg 10, Room 8C-101, Bethesda, MD 20892, USA
Tel.: +1 301 402 7834
Fax: +1 301 402 0374
sunitaa@mail.nih.gov

Most patients (70–90%) with the multiple endocrine neoplasia type 1 (MEN1) syndrome possess germline heterozygous mutations in *MEN1* that predisposes to tumors of multiple endocrine and nonendocrine tissues. Some endocrine tumors of the kinds seen in MEN1 that occur sporadically in the general population also possess somatic mutations in *MEN1*. Interestingly, the endocrine tumors of MEN1 are recapitulated in mouse models of *Men1* loss that serve as a valuable resource to understand the pathophysiology and molecular basis of tumorigenesis. Exploring these endocrine tumors in mouse models using *in vivo*, *ex vivo* and *in vitro* methods can help to follow the process of tumorigenesis, and can be useful for preclinical testing of therapeutics and understanding their mechanisms of action.

Keywords: cell cycle • epigenetic • MEN1 • menin • mouse models • tumor suppressor • tumorigenesis

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal-dominant tumor syndrome manifesting as endocrine tumors in adults (age 40–50 years) in the parathyroid (90%), pancreas/duodenum (50%) and pituitary (40%) [1,2]. MEN1 cases have at least two of the three main associated endocrine tumors, and familial MEN1 cases have at least one first-degree relative with tumor in at least one of these three tissues [3]. Other endocrine and nonendocrine tumors of MEN1 are: adrenal cortical tumors, foregut carcinoids, meningioma, uterine leiomyoma and skin lesions – angiofibromas, collagenomas and lipomas [4,5]. Most endocrine tumors of MEN1 are benign but they cause symptoms by producing and secreting excess hormones or by local mass effects, while some MEN1 tumors have the potential to be malignant (Table 1).

Most index cases with familial MEN1 (70–90%) have a heterozygous germline mutation in the *MEN1* gene (first hit) located on chromosome 11q13, and tumors show loss of the remaining normal copy of *MEN1* (second hit); thus *MEN1* functions as a tumor suppressor [6,7]. The frequency of *MEN1* germline mutation is much lower in MEN1 index cases without a family history of MEN1 (1–10%) [2]. MEN1 is rare but the sporadic counterpart tumors of MEN1 occur more commonly. Somatic *MEN1* mutation is observed in 30–40% of common tumors of the parathyroids or pancreas/duodenum, but in only 3% of common pituitary tumors [8–12]. More than 500 unique mutations have been identified in *MEN1* (mostly in the coding region and splice junctions) with no apparent genotype/phenotype correlations [13]. Few MEN1 cases (<2%) show rare germline mutations in the *CDKN1B/p27* gene (MEN4 syndrome) that encodes p27 one of the cyclin-dependent kinase inhibitors (CDKI), or other CDKI family members [14,15]. However, the clinical phenotype distinct from typical MEN1 with complete or incomplete disease features is not clearly defined. Somatic *CDKN1B/p27* mutations have been identified in 8–10% of sporadic small intestine neuroendocrine tumors [16]. Identification of other mechanisms of inactivating *MEN1*, and identification of mutations in other genes is necessary to elucidate

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the genetic cause of tumors in MEN1 cases and sporadic tumors that lack *MEN1* mutations.

The *MEN1* gene encodes a 610 amino acid protein named menin (NCBI Reference Sequence: NM_130799.2, human menin isoform-2) [17]. Menin is a ubiquitously expressed 67 kDa predominantly nuclear protein reported to undergo phosphorylation at six different amino acid residues – Ser394, Thr397, Thr399, Ser487, Ser543 and Ser583 [17,18]. Approximately 40 different proteins associated with diverse functions have been shown to partner with menin in the nucleus or cytoplasm [17]. Several lines of evidence show that menin is associated with the regulation of transcriptional activation or repression from its interaction with transcription factors or chromatin-modifying protein complexes [17]. However, the importance of phosphorylation and interactions of menin with various proteins in the context of endocrine tumorigenesis remains to be determined.

Mouse models can serve as effective experimental surrogates of autosomal dominant human genetic disorders, such as MEN1. Also, mouse models with tumors of MEN1 from manipulation of other relevant genes can provide insights about the genetic and molecular interactions that lead to tumor formation. This review surveys the mouse models that present with the endocrine tumors of MEN1 and describes the use of these mouse models to investigate the molecular and genetic interactions associated with endocrine tumorigenesis and the potential of preclinical studies in these mouse models in the investigation of therapeutics. This review contains information gathered from the existing literature, and does not contain any unpublished original results from the author.

Tumor spectrum in the conventional mouse models of MEN1 versus human MEN1 syndrome

Four different conventional germline *Men1* knockout mouse models have been generated that target and delete different *Men1* exons (labeled A, B, C and D in Table 1) [19–22]. The germline homozygous knockout mice are small in size and die during mid-gestation at embryonic days E10.5–E14.5 with craniofacial, neural tube, heart and liver developmental defects [19–25]. Similar to the human MEN1 syndrome, the germline heterozygous mice spontaneously lose the normal copy of *Men1* in the MEN1-associated endocrine tissues and show an endocrine tumor spectrum that resembles the human syndrome. After 12 months, the mice develop the main endocrine tumors of MEN1 in the parathyroids, pancreas, anterior pituitary and adrenals. Considering the differences in lifespan, the age at onset of tumors (12–18 months) in the mouse models with spontaneous second hit closely coincides with the age at onset of human MEN1 [26].

Similar to humans with MEN1, mice with MEN1 show anterior pituitary tumors predominantly in females [8,27]. In human MEN1, gastrinomas occur more frequently (40%) compared with insulinomas (10%) and nonfunctioning tumors (10%). However, in mouse models the pancreas/duodenum tumors are mainly insulinomas (28–88%) with significantly decreased blood glucose levels, and gastrinoma is observed in only one model (15%) (Table 1). The foregut carcinoids and nonendocrine tumors seen in human MEN1 cases are not observed in the mouse models except for the occurrence of a lipoma in one mouse model [22]. Some tumor types seen in mouse MEN1 do not occur in human MEN1 patients, for example, bilateral pheochromocytoma and gonadal tumors (in both male and female mice).

These mouse models confirm that germline heterozygosity of *MEN1* predisposes to tumorigenesis in multiple endocrine organs raising the possibility that these tissues could have similar mechanisms that control their growth and development. Exploring the tumors of MEN1 could provide insights about these endocrine cell-specific growth-control mechanisms. The conventional mouse models could serve as experimental surrogates of the human MEN1 condition to explore the *in vivo* progression of the MEN1 syndrome. Studies using genomic and proteomic approaches with tumors from the mouse models are warranted to explore tumor pathogenesis and pathways relevant to tumor formation and to identify other possible genetic/epigenetic hits after *Men1* loss that play a role in tumorigenesis. With advances in techniques that use very small amounts of RNA, DNA, protein or chromatin, such studies could be done with tumors and corresponding normal tissues from the mouse models of MEN1 tumors. For example, a recent study performed whole-genome sequencing of a tumor from a mouse model of acute myeloid leukemia and identified leukemia progression mutations relevant for human leukemia [28]. These mutations were previously only observed in single human cases. The driver nature of these mutations is now validated due to their occurrence in the mouse cancer genome.

Tumors of MEN1 in endocrine-targeted homozygous *Men1* knockout mouse models

Mouse models with conditional homozygous knockouts of *Men1* in MEN1-associated endocrine target tissues have been generated using standard Cre-Lox strategies, and these mice are viable. Mice carrying floxed *Men1* alleles ($Men1^{f/f}$) have been crossbred with mice expressing the Cre-recombinase from different tissue-specific promoters to selectively inactivate both copies

Table 1. Tumors associated with the MEN1 syndrome in man and mouse.

Tumors	Human (at age 40 years), %	Mouse (*Men1*$^{+/-}$ at age 12–18 months), %			
		A[†]	B[‡]	C[§]	D[¶]
Endocrine (hormone-secreting or NF)					
Parathyroid	90	24	47	9	85
Pancreas (entero-pancreatic neuroendocrine):					
• Gastrinoma[#]	40		15		
• Insulinoma	10	28–83	88	82	60
• NF, PPoma[#]	20				
• Glucagonoma[#], VIPoma[#],	2				
• Somatostatinoma[#], etc.					
Anterior pituitary:					
• Prolactinoma	20	26–43	12	10	
• GH + prolactin-secreting	5				32
• GH-secreting	5		7		
• NF	5				
• ACTH-secreting	2				3
Adrenal:					
• Cortex NF	25	20–43	35	10	10
• Medulla	1				
• Pheochromocytoma	–	7			1
Foregut carcinoid:					
• Gastric ECLoma NF	10				
• Thymic carcinoid[#] NF	4				
• Bronchial carcinoid[#] NF	2				
Nonendocrine					
Facial angiofibroma	85				
Truncal collagenoma	70				
Lipoma	30				3
Meningioma	5				
Barrett's esophagus	5				
Leiomyoma:					
• Uterus (in female)	30				
• Esophagus	5				
Ependymoma	1				
Lung (adenocarcinoma)	–	22			
Testis, Leydig cell (in male)	–	22	88	47	60
Ovary, sex-cord stromal (in female)	–	31	8		40

[†]Crabtree *et al.* 2001 [19], Balasubramanian and Scacheri 2009 [26], % from TSM exon3-8, % after hyphen from dNexon3-8.
[‡]Bertolino *et al.* 2003 [20], exon 3.
[§]Loffler *et al.* 2007 [21], exon 2.
[¶]Harding *et al.* 2009 [22], exon 1–2.
[#]Tumor type with malignant potential for 25% or more human cases. Mouse models and exons deleted.
ACTH: Adrenocorticotropic hormone; ECLoma: Tumor of enterochromaffin-like cells; GH: Growth hormone; NF: Nonfunctioning; PPoma: Tumor secreting pancreatic polypeptide; VIPoma: Tumor-secreting vasoactive intestinal polypeptide.
Adapted with permission from [8].

of endogenous *Men1* in the target tissues: parathyroid hormone promoter (PTH-Cre, for parathyroid cells), Villin promoter and Lgr5 promoter (Villin-Cre and Lgr5-CreERT2, for antral and intestinal epithelium, respectively), rat insulin promoter (Rip-Cre, for islet β-cells), glucagon promoter (GLU-Cre, for islet α-cells) and Pdx1 promoter (Pdx1-Cre for whole pancreas, exocrine and endocrine). Unlike the conventional germline *Men1* heterozygous mice, conditional mice are not dependent on the spontaneous tissue-specific second hit to the *Men1* gene; therefore, tumor formation in the conditional mice occurs at an earlier age.

Parathyroid hyperplasia and hypercalcemia are observed in the parathyroid-specific *Men1* knockout mouse model after 7–9 months [29]. The gastrointestinal epithelium-specific *Men1* knockout mice do not develop gastrinoma, but they show hypergastrinemia from antral G-cell hyperplasia and a hyperproliferative epithelium [30]. Insulinomas (adenoma or carcinoma) develop in most islets at 6–8 months in the pancreatic islet β-cell-specific *Men1* knockout mouse models, with islet hyperplasia as early as 4 weeks [31–33]. Although a pituitary-specific knockout of *Men1* has not been published, older (>12 months) Rip-Cre; *Men1*$^{f/f}$ female mice (18–56%) also develop prolactinomas due to leaky Cre expression from the Rip-Cre-promoter in the pituitary cells [31–33]. The occurrence of the tumors specifically in the lactoprophs shows that specific inactivation of *Men1* in this cell type can cause tumors. Surprisingly, two different mouse models of pancreatic islet α-cell-specific *Men1* loss show mostly insulinomas rather than the expected glucagonoma [34,35]. The unexpected phenotype has been attributed to transdifferentiation of α-cells into β-cells or from potential paracrine signals from the *Men1* knockout α-cells that induce β-cell proliferation. Another surprising finding is in the whole pancreas knockout *Men1* mouse model (Pdx-Cre; *Men1*$^{f/f}$), which develops a single tumor only in the endocrine pancreatic β-cells (insulinomas), rather than tumors of all pancreatic cells or the multiple insulinomas in the Rip-Cre; *Men1*$^{f/f}$ mice [36]. Therefore, mice knockout for *Men1* in the whole pancreas, in the α-cells or β-cells, all develop only β-cells tumors; underscoring the importance of menin in β-cell proliferation.

Although menin is haplosufficient for the development of all tissues, menin haploinsufficiency is observed in *Men1* knockout mouse islets (conventional and conditional knockout mice) leading to increased β-cell proliferation and hyperplasia prior to tumor formation [19–22,31–33]. This precursor stage of polyclonal hyperplasia is not observed in humans. Although loss of menin expression occurs early in embryogenesis in all the conditional *Men1* knockout mice, delayed tumor formation implicates other hits for tumor formation. The lack of chromosomal or microsatellite instability in the insulinoma of Rip-Cre; *Men1*$^{f/f}$ mice has suggested that mutations might occur at the nucleotide level or epigenetic mechanisms might be affected [37]. These conditional *Men1* knockout mice with accelerated tumorigenesis can be useful models to test drug treatments in younger animals and in specific endocrine tumors.

Tumors of MEN1 in mouse models of cell cycle control genes with or without *Men1*

Conventional mouse models engineered to express or knockout a single or combination of some cell cycle control genes develop the endocrine tumor types observed in MEN1. These include *Rb, Ccdn1/cyclinD1*, a cyclin-dependent kinase (*Cdk4*) and a few CDKIs, *p18*, *p21* and *p27*. Although mouse models with germline *Rb* loss (*Rb*$^{+/-}$) mainly show intermediate lobe pituitary tumors, *Rb*$^{+/-}$ mice in the C57 background show high penetrance for anterior pituitary tumors [38]. Mouse strain backgrounds did not have any effect on the development of prolactinomas in the MEN1 mouse models: mouse background C57/129 [21,22], C57/129SvTacFBR (TSM) or C57BL/6 (dN3–8) [19,26] or 129/Ola,129/Sv (Table 1) [20]. Mice with simultaneous germline loss of *p27* or *p21* with loss of *p18* develop an endocrine tumor spectrum that overlaps with both MEN1 and MEN2: hyperplasia and/or tumors of the parathyroid, endocrine pancreas, pituitary, adrenals, duodenum, stomach, testes and thyroid C cells [39]. Hyperplasia in the islet β-cells is observed in conventional mice expressing a constitutively active form of CDK4 (CDK4-R24C) and in mice with β-cell-specific cyclinD1 overexpression [40,41].

Men1$^{+/-}$ mice with combined loss of *Rb*, or *p27*, or *Cdk2* do not show any difference in the phenotype and tumor spectrum compared with *Men1*$^{+/-}$ mice [42–45]. While *Cdk4*$^{-/-}$ mice show hypoplasia of the pituitary and pancreatic islets, *Men1*$^{+/-}$ mice with combined loss of *Cdk4* do not develop any tumors and possess hypoplastic islets and pituitaries without loss of heterozygosity for *Men1* [45]. *Men1*$^{+/-}$ mice with combined loss of *p18* show tumors at an early age (3–12 months) with an increased penetrance for anterior pituitary tumors, insulinomas, parathyroid adenoma, adrenal cortical tumors and lung tumors [42]. However, these tumors do not lose the second copy of *Men1*, suggesting that p18 participates together with menin as a tumor suppressor in endocrine tissues. It is possible that the function of p27 is already maximally compromised in the *Men1*$^{+/-}$ mice and the additional loss of p27 is refractory to any effect on tumor formation; therefore, p27 overexpression in endocrine tumors of mouse models of *Men1* loss might be useful to test the hypothesis that p27 could block tumorigenesis in *Men1*$^{+/-}$ mice [26].

Reduced expression of CDKIs p15, p18, p21 and p27 has been observed in pancreatic islet tumors from *Men1*$^{+/-}$ mice [46]. The MENX syndrome in rat and the MEN4 syndrome in man are caused by germline mutations in *p27* [14]. Also, possible rare germline mutations in 3 other CDKI genes (*p15*, *p18*, and *p21*) are reported in MEN1 and MEN1-like disease [15]. These observations together with the mouse models described above highlight the importance of cell cycle regulators in endocrine tumorigenesis and warrant further studies to explore the therapeutic potential of CDKIs.

Tumors of MEN1 in mouse models with combined knockout of *Men1* with *p53* or *Rbp2*

To understand the contribution from interplay of different genes with *Men1* and their impact on MEN1 tumors, two other mouse models have been generated with combined loss of *Men1* with *p53* or *Rbp2*. Mice with knockout of the tumor suppressor *p53* (*Trp53*$^{+/-}$) in an *Men1*$^{+/-}$ background show independent and nonsynergistic effects on tumorigenesis, possibly indicating that disruption of the p53 pathway is not relevant for the endocrine tumors of MEN1 [43]. Menin partners with two similar multiprotein MLL-complexes, containing either MLL1 or MLL2 with an enzymatic activity (histone methyltransferase, HMTase) that catalyzes the trimethylation of histone H3 at lysine 4 [47,48]. This specific histone modification (H3K4me3) is associated with the activation of gene transcription. RBP2 (also known as KDM5B or JARID1B) is a histone demethylase that can remove the trimethylation at H3K4me3 [49]. Mice inactivated for *Men1* in β-cells are defective for H3K4me3 on specific genes, not only from loss of the menin–MLL complexes but also owing to increased activity of the demethylase Rbp2, which can act at H3K4me3. In such conditional β-cell-specific *Men1* knockout mice, β-cell-specific loss of *Rbp2* rescued tumorigenesis and enhanced median survival age from 45 weeks (*Men1* knockout in β-cells) to 69 weeks (combined knockout of *Men1* and *Rbp2* in β-cells) [50]. Therefore, inhibition of the histone demethylase activity of Rbp2 could be potentially therapeutic in insulinomas.

Exploring the tumors of MEN1 in mouse models for preclinical applications

Genetically engineered mouse models of disease can be useful for preclinical applications, such as for screening the potential safety, activity and efficacy of drug compounds, testing transgene delivery methods and for *ex vivo* tumor transplantation studies. Below are summarized five different studies published to date that have used different mouse models of MEN1 tumors.

The proliferation rates of the endocrine tumors of MEN1 have been investigated by long-term labeling with the thymidine analog 5-bromo-2-deoxyuridine (BrdU). The proliferation and kinetics of tumors of the pancreas, pituitary and adrenals was investigated in 18–21-month-old conventional *Men1* knockout mice [22,51]. Mice fed with BrdU in the drinking water, to allow continuous labeling of proliferating cells for 1–12 weeks, were assessed for tumor cell proliferation rate and apoptosis. The analysis using a mathematical model of neuroendocrine tumor cell proliferation predicted that the lifespan of mice was sufficient for studying tumors of the pancreatic β-cells, and two different pituitary cell types (lactotrophs and somatotrophs) but not for non-β-cell pancreatic neuroendocrine tumors (PNETs). This technique and analysis could be useful to assess the efficacy of novel treatments in mouse models to reduce tumor cell proliferation, such as monoclonal antibodies, gene replacement and other genetic/epigenetic modifying agents.

A preclinical evaluation of *MEN1* gene therapy has been conducted in the pituitary tumors of *Men1*$^{+/-}$ mice by transauricular intratumoral injection with Men1.rAd5 (a recombinant nonreplicating adenoviral serotype 5 vector with the murine *Men1* cDNA under the control of a cytomegalovirus promoter) [22,52]. Tumor growth characteristics in 55 *Men1*$^{+/-}$ female mice treated with Men1.rAd5 (and control mice) were studied by feeding BrdU in the drinking water for 4 weeks followed by MRI and immunohistochemical analysis. The Men1.rAd5-injected tumors showed significantly reduced daily proliferation rates. This study demonstrated that *MEN1* gene replacement therapy by direct injection in the tumor could effectively express ectopic menin to reduce tumor cell proliferation.

Germline *Men1*$^{+/-}$ mice [21] have been used to study the efficacy of an antiangiogenesis treatment with the anti-VEGF-A monoclonal antibody mAb G6–31 to inhibit the growth of pituitary tumors and insulinomas [53]. Anti-VEGF-A mAb G6–31 (or control antibody IgG) was administered to 125 female *Men1*$^{+/-}$ mice at age 11–13 months for 67 days or until mice were found moribund. Significant decrease in the mean pituitary tumor volume and prolactin levels was observed 39 days after treatment in the mAb G6–3 treated animals. A similar efficacy was observed against insulinomas. This study also established a transplantable model of MEN1 mouse pituitary tumors in BALB/c nude mice, which produced high levels of prolactin and responded well to anti-VEGF-A therapy. The availability of transplantable *ex vivo* models of MEN1 endocrine tumors can facilitate and hasten the investigation of such tumors that require many months to establish *in vivo* in the mouse models.

PNETs in human MEN1 and in the Pdx1-Cre; *Men1*$^{f/f}$ mouse model show increased vasculature and upregulation of VEGF expression, a known contributor to angiogenesis [36]. Using the insulinoma in the Pdx1-Cre; *Men1*$^{f/f}$ mice as a model, inhibition of VEGF signaling was investigated with sunitinib, a known small-molecule tyrosine kinase inhibitor of all VEGF receptors [36]. Four to eight sex-matched and weight-matched *Men1*$^{f/f}$ and Pdx1-Cre; *Men1*$^{f/f}$ mice at age 3 months were treated with vehicle or sunitinib via daily oral gavage for 3 months. Significantly reduced cell proliferation and reduced islet vascularity was observed in Pdx1-Cre; *Men1*$^{f/f}$ animals treated with sunitinib. This study demonstrated that VEGF signaling was a critical pathway in the PNET that developed in this mouse model of MEN1.

Using a novel single islet and tumor perfusion technique, wild-type or germline *Men1*$^{+/-}$ young (4–6 months, n = 7 per genotype) and older mice (>12 months, n = 7 per genotype) have been used to study molecular, morphological and physiological vascular alterations in the endocrine pancreas and in PNETs [21,54]. Differential regulation was observed for the vascular contractile responses to vasoactive agents (D-glucose and L-NAME) in tumor and normal islet-supplying capillaries. The vascular differences were accompanied by differential expression of multiple angiogenic factors (VEGF-A/VEGFR2/PIGF and FGF2/FGFR1 pathways) with some already expressed in the islets of normal looking *Men1*$^{+/-}$ islet tissue. These data implicate a possible haploinsufficient state of the *Men1*$^{+/-}$ islet that precedes tumor formation.

Unlike the other mouse models of *Men1* loss (conventional or Rip-Cre; *Men1*$^{f/f}$), the Pdx1-Cre; *Men1*$^{f/f}$ mouse model shows only a single insulinoma in the pancreas [36]. All somatostatin receptor types (SSTRs 1–5) are expressed in the PNET tissue of the Pdx1-Cre; *Men1*$^{f/f}$ mouse model [55]. Therefore, the efficacy of a somatostatin analog, Pasireotide (SOM230), was assessed [55]. Eight 12-month-old Pdx1-Cre; *Men1*$^{f/f}$ mice with insulinoma were treated with monthly subcutaneous injections of SOM230 or PBS. Significantly decreased serum insulin levels and a significant increase in serum glucose in the treatment group were observed on day 7. Also, a reduction in tumor size and increased apoptosis was detected in the treatment group. This study demonstrated the antisecretory, antiproliferative and proapoptotic activity of SOM230 in the Pdx1-Cre; *Men1*$^{f/f}$ MEN1 model of insulinoma.

Conclusion & future perspective

Studies of MEN1 tumors in mouse models have confirmed that germline heterozygosity of *MEN1* can predispose to tumorigenesis in multiple endocrine organs and have been useful to explore tumor progression in specific endocrine tissues, and menin's functional interaction with cell cycle regulators and with histone methylating and demethylating proteins. At the same time, several mechanistic and biological aspects remain to be determined, such as the reason for the second hit to the *Men1* locus in only certain selected endocrine tissues, the endocrine cell-type specificity of tumorigenesis in the pancreas and the reason for the delay in tumor formation even in the conditional mouse models. More than 30 different endocrine and nonendocrine tissues are affected in the human MEN1 syndrome (Table 1). It is not known why the nonendocrine tumors are not manifested in the conventional *Men1*$^{+/-}$mouse models. Although mouse background has been shown to affect the embryonic lifespan of the *Men1*-null mouse [25], similar data about the effect of mouse genetic background have not been formally gathered for the endocrine tumors of MEN1. The above aspects can be determined by developing new experimental mouse models.

Another aspect for future study in the mouse models is to test the treatment of endocrine tumors with drugs that mimic p18 to inhibit CDKs, or drugs that target epigenetic modifications. Menin's participation in histone-modifying protein complexes and the reduced islet tumor burden and increased lifespan of the compound conditional *Men1/Rbp2* knockout mouse model shows that epigenetic drug targets could be relevant for the treatment of MEN1 tumors.

The hyperplastic islets in the MEN1 mouse models retain their differentiated β-cell characteristic to produce and secrete hormones. Therefore, understanding the molecular basis of their proliferation capacity can help to develop methods for β-cell expansion/replacement in conditions of β-cell loss, such as in diabetes.

Future studies in the mouse models of MEN1 tumors will help to uncover molecular events that govern disease progression in the early stages that precede tumor formation. Such studies are not possible to implement in human patients. Elucidating the pathways perturbed during tumorigenesis in the tumors of MEN1 in mouse models will help to identify targets downstream of *Men1* loss that also could reveal genes causative for sporadic tumors that lack *MEN1* mutation. Ultimately it is very important to understand the manifestation of *Men1*/menin deficiency in murine models at the molecular level and how that relates to human patients.

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Executive Summary

- Mouse models with germline heterozygous loss of *Men1* resemble the endocrine tumor phenotype of the human multiple endocrine neoplasia type 1 (MEN1) syndrome.
- Mouse models with germline homozygous loss of *Men1* die during embryogenesis, whereas mouse models with homozygous loss of *Men1*, specifically in the MEN1-associated endocrine tissues, are viable and develop tumors.
- Mouse models of a few specific cell cycle control genes also show endocrine tumors of MEN1.
- Mouse models with combined loss of *Men1* and other relevant genes provide insights about the interplay of these genes with *Men1* to explore disease-associated pathways.
- Islet tumors from the mouse models of *Men1* loss reveal a multistep process of tumorigenesis.
- Tumors of MEN1 in mouse models serve as a valuable resource for preclinical testing of treatment options, such as menin replacement, angiogenesis inhibitors and a somatostatin analog.

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