REVIEW

Mouse models of endocrine tumors

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Abstract

Endocrine and neuroendocrine tumors comprise a highly heterogeneous group of neoplasms that can arise from (neuro)endocrine cells, either from endocrine glands or from the widespread diffuse neuroendocrine system, and, consequently, are widely distributed throughout the body. Due to their diversity, heterogeneity and limited incidence, studying in detail the molecular and genetic alterations that underlie their development and progression is still a highly elusive task. This, in turn, hinders the discovery of novel therapeutic options for these tumors. To circumvent these limitations, numerous mouse models of endocrine and neuroendocrine tumors have been developed, characterized and used in preclinical, co-clinical (implemented in mouse models and patients simultaneously) and post-clinical studies, for they represent powerful and necessary tools in basic and translational tumor biology research. Indeed, different in vivo mouse models, including cell line-based xenografts (CDXs), patient-derived xenografts (PDXs) and genetically engineered mouse models (GEMs), have been used to delineate the development, progression and behavior of human tumors. Results gained with these in vivo models have facilitated the clinical application in patients of diverse breakthrough discoveries made in this field. Herein, we review the generation, characterization and translatability of the most prominent mouse models of endocrine and neuroendocrine tumors reported to date, as well as the most relevant clinical implications obtained for each endocrine and neuroendocrine tumor type.

Endocrine and neuroendocrine tumors: origin, location, heterogeneity and outcome

Cancer and tumor pathologies represent one of the main health problems for the population worldwide, with incidence and death ratios still increasing (Rusner et al. 2013, Goswamy et al. 2016, Siegel et al. 2018). For this reason, more basic, translational and clinical studies are needed to identify novel markers to improve diagnostics and prognosis assessment and new therapeutic targets (Capdevila et al. 2017). Among tumor pathologies, endocrine and neuroendocrine tumors represent a unique group of neoplasms, which arise from (neuro)endocrine cells, either from endocrine glands or from the widespread diffuse neuroendocrine system. Therefore, endocrine and neuroendocrine tumors are widely distributed throughout the body and display a heterogeneous nature (Oberg 2018), with some of them exhibiting increasing incidence (Oberg et al. 2013, Dasari et al. 2017). Current
evidence indicates that, in addition to comprise an ample and diverse group of neoplasias, endocrine and neuroendocrine tumors are extremely heterogeneous, a feature that deeply influences their diagnosis, prognosis and medical management, and profoundly hampers the identification of novel and more general biomarkers and/or therapeutic targets. The basis for this heterogeneity resides in the variety of locations, cells of origin, genetic background, molecular fingerprints, functional abilities and clinical features exhibited by these tumors, which is translated to the patients bearing these diseases (Oberg et al. 2013, Capdevila et al. 2017, Giordano 2018, Oberg 2018, Pedraza-Arevalo et al. 2018).

In particular, endocrine and neuroendocrine tumors include those tumors arising mainly from cells located at the pituitary (including somatotropinomas, prolactinomas, corticotropinomas, etc.), thyroid (including medullary, papillary or follicular thyroid carcinomas), adrenal gland (including adrenocortical adenomas and carcinomas) or comprising the neuroendocrine system (which can be subdivided according to their location in foregut (lungs, thymus, esophagus, stomach, duodenum and pancreas), midgut (jejunum, ileum and cecum) and hindgut (distal colon and rectum) (Giordano 2018). This original location of the tumor is essential to define tumor characteristics and clinical management, as it greatly influences other additional tumor characteristics such as the possible cell types of origin, the molecular mechanisms underlying their development and/or progression or the accompanying clinical features. Regardless of their location, endocrine and neuroendocrine tumors can exhibit a variety of histological types, from well-differentiated and low-grade tumors to poorly differentiated and high-grade lesions. Likewise, they can course with diverse clinical features: from non-symptomatic tumors, only presenting non-specific symptoms at the time of diagnosis, to functioning tumors, which can present symptoms related to the specific hormone secreted by the tumor and, usually, translate into a corresponding disease or syndrome, such as acromegaly in somatotropinomas, Cushing’s disease in corticotropinomas or carcinoid syndrome in serotonin-producing neuroendocrine tumors. Endocrine and neuroendocrine tumors are also highly heterogeneous from a molecular and genetic perspective. In fact, although two molecular pathways have been found to be prominently altered in many endocrine and, particularly, neuroendocrine tumors (mammalian target of rapamycin (mTOR) and Notch pathways), other crucial pathways have been found to be altered in particular tumor types, as KRAS, HRAS and NRAS in non-small-cell lung carcinomas or retinoblastoma 1 (RB1) in small-cell lung carcinoma (SCLC) (Oberg 2018). In addition, endocrine and neuroendocrine tumors are among the neoplasms with a more marked heritable component, being associated with at least ten different genetic syndromes, including multiple endocrine neoplasia type 1 and 2 (MEN1 and MEN2), neurofibromatosis type 1 (NF1) or Von Hippel–Lindau (VHL) syndrome (Crona & Skogseid 2016).

All these levels of complexity found in endocrine and neuroendocrine tumors can be understood as a series of superimposed layers of heterogeneity, which have complicated their systematic and comprehensive study (Pedraza-Arevalo et al. 2018). Indeed, this heterogeneity, together with the limited incidence of some of these tumor types, drastically hampers the possibility of implementing high-scale translational and clinical studies. In this scenario, mouse models have emerged as a powerful and essential tool in basic and translational tumor biology research (Day et al. 2015, Gengenbacher et al. 2017, Kersten et al. 2017), specially in the case of endocrine and neuroendocrine tumors (Basham et al. 2016, Lines et al. 2016, Mohr & Pellegata 2017, Vitale et al. 2017). There are numerous examples of breakthrough discoveries in preclinical mouse tumor models that paved the way for clinical application in humans, such as the first demonstration of the efficacy of immune checkpoint blockade for the treatment of tumor pathologies (Leach et al. 1996). Indeed, this valuable association is further supported by the fact that preclinical models and cancer therapies have usually co-evolved accordingly, wherein this evolution was highly dependent on technical advances (Day et al. 2015). In this sense, studies in mouse models can contribute to improved clinical trials, identifying potentially effective therapies but also predicting positive or detrimental effects in molecular subtypes, being used in pre- or co-clinical studies (Pietras & Hanahan 2005, Raymond et al. 2011).

Mouse models for tumor pathologies

In vivo mouse models are potent and crucial tools in basic and translational tumor biology research that have being used to delineate the development, progression and behavior of human tumors, including endocrine and neuroendocrine tumors. These mouse models include cell line-based xenografts (CDX), patient-derived xenografts (PDX), environmentally induced models and genetically engineered mouse (GEMs) models (Day et al. 2015, Gengenbacher et al. 2017, Kersten et al. 2017).
Much of the current knowledge about cancer and its hallmarks derives from the establishment of long-term *in vitro* cultured tumor cell lines, and on their *in vivo* inoculation in mice. In fact, these models remain the most commonly used mouse models in basic and translational cancer research (Gengenbacher et al. 2017). Specifically, cell line-based tumor models represent classical prototypes of tumor development and have long been used in academic research and industry to analyze tumor growth and response to treatment. In these models, tumor cells can be injected ectopically (mostly subcutaneously), orthotopically (to mimic tumor growth in its organ of origin) or systemically (mostly intraperitoneally, intravenously or intracardially) to study metastatic spread by using syngeneic immunocompetent inbred mice (allografts) or immunocompromised mice ( xenografts). In all cases, these models exhibit important limitations, mainly derived from the strong selection process. First, CDX models exhibit a tissue architecture that is severely perturbed with clear alterations in the microenvironment compared with human tumors, including changes in the vascular, lymphatic and immune components, also due to their rapid growth (Sikder et al. 2003, Frese & Tuveson 2007). Second, these CDX models also present loss of genetic heterogeneity and irreversible changes in gene expression due to the long-term *in vitro* propagation (Daniel et al. 2009, Gillet et al. 2011). However, although CDX models are greatly reductionist, their easy technical manipulability, low cost and synchronous tumor growth enable the application of these models for quick identification and validation of relevant genes in tumor biology, as well as for preclinical evaluation of experimental treatments (Day et al. 2015, Gengenbacher et al. 2017, Kersten et al. 2017).

PDXs are also typically generated by subcutaneous implantation of fresh, surgically derived human tumor explants into immune-deficient mice (Tentler et al. 2012). However, PDXs have been shown to retain, at least during limited time of *in vivo* growth, the molecular, genetic and histopathological features of their originating tumors (Hidalgo et al. 2014). Indeed, the PDXs are the only current model system able to directly incorporate the enormous inter-patient and intra-tumor heterogeneity that is inherent to human cancer, and thus, represent a promising tool for personalized medicine. In this regard, inasmuch as PDXs are derived from human tumors, they may serve to directly evaluate clinically approved drugs and, in fact, they seem to be able to faithfully predict therapy responses when comparing retrospectively drug responses in patients and their corresponding xenografts (Siolas & Hannon 2013). For these reasons, the so-called co-clinical trials are aiming to establish PDXs from patients who are enrolled in clinical trials, which may be used concomitantly to explore numerous therapeutic alternatives from an individualized perspective. However, PDX studies remain hindered by several limitations. Likely, the most important handicap is the fact that the engraftment rate strongly varies between different tumor types and grades, and it is dependent on the recipient mouse strain and original patient sample quality (Landis et al. 2013). This engraftment variability limits the genetic complexity represented by PDXs, in that tumor samples from patients with poor prognosis exhibit higher engraftment rate and, therefore, this capacity may even serve as a predictive biomarker (Eirew et al. 2015). In addition, as mentioned above, in these mouse models, the human stroma is initially present in the tumor engrafted, but it is ultimately replaced by mouse stroma components following *in vivo* passaging (Sivanand et al. 2012). Finally, these tumor models require an immunocompromised background, which precludes their use to study immune system influence and to analyze immunotherapeutic strategies (Zitvogel et al. 2016).

Environmentally induced models are based on environmental cancer-causing agents, including chemicals, radiation and pathogens, which are identified to be carcinogenic in animals, through standardized approaches as the carcinogen bioassay (Kemp 2015). Remarkably, these environmental models exhibit *de novo* tumor growth, represent all stages of multistep carcinogenesis and closely recapitulate the phenotypic and genetic heterogeneity of their human counterparts, at least in many cases (Steele & Lubet 2010, Nassar et al. 2015, Westcott et al. 2015). However, despite their biological relevance, these models are scarcely used in cancer research (Gengenbacher et al. 2017), likely due to the inherently long latency and high variability of penetrance of the induced tumors, which precludes their use in experimental research.

Finally, genetically engineered mouse (GEM) models have emerged as the second most common type of mouse model in oncology research (Gengenbacher et al. 2017). This is due to the increasing understanding of the genetic aberrations underlying tumorigenesis, which has enabled the generation of different GEM models that reproduce the genetic events observed in human cancers, allowing *de novo* tumor formation in a native immunoproficient microenvironment. Accordingly, these models faithfully recapitulate the molecular and histopathological features of human cancers and exhibit a robust predictive power.
for drug response and resistance (Kersten et al. 2017). For these reasons, GEM models have been used in co-clinical trials, wherein parallel treatments of multiple genetically defined mouse cohorts may be used for patient stratification by identifying genetic biomarkers (Zitvogel et al. 2016).

### Mouse models of endocrine and neuroendocrine tumors

All the in vivo cancer-associated mouse models defined above have been or are being currently used to study different aspects of the biology of endocrine and neuroendocrine tumors. Though, it should be mentioned that the generation of CDX and PDX models depends on the existence of cell lines and patient samples available to be inoculated in the appropriate model, and the environmentally induced models are scarcely used to study these types of tumors. In contrast, several different GEM models have been generated and are widely used to model endocrine and neuroendocrine tumors as it is described below (Tables 1 and 2).

### Models of (neuro)endocrine tumor syndromes

The molecular bases of endocrine and neuroendocrine tumors are strongly related to genomic instability and mutations. Although the majority of them are sporadic, a significant percentage may arise in a context of familial inherited syndromes (Oberg 2013). In this sense, one of the first described syndromes associated to the presence of these tumors was the multiple endocrine neoplasia type 1 or MEN1, an autosomal dominantly inherited complex endocrine syndrome, caused by mutations in MEN1 gene, which encodes menin protein. MEN1 syndrome is mostly associated to the appearance of pancreatic, parathyroid and pituitary tumors (Thakker 2014). The mouse models developed to study this syndrome are exclusively based on mutations in Men1 gene (Mohr & Pellegrata 2017) and, therefore, the putative use of PDX models could represent a substantial advance in the near future (Table 1). Remarkably, all these mouse models of MEN1 syndrome show a remarkable overlap with the human syndrome in terms of pathologically relevant features, as recently reviewed (Mohr & Pellegrata 2017), and therefore represent ideal models to explore different aspects of this endocrine syndrome. The initial works were focused on Men1 systemic knockout models, which were generated by different combinations of exons 1–8 deletions, but these modifications resulted in embryonic lethality (Piret & Thakker 2011). For this reason, heterozygous mice were subsequently used, which presented tumors from 9 months of age in several glands, including pancreas, pituitary, parathyroid, thyroid, adrenal or gonads. These heterozygous Men1-knockout mouse models have been used to analyze different aspects of the disease, including microvascular alterations, since these tumors have been found to be one of the most vascularized type of tumors (Chu et al. 2013), and, more recently, to explore the

<table>
<thead>
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<th>Endocrine syndrome</th>
<th>Model name</th>
<th>Tumor type</th>
<th>Model type</th>
<th>Gene (promoter*)</th>
<th>References</th>
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<td>Men1</td>
<td>Piret &amp; Thakker (2011)</td>
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<td>Parathyroid</td>
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<td>Thyroid</td>
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<td>Adrenal</td>
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<td>Gonadal</td>
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<td>Homozygous knockout</td>
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<td>MTC</td>
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<td>Pheochromocytoma MTC</td>
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<td>NES-VHL</td>
<td>Paraganglioma</td>
<td>Homozygous knockout</td>
<td>Vhl (Nes*)</td>
<td>Merlo et al. (2017)</td>
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</tbody>
</table>

*Driver promoter in the case of knockout models.
### Table 2  Description of the main GEM models used in the study of endocrine and neuroendocrine tumors.

<table>
<thead>
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<th>Model name</th>
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<th>Model type</th>
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<td>Thyrotropinoma</td>
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<td>aGSU-PTTGxRb/+</td>
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<td>PTTG (αSU*)</td>
<td>Chesnokova et al. (2005), Donangelo et al. (2006)</td>
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<td></td>
<td>Gonadotropinoma</td>
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<td>Corticotropinoma</td>
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<td>Tp27</td>
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<td>McFadden et al. (2014a,b)</td>
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<td>CALC-MEN2B-RET</td>
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<td>Rb</td>
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<td>Nr5a1 (YAc*)</td>
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<td>Apc (F1-Cre*)</td>
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<td>Apc (PEPCK*)</td>
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<td>Transgenic</td>
<td>IGF2 (Akr1b7*)</td>
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<td>H1g12 (Ct/GnR)</td>
<td>ACC</td>
<td>Transgenic + homozygous knockout</td>
<td>Apc (H1g12)</td>
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<td>ACC</td>
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<td>Adc</td>
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<td>Transgenic</td>
<td>SV40-Tag (P450Scc*)</td>
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<td>ACC</td>
<td>Transgenic</td>
<td>SV40-Tag (Akr1b7*)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary neuroendocrine tumors</td>
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<td>SCLC</td>
<td>Double homozygous knockout</td>
<td>Rb</td>
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<td>Rb</td>
<td>Schaffer et al. (2010)</td>
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</table>

Continued
mouse models in endocrine

tumors). For MEN2B syndrome, which also develops

Met918Thr

Quinn

Gene

Transgenic

Pancreatic NET

under the control

Manoharan

CT/CGRP

Tumor type

Wiedemann & Pellegata 2016

Friis-Hansen

Hadoux

MyrAkt1 (Rat

However, these heterozygous models have

Libutti

Pancreatic NET

Jimenez & Gagel 2004

Ascl1 (Scgb11*)

Model type

Consequently, pancreatic knockout models of

Pacheco

Pancreatic NET

SV40-Tag (Rat

Pancreatic NET

SV40-Tag (Rat

Pancreatic NET

SV40-Tag (Glu*)

Pancreatic NET

SV40-Tag (RIPPyST1*)

Pancreatic NET

SV40-Tag (ITF*)

Table 2

Continued.

<table>
<thead>
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<th>Model name</th>
<th>Tumor type</th>
<th>Model type</th>
<th>Gene (promoter*)</th>
<th>Reference</th>
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<td>SCLC</td>
<td>Triple homozygous knockout</td>
<td>Rb Tp53</td>
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<td>Transgenic</td>
<td>Ascl1 (Scgb11*)</td>
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<td>GEP-neuroendocrine tumors</td>
<td></td>
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<td>INS-GAS</td>
<td>Stomach NET</td>
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<td>INS-GAS (INS*)</td>
<td>Wang et al. (1993)</td>
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<tr>
<td>Gastrin KO</td>
<td>Stomach NET</td>
<td>Homozygous knockout</td>
<td>Gast</td>
<td>Friis-Hansen et al. (1998)</td>
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<tr>
<td>RIP-Tag</td>
<td>Pancreatic NET</td>
<td>Transgenic</td>
<td>SV40-Tag (Rat Insulin-2*)</td>
<td>Hanahan (1985)</td>
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<td>RIP-MyrAkt1</td>
<td>Pancreatic NET</td>
<td>Transgenic</td>
<td>MyrAkt1 (Rat Insulin*)</td>
<td>Tuttle et al. (2001), Alliouachene et al. (2008)</td>
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<td>Glu-Tag</td>
<td>Pancreatic NET</td>
<td>Transgenic</td>
<td>SV40-Tag (Glu*)</td>
<td>Efrat et al. (1988)</td>
</tr>
<tr>
<td>RIP-Tag/RIPPyST1</td>
<td>Colon NET</td>
<td>Transgenic</td>
<td>SV40-Tag (RIPPyST1*)</td>
<td>Grant et al. (1991)</td>
</tr>
<tr>
<td>ITF-Tag</td>
<td>Colon NET</td>
<td>Transgenic</td>
<td>SV40 (ITF*)</td>
<td>Gum et al. (2004)</td>
</tr>
</tbody>
</table>

Chemo-preventive role of several drugs (i.e. aspirin or enalapril) in the progression of pancreatic neuroendocrine tumors in the context of MEN1 syndrome (Manoharan et al. 2018). However, these heterozygous models have the limitation of the unspecific and uncontrolled tumor appearance (Piret & Thakker 2011), and thus, currently, the models most commonly used are conditional knockouts, since they represent the most suitable models to study specific pathologies. Specifically, several conditional models have been generated by the knocking down of specific regions of Men1 gene in specific cell types. Among them, the most important mutants are those generated in parathyroid and alpha and beta pancreatic cells. In particular, conditional knockout mice of Men1 in parathyroid gland-produced parathyroid neoplasia and hypercalcemic hyperparathyroidism, which was useful to study their pathological consequences (Libutti et al. 2003). Similarly, pancreatic knockout models of Men1 are particularly important in that they are contributing to generate pivotal advances in these pathologies. For instance, the mouse model wherein Men1 was knocked out in beta cells under the regulation of Pdx1 promoter have been used to explore the effectiveness of pasireotide, a somatostatin analog, in the treatment of pancreatic neuroendocrine tumors in the context of MEN1 syndrome (Quinn et al. 2012).

Likewise, MEN2 syndrome is also an autosomal dominantly inherited disorder, which is specially associated to the development of medullary thyroid carcinomas (MTC), pheochromocytomas and parathyroid adenomas (Jimenez & Gagel 2004). MEN2 syndrome appears by the mutation of RET (rearranged during transfection), which may occur in different regions of the gene, leading to three different MEN2 syndrome subtypes, termed MEN2A, MEN2B and familial MTC (Pacheco 2016, Wiedemann & Pellegata 2016). Consequently, the mouse models associated to these pathologies have been developed imitating their respective mutations and exhibit remarkable similitudes (Table 1). Specifically, mouse models of MEN2A syndrome (mutation Cys634Arg) have been generated by developing transgenic mice with the expression of MEN2A Cys634Arg under the control of the rat calcitonin/calcitonin gene-related peptide (CT/CGRP) promoter, which led to the appearance of pheochromocytomas, MTCs and parathyroid tumors, and served, for instance, to improve the PET imaging techniques of MTC (Pestourie et al. 2010). Interestingly, PDX models have also been used for the study of this syndrome, wherein cells from a patient with MEN2A syndrome were reprogramed and injected in immunodeficient mice, maintaining their initial characteristics (Hadoux et al. 2016). For MEN2B syndrome, which also develops with the appearance of MTC and pheochromocytomas, mouse models with MEN2B characteristic Ret mutation (Met918Thr) have helped to corroborate molecular aspects of the syndrome, as well as the C-cell hyperplasia or the presence of sympathoadrenal malformations (Smith-Hicks et al. 2000). In the case of familial MTC, there is no mouse model available nowadays, which severely hampers the study of this syndrome (Wiedemann & Pellegata 2016).
Another syndrome associated to the appearance of endocrine tumors is the neurofibromatosis type 1 (NF1) syndrome, which is especially linked to the appearance of pheochromocytomas and duodenal neuroendocrine tumors, although parathyroid or other gastroenteropancreatic (GEP) tumors may also occur (Kalkan & Waguespack 2013). This syndrome is caused by a mutation in the NF1 gene, which encodes the main inhibitor of RAS in the MAPK pathway. The principal murine model for this syndrome is based in the heterozygous mutation of NF1 gene, generated by the deletion of the exon 31, which has been shown to develop pheochromocytomas in adults, while the homozygosity for the mutation of NF1 gene leads to embryonic lethality (Lepoutre-Lussey et al. 2016) (Table 1). This model has been used to provide important information regarding differential mRNA expression in the context of NF1 mutation, which indicates the possible existence of neural progenitors in pheochromocytomas (Powers et al. 2007).

There are a number of additional syndromes associated to endocrine and neuroendocrine tumors, but the knowledge gathered about them from mouse models is much more limited (Table 1). This is the case of MEN4 syndrome, which is caused by mutations in CDKN1B and is mainly related with the appearance of pituitary and parathyroid tumors (Lee & Pellegata 2013). Full elimination of this gene causes primarily pituitary tumors and other multiple organ hyperplasia in mice (Fero et al. 1996, Kiyokawa et al. 1996, Nakayama et al. 1996), but there are not yet conditional knockout models available. Thus, murine models of this syndrome are mainly based in heterozygous mutations of Cdkn1b gene. However, although these heterozygous models have been used to study the role of this gene in tumorigenesis, there are no studies about the context of these mutations in MEN4 syndrome.

Other examples are the VHL and tuberous sclerosis complex syndromes, which are associated with pheochromocytomas, paragangliomas, pancreatic and other neuroendocrine tumors. Complete ablation of genes causing these syndromes in knockout mice is embryonically lethal and, unfortunately, the heterozygous mutations are excessively dependent on the genetic background of the mouse strains. For these reasons, tissue- or cell type-specific knockout models are more commonly used (Table 1). This is the case of a mouse model generated by the knocking out of Vhl gene in neural crest-derived tissues, which resulted in paraganglioma development, and have served to show the relationship between Vhl, Hif1a and the microRNA miR-210 in this disease (Merlo et al. 2017). Alternatively, VHL syndrome has also been studied using PDX mice in the context of paragangliomas, which led to the demonstration that, in VHL disease, a therapy directed inhibiting the angiogenesis induced by constitutively expressed VEGF may constitute an effective medical treatment (Gross et al. 1999).

### Models of pituitary tumors

Pituitary adenomas (PAs) are generally benign adenomas from a mono/oligoclonal origin, which display heterogeneous clinical manifestations, derived from hormone over-secretion and/or mass effects due to excess growth. PAs are mainly classified depending on the cell type of origin as somatotropinomas, corticotropinomas, prolactinomas, non-functioning PAs and so forth (Melmed 2011). The pathophysiology of PAs seems to be associated to the imbalance of stimulatory and inhibitory signals or key genes regulating the proliferation and secretory function of the main hormonal cell types conforming the pituitary gland, and, therefore, most of the GEM models to study these pathologies have been generated modulating these factors (Table 2).

The vast majority (~90%) of existing PA GEM models have been established using gene knockout or overexpression methods in mice (Table 2). To date, homologous deletion of ten different genes (Men1, Cdkn1b, Prkar1a, Rb, Cdkn2b (encoding p19), Ddr2, Cdkn2c, Aip, Prl and Prlr) have been reported to yield mouse-knockout models for pituitary tumors. Of these ten genes, Men1, Cdkn1b, Prkar1a, Cdkn2b and Aip are tumor suppressors associated with human familial disorders that course with pituitary tumors. Indeed, Men1-knockout models described above develop PAs among other endocrine and neuroendocrine tumors (Crabtree et al. 2001, 2003, Biondi et al. 2002, 2004, Bertolino et al. 2003, Loffler et al. 2007a,b). In conventional heterozygous Men1 models, PAs occurred in 26–45% of mice by 18 months of age, with prolactinomas being the most common type, whereas, in conditional homozygous, the incidence rises up to 58% of mice (Crabtree et al. 2003). In addition, Cdkn1b+− and Prkar1a+− GEM models develop tumors consistent with MEN4 and Carney Complex syndromes, respectively. These mice are characterized by the presence of PAs (e.g. somatotropinomas) in association with other endocrine tumors (e.g. adrenal tumors and follicular thyroid adenomas) (Kiyokawa et al. 1996, Nakayama et al. 1996, Fero et al. 1998, Yin et al. 2008). In addition, Cdkn2c and Aip knockout resulted in representative acromegaly/gigantism GEM models. Cdkn2c−/− mice have been shown...
to develop somatotropinomas and exhibit gigantism and widespread organomegaly, in that the pituitary gland, spleen and thymus are disproportionately enlarged and hyperplastic (Franklin et al. 1998). Moreover, the phenotypic features of \( \text{Aip}^{-/-} \) mice are similar to those observed in familial isolated pituitary adenoma patients who have AIP mutations and predominantly develop GH-secreting adenomas, although some patients may also develop PRL- or ACTH-secreting and non-functioning PAs. Heterozygous mice developed normally but were prone to PAs, in particular to those secreting GH, while no excess of any other tumor type was found. A complete loss of \( \text{Aip} \) was detected in these lesions, and full penetrance of tumors was reached at the age of 15 months. Ki-67 analysis indicated that \( \text{Aip} \)-deficient tumors have higher proliferation rates compared with \( \text{Aip} \)-proficient tumors in mice, which is comparable to that found in human tumors, suggesting a more aggressive disease (Vierimaa et al. 2006, Leontiou et al. 2008, Raitila et al. 2010). These models have been shown to nicely recapitulate the features of human PAs and, for this reason, the latter model has been useful to elucidate the mechanisms causing PAs. Indeed, the Aip-deficient tumors showed a drastic reduction in the expression of aryhydrocarbon receptor nuclear translocator 1 or 2 (ARNT or ARNT2) protein, which are involved in the regulation of several genes associated with tumorigenesis and hypoxia process, thus suggesting mechanisms of AIP-related tumorigenesis (Raitila et al. 2010).

On the other hand, genes such as \( \text{Rb}, \text{Cdkn2b}, \text{Dnk2}, \text{Prl} \) and \( \text{Prlh} \) have not been previously associated with the prevalence of PAs, but studies in mouse models have revealed roles for such genes in pituitary tumorigenesis. Indeed, the absence of \( \text{Rb} \) protein caused by heterozygous germline mutation induces childhood-onset retinoblastoma in a high percentage of humans. Surprisingly, the same phenomenon in mice induced the development of pituitary carcinomas (Jacks et al. 1992, Vooijs et al. 1998, 2002). Similarly, the role of p19, encoded by \( \text{Cdkn2b} \), as a tumor suppressor in regulating pituitary anterior lobe proliferation has been revealed by a conventional knockout mouse model of \( \text{Cdkn2b} \), as these knockout mice developed multiple tumor types including PRL-\( \gamma \), GH- and FSH-secreting PAs (Bai et al. 2014).

As mentioned above, the transgenic overexpression of the main regulators of pituitary gland function has also been used to generate key GEM models of PAs (Table 2). This is the case of overexpression of the growth hormone releasing hormone (\( \text{Ghrh} \)) gene, which resulted in PAs that led to excessive GH secretion (Asa et al. 1992). These mice exhibited adenomas with variable ultrastructural appearances, ranging from cells that resembled somatotropes or mammosomatotropes to cells with features of the glycoprotein hormone cell line and have been useful to identify mechanisms that may prevent or delay adenoma formation in the presence of excess GHRRH (Luque et al. 2009). Similarly, transgenic overexpression of corticotropin-releasing hormone (\( \text{Crh} \)) or N-ethyl-N-nitrosourea-induced mouse mutant with a \( \text{Crh} \) promoter mutation have also been generated as models of Cushing’s disease (Stenzel-Poore et al. 1992, Bentley et al. 2014). In particular, \( \text{Crh} \)-transgenic animals exhibit endocrine abnormalities involving the hypothalamic–pituitary–adrenal axis, including elevated plasma levels of ACTH and glucocorticoids and display physical changes similar to those of patients with Cushing’s syndrome, such as excessive fat accumulation, muscle atrophy, thin skin and alopecia. These findings indicate that chronic production of excessive CRF results in sustained stimulation of pituitary corticotrope cells, resulting in elevated ACTH and consequent glucocorticoid overproduction, a condition that leads to the development of Cushing’s syndrome. A similar approach based on the modulation of the main regulators of pituitary gland function was implemented with pituitary tumor promoting genes as the pituitary transforming gene (\( \text{Pttg} \)) or the \( \text{Hmga} \) genes.\( \text{Pttg} \), which was first identified from rat pituitary tumor cells (Pei & Melmed 1997), and subsequently shown to be overexpressed in human pituitary tumors (Saez et al. 1999), encodes a securing protein that plays a role in cell transformation, aneuploidy, apoptosis and tumor microenvironment communication (Vlotides et al. 2007). Mice with targeted pituitary \( \text{Pttg} \) overexpression driven by the pituitary specific alpha subunit glycoprotein promoter have been reported to develop focal pituitary hyperplasia, while mice with \( \text{Pttg} \) inactivation have pituitary hypoplasia (Donangelo et al. 2006), consistent with a pituitary tumorigenic role of \( \text{Pttg} \) (Abboud et al. 2005, Donangelo et al. 2006). \( \text{Hmga1} \) or \( \text{Hmga2} \) transgenic mice have also been shown to develop mixed somatotrope/lactotrope PAs by 16 months of age (Fedele et al. 2002, 2005). In particular, \( \text{Hmga2} \) has been related to \( \text{E2f1} \) activity with the progression and development of PAs in these models (Fedele et al. 2006).

In addition, five double-mutant models derived from \( \text{Rb} \)-knockout mice have been reported hitherto (\( \text{Pttg}^{-/-}/\text{Rb}^{+/-}; \text{Rb}^{+/-}/\text{In1}^{-/-}; \text{Rb}^{+/-}/\text{Arf}^{-/-}; \text{Rb}^{+/-}/\text{Tp53}^{-/-}; \text{Men1}^{-/-}/\text{Rb}^{+/-} \)), which have served to explore the interactions between \( \text{Rb} \) and other genes important in PA tumorigenesis (Harvey et al. 1995, Tsai et al. 2002,
Chesnokova et al. 2005, Donangelo et al. 2006, Guidi et al. 2006, Loffler et al. 2007a,b). Other double mutants as Cdk4R245/Er3/Er3, Ccne1p27−/−, p18R−/−p27−/−, p18R−/−aSU−/−, Ink4c/Arf and Men1−/−/Cdkn2−/− have also allowed to investigate cyclin-dependent kinases (CDK) inhibitors (CKIs) and their links with CDKs, CKIs and their downstream pathway elements in this pathology (Franklin et al. 1998, Lloyd et al. 2002, Zindy et al. 2003, Sotillo et al. 2005, Roussel-Gervais et al. 2010).

Other methodological alternatives to induce GEM models of PAs include the use of viral particles (oncoviruses) and knock-in approaches (Table 2). Indeed, simian virus 40 (SV40) T antigen (SV40-Tag) is an oncovirus able to generate a mouse model with somatotropinomas when expressed under the control of bovine arginine vasopressin (AVP) gene promoter (Stefaneanu et al. 1992). In addition, mice harboring a SV40-Tag transgene under the control of Pomp promoter developed melanotroph tumors (Low et al. 1993). In the same way, SV40-Tag under the control of the Fshb promoter provided a model of non-functioning adenomas (Kumar et al. 1998). Likewise, the overexpression of the polyoma large T antigen linked to the polyoma early region promoter generated another model for Cushing's disease (Helseth et al. 1992).

In the case of PAs, the absence of established commercial cell lines from human PAs profoundly hampers the possibility of generating human CDXs. However, commercial murine cell lines such as ATT-20, GH3, GH4-C1 and non-commercial human cell lines have been used to date. For instance, ATT-20 and GH3 xenograft mice have been used in different studies to explore the sensitivity or sensitizing effect to current treatments, chemotherapeutics drugs and radiotherapy in PAs (Zeng et al. 2011, Dai et al. 2013, Li et al. 2014, Lin et al. 2015, Zhao et al. 2015). Indeed, in recent years, mice xenografts have been widely used to characterize the responses to drugs currently prescribed for these pathologies as lanreotide (Ning et al. 2009), thiazolidinediones (Mannelli et al. 2010), bromocriptine and/or cabergoline (Lin et al. 2017) or to alternative therapies such as metformin (An et al. 2017), histone deacetylase inhibitor SAHA (Lu et al. 2017), Liquiritigenin (Wang et al. 2014), Triptolide (Li et al. 2017) or Bafilomycin A1 (McSheehy et al. 2003), which have been shown to exhibit antitumoral roles in these pathologies. Likewise, PAs xenograft models have also been used to characterize the function of different pathways, genes and relevant mutations in several types of PAs, as Fgfr4 in intracranial xenograft mouse models (Ezzat et al. 2006, Jalali et al. 2016), inactivating mutations of Pit-1 (Roche et al. 2012), overexpression of Lrig1 (Cheng et al. 2016) or Meg3 (Chunharojith et al. 2015), persistent activation of the Ras/MAPK pathway (Booth et al. 2014), antitumoral actions of miR-524-5p (Zhen et al. 2017) and role of RSUME in PTTG protein stabilization (Fuertes et al. 2018). Finally, PDX models have been sparsely implemented in the case of PAs. However, a recent study has described the appropriate methodological approach to generate PDX models through the inoculation of CD15+ pituitary adenoma-initiating cells in human–mouse xenografts, which are capable of inducing tumor initiation (Manoranjan et al. 2016), and, therefore, providing a novel research line in the field of PAs.

Models of thyroid tumors

Thyroid cancer represents the most common endocrine tumor pathology and comprises different types of tumors, which are classified depending on the cells of origin. Specifically, epithelial thyroid cancers or non-MTC (NMTC) defines those thyroid cancers that arise from thyrocytes, comprising 95% of all the cases. NMTC tumors are subdivided in papillary (80%), follicular (10%) and anaplastic (5%) subtypes, based on their histological characteristics. On the other hand, the oncogenic dedifferentiation of c-cells originates MTC, which account for about 5% of all thyroid cancers. Thyroid cancers usually exhibit a slow growth rate and a good prognosis, being anaplastic the most aggressive subtype. However, it should be noted that if any tumor subtype reaches a metastatic stage, the prognosis of the patient worsens dramatically. According to the variety and complexity of this type of tumors, the mouse models of thyroid cancers available today are multiple and diverse, including GEM (Table 2), CDX and PDX models.

In the case of the papillary thyroid carcinomas (PTCs), the most abundant type of thyroid cancer, the vast majority of GEM models have been generated by thyroid-specific transgenic inclusion of a gene critical for the development and/or progression of PTC under the control of the thyroglobulin (TG) promoter, such as the RET-PTC1, RET-PTC3 or TRK-T1 models (Cho et al. 1999, Powell et al. 1998, Russell et al. 2000) or by the transgenic expression of a mutation-activated variant of different oncogenes, such as NRas<sup>S36K</sup>Glu or B Raf<sup>Val600Glu</sup>, under the control of the TG promoter (Vitagliano et al. 2006, Chakravarty et al. 2011) (Table 2). Among them, the tumors originated in the B Raf<sup>Val600Glu</sup> and RET-PTC3 models are the most similar to human PTCs from a histological perspective, since these tumors exhibit nuclear features and the ‘papillae’ structures characterizing PTC, respectively (Powell et al. 1998,
Charles et al. 2011). In addition, the NRasVal61Glu or BRAfVal600Gln mouse models are able to generate invasive PTC tumors (Vitagliano et al. 2006, Chakravarty et al. 2011), although none of them can produce metastasis. However, it should be mentioned that Powell et al. reported a 33% of advanced-age RET-PTC3 mice with metastatic lesions (Powell et al. 1998). Remarkably, these models are highly translational and representative of the disease, inasmuch as the same BRAfVal600Gln mutation and activating RAS mutations are found in about 60 and 10% of all human PTCs, respectively (Nikiforova & Nikiforov 2009, Cancer Genome Atlas Research Network et al. 2014), and the RTK rearrangement represents the second most common genetic dysregulation in human PTC tumors (Nikiforova & Nikiforov 2009). Indeed, these models have been used to describe the importance of p27 as regulator of the PTC phenotype by using the TRK-T1 transgenic model (Fedele et al. 2009), while the BRAfVal600Gln model was crucial to unveil the role of Gsα and TSH in the oncogenesis and aggressiveness of PTC (Franco et al. 2011). Furthermore, the BRAfVal600Gln model has also been used to test the effectiveness of MEK inhibitors (Chakravarty et al. 2011), wherein these studies served as precedents for clinical trials with MEK inhibitors (selumetinib), in which the effectiveness of the drug to decrease tumor volume and increase the radioiodine uptake was demonstrated (Ho et al. 2013).

On the other hand, models of follicular thyroid cancer (FTC) have been mainly generated by knock-in of mutated and inactivated isoform of thyroid hormone receptor β (Thrb) (Suzuki et al. 2002), knockout of Prkar1a (Pringle et al. 2012) or by treating the transgenic mice that express the constitutively active variant Rap1bGlu12Val with goitrogens for 1 year (Ribiero-Neto et al. 2004) (Table 2). Strikingly, although PAX8-PPARG fusion (PPFP) appears in 35% of FTC cases, mutations of RAS gene in 45%, and mutations in PIK3CA and PTEN in 5%, none of the models generated by any of these mutated genes are able to produce an evident tumor phenotype (Yeager et al. 2007, Diallo-Krou et al. 2009, Charles et al. 2014, Pringle et al. 2014). Indeed, the results from PTEN models are quite controversial, since some groups have reported that tissue-specific knockout of Pten in the thyroid gland is sufficient to generate metastatic FTC (Antico-Arciuch et al. 2010), while other groups have reported hyperplasia in response to loss of Pten in the thyroid (Yeager et al. 2007, Pringle et al. 2014). For these reasons, double-hit GEM models have also been generated. Among them, Pten knockout into the PPFP model has emerged as an ideal model of aggressive FTC tumors (Dobson et al. 2011). In fact, the Rap1bGlu12Val, Prkar1a, TRβ-PV and Pten-PPFP models develop locally invasive FTC tumors, although only the latter two generate distant metastasis (Dobson et al. 2011). Even though these mouse models recapitulate human thyroid cancer features, they exhibit some differences with human FTCs, such as elevated circulating T3 and T4 levels in the case of TRβ-PV (Kato et al. 2004). In any case, these models have served to provide relevant basic and clinical information regarding the biology of FTCs and, indeed, the TRβ-PV model has been used in the first preclinical study testing the PI3K inhibitor (LY294002) in FTC, wherein a significant improvement in survival was found (Furuya et al. 2007).

In the case of anaplastic thyroid carcinoma (ATC), it seems that two different genetic hits are necessary to generate appropriate GEM models (Table 2), since the only model developed with a single genetic hit caused severe hypothyroidism (Zhu & Cheng 2012). This single-hit model is based on the expression of SV40-Tag under the control of TG promoter (Ledent et al. 1991). Conversely, the double-hit models that generate ATC are the result of deleting Pten and inactivating Tp53 (Antico Arciuch et al. 2011), crossing RET-PTC1 or RET-PTC3 mice with Tp53+/− mice (La Perle et al. 2000, Powell et al. 2001), cross-breeding TRβ-PV mice with KasGlu12Val mice (Zhu et al. 2014) or combining Braf activation with constitutively active Pik3ca (Charles et al. 2014). Among them, the models that better recapitulate APC tumors observed in humans are the Pten/Tp53 GEM mice, since they present features such as pleomorphism, epithelial-mesenchymal transition, aneuploidy, local invasion and metastases (Antico Arciuch et al. 2011), and the Braf/Tp53 GEM mice, which generate micrometastases similar to those observed in human ATC (McFadden et al. 2014b). Indeed, these two GEM models have been used in preclinical studies to demonstrate the effectiveness of the PLK1 inhibitor GSK461364A and the additive antitumor effect of MEK inhibitor and BRAF in APC (McFadden et al. 2014b, Russo et al. 2013).

For the study of MTC, conditional transgenic mice expressing mutated Ret isoforms, such as RetVal664Arg and RetVal1081Thr under the control of calcitonin/calcitonin gene-related peptide (Ct/CGRP) promoter, have been used (Michiels et al. 1997, Acton et al. 2000). Moreover, the transgenic overexpression of P25 cell cycle regulator under the control of enolase promoter, in response to doxycycline, also generates strongly invasive and metastatic MTC (Pozo et al. 2013). Finally, it should be mentioned that the genetic double hit of heterozygous mutation of Tp53 added to heterozygous mutation of Rb1 induces the production of MTC (Harvey et al. 1995).
Apart from the GEM models, CDX and PDX models have also been used to study different aspects of thyroid cancer pathophysiology. Indeed, several thyroid cancer-derived cell lines have been shown to be able to generate tumors in SCID mice, such as FTC-133, FTC-236, THJ-11T, TT or CAL-62, among others. In general, these thyroid cancer-derived CDX models have been commonly used for the study of new therapeutic strategies (Lee et al. 2018, Lin et al. 2018, Lombardo et al. 2018) or to describe the role of specific genes on tumor development and progression (Kim et al. 2014, Lou et al. 2018, Ma et al. 2018). Finally, it should be noted that establishing PDX models of thyroid cancer is, unfortunately, particularly problematic and laborious, mainly due to the slow growth rate of these tumors. The first PDX model of thyroid cancer was generated by Gyory et al. in 2005, and these models have been later used to unveil mechanistic aspects and to identify diagnostic markers of these tumor types (Gyory et al. 2005, Chen et al. 2017).

Models of adrenal gland tumors

Adrenal gland neoplasms are commonly diagnosed as incidental endocrine findings, being the incidence of these adenocortical tumors (ACTs) relatively high (Siegel et al. 2018). In addition to benign adenocortical adenomas (ACAs), malignant carcinomas may also arise in the adrenal cortex, being these adenocortical carcinomas (ACCs) highly aggressive and routinely fatal. Given the prevalence and severity of ACAs and ACCs, respectively, the treatment and management of adrenal gland tumors remain a significant public health challenge. In that sense, murine models have become essential for the study of this pathology (Table 2).

Different GEM models have been generated and characterized in order to further explore the role of certain molecular factors that are involved in the development and progression of ACTs, including steroidogenic factor-1 (SF1/NR5A1), WNT and β-catenin (CTNNB) or TP53 (Table 2). In the case of SF1/NR5A1, the gene dosage is especially critical for its function, as demonstrated by the fact that Nr5a1+/− mice present smaller adrenal glands (Bland et al. 2000). In this sense, a GEM model of increased Nr5a1 expression has been generated by the use of a 500-kb yeast artificial chromosome (YAC) containing the rat Nr5a1 gene (Dohghan et al. 2007). The Nr5a1 transgenic model, termed YAC-TR mice, developed ACTs that first appeared as nodular hyperplasia and subsequently progressed to tumors with complete penetrance. Similarly, one model that is closely related to the pediatric ACTs is the FAdE-SF1 transgenic mice, which express multiple copies of a transgene engineered to express Nr5a1 using a fetal enhancer (Zubair et al. 2009). These mice had larger adrenal glands as well as ectopic adrenal tissue in the thorax, but did not develop adrenal tumors. In all cases, these Nr5a1 mouse models have been used to further explore the role of this crucial factor in the development of ACTs, particularly in childhood ACTs, as 90% of cases display gains of 9q, which is the chromosomal region containing NR5A1 gene (Figueiredo et al. 2005).

The modulation of the classical WNT/β-catenin or associated pathways has also been used for the generation of different GEM models of ACTs and to explore the implication of these pathways in their development and progression (Table 2). First, 0.5 Akr1b7-Cre mice, which express Cre recombinase in all steroidogenic cells of the adrenal cortex (Lambert-Langlais et al. 2009), were crossed with Catnblox(ex3) mice (Harada et al. 1999). Histological analysis of these mice, termed Acat mice, showed severe defects in adrenal architecture with progressive dysplasia and hyperplasia. Second, ectopic Wnt activation in the adrenal cortex has been implemented through conditional loss of Apc, an upstream inhibitor of the Wnt pathway, similar to that observed clinically in some sporadic ACC patients (Assie et al. 2014). To this end, mice harboring an Apc allele floxed at exon 14 (Apclox/lox) were crossed with mice expressing the Sf1-Cre transgene (Heaton et al. 2012). Particularly, a stochastic Sf1-Cre driver was used, resulting in Cre-mediated recombination in a subset of adenocortical cells (Bingham et al. 2006). In any case, both models have been used to demonstrate that exacerbated WNT/β-catenin signaling in cells of the adrenal cortex is associated with surprisingly low penetrance of adrenal tumors, which displayed slow progression. Remarkably, the WNT/β-catenin signaling has been shown to interact with insulin-like growth factor 2 (IGF2), which has also been thought to be associated with adrenal tumorigenesis. Indeed, a possible connection between IGF2 and ACC was first suggested by a familiar cancer susceptibility syndrome, which is characterized by alterations at 11p15, an imprinted locus where IGF2 gene resides. However, clinical trials in advanced ACC patients have found slight benefit of IGF1R inhibitors (Fassnacht et al. 2015). Consistent with this observation, Igf2 transgenic mice generated using the rat phosphoenolpyruvate carboxykinase (Pepck) promoter presented elevated Igf2 serum levels and hyperplastic growth leading to increased overall adrenal weight, but adrenal tumors were not seen in animals up to 18 months of age (Weber et al. 1999). In addition, Igf2 overexpression...
in the adrenal cortex using regulatory regions from the Akr1b7 gene in the so-called AdIgf2 mice resulted in almost seven-fold higher basal levels of Igf2 and increased infiltration of mesenchymal cells in the adrenal cortex but again no tumor development (Drelon et al. 2012). Altogether, these GEM models have served to suggest that the single alteration of WNT/β-catenin or IGF2 pathways is not sufficient to drive adrenal cortex tumor formation. Nevertheless, these observations led to propose a model of cooperation between the WNT pathway and IGF2, which was tested in two independent mouse models. First, mice were generated with joint loss of Apc and overexpression of Igf2, by mating the Apc-knockout and H19ΔDMD mice (Heaton et al. 2011). This resulted in a more aggressive tumor phenotype, marked by earlier onset of tumor formation, a higher tumor penetrance and formation of one overt carcinoma. A second model combining the described ΔCat and AdIgf2 mice confirmed these results and also found a higher tumor Weiss score (the most commonly used method for assessing malignancy of ACTs) when both pathway alterations were present (Drelon et al. 2012).

Mutations in TP53 exhibit a high prevalence in ACC; however, no direct GEM models of adrenal restricted TP53 loss have been developed to date. Nonetheless, there are several GEM models that indirectly study the functional consequences of TP53 loss during adrenal tumorigenesis. The first approach is based on the crossbreeding of the Acd mouse, which contains a spontaneous, recessively hereditary mutation in the adrenocortical dysplasia (Acd) gene that results in adrenal insufficiency (Beamer et al. 1994), with mice from a Tp53-null background (Else et al. 2009). This results in a significant increase in carcinoma formation, including ACC in 5% of animals, suggesting that the adrenal hypoplasia seen in Acd mice results from Tp53-dependent senescence and apoptosis and that release from Tp53-sensitive checkpoints is a critical step in the adrenal tumorigenesis. In addition, two transgenic lines expressing SV40-Tag, which acts in part by binding and inactivating Tp53, in the adrenal cortex have been generated. In these models, the promoter of the human P450 cholesterol side-chain cleavage gene (Mellon et al. 1994) or a 0.5 kb region of the Akr1b7 promoter (Sahut-Barnola et al. 2000) were used to express an SV40-Tag construct. This approach resulted in the generation of ACTs that were subsequently used to generate ACTH-responsive cell lines (ATC1 and ATC7-L) (Sahut-Barnola et al. 2000).

In addition to the GEM models, different CDX and PDX models have been used to investigate different aspects of ACT pathophysiology. Among them, the most commonly used are the CDX models obtained from the inoculation of the human ACC cell line NCI-H295 (Logie et al. 2000). These mice exhibit elevated plasma steroid levels as well as an overexpression of Igf2 and IGF2-binding protein 2 (Igfbp2). Moreover, a significant inhibition of tumor growth and increase in survival time could be observed after treatment with IGFIIR antagonistic compounds (Hantel et al. 2012). Similar preclinical ACC model has been generated by the subcutaneous injection of SW13 cells. These models have served to provide the first evidence regarding the usefulness of local gene transfer therapy of an HSV thymidine kinase expressing adenoviral shuttle followed by ganciclovir treatment (Wolkersdorfer et al. 2002), to investigate the antitumor efficacy of different drugs such as sorafenib and everolimus (Mariello et al. 2012) or to facilitate the enrichment of cancer stem cells from this tumor model (Zeng et al. 2014). Finally, PDX models of ACT have also been generated. Specifically, Pinto et al. reported in 2013 the establishment of the first pediatric tumor model for ACC. In this case, the original patient tumor derived from an 11-year-old boy with an incidentally found right adrenal mass resected and subsequently confirmed as an ACC (Pinto et al. 2013). For inoculation of tumor xenografts, surgical specimens were implanted as tumor pieces without mincing in CB17 scid−/− mice (SJACC3). Similarly, Hantel et al. in 2014 used female athymic NMRI nu/nu mice to successfully generate NCIh295 cell line xenografts and PDXs to test different treatment regimens, which indicates that these xenografts could be utilized as standardizable tumor model for multiple preclinical settings (Hantel et al. 2014).

Models of neuroendocrine tumors
GEP-neuroendocrine tumors
GEP-neuroendocrine tumors (GEP-NETs) are the most common type of neuroendocrine tumor and have been classified by the World Health Organization into three categories (G1, G2 and G3) based on tumor size, histopathological differentiation, proliferation index (Ki-67), hormonal behavior, neuroendocrine biomarkers (such as serotonin and chromogranin A), direct invasion and distant metastasis (Oberg 2018). GEP-NETs comprise a heterogeneous group of rare neoplasias with increasing incidence and prevalence that arise in almost all regions of the GEP tract, although some of them are not very frequent or are associated to the existence of specific syndromes (e.g. MEN1). For this reason, although
the development of GEM models for certain types of GEP-NETS is quite limited, in general terms, there are a wide variety of GEP-NETS GEM models (Capdevila et al. 2017) (Table 2).

The stomach may be the origin of different types of GEP-NETS, which represent about 7–8% of the total (Yang et al. 2018b). The most common of these tumors are related with an abnormal production of gastrin, which could be linked with a chronic H. pylori infection. One of the most typical stomach GEP-NET models is the insulin-gastrin (INS-GAS) mouse, which overexpresses the human gastrin gene under the control of the mouse insulin promoter (Wang et al. 1993). These mice exhibit elevated serum levels of human amidated gastrin and spontaneously develop gastric atrophy, metaplasia, dysplasia and eventually progress to invasive gastric tumors in the corpus by 20 months of age without lymph node invasion or distant metastasis. Because of this, it is considered a valuable model of stomach GEP-NET development in combination with other agents, like H. pylori or H. felis. Strikingly, the genetic ablation of gastrin in the gastrin-deficient mice (Friis-Hansen et al. 1998) also results in the development of spontaneous gastric cancer at few weeks of age (Friis-Hansen 2002), suggesting that any dysregulations of gastrin production may lead to oncogenic growth.

In the case of pancreatic neuroendocrine tumors (panNETs), the first GEM model reproducing this pathology, the so-called RIP-Tag model, was generated in 1985 (Hanahan 1985). Due to the exclusivity of the pancreatic β-cells to synthesize insulin hormone, the authors used the promoter of the insulin gene (Rat Insulin-2 (RIP)) to mediate the expression of the SV40-Tag oncogene (Table 2). This model results in an invasive carcinoma that nicely recapitulates some of the typical panNET characteristics, including β-cell hyperplasia, dysplasia and angiogenesis. The RIP-Tag mouse model is considered a valuable model of panNETs development, and it has been used in numerous preclinical studies to test the potential therapeutic effects of several drugs (Casanovas et al. 2005, Chiu et al. 2010). Furthermore, Hanahan et al. described the role of different promoters to induce the overexpression of the SV40-Tag and, among them, the most commonly used GEM models derived from the RIP-Tag have been RIP1-Tag2 and RIP1-Tag5 (Onrust et al. 1996), which are used as models of angiogenesis and immune response to tumorigenesis, respectively. After this, several groups followed the strategy of the RIP method, to study different panNETs characteristics (Table 2). One of these is the RIP-MyrAkt1 mouse model, which develops a moderate insulinoma and has been used to explore the role of Akt in the β-cell activation. This model demonstrated that Akt1 is a key regulator of pancreatic β-cell functions, for these mice developed β-cell hyperplasia and dysplasia, which progress to insulinomas in older mice (Tuttle et al. 2001, Alliouachene et al. 2008).

Using a similar approach, in 1988, a new model for glucagonomas was published (Table 2). In this case, the SV40-Tag was overexpressed under the control of the glucagon promoter (Glu), so that it was exclusively expressed in pancreatic α-cells (Efrat et al. 1988). Curiously, despite the fact that it could be possible to find expression of Glu-Tag in the central nervous system, it only had the capacity of establish glucagonomas. The hyperplasia of α-cells in Langerhans islets led to the formation of solid tumors in 9–12 months-old mice, which showed several similarities with sporadic human glucagonomas (Rindi et al. 1991). Subsequently, a Glu-Tag mouse model with a more aggressive tumor was also described, but it was not a specific model as they also presented poorly differentiate intestinal neuroendocrine tumors (Lee et al. 1992). In contrast to insulinomas and glucagonomas, there are no well-established GEM models for the study of somatostatinomas.

Although intestine neuroendocrine tumors are one of the most incident GEP-NETS, there are not many GEM models that resemble this group of pathologies, except for colon neuroendocrine tumors (Table 2). Although colon adenocarcinoma is the pathology that more predominantly affects this organ, colon neuroendocrine tumors have also been described and, in this sense, it is worth noticing that the previously described models Glu-Tag and a version of RIP-Tag mice, the RIP-Tag/RIPpyST1, also develop colon neuroendocrine tumors in addition to panNETs. These intestinal tumors, which first appear in submucosal areas, metastasize with high frequency to the lymph nodes and liver. In these mice, preneoplastic mucosal lesions were not observed, indicating that invasiveness is acquired early in the tumorigenic progression of these cells (Grant et al. 1991). Additionally, using a more precise approach, the ITF-Tag mouse model was generated by expressing the SV40-Tag under the control of the intestinal trefoil factor (ITF) promoter, which led to the generation of a colon neuroendocrine tumors model. This caused tumor formation in the proximal colon with remarkable efficiency (multifocal and invasive tumors) (Gum et al. 2004).

Finally, there are different CDX and PDX models of GEP-NETS that could complement the GEM models described earlier. In the case of CDXs, the development of
an esophagus neuroendocrine carcinoma (NEC) cell line, NEC-DUE (Krieg et al. 2014), and a glucagonoma cell line, GLU-Tag cells (Drucker et al. 1994), provided new tools to design and generate preclinical models, based on the initial evidence that establishment of xenografts revealed their validity as model of these rare pathologies. In the case of somatostatinomas, although different studies have isolated cells from different mouse models that develop this type of pathology, like the Tg(Ela-1-SV40E)Br18 mice (Pettengill et al. 1994), the most commonly used models areCDXs with specific cell lines, such as the QGP-1 human panNET cell line (Fraedrich et al. 2012). Furthermore, CDXs developed with some cell lines derived from intestinal neuroendocrine tumors (ileum or rectum), such as KRJ-I or GOT1, have been used to explore the role of different drugs in these pathologies as the response in CDXs has been found to be consistent with the clinical response (Pfragner et al. 1996, Kolby et al. 2001, Nilsson et al. 2004, Stilling et al. 2007). In contrast, the establishment of relevant GEP-NET PDX models has been historically highly problematic (Tanaka et al. 1999, Yang et al. 2016). Nevertheless, some authors have reported the generation of a GEP-NET PDX collection from 106 patients’ tumors (Tanaka et al. 1999, Yang et al. 2016) or the generation of a gastric NEC PDX model, GA0087, which was used to successfully validate the extended survival rate in response to cisplatin relative to untreated controls, which is concordant with clinical results (Jiang et al. 2015).

Pulmonary neuroendocrine tumors

Pulmonary neuroendocrine tumors arise from either individual or clusters of neuroendocrine cells that reside in the lung and often display an active secretory phenotype. They comprise the second most common type of neuroendocrine tumors, accounting for 25% of primary lung tumors. These tumors can be subdivided in four types, depending on their histology, and in three groups, regarding their grade: low-grade or typical carcinoids, intermediate-grade or atypical carcinoids and two high-grade tumors, large cell neuroendocrine carcinoma (LCNEC) and SCLC (Dincer et al. 2015). In this review, we will mainly focus on high-grade tumors, although the few mouse models available for typical or atypical tumors will be briefly commented (Table 2).

In terms of the high-grade SCLCs and LCNECs, due to the fact that they are rarely resected, material from human source is very limited. Therefore, translational research of these pathologies is mainly based in GEM models, which represent an essential tool in this field (Gazdar et al. 2015). These tumors are strongly associated with smoking habits and are characterized by high proliferation and mitotic rates, as well as other aggressiveness-related features. In addition, the development of these tumors is closely related to the inactivation of TP53 and RB1 genes (Gazdar et al. 2017, Pelosi et al. 2017). For this reason, all the GEM models used for the study of high-grade pulmonary neuroendocrine tumors are based in adenoviruses-based conditional rb1 and/or Tp53 double knockout, which have been combined with other knockout models (Gazdar et al. 2015). The first model developed was the Rb1/Tp53 double knockout, which develops both types of high-grade pulmonary neuroendocrine tumors, being the SCLC the most predominant (Meuwissen et al. 2003). However, due to the extended latency of these tumors, the first triple knockout was developed seven years later, which included the Rbl2 mutation (p130 protein). Interestingly, these mice displayed a shorter latency time and presented LCNEC first and SCLC later, which could progress to metastases (Schaffer et al. 2010). These models have been shown to closely recapitulate the typical features of high-grade pulmonary neuroendocrine tumors in human and have been used in the study of the genomic landscape of SCLCs (George et al. 2015), in the identification of the tumorigenic population of SCLC (Jahchan et al. 2016) or to demonstrate the existence of distinct metastatic programs attributable to the cell type of origin (Yang et al. 2018).

Subsequently, additional Rb1/Tp53/Pten triple-knockout models have been generated by the use of different experimental approaches, but in all cases, the pathology of these new models was considerably more complex than the previous models (Table 2). Indeed, these mice developed the tumors and died faster than the previous, but the non-endocrine component of the tumors was also higher, which could be associated to the modification of the driver promoter. These tumors exhibited LCNEC, SCLC and non-SCLC components, with more SCLC constituents in metastases (Cui et al. 2014, McFadden et al. 2014a). Moreover, additional GEM model of pulmonary neuroendocrine tumors have been generated with different genetic modifications. In this case, the co-expression of SV40-Tag and Ascl1 under the constitutive Scybg1a1 promoter generated a model that developed adenocarcinoma with neuroendocrine characteristics and neuroendocrine differentiation in tumors (Linnoila et al. 2000).

In addition to these GEM models, other pulmonary neuroendocrine tumor models based on CDX and PDX approaches have been described in several studies. Indeed, different SCLC-derived cell lines have been used in CDX
models to explore different aspects of SCLC biology. For instance, xenografted NCI-H510A cell line has been used to investigate LSD1 inhibitors as therapeutic strategy (Takagi et al. 2017) or to study the role of miR-450 in the progression of SCLC (Liu et al. 2016). As well, the SCLC cell lines NCI-H146 and NCI-H446 have been used in CDX models to test new therapies (Zhang et al. 2013, Kloos et al. 2015) and xenografted NCI-H1688 cells have been useful in the search of new therapeutic targets for SCLC, as NOTCH1 (Wael et al. 2014). Interestingly, and despite the very limited human material, there are several studies using PDX mice of SCLC. The importance and fidelity of PDX in SCLC has been very recently reported (Drapkin et al. 2018) and, few years ago, Gao et al. used more than 1000 samples from human tumors, including more than 50 from lung cancer, for PDX models, in order to predict drug response in several pathologies, demonstrating that these models are more accurate than CDX (Gao et al. 2015). In fact, these models have been used to evaluate new therapies, as is the case of PARP inhibitors (Lok et al. 2017), DLL3-antibody (Saunders et al. 2015) or the confirmation in a Phase II clinical trial with arsenic trioxide (Owonikoko et al. 2016). In the case of LCNECs, the studies with xenograft models are less numerous and, often, are simultaneous to those of SCLC, such as the DLL3-antibody based study, mentioned above. However, the LCNECs-derived cell line NCI-H810 has also been used in CDX models in order to find novel therapeutic targets, such as TRKß (Odate et al. 2013).

Finally, it is worth noting that there are several works using mouse models for the study of low-grade pulmonary neuroendocrine tumors (Table 2). In two different studies, xenografted mice with NCI-H727 typical tumors cell line were used to explore the role of carbonic anhydrase in these tumors (Mokhtari et al. 2013, Zhou et al. 2015). This cell line, the most used for the study of typical pulmonary neuroendocrine tumors, has been also xenografted for the screening of everolimus effect (Johnbeck et al. 2014), to compare different image techniques (Oxboel et al. 2014), to evaluate the role of certain viruses (Randle et al. 2013) or radiolabeled peptides treatments (Petersen et al. 2012, Wu et al. 2013) or even to test new isotopes, such as neodinium-140 (Severin et al. 2017).

Conclusions

Endocrine and neuroendocrine tumors represent a highly heterogeneous group of neoplasms arising from endocrine and neuroendocrine cells that exhibit a limited incidence. For these reasons, the comprehensive study of the molecular and genetic alterations underlying their development and progression represents a highly elusive task and hinders the discovery of novel biomarkers and therapeutic options. In this scenario, this review reveals that mouse models represent powerful and indispensable tools in basic and translational studies exploring the biology of endocrine and neuroendocrine tumors. Indeed, there is a growing wealth of endocrine and neuroendocrine tumors mouse model systems that continuously expand the toolbox for cancer research. Among them, the CDXs comprise, hitherto, the most commonly used models, but the PDX and GEM models provide the best representation of tumor microenvironments, physiological responses and disease pathology. In addition, GEMs further allow for evaluation of immune system interventions and of responses in situ developed diseases. Therefore, the use of this ample variety of available mouse models, together with the continuous efforts to improve them or to develop new, more sophisticated and valuable models, will drive preclinical studies to a new level of cancer research in the field of endocrine and neuroendocrine tumors.

Declaration of interest

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

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in follicular tumors that progress to poorly differentiated carcinomas.


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