

Animal models of MEN1

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Abstract

Animal models of cancer have been instrumental in advancing our understanding of the biology of tumor initiation and progression, in studying gene function and in performing preclinical studies aimed at testing novel therapies. Several animal models of the MEN1 syndrome have been generated in different organisms by introducing loss-of-function mutations in the orthologues of the human *MEN1* gene. In this review, we will discuss MEN1 and MEN1-like models in *Drosophila*, mice and rats. These model systems with their specific advantages and limitations have contributed to elucidate the function of Menin in tumorigenesis, which turned out to be remarkably conserved from flies to mammals, as well as the biology of the disease. Mouse models of MEN1 closely resemble the human disease in terms of tumor spectrum and associated hormonal changes, although individual tumor frequencies are variable. Rats affected by the MENX (MEN1-like) syndrome share some features with MEN1 patients albeit they bear a germline mutation in *Cdkn1b* (p27) and not in *Men1*. Both *Men1*-knockout mice and MENX rats have been exploited for therapy-response studies testing novel drugs for efficacy against neuroendocrine tumors (NETs) and have provided promising leads for novel therapies. In addition to presenting well-established models of MEN1, we also discuss potential models which, if implemented, might broaden even further our knowledge of neuroendocrine tumorigenesis. In the future, patient-derived xenografts in zebrafish or mice might allow us to expand the tool-box currently available for preclinical studies of MEN1-associated tumors.

Key Words

- ▶ multiple endocrine neoplasia type 1
- ▶ endocrine tumors
- ▶ menin
- ▶ animal models

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Introduction

Multiple endocrine neoplasia type 1 (MEN1) is a complex syndrome defined by the neoplastic transformation of at least two endocrine organs, most frequently parathyroid glands, pancreatic islets, anterior pituitary and endocrine pancreas. Less frequently, patients present with adrenal cortical tumors, carcinoids, facial angiofibromas, collagenomas and lipomas (reviewed in [Thakker 2014](#)). In 1997, by linkage analysis and tumor deletion mapping, the *MEN1* gene located on chromosome 11q13 was identified as the gene responsible for the MEN1 syndrome ([Chandrasekharappa et al. 1997](#), [Lemmens et al. 1997](#)). The encoded 610-aa long protein named Menin is a

tumor suppressor, and tumors of MEN1 patients usually show loss-of-heterozygosity (LOH), which leads to loss of Menin function. The protein plays a role in cell division, genome stability and transcriptional regulation ([Thakker 2014](#)). Menin binds to a MLL-containing complex with histone methyltransferase activity and recruits this complex to the promoters of the cyclin-dependent kinase (CDK) inhibitors *Cdkn1b* (p27) and *Cdkn2c* (p18), thereby activating their transcription ([Karnik et al. 2005](#)). The binding to JunD, a member of the Jun family of transcription factors, suppresses Menin's ability to activate transcription. Not surprisingly, missense mutations in



Genetically modified and spontaneous models	Mnn1 ^{e200} (Busygina, 2004) Mnn1 ^{e173} and Mnn1 ^{e90} (Papaconstantinou, 2005) Mnn1- (Cerrato, 2006)	Not described yet	MEN1 $\Delta 3-8/+$ (Crabtree, 2001) MEN1 1/T (Bertolino, 2003) MEN+/- (Loeffler, 2007) MEN+/- (Harding, 2009)	MENX (<i>Cdkn1b</i>) (Fritz, 2002)
Xenograft models	no	yes	yes	no

Figure 2

Animal models of MEN1 currently available and potential alternative models.

regulatory regions. Most of the reported alterations are frameshift or nonsense mutations leading to lack of Menin expression or to a truncated (non-functional) protein variant (Lemos & Thakker 2008). Most missense mutations (68%) and the most frequently reported human mutations occur within highly conserved regions between human, rat, mouse, zebrafish and *Drosophila* (Marini *et al.* 2009, <http://www.umd.be/MEN1/>) (Fig. 1B). No genotype–phenotype correlation has been found in MEN1 as individuals with the same *MEN1* mutation may have different clinical presentations.

To date, several animal models of MEN1, or having a MEN1-like phenotype, have been described. We here review the existing models but we also discuss model organisms that could potentially be useful to study MEN1-associated pathogenesis (Fig. 2).

***Drosophila melanogaster*: a model to study Menin's function**

The fruitfly *Drosophila melanogaster* has a long history as model organism and it has helped elucidate the basic principles of inheritance before DNA was discovered to be the carrier of genetic information (Beller & Oliver 2006). Genetic screening in *Drosophila* revealed that several genes important in tumorigenesis are conserved between fly and man, including *Notch*, *Shh* (sonic hedgehog), *Wnt* (Wingless) and *Men1*. The advantages of this model organism are on the one hand extremely short life span and generation time, numerous progeny, low maintenance costs, and, on the other hand, well-established methods to modify its genome. Disadvantageous or unfavorable characteristics are the organism's lower complexity, as well as the necessity to keep mutant fly strains as living stocks, as embryos cannot be frozen. Due to their short life

span and limited cell divisions, flies do not spontaneously develop cancer. However, transgenic flies show hallmarks of cancer such as evasion from apoptosis, sustained proliferation, metastasis, survival, genomic instability and metabolic reprogramming when cancer-associated genes are mutated (Tipping & Perrimon 2014).

The *Mnn1* gene is the *Drosophila* orthologue of the human *MEN1* gene and both genes share a similar genomic organization. *Mnn1* consists of two transcripts, one which is found only in embryos while the other is expressed in adult tissues and encodes a 763 aa long protein. In humans, six different *MEN1* transcripts were reported having a different 5' UTR; however, only two with substantial differences in the coding sequence (Marini *et al.* 2009). *Mnn1* encodes a protein having 46% overall identity with human Menin (Guru *et al.* 2001, Marini *et al.* 2009) (Fig. 1). Although the overall identity is rather low, the N-terminal part, which harbors the binding sites to several Menin interaction partners, has higher homology. Moreover, sequences at the C-terminus that were shown to be important for Menin's nuclear localization are conserved between human and fly. Although initial studies could not demonstrate an interaction between fly Menin and human JunD in yeast two hybrid assays, this binding was then confirmed when the fly homologue of JunD was used (Cerrato *et al.* 2006).

Three transgenic *Drosophila* strains have been generated by introducing slightly different deletions in *Mnn1* that result in loss of Menin expression (Busygina *et al.* 2004, Papaconstantinou *et al.* 2005, Cerrato *et al.* 2006). All flies having a homozygous *Mnn1* deletion were viable, suggesting that the Menin orthologue is dispensable for *Drosophila* development. However, deletion of *Mnn1* resulted in a 5–7% reduction in viability, which was identified by screening 6000 flies. Such progeny numbers

cannot be obtained using rodent models, thereby making *Drosophila* a useful model organism to study genetic alterations causing subtle changes in survival.

As stated previously, *Mmn1*-deleted flies developed rather normally, yet when exposed to DNA-damaging agents or ionizing radiation, they displayed a higher sensitivity than wild-type flies (Busygina *et al.* 2004). The authors ascribed this phenotype to a defect in nucleotide excision repair in the transgenic flies, which resulted in loss of genomic integrity. Interestingly, *MEN1* expression is frequently lost in human melanomas due to epigenetic silencing and deletion of the gene in melanocyte cell lines impairs homologous recombination-directed DNA repair while concomitantly inducing the error prone mechanism of non-homologous end-joining (Fang *et al.* 2013). Thus, *Mmn1*-deleted flies share molecular mechanisms with *MEN1*-associated human cancers.

In another *Mmn1*-knockout strain, flies were found to be more sensitive to a variety of different stressors (Papaconstantinou *et al.* 2005). Mutant flies exposed to heat shock, hypoxia, hyperosmolarity and oxidative stress had a higher degree of developmental arrest and lethality when compared with wild-type flies. Mechanistically, it was shown that Menin activates the transcription of the heat shock protein genes *Hsp70* and *Hsp23*. This induction was abolished in the knockout flies, thereby impairing their response to stress. In a follow up study, the same authors showed that the lack of heat shock proteins induction caused by *Mmn1* deletion can be linked to genome maintenance (Papaconstantinou *et al.* 2010). These studies broadened our knowledge of the role of Menin in regulating stress response and genomic stability.

Collectively, these *Drosophila* models provided us with valuable information about the function of Menin as a regulator of transcription and DNA repair, and the potential implications of these characteristics for tumorigenesis. Although *Drosophila* as a model organism may be a bit old fashioned, it can still be useful to elucidate the genetic events leading to secondary mutations or genomic instability or to screen for drugs that might counteract the abnormal DNA repair due to loss of Menin.

Zebrafish as a potential model of MEN1

Transgenic fish strains to study NET-associated genes

While the zebrafish *Danio rerio* has been used as animal model of developmental disorders for over 50 years, only recently it became a focus in cancer research. The overall advantages of the model lie in the high fecundity

(up to 200 progenies/week), the simple assessment of the transparent embryos that develop outside the mother, and the conservation of most of the vertebrate organs. Also the endocrine system is conserved between human and zebrafish, with relevant orthologues of neurohormones being present (Vitale *et al.* 2014). In addition, a very broad genetic tool box allows easy manipulation of the zebrafish genome for large genetic screens or for specific site-directed mutagenesis (nicely reviewed in Gut *et al.* 2017). A few years ago, a global initiative was set in motion with the aim to target every gene in the zebrafish genome and provide researchers with the resulting mutants (www.zfin.org/). Unfortunately, although 36,284 mutant alleles have been generated so far, no *Men1* mutation has been described. Yet, a functional orthologue of human *MEN1* exists, named *Men1*, which encodes a 617 aa long protein with 67% identity and 80% similarity to human Menin (Manickam *et al.* 2000) (Fig. 1). Expression patterns of Menin in fish larvae correlated with those in murine tissues, and the binding ability of fish Menin to mouse and human JunD was also conserved (Manickam *et al.* 2000).

The generation of transgenic strains with defective Menin could provide us in the future with a promising *MEN1* model especially since zebrafish develop tumors that quite well resemble human cancers at histological and molecular levels (White *et al.* 2013). Although such strains are currently not yet available, several zebrafish mutants exist that develop tumors belonging to the *MEN1* spectrum including parathyroid, pancreatic and pituitary tumors. These models may be useful to study specific characteristics associated to NETs (e.g. interaction with the tumor microenvironment, metastatic potential, angiogenesis).

Primary hyperparathyroidism (1°HPT) is the most common phenotypic manifestation of *MEN1*. It is defined by an excess of parathyroid hormone (PTH), which results in hypercalcemia and ultimately in bone thinning and formation of kidney stones (Giusti *et al.* 2012). Fish do not possess a typical parathyroid gland; yet, studies from Okabe and Graham (2004) proved that the gills of fish are evolutionarily related structures that express calcium-sensing receptors and PTH. A transgenic zebrafish strain deleted for *cdc73* has been suggested as a model for parathyroid tumors, as the human homologue HRPT2/CDC73 is responsible for the hyperparathyroidism–jaw tumor syndrome (Carpten *et al.* 2002, Bourque & Houvras 2011).

Pancreatic NETs were observed in zebrafish overexpressing the human *MYCN* gene under control

of the core-zymod-promoter (Yang *et al.* 2004). The few analyzed transgenic fish mostly expressed insulin in the tumors, with glucagon expression found in one case. The tumor morphology indicated a malignant phenotype. In MEN1 patients, insulinomas are also more frequent than glucagonomas; however, all these tumors are usually benign (Tonelli *et al.* 2012). Interestingly, although the overexpression of MYCN was ubiquitous, only tumors in the pancreas arose. This establishes a parallel with *MEN1*, which is also ubiquitously expressed, but its defective function causes tissue-selective tumorigenesis. The possibility to perform large genetic screens is among the strengths of the zebrafish model, and this could be applied to search for genes associated with tissue specificity of pancreatic cancer.

Extensive work has been done to analyze the temporal and spatial development of the zebrafish pituitary gland, which shares with the organ in higher vertebrates the organization into intermediate and anterior lobe (Pogoda & Hammerschmidt 2009). At the 96-h embryonic stage, the pituitary gland is already fully developed, thereby facilitating studies focusing on alterations that affect pituitary development. Observing anomalies during development is further simplified by the transparency of the embryos and by several well-established imaging techniques (Ignatius & Langenau 2011). Mutation of the ubiquitin-specific peptidase 39 (Usp39), a protein involved in RNA splicing, promotes the expansion of anterior pituitary cells (hyperplasia), making of this transgenic zebrafish a potential model for these lesions (Rios *et al.* 2011). Liu and coworkers generated a transgenic zebrafish overexpressing the pituitary tumor-transforming gene (*Pttg*) under the control of the proopiomelanocortin (POMC) promoter (Liu *et al.* 2011). These animals (Tg: Pomc-Pttg) develop corticotroph adenomas associated with decreased glucocorticoid sensitivity, oversecretion of the corticotroph hormone (ACTH) and subsequent metabolic disturbances similar to the hypercortisolism seen in Cushing's disease patients (Lacroix *et al.* 2015). Although ACTH-secreting tumors represent a minority (4%) of the pituitary adenomas occurring in MEN1 patients (Uraki *et al.* 2017), this zebrafish model might be a valuable tool to identify molecular pathways associated to pituitary tumorigenesis or to screen for potential antitumor drugs. Indeed, zebrafish embryos can be maintained in cell culture dishes, thus simplifying large-scale screens for therapeutic agents in a cost-effective way. In the study of Liu and coworkers (Liu *et al.* 2011), R-roscovitine, a CDK inhibitor, was found to suppress

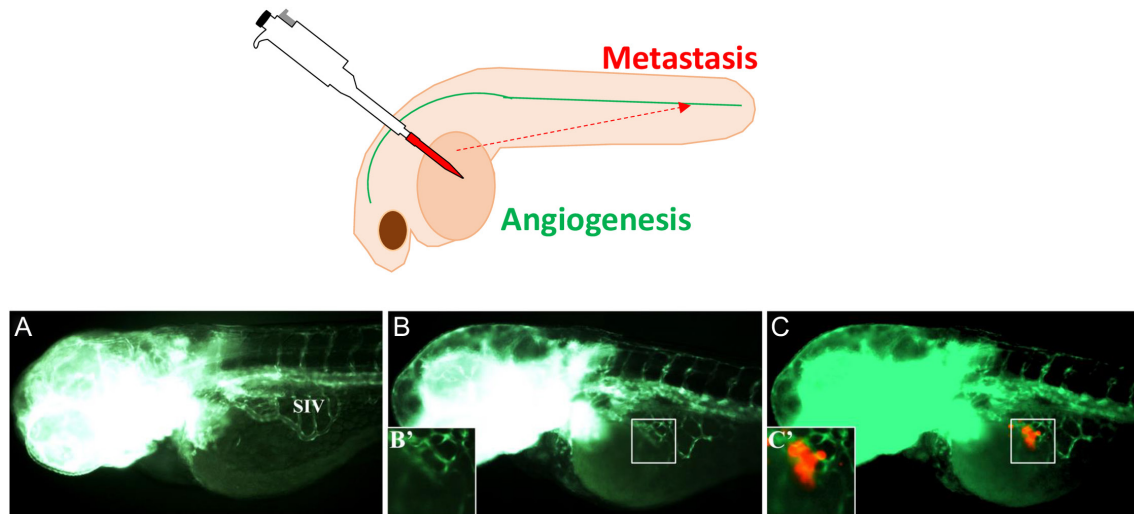
corticotroph expansion in the transgenic zebrafish embryos. The effect of this drug was also validated in a mouse model of ACTH-secreting pituitary tumors, and R-roscovitine was therefore proposed as a potential therapeutic option for Cushing's disease (Liu *et al.* 2011).

Patient-derived xenografts (PDXs) in zebrafish

Another important use of zebrafish in cancer research involves xenotransplantation studies. A hallmark of tumors is the ability to engraft after transplantation into an appropriate recipient animal. While xenografts in mice are still the gold standard, xenografting in zebrafish is increasing in popularity and examples of transplanting human cancer cell lines (Stoletov *et al.* 2007, Lara *et al.* 2011), patient-derived cancer cells (Gaudenzi *et al.* 2016) or tissues (Marques *et al.* 2009) have been reported. Tumor cells can be engrafted in zebrafish embryos, juveniles or adults, and working protocols have been established that outline the advantages and disadvantages of each approach. The problem posed by the fact that for optimal growth human cells and zebrafish require the temperature of 37 or 28°C, respectively, can be overcome by using 31°C for embryos and 35°C for adult zebrafish (Haldi *et al.* 2006).

From the early embryonic stages up to 1 month after birth, zebrafish do not possess a completely developed immune system, so that immune suppression to engraft xenotypic tissues is not necessary (Tobia *et al.* 2011). Furthermore, in the first 3–4 days of life, the embryos do not need an established blood circulation system as the oxygen can perfuse through the tissues (Pelster & Burggren 1996). Thus, transplanted cells can survive until they are able to induce neovascularization. Monitoring angiogenesis in zebrafish is simplified by the availability of several transgenic strains where blood vessels are fluorescently labeled (e.g. Tg(fli1-eGFP), Tg(flk1:mCherry), Tg(vegfr2:g-rGFP)) (Lawson *et al.* 2002, Jin *et al.* 2005). In the translucent embryo, the newly forming blood vessels can be measured in real time by confocal fluorescence microscopy. In Fig. 3, the example of a xenograft of a prolactin-secreting pituitary adenomas in zebrafish embryos inducing the growth of the new vessels (green fluorescent-labeled endothelial cells), which sprout toward the transplanted tumor cells (chemically labeled in red) is illustrated.

Wurth and coworkers transplanted human pituitary adenoma stem cells (hPASCs) in zebrafish and in NOD/SCID mice (Wurth *et al.* 2016). While there was no

**Figure 3**

Xenografting human cancer cells into zebrafish embryos to follow angiogenesis and migration. (A, B and C) Example of xenografting human pituitary adenoma cells in zebrafish. Red stained primary cells isolated from a human prolactin-secreting pituitary adenoma were xenografted in 48h post fertilization $Tg(fli1:EGFP)^{+1}$ zebrafish embryos, that express the enhanced green fluorescent protein (EGFP) in the vascular endothelium. In comparison to PBS-injected control embryos (A), 24h post injection tumor-grafted embryos (B and C) showed alterations in the pattern of sub-intestinal vessel (SIV) plexus and the presence of tumor induced-endothelial structures that sprouted from the SIV and reached the implant. The red channel was omitted in images B and B' to highlight the newly formed microvascular network. B' and C' represent digital magnification of white box in B and C, respectively. All images are oriented so that rostral is to the left and dorsal is at the top. Source: Picture kindly provided by Dr Germano Gaudenzi and Prof. Giovanni Vitale (University of Milan, Italy).

proliferation of hPASCs in murine hosts up to 8 months after injection, these cells readily engrafted into zebrafish embryos. In the latter system, neoangiogenesis toward the tumor mass could be detected 2–3 days after injection. Taking into account that $2\text{--}5 \times 10^6$ cells had to be injected into mice against 0.5×10^3 cells in zebrafish embryos and that the time required for cell engraftment in the embryos was a few days, exploiting this model organism for xenotransplantation experiments may bring personalized cancer therapy within reach.

Xenograft studies in zebrafish suggest that the aggressiveness of human primary tumors correlates with their ability to spread from the initial implantation site (Marques *et al.* 2009). To conduct these studies, tumor cells are labeled with a fluorescent dye before implantation and then invasion, migration and formation of micrometastases are followed in the translucent embryo by laser scanning confocal live imaging (Fig. 3). This approach has also been tested for NETs. In a proof-of-concept study, using eight different human primary NET samples, Gaudenzi and coworkers evaluated angiogenesis and cell migration in zebrafish xenotransplants (Gaudenzi *et al.* 2016). The tumors engrafted in six of eight cases and tumor originating from metastases showed a higher migration capacity, thereby proving the general applicability of the concept. In MEN1 patients, the leading

cause of death is the malignant potential of pancreatic endocrine tumors (Ito *et al.* 2013). Using this assay in future applications might allow us to assess pancreatic NETs for their propensity to metastasize, with important implications for predicting disease outcome and selecting appropriate therapeutic interventions.

Murine MEN1 models

Constitutive and conditional *Men1*-knockout mice

Not always murine models of cancer recapitulate the corresponding human disease. This is not the case for mouse strains with defective *Men1* function, which possess a remarkable phenotypic overlap with the human MEN1 syndrome. This despite the fact that most MEN1 mutations in patients are point mutations leading to truncated peptides, whereas the mouse models were generated by deleting entire exons of the *Men1* gene. Therefore, to mimic the human disease, it is not necessary to have a specific genetic mutation as long as Menin's function is abolished.

We will here focus on the phenotypic differences among the various *Men1*-knockout models in comparison to the human disease (Fig. 4).

Four different transgenic mouse lines were created by constitutive deletions of the *Men1* gene. In each model,

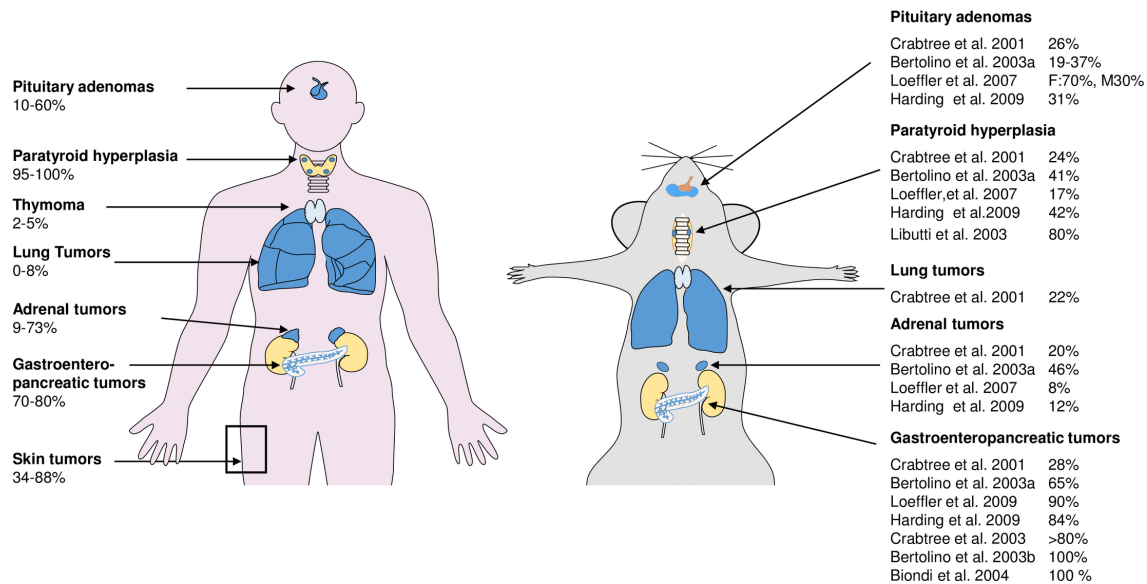


Figure 4

Tumor spectrum of the MEN1 syndrome and phenotype of the *Men1*-knockout mouse strains. The incidence of the various tumor types both in MEN1 patients and in the different mouse lines are reported.

different exons of *Men1* were targeted, resulting in loss of *Men1* transcription (e.g. deletion of exon 1–2, *Men1*^{Δ1–2} (Harding *et al.* 2009), and deletion of exon 2, *Men1*^{Δ2} (Loeffler *et al.* 2007)) or in truncated *Men1* transcripts (e.g. deletion of exon 3, *Men1*^{Δ3} (Bertolino *et al.* 2003a) or of exons 3–8, *Men1*^{Δ3–8} (Crabtree *et al.* 2001)). Regardless of the targeting site, these mouse strains share a similar tumor spectrum, albeit the frequency of the individual tumor types differs among them. In all four transgenic lines, homozygous *Men1*-knockout mice died at embryonic stages E10.5–E13.5. Therefore, studies were performed on heterozygous knockout animals. In all mouse lines, loss of the wild-type *Men1* allele was found in the tumors. This closely resembles the situation in MEN1 patients whose tumors usually show LOH (Valdes *et al.* 1998). In addition to these conventional knockout models, conditional mouse lines with tissue-specific deletion of *Men1* were generated and are here presented and compared with the constitutive models.

Parathyroid glands In MEN1 patients, primary hyperparathyroidism is the most prevalent, and often the first, symptom with an incidence of 95–100% and is usually due to hyperplasia or adenoma in the parathyroid glands (Giusti *et al.* 2012). In the constitutive knockout mouse lines, this phenotype occurred with an incidence of only 17–42%. In *Men1*^{Δ3–8/+} mice, although serum calcium levels were not elevated, the incidence of parathyroid adenomas was 12 fold higher

than that in wild-type animals. In one case, progression to parathyroid carcinoma was detected. In *Men1*^{Δ3/+} mice, PTH levels were not significantly elevated, yet a few animals had enhanced secretion of the hormone (Bertolino *et al.* 2003a). Serum calcium levels were not assessed. In these mice, parathyroid adenomas were observed starting at 12 months (41% of mice) and by 19+ months they reached the frequency of 64%. Increased incidence of parathyroid tumors with age was also a characteristic of *Men1*^{Δ2/+} mice but the rates were lower, reaching only 15% in 2-year-old mice (Loeffler *et al.* 2007). In the *Men1*^{Δ1–2/+} model, hypercalcemia and hypophosphatemia were observed, which were caused by overactivity of PTH, and not by increased levels of the hormone (Harding *et al.* 2009). In conclusion, similar to MEN1 patients, all *Men1*-knockout lines show abnormalities in the parathyroid glands, albeit with variable frequencies.

In a conditional mouse model obtained by crossing *Men1*^{Δ3–8flox/flox} mice with animals carrying Cre under control of the PTH promoter, up to 80% of the homozygous mice developed hyperparathyroidism (Libutti *et al.* 2003). Elevated serum calcium levels were detected by 7 months of age, and an enlargement of the parathyroid glands was visible by 9 months. By 14 months, the size of the glands was 5-fold bigger in transgenic than in control animals. The specificity of the Cre recombinase expression was very high, as no tissue other than the parathyroid glands was affected. Consequently, these mice are a suitable

model to study primary hyperparathyroidism without the interference of other hormonal imbalances.

Gut and pancreas Gastroenteropancreatic (GEP) NETs are the second most common neoplasm in MEN1 patients. They can be subdivided into functioning tumors (hormone secreting, frequency up to 40%) and nonfunctioning tumors (60–100%). Functioning GEP-NETs are defined based on the hormone they secrete. Insulinomas, pancreatic tumors secreting insulin, occur in 21% of patients, glucagonomas in 3%, while somatostatinomas, VIPomas and GHRH-omas are quite rare (1% of cases) (Tonelli *et al.* 2012). In MEN1 patients, gastrin-producing tumors (gastrinomas) mainly occur in the duodenum wall with a frequency of 50%, but micro-gastrinomas have also been observed in the pancreas (Pritchard *et al.* 2007). GEP-NETs in MEN1 patients present as multiple lesions and tend to metastasize.

In *Men1*^{Δ3-8/+} mice, pancreatic islet tumors developed at high frequency and correlated with elevated serum insulin levels, suggesting that they are insulinomas (Crabtree *et al.* 2001). The *Men1*^{Δ3/+} model showed islet cell hyperplasia in 65% of cases at the age of 8–12 months, but also adenomas (5%) and carcinomas (9%), whereas gastrinomas occurred in 19% of the analyzed mice. All pancreatic NET subtypes could be detected, with the occasional simultaneous overexpression of two hormones, a feature also observed in microadenomas of MEN1 patients (Anlauf *et al.* 2006). In this model (i.e. *Men1*^{Δ3/+}), GEP-NETs had a higher incidence than parathyroid tumors (41%).

Also in the *Men1*^{Δ2/+} model of Loffler *et al.* (2007) over 80% of mice harbored pancreatic lesions of different grades up to adenomas. No gender difference was found regarding the incidence of GEP-NETs. It should be noted that in this study over 130 mice were analyzed thereby reaching more statistical power when compared with reports where fewer animals were studied. Most of the adenomas were insulinomas, some were glucagonomas, but gastrin immunoreactivity was usually absent. In line with other models, pancreatic islet hyperplasia and pancreatic adenomas were found at high incidence in *Men1*^{Δ1-2/+} mice. Given that gastrinomas in patients develop in stomach and duodenum, 36 knockout mice between the ages of 18–21 months were intensively screened for the presence of these extrapancreatic gastrinomas, but none were found.

Altogether, the whole-body knockout mouse lines recapitulate the high incidence of pancreatic NETs seen in MEN1 patients but not that of the gastrinomas, much more rare.

Due to the underrepresentation of these tumors in the various mouse models, Veniaminova and coworkers (Veniaminova *et al.* 2012) addressed the question as to whether the deletion of *Men1* is sufficient to induce gastrinomas by specifically deleting Menin in antral and intestinal epithelium. To this aim, the authors crossed *Men1*^{Δ3-8flox/flox} mice with Villin-Cre or leucine-rich repeat containing G protein-coupled receptor 5 (Lgr5)-Cre mice. The resulting conditional knockout mice developed hypergastrinemia, but again no gastrin-secreting tumors. It needs to be noted that the recombination of the floxed sites was not complete, so that residual Menin expression was still present in the targeted tissues and may have prevented tumor formation.

Three studies addressed the effect of tissue-specific deletion of Menin in pancreatic β-cells by crossing four different rat insulin promoter (Rip)-Cre mouse lines with three different floxed *Men1* lines (Fig. 4). In order to easily distinguish these models, we will name them *Men1*^{Δ3-8flox/flox} (Crabtree *et al.* 2003), *Men1*^{Δ3flox/flox} (Bertolino *et al.* 2003b) and *Men1*^{Δ2flox/flox} (Biondi *et al.* 2004) based on the exonic region excised after recombination. Several versions of the Rip were used in these studies, leading to the conclusion that the stronger the promoter, the earlier and more pronounced was the phenotype. In all studies, homozygous tissue-specific deletion of *Men1* resulted in larger islet sizes compared to the heterozygous-deleted mice, as well as hyperplasias and insulinomas. In conditional *Men1*^{Δ3-8flox/flox}/Rip-Cre mice, the size of the insulinomas correlated with the secretion of insulin, the level of blood glucose and the survival rate (Crabtree *et al.* 2003). Tumor latency in the conventional heterozygous knockout mice was dependent on the complete loss of *Men1*, which represented the rate-limiting step, whereas in the conditional floxed mice, both alleles are lost upon recombination and thus the tumors develop earlier. Tumor progression was characterized by dedifferentiation, angiogenesis and multistage tumorigenesis (Bertolino *et al.* 2003b). Studies of *Men1*^{Δ3flox/flox}/Rip-Cre and *Men1*^{Δ2flox/flox}/Rip-Cre models revealed a poor tissue specificity of Rip-mediated Cre expression, and consequently, these mice developed pituitary tumors too, which were mainly prolactinomas. Different chromosomal rearrangements were found in pancreatic and pituitary tumors of *Men1*^{Δ3flox/flox}/Rip-Cre animals, with duplication of chromosome 11 and of chromosome 15, respectively, being the most frequent alterations. One possible explanation for these findings is that loss of *Men1* increases the susceptibility to a second mutagenesis hit in a tissue-specific manner.

To study the early events associated with *Men1* deletion in pancreatic islets, two conditional and inducible mouse models were generated. Schnepf and coworkers (Schnepf *et al.* 2006) crossed the *Men1*^{Δ3-8flox/flox}/Rip-Cre mice described above (Crabtree *et al.* 2003) with Cre-ER (estrogen receptor) transgenic mice expressing Cre under the control of a ubiquitously active human ubiquitin carrier 9 (UBC9) promoter. In the resulting mice (named *Men1*^{fl/fl};Cre-ER), the excision of *Men1* can be induced by tamoxifen in a controlled fashion. *Men1*^{fl/fl};Cre-ER mice at 12 weeks of age were treated with tamoxifen and their pancreata were analyzed 7, 14 and 30 days later. Loss of *Men1* caused an increase in islet cell proliferation detectable already 7 days post-treatment, which resulted in islet enlargement and hyperplasia at the 14-day time point (Schnepf *et al.* 2006). One limitation of this study is that mice were analyzed before tumor development could take place. Moreover, the *Men1* gene was deleted not only in islet cells but also in the exocrine pancreas and in other mouse tissues due to the broad expression of the Cre-ER transgene. Subsequently, another conditional, inducible mouse line was established by crossing *Men1*^{fl/fl} mice (see above) with mice expressing the tamoxifen-inducible Cre-ER driven by the rat insulin promoter (*RIP2-Cre-ER*) for a Cre expression restricted to pancreatic endocrine cells (Lines *et al.* 2017a). The resulting *Men1*^{fl/fl};RIP2-Cre-ER transgenic mice were treated with tamoxifen at 12 weeks of age and analyzed up to 5 months later (age 7, 5–8, 5 months) when pancreatic endocrine tumors (insulinomas) were present. Loss of Menin in the islets was observed in transgenic mice at all ages, along with a rise in both proliferation of pancreatic β-cells and islet area (Lines *et al.* 2017a). These inducible mouse models further strengthen the hypothesis that increased cell proliferation is the first effect of Menin loss.

Pituitary gland Pituitary tumors were a feature of all conventional knockout mice. Interestingly, the gender difference that is seen in men is recapitulated in mice: females have a higher prevalence of pituitary adenomas. In the *Men1*^{Δ2/+} line, pituitary tumors were present in 78% of the female mice vs 42.0% of the males (Loffler *et al.* 2007) (Fig. 4). In these mice, both anterior pituitary microadenomas and macroadenomas were observed, which were highly vascularized and showed signs of necrosis. Mass effects due to the significant increase in pituitary size were seen. Prolactin-positive and nonfunctioning tumors were found in these mice, but no ACTH-secreting adenomas. Pituitary adenomas were more frequent in females also in *Men1*^{Δ3-8/+}

mice, and all analyzed tumors were prolactinomas (Crabtree *et al.* 2001). In the *Men1*^{Δ1-2/+} line (Harding *et al.* 2009), the incidence of pituitary adenomas was only 31.4%, and all tumor subtypes were seen (i.e. prolactin-, GH- and ACTH-secreting adenomas, as well as nonfunctioning ones). Considering pituitary adenomas, the various constitutive knockout mice are a relatively faithful model of MEN1, although differences in tumor incidence and subtypes were observed between mice and men.

Adrenal gland All conventional *Men1*-deficient models developed tumors in the adrenal gland with frequencies ranging from 10 to 43%: not only adrenocortical tumors (20–43%) but also bilateral pheochromocytomas (7%) were found in *Men1*^{Δ3-8/+} mice (Crabtree *et al.* 2001). *Men1*^{Δ1-2/+} animals developed cortical hydroxysteroid hydroxylase-positive adenomas at low frequency (7%) and pheochromocytoma was detected in one case (Harding *et al.* 2009). Surprisingly, only male knockout mice developed adrenocortical adenomas. While gender differences in pituitary adenomas are a feature of MEN1, no differences in adrenal tumors development between genders have been reported in patients (Goudet *et al.* 2011).

Other tissues MEN1 patients suffer from a variety of skin lesions that occur in 33–84% of the cases. The presence of multiple collagenomas and angiofibromas is a good indicator of the MEN1 syndrome (Ashgarian *et al.* 2004). Cutaneous tumors were not observed in any of the above described mouse models. In contrast, all constitutive knockout mice developed tumors in the gonads. Leydig tumors of the testis were found in 22–88% of the male mice and sex-cord stromal tumors in the ovary were found in *Men1*^{Δ3-8/+}, *Men1*^{Δ3/+} and *Men1*^{Δ1-2/+} females with incidences ranging from 8 to 40%. These tumors do not belong to the MEN1 tumor spectrum. Detailed studies revealed that the wild-type *Men1* allele was not lost in the Leydig cell tumors of the heterozygous knockout mice (Loffler *et al.* 2007) and thus molecular events other than Menin loss of function might account for the tumorigenesis in these cells.

Non-small-cell lung cancer (NSCLC) was observed in *Men1*^{Δ3-8/+} mice with an incidence varying from 22% (Crabtree *et al.* 2001) to 42% (Pei *et al.* 2007) depending on the study. Lung tumors are rarely seen in MEN1 patients (8% of cases). The different frequency of NSCLC occurring in the same transgenic model might be due to the background strain of the mice, as Pei and coworkers backcrossed *Men1*^{Δ3-8/+} animals to the C57/BL6 background.

For the sake of completeness, it needs to be mentioned that *Men1*^{Δ3} mice older than 19 months of age develop mammary carcinomas at low frequency (8.3%) (Bertolino *et al.* 2003a), as well as carcinomas of the prostate in 12.8% of cases (Seigne *et al.* 2010). These neoplasms do not belong to the tumor spectrum of the MEN1 syndrome.

It should be taken into account that for the models described previously, mouse lines were usually a mixture of different background strains. This hampers the comparison of the different murine MEN1 models, due to strain-specific sensitivity to tumor development (Brayton 2007).

Preclinical studies with MEN1 mouse models

As stated above, the *Men1* knockout models recapitulate several of the key phenotypic features of the human MEN1 syndrome. Hence, it is reasonable to exploit them for preclinical studies aimed at identifying novel effective therapies against NETs, and a few such studies have indeed been conducted so far. We here discuss several examples of such studies which evaluated different therapeutic approaches.

As Menin-dependent tumorigenesis starts with the loss of both functional *Men1* alleles, Walls and coworkers (Walls *et al.* 2012) explored the feasibility of a *Men1* gene replacement therapy *in vivo* using a recombinant replication-deficient adenoviral vector containing the mouse *Men1* gene under the control of a cytomegalovirus promoter (Men1.rAd5). The virus was injected into the pituitary adenomas of *Men1*^{Δ1-2/+} female mice (Walls *et al.* 2012). Although the proliferation rates of pituitary tumors decreased, no changes in tumor mass or apoptosis were detected 4 weeks after injection of the recombinant adenoviruses.

Promising results were obtained by treating *Men1*^{Δ3-8/+} mice with the monoclonal antibody mAB-G6-31 directed against vascular endothelial growth factor A (VEGF-A), the best characterized pro-angiogenic factor (Korsisaari *et al.* 2008). Pituitary tumors are highly vascularized and thus by inhibiting angiogenesis, tumor progression should be suppressed. Indeed, Korsisaari and coworkers (Korsisaari *et al.* 2008) could nicely demonstrate that both tumor size and vascularization were reduced by the drug both *in situ* in *Men1*^{Δ3-8/+} mice and in syngeneic models of the mouse pituitary adenomas. Upon treatment with the anti-VEGFA antibody, mice bearing pituitary tumors showed a reduction in serum prolactin levels. In contrast, the insulin levels, elevated due to the insulinomas, were unaffected by the drug. Targeting angiogenesis with sunitinib, a multikinase inhibitor, reduced the

proliferation rates of the insulinomas developing in *Men1*^{Δ3-8flox/flox/Pdx1-Cre} mice, which was not accompanied by changes in tumor vascularization (Shen *et al.* 2009). Effects on insulin secretion were not assessed in this study. Thus, both studies suggest that the treatment of MEN1-associated tumors with drugs targeting angiogenesis might be effective at reducing tumor size.

NETs often express somatostatin receptors (SSTRs) on their cell membrane, and this has been exploited for diagnostic imaging, radiotherapy and pharmacological treatment using stable somatostatin analogues such as octreotide. Taking advantage of SSTR expression on pancreatic NET cells, Smith and coworkers (Smith *et al.* 2016) used an adeno-associated virus displaying octreotide on the surface to deliver tumor necrosis factor (TNF) to the tumor cells in order to induce apoptosis. These viral particles were tested in the conditional *Men1*^{Δ3-8flox/flox/Pdx1-Cre} mice (Shen *et al.* 2009) *in vivo* and found to reduce tumor size, to lower tumor metabolism and insulin secretion thereby leading to improved survival (Smith *et al.* 2016).

The efficacy of pasireotide (SOM230), a multi-ligand somatostatin analogue, against pancreatic NETs was evaluated in the *Men1*^{Δ3-8flox/flox/Pdx1-Cre} mouse model (Quinn *et al.* 2012). This agent reduced tumor volume of the insulinomas by activating apoptosis. Circulating insulin levels decreased in the treated mice and, as a consequence, blood glucose reached more physiological levels. Adjustment of the blood glucose levels had a positive effect on the survival rates of the treated mice, but due to small group sizes, no statistical analyses were performed. In another study, treatment of *Men1*^{Δ1-2/+} mice with pasireotide was found to reduce pancreatic tumor volume and frequency, to suppress pancreatic islet cell proliferation and induce apoptosis (Walls *et al.* 2016).

Chromatin remodeling via histone modifications has been shown to play an important role in tumorigenesis and several drugs have been developed that target epigenetic pathways (Jones *et al.* 2016). Menin interacts with histone methyltransferases in pancreatic β-cells thereby initiating specific transcriptional programs that promote cell proliferation (see above). Moreover, human sporadic and familial pancreatic NETs show mutations in chromatin remodeling genes such as *DAXX* and *ATRX* (Jiao *et al.* 2011). These findings provide the rationale for the evaluation of compounds targeting epigenetic regulatory proteins in these tumors. Recently, Lines *et al.* (2017b) tested several epigenetic drugs for efficacy on pancreatic NET cells *in vitro*, and the most promising one, JQ1, was then evaluated *in vivo* in *Men1*^{fl/fl};RIP2-Cre-ER mice. JQ1 is

an inhibitor of the bromo and extra-terminal motif (BET) proteins that bind acetylated lysine residues. This agent was able to reduce proliferation and promote apoptosis in pancreatic NETs of the transgenic mice, suggesting that targeting epigenetic pathways might be an effective strategy for the treatment of these tumors.

Based on the observation that active β -catenin accumulates in pancreatic NETs of *Men1*-deficient mice, conditional *Men1* ^{Δ 3^{flox/flox}}/*Rip-Cre* mice were treated with a small molecule antagonist of the T cell factor/ β -catenin complex, i.e. PKF115-584 (Jiang *et al.* 2014). Mice at the age of 14 months were treated for 8 weeks with PKF115-584 and then their pancreatic tumors were assessed for proliferation, which was suppressed by the drug. In addition, PKF115-584 treatment improved hypoglycemia in these mice by reducing insulin secretion.

Altogether, these studies emphasize the potential of the various *Men1*-knockout mouse models as translational platforms to identify effective therapies for MEN1 patients.

Patient-derived xenografts (PDXs) in mice

The variety of cell types, stages of progression and sequential mutational events contribute to the tumor heterogeneity typically seen in most human tumors, including NETs (Hessman *et al.* 2001). It has been suggested that one of the reasons behind drug failure in clinical testing is the lack of complexity of the models used for preclinical testing. A possibility to circumvent the potential lack of predictive power of various lower model organisms could be the use of patient-derived xenografts (PDXs) in preclinical studies.

Transplantation of human tumor samples into appropriate immunocompromised murine hosts allows the propagation of the primary tumors while maintaining their histological and genetic characteristics (Cassidy *et al.* 2015). Protocols for successfully engrafting human tumor cells/tissues into mouse recipients have been established (Mattar *et al.* 2017). The engraftment rate is strongly dependent on the intrinsic characteristics of the primary tumor, with aggressive tumors having in general higher rates (Siolas & Hannon 2013). The slow proliferation rate of NETs has historically hampered the generation of tumor cell lines (Grozinsky-Glasberg *et al.* 2012) and poses a problem also for their xenotransplantation. Only a few studies so far have reported the engraftment of NETs but with rather low success rates. In a proof-of-concept study Powers *et al.* (2017) demonstrated for the first time, the successful engraftment of pheochromocytomas and paragangliomas in the NSG mouse model. Histological

analysis proved the conservation of tumor features in the PDXs, and BrdU labeling demonstrated the proliferation of tumor grafts in the host. The NSG mouse strain is more immunocompromised than the better-known NOD/SCID strain, and the lack of thymoma formation allows the mice to age up to 1.5 years in appropriate housing conditions. Therefore, NSG mice represent the ideal strain to engraft slow-growing tumors (Shultz *et al.* 2005).

In another study, using fragments of 39 well-differentiated grade 1 and 2 pancreatic NETs, only one tumor generated xenografts in 90% of the host NSG mice over multiple passages (Krampitz *et al.* 2016). In a bigger tumor cohort, only 7 of 106 gastrointestinal NETs were successfully engrafted. Of these seven xenografts, only one tumor could be passaged several times and maintained for 2 years in NSG mice. Remarkably, the tumor retained its morphology and molecular characteristics over time (Yang *et al.* 2016). With this established PDX model, the authors plan to carry out preclinical drug studies. Francois and coworkers have already used PDX models of pancreatic NETs in drug efficacy tests (Francois *et al.* 2015). Treating the PDX models with an inhibitor of focal adhesion kinase (FAK), they could show a reduction in tumor progression over time. Interestingly, the tumor volume of the untreated control PDX doubled in 2 weeks, indicating a faster proliferation of the tumor cells when compared with the aforementioned studies.

MEN1 is a rare disease with 1 in 30,000 people being affected (Marini *et al.* 2009). Accessibility to patient material is therefore limited. This precious material could be preserved by subsequent passaging in nude mice to then conduct biomarker screening and drug testing using the same tissue. The finding that loss of Menin increases cell proliferation might come in handy for the engraftment of MEN1 patient-derived tumors that usually have no expression of the protein. Indeed, these tumors might grow and progress faster than usual NETs, thereby resulting in a higher engraftment rate. In a xenotransplantation study, A549 cells derived from a human lung carcinoma cell line were stably transfected with either control or Menin-overexpressing constructs. These cells were then injected into nude mice, and it was demonstrated that Menin levels negatively affect the engraftment and growth of the cells (Gao *et al.* 2009).

MENX rats as a MEN1-like model

The MENX multi-tumor syndrome was discovered by serendipity in a rat colony that spontaneously started to develop multiple NETs. Affected rats present with

anterior pituitary adenomas, adrenal and extra-adrenal pheochromocytomas, thyroid C-cell hyperplasia, parathyroid hyperplasia and pancreatic islet cells hyperplasia (Fritz *et al.* 2002, Wiedemann & Pellegata 2016). The MENX tumor spectrum shares features with both MEN1 and MEN2 human syndromes. However, in contrast to the human syndromes, MENX is inherited as a recessive trait and affected rats are homozygous for the underlying mutation. Rat tumors develop at high frequency (often 100%) with a progression from hyperplasia to neoplasia with time. Although wild-type rats live approximately 24–30 months, the average life span of MENX-affected rats is 10 ± 2 months.

Following classical linkage studies (Piotrowska *et al.* 2004) and candidate gene analysis, a tandem duplication of 8 nucleotides in exon 2 of *Cdkn1b* (p27), which causes a frameshift, was identified as the causative mutation responsible for MENX (Pellegata *et al.* 2006). The mutant *Cdkn1b* allele encodes a very unstable p27, which is rapidly degraded *in vitro* (Molatore *et al.* 2010a) and virtually not detected in the mutant rat tissues *in vivo* (Pellegata *et al.* 2006). Therefore, we refer to it as a loss-of-function mutation. As mentioned earlier, the CDK inhibitor p27 was demonstrated to be a transcriptional target of Menin in pancreatic β cells (Karnik *et al.* 2005). Noteworthy, germline mutations in the human homologue, the *CDKN1B* gene, were discovered in patients with a MEN1-like phenotype (Pellegata *et al.* 2006). These findings identified a novel MEN syndrome, named MEN4, caused by alterations of p27 (Lee & Pellegata 2013).

Pituitary gland

MENX-affected rats develop multifocal tumors in the anterior pituitary (frequency 100%), which belong to the gonadotroph lineage and are histologically and ultrastructurally similar to human gonadotroph adenomas (Marinoni *et al.* 2013). In patients, gonadotroph adenomas are clinically nonfunctioning (named NFPAs). These tumors occur in about 5% of MEN1 patients. The lesions in affected rats start from 4 months of age as multiple neoplastic nodules and progress to become large adenomas that efface the gland. Rat adenomas express the glycoprotein alpha-subunit (α GSU) at all stages of progression. Similar to NFPA patients, the expression of LH β and FSH β subunits is present in the early lesions but is then lost in the larger tumors, accordingly serum LH β levels in the mutant rats decrease with tumor progression. Rat adenomas show mitotic activity and relatively high

Ki67 labeling index (average 8% at >8 months) (Marinoni *et al.* 2013). In addition to morphology and hormone expression, rat and human pituitary adenomas also share common genetic signatures. Transcriptome analysis identified dysregulated genes in both species that are involved in tumorigenesis and may represent novel biomarkers for future clinical applications (Lee *et al.* 2015).

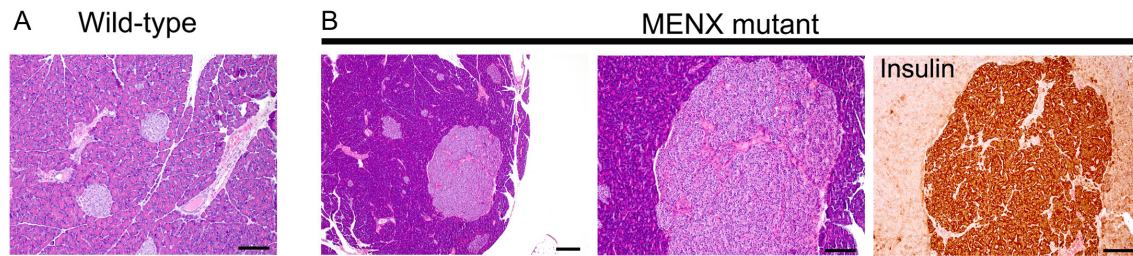
MENX rats are the only spontaneous model of NFPAs. Moreover, adenomas develop in all affected animals. Considering these two aspects, MENX rats are the ideal model to evaluate novel antitumor drugs for their efficacy against NFPAs. Studies were thus performed to test BEZ235, a dual PI3K/mTOR inhibitor, *in vitro* (on 3D cultures of rat primary pituitary adenoma cells) and *in vivo* in the rats. The results demonstrated that BEZ235 has anti-proliferative and pro-cell death activities against rat pituitary adenomas tumors both *in vitro* and *in vivo*. Diffusion-weighted magnetic resonance imaging (DW-MRI) was used to monitor treatment efficacy and emerged as a useful modality to assess early therapy response (Lee *et al.* 2015). These findings provided a rationale for the clinical investigation of PI3K/mTOR inhibition in NFPA patients.

Pancreas

MENX rats develop pancreatic islet hyperplasia (100%), which leads to an increase in islet mass already detectable 2 weeks after birth (Wiedemann *et al.* 2016a). The pancreatic islets consist of five types of cells each producing a specific hormone. In mutant rats, all five cell populations are increased in number. The islet hyperplasia occasionally progresses to insulinomas (Fig. 5). Following oral glucose stimulation test, mutant female rats showed increased insulin output when compared with wild-type littermates, compatible with their islet hyperplasia (Pellegata, unpublished).

Adrenal gland

Adrenal tumors belong to the spectrum of the MEN1 syndrome but usually arise in the cortex. Tumors of the adrenal medulla (pheochromocytomas) occasionally occur in *Men1*-deficient mice (see above). MENX rats develop adrenomedullary hyperplasia at 3–4 months of age, which progresses to pheochromocytoma by 6–8 months (frequency 100%). The histology of these lesions resembles that of human pheochromocytoma. The rat tumors show high mitotic counts and elevated Ki67

**Figure 5**

Pancreatic islets in wild-type and MENX mutant rats. (A) Hematoxylin and eosin (H&E) staining of an area of the pancreas of a wild-type adult rat containing two islets. (B) H&E staining of an area of the pancreas of an adult MENX mutant rat. The endocrine tumor is composed mostly of insulin-expressing cells, as demonstrated by immunohistochemistry with an anti-insulin antibody (right figure). Size bars: panel A and two most right hand panels in B: 100 μ m; left hand panel in B: 200 μ m.

labeling index (Miederer *et al.* 2011). Pheochromocytoma in patients secretes an excess of catecholamines. To verify whether the same occurs in rats, urine catecholamine levels were measured longitudinally in both MENX-affected and wild-type animals by high-performance liquid chromatography. Mutant rats at 8 months of age show increased urinary concentrations of norepinephrine, normetanephrine, 3-methoxytyramine and dopamine compared to wild-type age-matched rats; hence, their tumors are noradrenergic (Wiedemann *et al.* 2016b). This is consistent with the lack of expression of phenylethanolamine N-methyltransferase (PNMT), the enzyme that converts noradrenaline to adrenaline, in these tumors (Molatore *et al.* 2010b). In patients with pheochromocytoma, high catecholamine secretion associates with an increase in blood pressure, which, if not controlled, can cause severe symptoms. Non-invasive measurements performed on mutant and wild-type rats over time showed that blood pressure increases in the MENX animal model together with tumor progression, as in patients. Moreover, mutant rats show pathological changes in organs such as heart and kidney, similar to those observed in the patients if blood pressure is not controlled (Wiedemann *et al.* 2016b). Rat and human pheochromocytomas also share gene expression signatures (Molatore *et al.* 2010b, Leinhauser *et al.* 2015). For the diagnosis and staging of pheochromocytoma, functional imaging plays a crucial role. In addition to $^{131}\text{I}/^{123}\text{I}$ -metaiodobenzylguanidine (MIBG) scintigraphy, a gold standard procedure, a variety of tracers have been developed for the detection of pheochromocytoma using positron emission tomography (PET), including ^{18}F -fluorodopamine (DOPA), ^{11}C -hydroxyephedrine (HED) a norepinephrine analog and ^{68}Ga -DOTATOC targeting somatostatin receptors. Noteworthy, also the rat tumors show uptake of these radiotracers (Miederer *et al.* 2011, Gartner *et al.* 2013). MENX rats were used to test a novel

norepinephrine analog (e.g. LMI1195) for its ability to detect pheochromocytoma *in vivo* by PET imaging. High and specific accumulation of ^{18}F -LMI1195 in the adrenals of tumor-bearing mutant rats was seen over time. Its favorable biodistribution makes it a promising PET tracer for pheochromocytoma imaging (Gartner *et al.* 2013). Given that in MENX rats pheochromocytoma develops with complete penetrance and recapitulates several key features of the human tumors, therapy-response studies were conducted to test the efficacy of BEZ235 (dual PI3K/mTOR inhibitor) in this model *in vivo*. The results showed that this agent holds promise for the treatment of pheochromocytoma (Lee *et al.* 2017).

Other organs

Parathyroid hyperplasia has been observed in MENX-affected rats. The incidence was 65% when considering macroscopically visible tumors at the time of death (Fritz *et al.* 2002). It is not known whether the blood levels of PTH are elevated in affected rats as a consequence of the parathyroid hyperplasia. MENX rats also present with bilateral hyperplasia of the thyroid C-cells, a feature exclusively associated with MEN2.

Concluding remarks

In cancer research, animal models are used to understand the underlying pathogenetic mechanisms and to develop strategies to diagnose and treat the corresponding human disease. Depending on the specific questions to be addressed, not necessarily model organisms with higher complexity represent the most appropriate option. For instance, high-throughput screening of new putative targets or antitumor agents might be more easily and cost-effectively carried out in *Drosophila* or zebrafish, whereas rodents or PDX models might be better suited for an

in-depth characterization of tumor biology. The available Menin-deficient transgenic *Drosophila* strains have shed light on the protein's function. The use of PDX models of NETs (in mice) is still in its infancy, as only a few studies have been performed so far and with variable success. However, this platform could allow to preserve and to propagate the rare tumors of MEN1 patients for further studies. Although tumors cannot be passaged in zebrafish xenografts, the implantation and characterization of primary patient samples are quite promising.

Interestingly, although mouse models of MEN1 show an impressive overlap of pathologically relevant features with the human syndrome, they have been underused for molecular and preclinical studies. Serum profiling of these mice or tumor transcriptome analysis could provide us with new therapeutic targets or biomarkers, whereas the evaluation of novel drugs or existing ones in off-label use in these models could identify effective therapies for MEN1 patients. Moreover, these mouse models might be suitable to establish imaging modalities for diagnosis and therapy-response monitoring. Therefore, investing time and effort in further characterizing the existing MEN1 models has the potential for a big return if we thereby create more reliable *in vivo* platforms for therapy assessment studies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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