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

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“Over the past two decades, many different mouse models of *Men1* loss have been generated in order to determine causality of *Men1* loss as a driver of tumorigenesis, and utility as experimental surrogates.”

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Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominantly inherited tumor syndrome characterized by the development of endocrine and nonendocrine tumors (Online Mendelian Inheritance in Man, MIM number: 131100). MEN1-associated endocrine tumors mainly arise from the cells in the anterior pituitary, the parathyroids and the duodenopancreatic neuroendocrine tissues. Other tumors of the MEN1 syndrome include adrenal cortical tumors, foregut carcinoids, meningioma, uterine leiomyoma, and skin lesions – angiofibromas, collagenomas and lipomas. Patients with the MEN1 syndrome are predisposed to develop these tumors because they carry heterozygous germline inactivating mutations in the *MEN1* gene that encodes menin (first hit). Tissue-specific somatic loss of the remaining normal *MEN1* allele (the second hit, from loss of heterozygosity at chromosome 11q13) is

responsible for subsequent tumor formation. A significant fraction (30–40%) of sporadic parathyroid and sporadic non-functioning pancreatic neuroendocrine tumors (PNETs or PanNETs) also show *MEN1* mutations (see [1] [2]). Over the past two decades, many different mouse models of *Men1* loss have been generated in order to determine causality of *Men1* loss as a driver of tumorigenesis, and utility as experimental surrogates [3]. Germline homozygous knockout (KO) of *Men1* in mice causes embryonic lethality. The conventional germline heterozygous *Men1* KO mice depend on a spontaneous tissue-specific second hit to the nontargeted *Men1* allele for MEN1-associated tumor development, thus mimicking the condition in the human MEN1 patient. Although the endocrine tissue-specific conditional *Men1* KO mice do not depend on a spontaneous second hit for homozygous loss of *Men1*, tumor formation still takes many

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months and the precise reasons for the delay are not known. Interestingly, mice with *Men1* loss in the whole pancreas, or the endocrine α -cells or β -cells mostly present with β -cell tumors. These mouse models are valuable experimental surrogates of the tumors of MEN1 for understanding tumor initiation, maintenance and progression/evolution. The fact that it is possible to make mouse models that mimic the tissue-specific genotype and pathology of human MEN1-associated tumors has presented opportunities to test potential therapeutic options and to direct research in these mouse models to test relevant hypotheses. This editorial provides highlights of the key recent findings in this field after the publication of the article ‘Exploring the tumors of MEN1 in mouse models for basic and preclinical studies’ in the International Journal of Endocrine Oncology [3].

Gastrinoma is the most common functional neuroendocrine tumor in MEN1 that is usually found in the duodenum and rarely in the pancreas of MEN1 patients [4]. However, gastrin-secreting neuroendocrine tumors were observed only in one of four different *Men1*^{+/-} mouse models of MEN1 [5]. Further characterization in cell-type-specific KO mouse models has shown that pancreatic gastrin-expressing lesions develop after 12 months upon specific deletion of *Men1* in pan-pancreatic progenitors (PancMen1 KO, using *Men1*^{fl/fl} and Ptfla-Cre), or in pancreatic endocrine progenitors (PancEndoMen1 KO, using *Men1*^{fl/fl} and Ngn3-Cre) [6]. Also, careful observation showed gastrin coexpression (together with glucagon or insulin) in the PNETs of 12-month-old mice with α -cell or β -cell-specific *Men1* loss [6]. These mouse models can prove to be very useful to study the cell of origin of pancreatic gastrinomas and for developing diagnosis and treatment of these tumors.

The development of gastrinomas was also explored by targeted deletion of *Men1* in intestinal epithelial cells (IEC) of the gastrointestinal mucosa using Villin-Cre (*Men1*^{AIEC}) [7]. However, this mouse model did not develop gastrinomas. When this mouse line (*Men1*^{AIEC}) was crossed with mice that lacked somatostatin (*Sst*^{-/-}), after 2 years the *Men1*^{AIEC}; *Sst*^{-/-} mice developed gastric carcinoids (polyps in the corpi of the stomach) [8]. Acid suppression in these mice by feeding omeprazole-laced chow caused carcinoid formation at an earlier age [8]. This mouse model shows that *Men1* deletion may not be sufficient to favor carcinoid development but

rather additional hits to other loci are essential, such as somatostatin deficiency. Characterization of gastric carcinoid development and potential preclinical approaches can be investigated in this mouse model.

The contribution of additional genetic hits after *Men1* loss has been explored in the context of PNETs by deleting candidate genes whose expression is upregulated upon menin loss (ARC and ActivinB), or candidate genes that are important functional partners of menin (β -catenin and MLL1). Compound-mutant mice with germline homozygous deletion of the gene that encodes for the apoptosis repressor with CARD (ARC; *Nol3*^{-/-}) and *Men1* deletion in the whole pancreas (*Men1*^{fl/fl}; Pdx1-Cre) did not alter PNET (insulinoma) burden [9]. Therefore, ARC upregulation may not be a critical second hit in insulinoma formation. Compound-mutant mice with germline homozygous deletion of the gene that encodes for a TGF- β -related ligand ActivinB (*Inh β B*^{-/-}) and β -cell-specific deletion of *Men1* (*Men1*^{fl/fl}; Rip-Cre) did not affect insulinoma tumor growth but increased the survival of this mouse model by blocking the dedifferentiation of β -cells in the tumors [10]. Conditional deletion of *Men1* together with β -catenin (*Men1*^{fl/fl}; *Ctnnb1*^{fl/fl}; Rip-Cre) or pharmacological inhibition of β -catenin signaling (with PFK115-584) in *Men1*^{fl/fl}; Rip-Cre mice suppressed insulinoma tumorigenesis and prolonged survival [11]. Mice with deletion of the gene that encodes MLL1 in β -cells (*Kmt2a*^{fl/fl}; Rip-Cre) showed hyperplastic islets but no frank tumor [12]. However, compound mutants that lacked menin and MLL1 (*Men1*^{fl/fl}; *Kmt2a*^{fl/fl}; Rip-Cre) lead to earlier onset of tumor formation (4 vs 6 months) and shortened the lifespan of these mice by promoting tumor progression and angiogenesis [12]. These four mouse models have helped to gain insights into the molecular pathology of PNET development and can be useful for the analysis of potential therapeutic interventions.

Two different studies had shown that conditional deletion of *Men1* in the glucagon-expressing α -cells did not result in glucagonomas but rather β -cell insulinomas [13,14]. These data suggested that menin loss might promote cross communication between α - and β -cells and/or α -cell plasticity to transdifferentiate into β -cells. Recently, transdifferentiation of β -cells into α -cells was observed in older mice with β -cell-specific *Men1* loss wherein these mice developed

glucagon-expressing tumors (35% mice at 18 months of age) [15]. Further studies in these mouse models can help to elucidate the genetic events that may regulate cross-communication and the plasticity of pancreatic endocrine cells.

One of the important interactions of menin is with the trithorax-like complexes containing the histone methyltransferases MLL1 (KMT2A) or MLL4 (KMT2B; previously also known as MLL2) that catalyze the addition of the histone H3 lysine-4 trimethyl mark (H3K4me3). This chromatin mark activates gene expression, and loss of this chromatin mark promotes the recruitment of histone-modifying complexes that deposit H3K27me3 to repress gene expression. Pancreatic islets with *Men1* loss (*Men1^{flf}*; Rip-Cre) did not show a significant difference in the level of H3K4me3 or H3K27me3, as determined by immunohistochemistry. However, ChIP-seq analysis of *Men1* KO islets with anti-H3K4me3 or anti-H3K27me3, and gene expression analysis identified a number of menin-dependent target genes that showed loss of H3K4me3 and gain of H3K27me3 [16]. Exploring this dynamic epigenetic regulation of gene expression during the development of *Men1* KO tumors can help to study the molecular underpinnings of tumor progression that may help to identify therapeutic targets that are induced by menin loss.

Regarding preclinical studies using the mouse models of MEN1 tumors, a somatostatin analog (pasireotide, SOM230) was shown to be effective for the treatment of insulinoma in 12-month-old *Men1^{flf}*; Pdx1-Cre mice who received 160 mg/kg per month of SOM230 by subcutaneous injection every month for 4 months [17]. Another study has shown that pasireotide treatment in the conventional mouse model of *Men1* loss (*Men1^{+/-}*) could increase survival by 20%, and inhibited PNET and pituitary tumor growth [18]. These mice were treated at the age of 12 months with 40 mg/kg pasireotide long-acting release (SOM230LAR) by intramuscular injection every month for 9 months [18]. These studies concluded that pasireotide or similar somatostatin analogs could

serve as potential treatment options for human MEN1-associated PNETs and pituitary tumors.

Future perspective

In the past few years, several mouse models have been generated to investigate the consequence of the combined loss of *Men1* and relevant target genes to understand the molecular pathology of gastrinomas and PNETs. Future studies in these mouse models can be directed toward the identification of pathways affected by the combined loss to provide insights into the tissue selectivity of MEN1-associated tumor development. In a recent study, whole-genome sequencing of a large number of sporadic PNETs (n = 98) identified mutations in novel genes that may cooperate with the inactivation of known genes that are frequently associated with PNETs [19]. New mouse models with combined targeting of the specific genes can be generated to explore these findings. It is important to note that PNETs that develop in the *Men1^{+/-}* mouse model are mainly insulinomas although insulinomas are not the most frequent PNETs in MEN1 patients, and the vast majority of sporadic PNETs are nonfunctioning. Regardless of these differences, the mouse models of MEN1 tumors (*Men1^{+/-}* and others) can continue to serve as valuable experimental surrogates for preclinical studies, for example, to explore the recently discovered druggable targets in PNETs such as CD47 and MET [20,21]. Also, understanding the molecular underpinnings of β -cell proliferation in insulinomas has the potential to impact diabetes treatment for developing strategies to expand functional β -cell mass.

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